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Protective capacity of gum Arabic, maltodextrin, different starches, and fibers on the bioactive compounds and antioxidant activity of an orange puree (*Citrus sinensis* (L.) Osbeck) against freeze-drying and *in vitro* digestion

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ABSTRACT

Dehydrated fruit puree may be a convenient way to promote the healthy consumption of fruit based foods. Drying carriers, highly used by the food industry to stabilize dried fruit products, may show a potential encapsulating capacity of the biocompounds, that could also limit their bioaccesibility. This study analyzed the impact of gum Arabic (GA), bamboo fiber (BF), native corn starch, starch substituted with octenylsuccinic groups, pea fiber, and maltodextrin on the *in vitro* bioaccessibility of vitamin C (VC), total phenols (TP), and β-carotene, as well as on the antioxidant capacity during the freeze-drying and *in vitro* digestion of an orange puree. Amongst the formulations studied, GA+BF was the most effective for phytochemicals protection of the freeze-dried orange puree during the intestinal stage of digestion, resulting in a higher TP and VC bioaccessibility (59% and 36%, respectively).

Keywords: total phenolic compounds, vitamin C, β-carotene, *in vitro* bioaccessibility

Abbreviations

AA, ascorbic acid; ANOVA, analysis of variance; AOA, antioxidant activity; BC, β-carotene; BF, bamboo fiber; B_x, concentration of each bioactive compound (x) analyzed; DPPH, 2,2-diphenyl-1-picryl-hydrazyl-hydrate; FDP, freeze-dried purees; FRAP, ferric reduction antioxidant power; GA, gum Arabic; GD, gastric digestion; I_eD, external intestinal digest; I_iD, internal intestinal digest; MD, maltodextrin; NCS, native corn starch; O, orange puree; OD, oral digestion; OS, orange's own solutes; OSA, starch modified with octenylsuccinic anhydride; P, purees before being freeze-dried; PF, pea fiber; Tg, glass transition temperature; TP, total phenolic compounds; VC, vitamin C.

1. Introduction

The consumption of fruit is of great significance due to its nutritive and functional properties, which helps in the prevention of different illnesses. Therefore, the reports from the World Health Organization and the Food and Agriculture Organization of the United Nations recommend including a daily minimum consumption of 400 g of fruits and vegetables (WHO, 2020). Citrus fruit is a primary crop worldwide, with a production of around 50 million tons per year, with Brazil, U.S.A., and the Mediterranean countries as the main producers (USDA, 2020). As regards the nutritional characterization, citrus fruit is mainly composed by simple carbohydrates (fructose, sucrose and glucose) and by non-starchy polysaccharides (dietary fiber). With respect to the bioactive compounds present in citrus, their potential contribution to an improvement in people's health is a major research topic. Specifically, vitamin C, phenolic compounds and carotenoids appear to contribute to the prevention of diseases, such as cancer, cardiovascular disease and cataracts. These protective effects seem to be related to their antioxidant activity because of their capacity to prevent the harmful effects of free radicals (Gorinstein et al., 2001).

For the purpose of promoting fruit consumption among the population, new attractive, safe, and healthy fruit products may be developed. The design of food matrices that can regulate the release of fruit bioactive compounds may be useful not only for researchers, but also for food consumers. In this regard, agri-food industry finds it crucial to develop processing technologies able to preserve the nutritional value of the fruit and to ensure the bioaccessibility of its bioactive compounds (Barba et al., 2017). Offering a freeze-dried orange puree to be consumed as a snack could cover all these aspects and represent a feasible option.

Freeze drying is considered to be the best drying technique for heat labile food materials compared to other conventional drying techniques (Hammami et al., 1999). It is a

preferred method for drying foods containing compounds that are thermally sensitive and prone to oxidation since it operates at low temperatures and under high vacuum. In the food industry, it is not only applied to high-value products, such as those intended for mountaineers, astronauts, babies, the military, and sporting activities, but also to those products in which it is particularly necessary to maintain the organoleptic quality, such as coffee, tea, and fruit, among many others. Despite the advantages of obtaining freeze-dried foods, such as the preservation of the flavor, color and nutrients and microbiological stabilization, dried fruits can present problems related to the glass transition of their amorphous matrix, mainly the development of collapse phenomena (Ratti, 2013; Telis and Martínez-Navarrete, 2012). The high content of simple sugars and organic acids in fruits makes the glass transition temperature (Tg) of dried fruits very low, so it is common to find them in a rubbery state at room temperature (Telis and Martínez-Navarrete, 2012). Since the Tg increases with the average molecular weight of the solutes, some biopolymers, such as gums, maltodextrins, or starches, have been used as carriers for the stabilization of dried products (Telis and Martínez-Navarrete, 2012). In addition, some other biopolymers have been described to act as fillers, with a steric role avoiding the formation of interparticle bridges in the food matrix, thus also delaying structural collapse (Silva-Espinoza et al., 2020a).

In this particular study, gum Arabic (GA), maltodextrin (MD), starch modified with octenylsuccinic groups (OSA), bamboo fiber (BF), pea fiber (PF) and native corn starch (NCS) have been used to provide stability to the freeze-dried orange puree. GA, MD, and OSA have been used as to increase the Tg while BF, PF and NCS as fillers. All these biopolymers are widely used in the food industry. GA, MD, and OSA have been applied as thickeners, stabilizers, emulsifiers and flavor encapsulants of confectionary and different beverages (Agama-Acevedo & Bello-Perez, 2017; Kennedy et al., 2012;

Williams & Phillips, 2021). The selection of fibers responds to the growing interest of the food industry in increasing the fiber content of foods, as numerous studies have demonstrated the beneficial effects of its consumption in protecting against heart related disease and cancer, fat lipid regulation, regulation of glucose absorption and insulin secretion and prevention of intestinal diseases (Mckee & Latner, 2000). In this regard, bread and breakfast cereals have been enriched with PF (Vetter, 1984), while BF is a common ingredient in breakfast cereals, pasta, cheeses, sauces, mustards, ketchup, beverages, fruit juices, snacks, frozen desserts, and pastries (Chongtham et al., 2011). For its part, NCS contributes to stabilize food, helps emulsification, and improves texture (Luallen, 1985).

On the other hand, different biopolymers have been described to act as encapsulating agents, helping to prevent the degradation of bioactive compounds. The encapsulated compounds maintain their functional activities during the food processes and storage (Rascón et al., 2011). Additionally, one of the objectives of encapsulation is to protect the bioactive compounds from the drastic conditions of the gastrointestinal tract (Hu et al., 2017). The encapsulating agents should also allow a controlled release of the compounds, so they can be bioaccessible and, therefore, potentially bioavailable. In this sense, the nature and functionality of the biopolymers may affect, to a greater or lesser extent, the release and absorption of these compounds by the organism. Bioaccessibility can be defined as the fraction of an ingested biocomponent released from the food matrix during digestion becoming potentially accessible for absorption into the mucous membrane (Dima et al., 2020; Minekus et al., 2014). Bioaccessibility can be measured *in vitro* by simulating gastrointestinal digestion steps, through a series of treatments using characteristic enzymes from each digestion step and adjusting the temperature and pH conditions. *In vitro* digestion models are widely used in food and nutritional sciences for

the purposes of predicting compound bioaccessibility because they offer several advantages with respect to the *in vivo* models: they are relatively inexpensive, simple and faster, and, what is more, they present no ethical restrictions, the conditions can be controlled, sampling is easy and the results are reproducible. Furthermore, the evaluation of bioaccessibility using this type of model is well correlated with the data obtained in animal and human studies (Minekus et al., 2014).

