UNIVERSITAT POLITÈCNICA DE VALÈNCIA

Departamento de Tecnología de Alimentos

Ph.D. THESIS

Design of the process of obtaining a freeze-dried orange puree. Formulation, freeze-drying variables, and storage conditions

Presented by:

Marilú Andrea Silva Espinoza

Supervised by:

Nuria Martínez Navarrete

Valencia, May 2021

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Dña. NURIA MARTÍNEZ NAVARRETE, DOCTORA EN CIENCIAS BIOLÓGICAS Y CATEDRÁTICA DE UNIVERSIDAD EN EL DEPARTAMENTO DE TECNOLOGÍA DE ALIMENTOS (DTA) DE LA UNIVERSITAT POLITÈCNICA DE VALÈNCIA (UPV).

Hace constar:

Que Doña MARILÚ ANDREA SILVA ESPINOZA, Tecnóloga de Alimentos, ha realizado bajo mi dirección el trabajo que con el título “Design of the process of obtaining a freeze-dried orange puree. Formulation, freeze-drying variables, and storage conditions”, presenta para optar al grado de Doctora por la Universitat Politècnica de València.

Para que así conste y sirva a los efectos oportunos, firmo el presente escrito en Valencia, a 19 de mayo de 2021.

NURIA| MARTINEZ| NAVARRETE

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Fdo.: Directora de la Tesis

There is a single light of science,
and to brighten it anywhere
is to brighten it everywhere.
Isaac Asimov

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A mi querida madre,

María Rosa,

mi ejemplo de lucha y fortaleza.

ABSTRACT

Currently, the growing consumer demand for healthy and sustainable products is a fact. The undeniable role of fruit as part of a balanced and healthy diet has led various institutional and non-institutional organisms to promote its consumption in recent years. The food industry has found in this sector a niche of opportunities to develop new fruit-based products as a way to attract the attention of consumers. In this sense, offering a freeze-dried orange puree could represent a feasible option. Freeze-drying the puree results in a cake that can be consumed directly as a snack, or it can be crushed to obtain a powder that can be used for the preparation of juices, infusions, desserts, salads, among others, and even for the enrichment in bioactive compounds of almost any foodstuff. Freeze-drying is known to yield high quality dehydrated foods. However, despite the possibility offered by this technique to use all the edible part of the fruit and its very high yield, it is a slow and energetically expensive process. A suitable optimisation of the process conditions could help to reduce the duration of the process without affecting the characteristics of the final product obtained. Despite the advantages offered by dehydrated fruit (microbiological stability and lower volume), it presents problems of structural collapse related to its low glass transition temperature. In this sense, a frequent approach for the stabilisation of dehydrated products is the incorporation of high molecular weight biopolymers. In addition, some of these agents can act as encapsulants that prevent degradation of bioactive compounds during processing, food storage, and the drastic conditions of the gastrointestinal tract. However, biopolymers must also allow the controlled release of these compounds during digestion, so that they can be bioaccessible and therefore bioavailable.

The aim of this Thesis has been the design of the freeze-drying process to obtain an orange snack. On the one hand, the influence of different combinations of biopolymers on the physical stability of the freeze-dried orange puree (orange snack) and on the bioaccessibility of its bioactive compounds by in vitro digestion has been studied. Their effect on the air flow and rehydration properties of an orange powder obtained after crushing the snack has also been evaluated. Specifically, different combinations of gum Arabic, maltodextrin, starch substituted with octenyl succinic groups, native corn starch, pea fibre and bamboo fibre were used. The results showed the need to incorporate these biopolymers to increase the critical water activity and the critical water content for the glass transition, the onset of which has been related to the loss of snack texture. In this sense, the stability map allows recommending the relative temperature-humidity condition for product storage. Regarding the different biopolymer mixes studied, although none of the biopolymer combinations was better than the others in terms of hygroscopicity, anti-plasticising character, colour, and mechanical properties of the snack, the gum Arabic (GA) mixed with bamboo fibre (BF) was the one that improved the bioaccessibility of vitamin C (VC) and total phenolic compounds (TP). Moreover, it was this same combination that gave the powdered product one of the shortest wetting times and the lower viscosity of the rehydrated product, which is desirable for a juice-type product. On the other hand, the impact of the freeze-drying conditions on the total energy consumption of the process and on the quality of the snack formulated with GA and FB has also been studied. The process variables considered were freezing rate (conventional and blast freezer), shelf temperature (30, 40, 50 ºC) and working pressure