In the interest of offering a healthy fruit product, the efficacy of different biopolymers used to stabilize a freeze-dried orange puree, on the protection of its bioactive compounds and antioxidant activity through the freeze-drying and the different stages of *in vitro* gastrointestinal digestion was studied. As the different chemical and functional nature of the biopolymer added to food may condition the degree of release of bioactive compounds from the food matrix into the body, the main objective was to evaluate the bioaccessibility of vitamin C, β-carotene, and total phenolic compounds of the freeze-dried orange puree.

2. Material and methods

2.1. Raw material

Oranges (*Citrus x sinensis* (L.) Osbeck var. Lane late) were obtained in a local supermarket in the city of Valencia (Spain). Their selection was carried out by visual inspection based on homogeneity of size, color, and good physical integrity (no external damage). The biopolymers used as carriers were gum Arabic (GA, Scharlab, Sentmenat, Spain), pea fiber (PF, Roquette, Lestrem, France), bamboo fiber (BF, VITACEL®, Rosenberg, Germany), starch modified with octenylsuccinic anhydride (OSA, Roquette, Lestrem, France), maltodextrin (MD, Roquette, Lestrem, France) and native corn starch (NCS, Roquette, Lestrem, France).

2.2. Chemicals and Reagents

All the enzymes used throughout the *in vitro* digestion, α-amylase from porcine pancreas (Type VI-B 12 units/mg solid), pepsin from porcine gastric mucosa (≥400 units/mg protein), and pancreatin from porcine pancreas (4 x U.S. Pharmacopeia specifications), also as the gallic acid used for analyzing the total phenolic compounds, the DL-dithiothreitol reagent for the vitamin C analysis and the Trolox used for the antioxidant activity, were obtained from Sigma-Aldrich (Saint Louis, MO, USA).

The β-carotene used for the calibration curve for the β-carotene analysis was obtained from Dr. Ehrenstorfer (Augsburg, Germany).

The solvents, such as methanol, acetone, ethanol and hexane for the extraction of the bioactive compounds, were obtained from VRW International (Barcelona, Spain).

L (+) ascorbic acid, used for the calibration curve for the vitamin C analysis, was obtained from Scharlau SL (Sentmenat, Spain).

2.3. Sample preparation and freeze-drying conditions

The oranges were peeled and cut and the pulp was triturated in a bench top electrical food processor for 40 s at 2000 rpm followed by 40 s at 9100 rpm (Thermomix TM 21, Vorwerk, Madrid, Spain), obtaining an orange puree (O). Different mixtures of biopolymers were added to the O and mixed for 10 min at 1000 rpm using the same Thermomix to obtain a homogeneous blend. In this way, five different puree samples (P) were considered, as shown in Table 1, before the freeze-drying process. The ratio puree:Tg modifier:filler was selected to obtain freeze-dried fruit products with adequate physical and functional quality as proposed by Agudelo et al., (2017) and Silva-Espinoza et al., (2020a).

For the freeze-drying, each of the five P samples was distributed (0.5 cm thickness) on aluminum plates of 10.5 × 7.8 cm and frozen (Liebherr Mediline LGT 2325, Baden-

Wurtemberg, Germany) for 7 days at -45ºC. The frozen samples were dried (Telstar Lyo Quest-55-, Telstar, Terrassa, Spain) at 5 Pa, -48ºC in the condenser and at 40ºC on the shelves of the chamber for 20 h to obtain the corresponding freeze-dried orange puree samples (FDP). The final water content (x_w, g water/ 100 g product) of each sample before being freeze-dried was determined by drying in a vacuum oven (Vaciotem, J.P. Selecta) at 60 °C ± 1 °C, under pressure < 100 mmHg until constant weight (AOAC 2000, method 934.06). The x_w of each freeze-dried sample was determined by using an automatic Karl Fisher titrator (Mettler Toledo, Compact Coulometric Titrator C10S, Worthington, OH, USA).

2.4. *In vitro* digestion

The *in vitro* digestion of the samples was performed to imitate the human physiological digestion conditions during oral, gastric, and intestinal steps. The methodology proposed by Miller et al. (1981), adapted to include the oral step described by Huang et al. (2014), was followed. Three replicates of each digestion stage per sample were carried out. The steps of the *in vitro* digestion are summarized in Fig. S1 (Supplementary Material). In brief, for the oral step, 120 mL of each P or 12 g of the corresponding FDP samples + 120 mL water, were mixed with 250 µL of a α-amylase/$CaCl_2$ solution (130 mg α-amylase /100 mL $CaCl_2$ 1 mM, pH 7) per gram of solid. Samples were incubated at 37 °C in glasses with a thermostatic jacket (Vidrafoc, Valencia, Spain) for 10 min at 200 rpm (C-MAG HS 7, Ika Labortechnik, Staufen, Germany). Once the oral step was finished, 100 mL of oral digest was removed for the next gastric step and the remaining 20 mL were stored in sterile vessels and frozen at -45 °C for the subsequent analysis of the different bioactive compounds and antioxidant capacity. The samples analyzed after the oral digestion step were coded as OD.

For the gastric step, the pH of the oral digest was adjusted to 2 with 2M HCl. Next, 0.1 g of pepsin of porcine origin (40,000 units) was added to 100 mL of the gastric digest and incubated for 2 h at 37 ºC under continuous shaking at 200 rpm (C-MAG HS 7, Ika Labortechnik, Staufen, Germany). Once finished, 20 mL of the sample was used for the intestinal step and the remaining amount was stored in sterile vessels and frozen at -45ºC for the subsequent analysis. The analyzed samples after gastric digestion were coded as GD.

Finally, a dialysis membrane with a pore size of 14000 D, filled with 25 mL of 0.5N $NaHCO_3$ was used to simulate intestinal digestion. The sample from the gastric step was dialyzed under agitation (Ovan, Barcelona, Spain) at 37ºC (J.P. Selecta, S.A., Barcelona, Spain). Once the sample reached pH 5, a volume of 5 mL of a mixture of pancreatin (4 g/L) and bile extract (25 g/L) in 0.1N $NaHCO_3$ was added to 20 mL of the gastric digest and incubation was continued for 2 h until pH 7.5 was reached. After the intestinal step, two fractions were collected: the external and internal parts of the dialysis membrane. The external content of the dialysis tube was considered to be the part of the digesta that reached the colon, while the internal dialysis membrane contained the compounds capable of crossing the membrane, which was considered as the bioaccessible fraction. Both were stored in different sterile vessels at -45ºC to inactivate the enzymes and to be analyzed. The external intestinal digest was coded as I_eD and the *in vitro* bioaccessibility of the different bioactive compounds was calculated from the internal intestinal digest (I_iD) following Eq. 1 (Rodríguez-Roque et al., 2013a).

$$\text{Bioaccesibility}(\%) = \frac{[B_x]\,(I_iD)}{[B_x]\,(P\ or\ FDP)} \text{ x } 100 \qquad \text{(Eq. 1)}$$

Where, for each sample, P is the puree sample before being freeze-dried and FDP is each of the freeze-dried P. [B_x] is the concentration of each bioactive compound (x) analyzed; I_iD is the internal intestinal digest.

To evaluate the impact of the different digestion steps on the different bioactive compounds and antioxidant activity, the results were expressed as the ratio of each compound present after each digestion step referred to that present in the corresponding P or FDP samples (Table 2), following Eq. 2.

$$\text{Ratio} = \frac{[B_x]\ (\text{OD, GD, } I_eD)}{[B_x]\ (\text{P or FDP})} \quad \text{(Eq. 2)}$$

Where [B_x] is the concentration of each bioactive compound (x) in the oral (OD), gastric (GD), or external intestinal digests (I_eD) related to that present in the puree samples before and after freeze-drying (P and FDP, respectively).

2.5. Analytical determinations

The total phenolic compounds (TP), vitamin C (VC), β-carotene (BC) and antioxidant activity (AOA) of each of the five formulations (Table 1), both P and FDP, were analyzed, as well as after each different step of the *in vitro* digestion. The analysis were carried out as described below for every sample, except for digests which previously were centrifuged at 11,515× g at 20 °C for 10 min (GYROZEN Co., 1236R, GYROZEN, Daejeon, Korea) and the supernatant was filtered by a 45 μm nylon filter before each analysis. Since the samples evaluated had both different water contents and quantities of added biopolymers, the results were referred to the orange's own solutes (OS) as calculated by Agudelo et al. (2017).

The extraction of TP was carried out by mixing 3 g of each P, 0.6 g of each FDP sample or 1-4 g of the corresponding digests (OD, GD, I_eD, I_iD), with 9 mL of methanol: water (70:30 v/v) solution using a magnetic multi-stirrer at 200 rpm (JEIO TECH Lab Companion MS-51M, JEIO TECH Lab Companion, Seoul, Korea), in darkness and at room temperature for 30 min. The extraction beakers were sealed with parafilm (PM-996, Parafilm® M, Bemis Company Inc., Wisconsin, USA) to avoid volatilizations. The homogenates were centrifuged at 11515×g at 4 °C for 10 min (GYROZEN Co., 1236R, GYROZEN, Daejeon, Korea). The upper layer was collected and TP was analyzed using the Folin–Ciocalteu method, which was adapted from Benzie et al. (1999) with some modifications (Igual et al., 2016). The TP content was calculated as mg of gallic acid equivalents (GAE)/100 g_{OS} using a standard curve of gallic acid in the range of 0–1000 ppm.

The BC determination was carried out by spectrophotometry according to Silva-Espinoza et al., (2020b) for extraction and the method of AOAC (2000) for quantification. First, the extraction was performed under the same conditions as for TP but using a solution of hexane: acetone: ethanol (50:25:25, v/v) as solvent extractor. The absorbance was measured at 446 nm (spectrophotometer V-1200 VWR, VWR, Radnor, PA, USA). The BC was calculated as mg BC/100 g_{OS} using a β-carotene calibration curve in the range of 0.5–7 ppm.

The determination of VC consisted of the reduction of dehydroascorbic acid to ascorbic acid (AA) by means of DL-dithiothreitol, according to Sánchez-Moreno et al. (2003) and a subsequent high-performance liquid chromatography determination, according to Xu et al. (2008). The conditions were: Kromaphase100-C18, 5 mm (4.6 × 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. VC content was calculated as mg AA/100 g_{OS}. A standard solution of L (+) ascorbic acid (in the range of 5–200 ppm) was prepared.

The AOA was determined in the extracts obtained for the TP determination. Two complementary assays, FRAP and DPPH, were used. The results for both methods were converted to mmol Trolox equivalent (TE)/100 g_{OS} using a calibration curve of Trolox of 0-250 ppm. All the measurements were taken in a UV-visible spectrophotometer (V-1200 VWR, VWR, Radnor, PA, USA).

The FRAP assay was carried out as described by Benzie et al. (1999) and the DPPH scavenging capacity assay was carried out following Brand-Williams et al. (1995), with minor modifications. For this analysis, absorbance was recorded at 515 nm at initial time ($A_{control}$) and 15 min later (A_{sample}), when the reaction had reached the steady state. The percentage of DPPH was calculated following Eq. 3.

$$\%\text{DPPH} = \frac{(A_{control} - A_{sample})}{A_{control}} \times 100 \qquad \text{(Eq. 3)}$$

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. Effect of freeze-drying on the bioactive compounds and antioxidant activity

In order to characterize the samples, the water content before and after being freeze-dried was analyzed (Table 1). The effect freeze-drying had on each analyzed compound and on the antioxidant activity of each sample appears in Table 2. The results revealed a high VC and TP stability after freeze-drying. The fact that the FDP samples had higher values than the P can be justified by assuming an easier extraction of the compounds in the freeze-dried products, related to their higher porosity (Michalczyk et al., 2009).

As regards VC in particular, O was the only sample where a loss in VC was observed, indicating the encapsulating role of the biopolymers added to the puree, especially when either of the fibers (BF or PF) were used. This protective effect was also found by Agudelo et al. (2017), who indicated a slightly better retention of VC in grapefruit powder obtained by spray-drying when BF was added to the formulation. In the case of TP, it was PF which seemed to promote this protective effect, to a greater extent when it was combined with MD. The worst affected compound was BC with losses in the range of 58-33% for every sample, except for OSA+PF, whose loss was only of 18%. The amphiphilic character of OSA (Sweedman et al., 2013) may have created lipidic unions with BC, developing a protective barrier against degradation. In general, carotenoids have been found to be susceptible during and after the freeze-drying process due to their high sensitivity to oxygen, even when the oxygen content is minimum when working at low pressure (Silva-Espinoza et al., 2020b).