(5 and 100 Pa) during drying. The results showed that the lower pressure and higher temperature promoted slightly higher drying of the samples, which resulted in a crispier product, as well as a less intense yellow colour than higher pressure and lower temperature. However, at the sensory level, there was no significant preference for any of the samples processed under the different conditions studied. In addition, VC and β-carotene (BC) were better preserved under these conditions. It should be emphasized that working under these conditions resulted in a significant reduction, up to 75%, in the total energy consumption during drying, due to the reduction of the process time. The freezing rate had no significant impact on any of the properties evaluated. Therefore, the recommended conditions for freeze-drying in order to maximise the preservation of bioactive compounds, with a lower energy consumption, and to provide a snack with appealing structural properties, perceived as a crispy product by consumers, are 5 Pa pressure and 50 ºC as shelf temperature. Finally, this work has evaluated the physical stability (colour, humidity, water activity, texture, hygroscopicity) and the stability of bioactive compounds (VC, BC, TP) and antioxidant activity of the snack stored in zip bags at 4 and 20 ºC for six months, simulating domestic storage conditions. As a result, a certain moisture gain of the sample was observed, with a consequent loss in porosity and crispness after 2 months. Also, the luminosity of the snack stored at 20°C decreased after 2 months, probably due to browning reactions, including degradation of VC (20%). BC suffered a large decrease, from the beginning of storage and more so at the higher the temperature. Therefore, refrigerated storage is recommended for better preservation of the bioactive compounds of this product.

RESUMEN

Actualmente, es un hecho la creciente demanda por parte de los consumidores por productos sanos y sostenibles. El innegable papel de las frutas como parte de una dieta equilibrada y sana ha hecho que variados organismos institucionales y no institucionales promocionen su consumo a lo largo de los últimos años. La industria alimentaria ha encontrado en este sector un nicho de oportunidades para elaborar nuevos productos a base de frutas como forma de atraer la atención de los consumidores. No obstante, no es habitual la comercialización de fruta en forma de puré liofilizado. La liofilización del puré supone la obtención de una torta que puede consumirse directamente como snack, o puede triturarse para la obtención de un polvo que puede utilizarse para la preparación de zumos, infusiones, postres, ensaladas, entre otros, e incluso para el enriquecimiento en compuestos bioactivos de casi cualquier alimento. La liofilización permite obtener alimentos deshidratados de alta calidad. Sin embargo, a pesar de la posibilidad que ofrece esta técnica de aprovechar toda la parte comestible de la fruta y de su muy elevado rendimiento, se trata de un proceso lento y energéticamente costoso. Una adecuada optimización de las condiciones del proceso podría contribuir a reducir la duración del mismo sin afectar a las características del producto final obtenido. Por otra parte, a pesar de las ventajas que ofrece la fruta deshidratada (estabilidad microbiológica y menor volumen), ésta presenta problemas de colapso estructural relacionados con su baja temperatura de transición vítrea. En este sentido, una técnica frecuente para la estabilización de estos productos deshidratados es la incorporación de biopolímeros de alto peso molecular. Asimismo, algunos de estos agentes pueden actuar como encapsulantes que previenen la degradación de los compuestos bioactivos durante el procesado, almacenamiento de los alimentos y de las drásticas condiciones del tracto gastrointestinal. Sin embargo, los biopolímeros también deben permitir la liberación controlada de dichos compuestos durante la digestión, para que puedan ser bioaccesibles y por tanto biodisponibles.