The AOA of fruit depends on the concentration of antioxidant compounds, such as vitamins, phenolic compounds, and carotenoids. Different studies indicate that the AOA of citrus is mainly due to its hydrophilic fraction, such as VC and some phenols (Rodríguez-Roque et al., 2013a). Since this activity is due to synergistic reactions between different compounds, the use of more than one method is recommended to correctly measure that activity. Nevertheless, the AOA results can only be compared for

the same method since the antioxidant mechanism studied is different (Pérez-Jiménez et al., 2008). Therefore, the FRAP test is based on the power of a substance to reduce Fe^{3+} to Fe^{2+}, while the DPPH assay studies the availability of a substance to reduce the DPPH˙ radical. The ferric reducing capacity of samples was not significantly affected ($p>0.05$) by the freeze-drying (Table 2). The high degree of preservation of TP, and especially of VC, exhibited by every formulation after freeze-drying may be responsible for the high AOA, since a close correlation of VC and TP with the AOA determined by the FRAP assay on orange juice has been found (Gardner et al., 2000). The ability of both many phenolic compounds and VC to donate hydrogen atoms from the hydroxyl groups in their ring structures is related to their reducing capacity (Scott, 1997). However, the freeze-drying process was observed to have a significant impact ($p<0.05$) on the antioxidant activity of every samples when measured by DPPH assay. The decrease in the DPPH˙ free radical scavenging activity after freeze-drying may be related mostly to the loss in BC, among other bioactive compounds present in oranges not evaluated in this study. In this sense, carotenoids such as β-carotene, cryptoxanthin, lutein or zeaxanthin, present in oranges, have demonstrated a scavenging capacity of the DPPH˙ free radical (Liu et al., 2008) but no similar association was found between carotenoids and FRAP (Gardner et al. 2000). Of the formulations, GA+BF sample was the one that best preserved the antioxidant activity determined by DPPH (77% retention).

3.2. Effect of *in vitro* digestion on the bioactive compounds and antioxidant activity

Figures 1 and 2 show the *in vitro* bioaccessibility (Eq. 1) and the Ratios (Eq. 2) of TP and VC, respectively, for both the P and FDP samples of each formulation.

As can be observed in Figure 1, the oral and gastric steps did not have a remarkable impact on the TP content for either the P or FDP products, all the formulations showing Ratio

values near 1. These results coincide with other studies in which different phenolic compounds present in foods demonstrated their stability under gastric conditions (Rodríguez-Roque et al., 2014). This stability results from the short time of the oral digestion, as the polyphenol degradation is usually a time-dependent process. Moreover, it has also been described that an acid pH during the gastric step protects polyphenols against degradation (Pineda-Vadillo et al., 2017). Oscillations observed in the TP stability of the different samples may be due to the interaction of the biopolymer/matrix/digestion conditions. The increases could be due to the pH of the stomach and the effect of enzymes, which break down structures and release phenolic compounds. Furthermore, depending on their nature, gastric conditions can favor or protect these compounds from chemical or enzymatic oxidation. In this sense, it has been described how hesperidin, quercetin or catechin decrease during gastric digestion while naringenin and routine increase (Rodríguez-Roque et al., 2013a). In this case, in which every sample contained orange, the different effect that was observed could be due to small variations in the protective capacity of the biopolymers used in each formulation. Our results point to a higher compound degradation at intestinal level. Furthermore, some differences were found to be dependent on the sample matrix. In this sense, a slightly higher Ratio was observed in FDP than P, which may indicate a lower degree of degradation of the dried matrix in that digestion step. The alkaline pH of the intestinal phase causes phenolic compounds to undergo different chemical reactions, mainly oxidation and polymerization, favoring the formation of other derived phenolic compounds (such as chalcones) that cannot be absorbed due to their low solubility and high molecular weight (Rodríguez-Roque et al., 2013a). Also, it has been described that the interactions between phenolic compounds and other orange compounds (minerals, fiber) may favor the formation of complexes incapable of crossing the dialysis membrane (Rodríguez-Roque et al., 2013b). However,

this does not mean that the ingested phenols remaining in the external intestinal fraction have no role in health protection, as these compounds, if they are not absorbed in the small intestine, can reach the large intestine, where they can be transformed and/or degraded by the colon microbiota.

VC can be observed to follow the same trend as TP (Figure 2) as concerns oral and gastric digestion, which did not have a great effect on the VC, with the exception of OSA+PF in the case of the FDP sample. Other authors also reported that oral and gastric steps had a mild effect on VC (Rodríguez-Roque et al., 2013a). The acid conditions of the gastric phase protect the VC against its chemical and enzymatic oxidation: the ascorbic acid molecule is protonated at low pH which protects it from the attachment of oxygen. The lower Ratio observed in both digestion steps of OSA+PF FDP sample as compared with the other FDP samples suggests a high encapsulation efficiency of OSA as commented by other authors (Agama-Acevedo & Bello-Perez, 2017), that hampers the extraction of VC. Given the amphiphilic behavior of OSA, the encapsulation promoted by the hydrophilic interaction between both OSA and VC could have promoted the flocculation or coalescence among the hydrophobic binding sites of OSA. This effect of OSA+PF in FDP sample was not observed for TP in both the oral and gastric steps (Fig. 1), since TP have both hydrophilic and hydrophobic groups (Khoddami et al., 2013), that could form a more stable emulsion with OSA. This fact may result in a better sample dispersion in the aqueous phase characteristic of our gastrointestinal tract, allowing the interaction with the enzymes and the release of TP. Samples were observed to demonstrate greater variability in the Ratio of the intestinal phase (Figure 2), which showed the instability of VC under intestinal conditions, such as the alkaline pH and the action of the enzymes of this step. In this sense, Jeney-Nagymate and Fodor (2008) observed that the ascorbic acid concentration fell when the pH was >4. In addition, Ball (2006) reported that vitamin C

oxidation in the gastrointestinal tract occurs due to its prooxidant behavior, maintaining the reduced state of other nutrients, such as iron. This author also related the VC degradation to the formation of metal-oxygen-ascorbate complexes.

As far as the bioaccessibility is concerned, what is remarkable is the ready bioaccessibility of TP for every sample, ranging from 39-59%, as compared with similar studies that offer results of 12-30% for fruit-based foods (Buniowska et al., 2017; Quan et al., 2020; Rodríguez-Roque et al., 2013a). The high bioaccessibility of the TP may be related to the antioxidant function of VC in the stabilization and protection of polyphenolic compounds from auto oxidation at alkaline pH (Green et al., 2007). In any respect, the differences among studies may generally result from the effect of the food matrix and also from the different experimental conditions applied. When comparing all samples, FDP GA+BF showed a greater bioaccessibility of TP ($p<0.05$, Fig. 1), which may be related to an increased protective effect of this biopolymer mixture at this digestion step. In this sense, some authors observed a more efficient TP encapsulation in food powders when GA was added to the formulation rather than using just maltodextrin (Dag et al., 2017). Zhang et al. (2020) also observed a better bioaccessibility of TP in fruit powder as the GA content increased as compared with MD, which implied that GA could make more contribution than MD for bioactive protection during the digestion process.

As regards VC bioaccessibility, values in the range of 10-37% were obtained, similar to data shown by Rodríguez-Roque et al. (2013a, 2014) for fruit juice products. The P samples containing MD showed greater bioaccessibility. However, when they were freeze-dried, their bioaccessibility worsened. As already stated, the food matrix significantly affects the release of the bioactive compounds during digestion (Donhowe et al., 2014). MD is known to confer high levels of viscosity to solutions (P sample) or even form a hydrogel. In this way, the viscous foods may presumably play a steric role,

avoiding contact between VC and the medium (enzymes and pH), as also suggested by Donhowe et al. (2014). That effect may prevent their structural degradation in the small intestine, promoting greater bioaccessibility. Otherwise, when this sample is dried, there is no effect of the maltodextrin on the viscosity, so VC is more exposed to the drastic digestion conditions, lowering its bioaccessibility. As it was observed with TP, the FDP GA+BF sample also presented great VC bioaccessibility, suggesting these biopolymers exerted an efficient VC encapsulation effect than the other ones. The high encapsulation efficiency of this formulation could be attributed to the good emulsifying properties of GA due to the polypeptide glycated on the saccharide chain, and good solubility over a wide range of pH (Batalha et al., 2010). As already mentioned, in addition to the fact that GA is widely used, these properties may be of interest to industry for the design of functional foods that enhance the TP and VC bioaccessibility and so its potential availability. The lowest bioaccessibility of VC was shown by FDP OSA+PF sample (10%, Fig. 2) which may be due to the less stable emulsion when encapsulating the VC due to the hydrophilic nature of VC as commented above, hampering the release and therefore, the potential absorption of VC.

BC was not detected in the oral, gastric, or dialyzed fractions. It has been reported that the α-amylase and the pepsin affect the structure of lipophilic molecules as BC, resulting in a greater aggregation (Mun & McClements, 2017; Nik et al., 2010). This could hamper the extraction of this biocompound for its analysis. In addition, some authors also observed the instability of carotenoids under the acid conditions of gastric digestion, mainly undergoing oxidation reactions and structural changes (Rodríguez-Roque et al., 2013a). As regard the no detection of BC in the dialyzed fraction, it may be due to its low concentration, being below the detection limit of the analytical method used for its quantification. Thus, just a small fraction of carotenoids would be bioaccessible. Their

lipidic nature makes their dispersion in the aqueous medium of the digestive tract difficult. Furthermore, their absorption is also hampered by the fact that they are substances that have a limited capacity of both release from the food matrix and solubilization (Fernández-García et al., 2009). It has also been reported that the soluble fiber present in plant-based foods may decrease the carotenoids bioaccessibility by interacting with the bile and thus diminishing the micelle formation, or it can interfere the contact of micelle with intestinal mucosal cells by increasing the viscosity of the intestinal contents (Priyadarshani, 2017).

The BC after the intestinal digestion step (the external part of the dialysis membrane) ranged from 30-57 to 6-28 mg BC/ 100 g_{OS} for P and FDP, respectively. The fact that the BC content after the intestinal step was lower in FDP samples than in P may be related to the effect of the freeze-drying process which, as previously mentioned, reduced BC (Table 1). The highest BC content was exhibited in samples with maltodextrin, especially when combined with pea fiber (57 and 28 mg BC/ 100 g_{OS} for P and FDP, respectively), and the lowest in O (30 and 6 mg BC/ 100 g_{OS} for P and FDP, respectively). In the intestinal step, pancreatic and bile enzymes help to emulsify fat-soluble substances, facilitating their solubilization and subsequent absorption in the large intestine.

The results of AOA assessed by FRAP and DPPH methodologies are shown in Figures 3 and 4, respectively. AOA exhibited less stability under oral and gastric conditions than that observed in VC and TP, especially among P analyzed by DPPH assay. Of P, those samples containing maltodextrin had a higher Ratio of AOA measured by FRAP in these digestion steps. Slight differences were observed when comparing FDP with the corresponding P samples, except in the case of OSA+PF (Fig. 3). For DPPH scavenging activity, FDP showed higher values than P (Fig. 4), which pointed to the protective effect of biopolymers on the compounds responsible for the DPPH free-radical scavenging

activity when the digestion is carried out in the dried matrix. In this case, biopolymers would not have that favorable response in the FRAP reducing test.

The general decrease in the AOA in the intestinal step may be due to the action, already commented on, of the enzymes and pH present in this digestion step on the chemical structures of some bioactive compounds (Bouayed et al., 2011). In this sense, the change in some chemical structures and/or the formation of complexes with other substances present in the sample could lead to a decrease, not only in the concentration, but also in the AOA exerted by these compounds.

Since the bioaccessibility concept is only applicable for compounds, there is no bioaccessibility for AOA. But if the same equation (Eq. 1) is used for AOA, an estimation may be made of the % of AOA provided by each product that could finally exert its effect at body level (Figures 3 and 4). From the FRAP test results, it was remarkable that only the O samples (formulated without biopolymers) showed a significant decrease in the % of AOA that finally had an effective role on the organism ($p<0.05$) when the samples were freeze-dried (Figure 3). This confirmed the protective effect of the biopolymers added to the orange puree on the different biocompounds with antioxidant reducing activity throughout the freeze-drying process. GA+BF and OSA+PF were the combinations with the greatest protective effect (Fig. 3). The compounds that provided antioxidant reducing activity were more readily absorbed than those that provided free radical scavenging, since the DPPH values were lower than the FRAP in every case. As regards the DPPH values (Fig. 4), FDP OSA+PF was significantly ($p<0.05$) higher, which may be linked to the effectiveness of OSA on the encapsulation of the bioactive compounds with free radical scavenging activity during the freeze-drying process. This greater OSA+PF encapsulation capacity may be related to the ease with which it joins and facilitates the absorption of lipophilic compounds, such as carotenoids with $DPPH^{\cdot}$

free radical scavenging capacity, as commented in section 3.1. Moreover, the absorption of other lipophilic antioxidant compounds present in orange, such as vitamin E (Jeney-Nagymate and Fodor, 2008), not analyzed in this study, could also contribute to this result.

4. Conclusions

Any of the studied biopolymer mix added to the orange puree helped to obtain a freeze-dried orange puree with high vitamin C and total phenolic content, just being the sample with modified starch and pea fiber the mix that enhanced the protection of the more labile β-carotene. Vitamin C and total phenolic compounds also showed high stability against oral and gastric stages of digestion, being more sensitive to the conditions of the intestinal stage, especially vitamin C. The bioaccessibility results lead to the conclusion that the sample with gum Arabic and bamboo fiber provided a clear protective effect of vitamin C and total phenolic compounds in the freeze-dried sample. This indicator increased to 36 and 59 %, respectively, which guarantees an excellent nutritional contribution of consuming fruit in this format. In this regard, the actual study provides useful approaches for agri-food industries seeking to develop innovative and added nutritional value fruit-based products.

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Figure captions

Figure 1. Ratio value (Eq. 2) between total phenolic content after the oral (OD), gastric (GD) and intestinal (I_eD) digestion and that of the non-digested sample, for each formulation before (P) and after being freeze-dried (FDP) (nomenclature according to Table 1). Percentages indicate the bioaccessibility (Eq. 1). Different letters (a,b,c) indicate different homogeneous groups established by Tukey HSD ANOVA for the bioaccessibility.

Figure 2. Ratio value (Eq. 2) between vitamin C content after the oral (OD), gastric (GD) and intestinal (I_eD) digestion and that of the non-digested sample, for each formulation before (P) and after being freeze-dried (FDP) (nomenclature according to Table 1). Percentages indicate the bioaccessibility (Eq. 1). Different letters (a,b,c,d) indicate different homogeneous groups established by Tukey HSD ANOVA for the bioaccessibility.

Figure 3. Ratio value (Eq. 2) between FRAP values after the oral (OD), gastric (GD) and external (I_eD) digestion and that of the non-digested sample, for each formulation before (P) and after being freeze-dried (FDP) (nomenclature according to Table 1). Estimation of % of antioxidant activity preserved in the small intestine of each sample is showed at the top of the figure. Different letters (a,b,c) indicate different homogeneous groups established by Tukey HSD ANOVA for that fraction.

Figure 4. Ratio value (Eq. 2) between DPPH values after the oral (OD), gastric (GD) and external (I_eD) digestion and that of the non-digested sample, for each formulation before (P) and after being freeze-dried (FDP) (nomenclature according to Table 1). Estimation of % of antioxidant activity preserved in the small intestine of each sample is showed at the top of the figure. Different letters (a,b,c) indicate different homogeneous groups established by Tukey HSD ANOVA for that fraction.

Figure S1. *In vitro* digestion protocol.

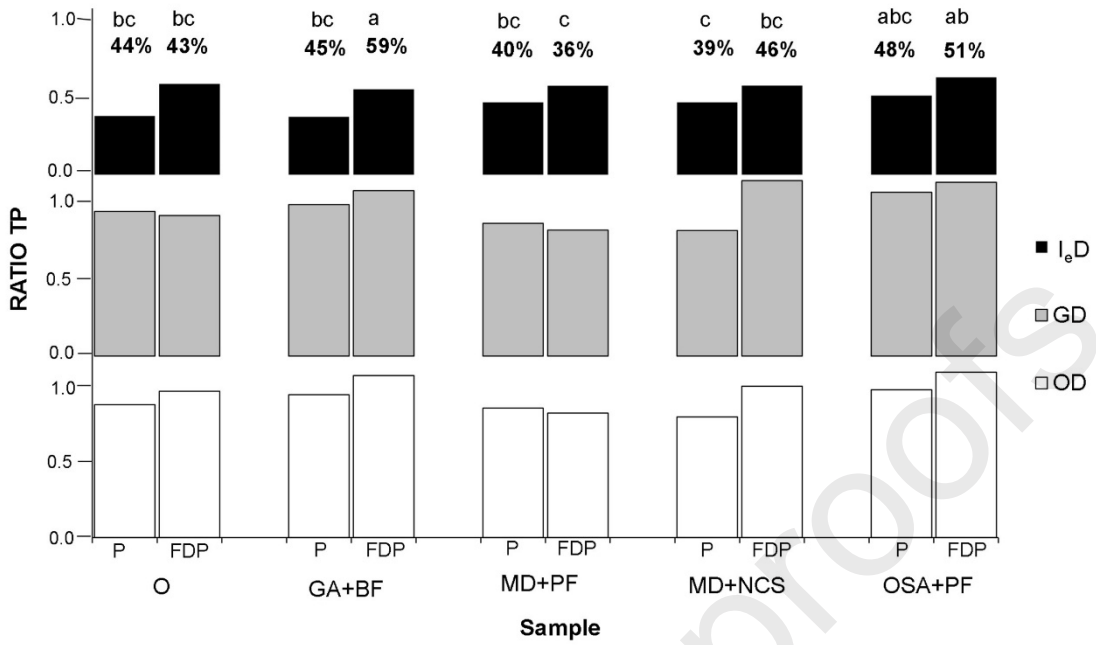
Table 1. Biopolymers added to the orange puree, codes assigned to the orange puree samples, and water content of each sample.

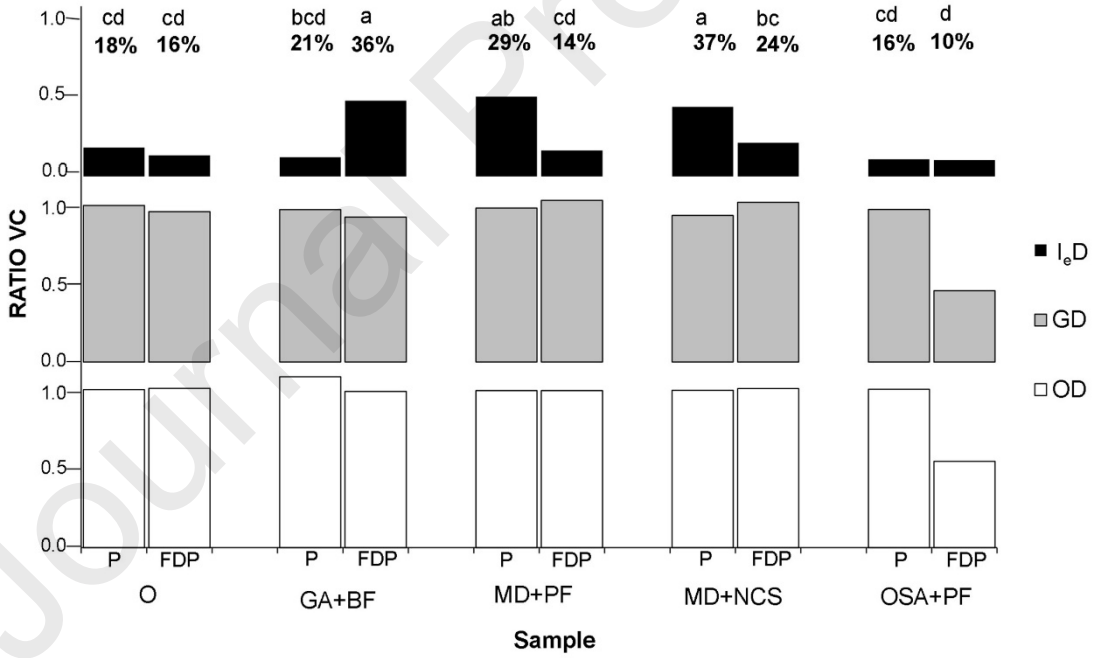
Sample	Biopolymers concentration		Water content (g water/ 100 g sample)	
	5g/100 g orange puree	1g/100 g orange puree	Puree sample (P)	Freeze-dried puree sample (FDP)
O	-	-	88.15	2.60
GA+BF	Gum Arabic	Bamboo fiber	83.37	2.35
MD+PF	Maltodextrin	Pea fiber	81.97	2.00
MD+NCS	Maltodextrin	Native corn starch	82.16	2.17
OSA+PF	Starch modified with octenylsuccinic anhydride	Pea fiber	82.26	2.05

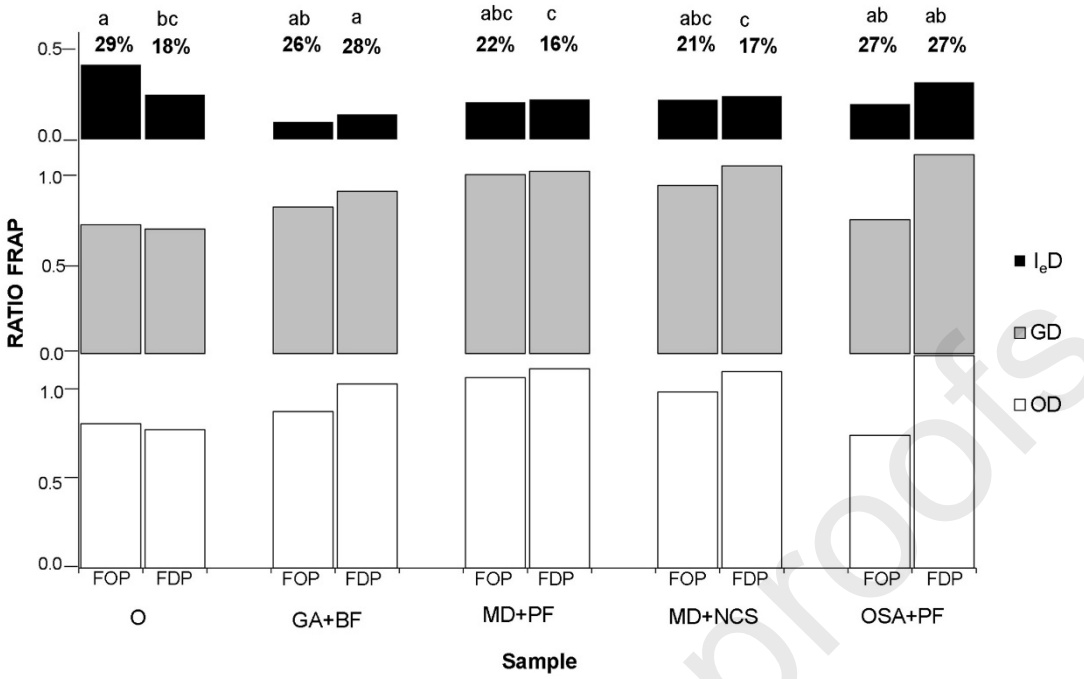
Table 2. Values (mean ± standard deviation) of each bioactive compound and the antioxidant activity of both the orange puree (O) and those formulated with gum Arabic and bamboo fiber (GA+BF); maltodextrin and pea fiber (MD+PF); maltodextrin and native corn starch (MD+NCS) and starch modified with octenylsuccinic anhydride and pea fiber (OSA+PF), before and after being freeze-dried (P and FDP, respectively).

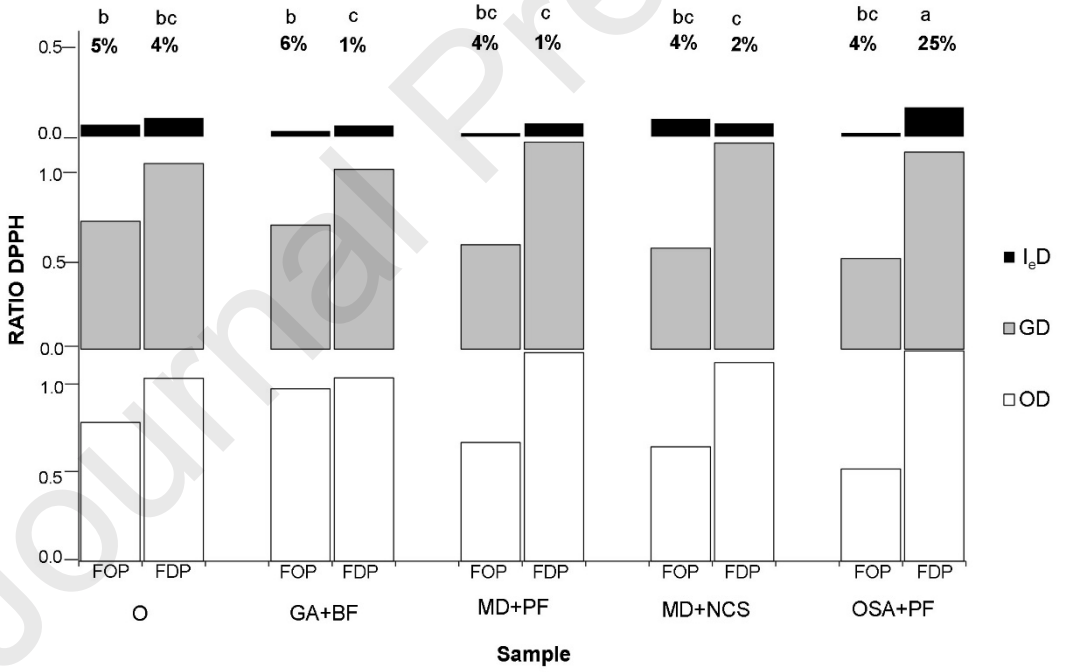
Sample		TP[1] (mg GAE/100 g_{OS})	VC[2] (mg AA/100 g_{OS})	BC[3] (mg BC/100 g_{OS})	FRAP[4] (mmol TE/100 g_{OS})	DPPH[4] (mmol TE/100 g_{OS})
O	P	638±10^{a}	503.9±0.9^{a}	58±6^{a}	5.4±0.2^{a}	3.2±0.2^{a}
	FDP	565±13^{b}	499.1±0.9^{b}	39±3^{b}	5.4±0.2^{a}	2.150±0.008^{b}
GA+BF	P	762±34^{a}	515.8±0.3^{b}	55.2±0.5^{a}	5.5±0.2^{a}	2.91±0.11^{a}
	FDP	611±46^{b}	531±9^{a}	37±4^{b}	5.4±0.6^{a}	2.26±0.04^{b}
MD+PF	P	548±14^{b}	513±8^{b}	70±4^{a}	4.6±0.2^{a}	3.49±0.03^{a}
	FDP	611±14^{a}	531±6^{a}	30±3^{b}	4.4±0.3^{a}	2.30±0.09^{b}
MD+NCS	P	563±6^{a}	498±11^{a}	68.3±1.8^{a}	4.6±0.3^{a}	3.7±0.3^{a}
	FDP	575±15^{a}	495±15^{a}	29±3^{b}	4.2±0.2^{a}	2.29±0.06^{b}
OSA+PF	P	593±17^{a}	512±5^{b}	40±2^{a}	5.1±0.2^{a}	4.35±0.16^{a}
	FDP	628±23^{a}	531±7^{a}	33.3±1.2^{b}	4.3±0.2^{b}	2.29±0.13^{b}

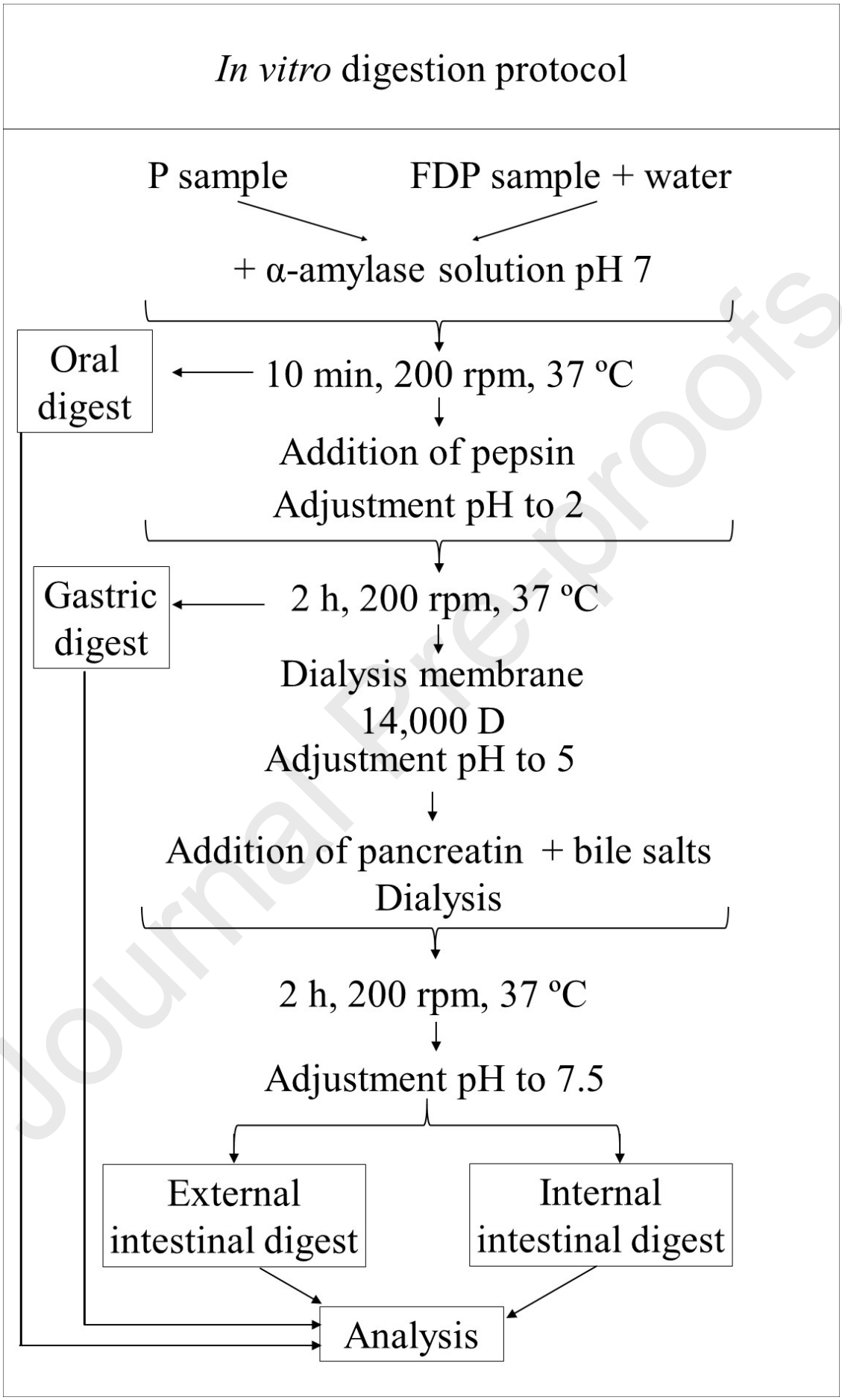
Different letters indicate different homogeneous groups established by Tukey HSD ANOVA between the P and the FDP for each formulation and parameter. [1]Total phenolic compound, GAE: gallic acid equivalent; [2]Vitamin C; [3]β-carotene; [4]FRAP: Ferric reducing antioxidant power; [5]DPPH: DPPH$^{\cdot}$ scavenging capacity assay, TE: Trolox equivalent. OS: orange's own solutes.

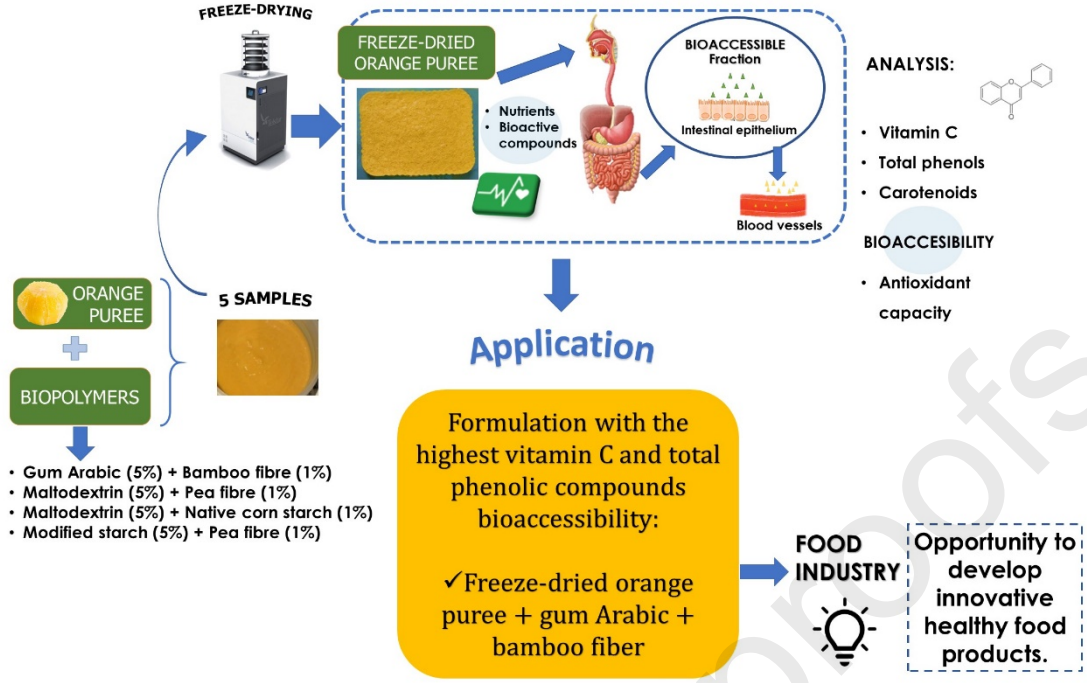












Any of the biopolymer protected the freeze-dried orange puree phenolics and vitamin C.

Sample with modified starch enhanced the protection of the more labile carotenoids.

Gum Arabic and bamboo fiber most favored bioaccesibility of phenolics and vitamin C.