El objetivo de esta Tesis ha sido el diseño del proceso de liofilización para la obtención de un snack de naranja. Para ello se ha estudiado la influencia de diferentes combinaciones de biopolímeros en la estabilidad física del puré de naranja liofilizado (snack de naranja) y en la bioaccesibilidad de sus compuestos bioactivos por digestión in vitro. Asimismo, se ha evaluado su efecto en las propiedades de flujo en aire y de rehidratación del polvo de naranja obtenido tras la trituración del snack. En concreto se ha trabajado con diferentes combinaciones de goma Arábiga, maltodextrina, almidón sustituido por grupos octenil succínico, almidón nativo de maíz, fibra de guisante y fibra de bambú. Los resultados mostraron la necesidad de incorporar estos biopolímeros para aumentar la actividad de agua crítica y el contenido de agua crítico para la transición vítrea, el inicio de la cual se ha relacionado con la pérdida de la textura del snack. En este sentido, el mapa de estabilidad permite recomendar la condición temperatura-humedad relativa para el almacenamiento del producto. En cuanto a las diferentes mezclas de biopolímeros estudiadas, si bien ninguna de ellas fue mejor que las otras en términos de higroscopicidad, carácter anti-plastificante, color y propiedades mecánicas del snack, la mezcla GA con FB fue la que mejoró la bioaccesibilidad de la vitamina C (VC) y de los compuestos fenólicos totales (TP). Además, esta misma combinación fue la que confirió al producto en polvo uno de los tiempos de mojado más cortos y una

menor viscosidad del producto rehidratado, deseado para un producto tipo zumo. Por otra parte, se ha estudiado el impacto de las condiciones de liofilización en el consumo total de energía del proceso y en la calidad del snack formulado con GA y FB. Las variables del proceso consideradas han sido tanto la velocidad de congelación (convencional y abatidor), como la temperatura de bandeja (30, 40, 50 ºC) y presión de trabajo (5 y 100 Pa) durante el secado. Los resultados mostraron como la menor presión y la mayor temperatura promovieron un ligero mayor secado de las muestras, que supuso la obtención de un producto más crujiente, además de con un color amarillo menos intenso. No obstante, a nivel sensorial, no hubo preferencia significativa por ninguna de las muestras procesadas bajo las diferentes condiciones estudiadas. Además, VC y β-caroteno (BC) se preservaron mejor en estas condiciones. A su vez, trabajar en estas condiciones supuso una reducción significativa, de hasta un 75%, en el consumo de energía total durante el secado, debido a la reducción del tiempo del proceso. La velocidad de congelación no tuvo impacto significativo sobre ninguna de las propiedades evaluadas. Por tanto, las condiciones recomendadas para el secado por liofilización de manera que se maximice la preservación de compuestos bioactivos, con un menor consumo de energía, y que proporcionen un snack con propiedades estructurales interesantes, percibido como un producto crujiente por los consumidores, son 5 Pa de presión y 50 ºC como temperatura de bandeja. Por último, en este trabajo se ha evaluado la estabilidad física (color, humedad, actividad del agua, textura, higroscopicidad), de los compuestos bioactivos (VC, BC, TP) y de la actividad antioxidante del snack almacenado en bolsas zip, a 4 y 20 ºC, durante seis meses, simulando condiciones domésticas de almacenamiento. Como resultado, se observó una cierta ganancia de humedad de la muestra, con la consecuente pérdida en porosidad y carácter crujiente a partir de los 2 meses. Asimismo, la luminosidad del snack almacenado a 20 ºC disminuyó pasados 2 meses, probablemente debido a las reacciones de pardeamiento, que incluyen la degradación de la VC (20%). BC sufrió una gran disminución, desde el principio del almacenamiento y más cuanto mayor fue la temperatura. Por lo tanto, para este producto se recomienda un almacenamiento en refrigeración para una mejor preservación de los compuestos bioactivos.

RESUM

Actualment, és un fet la creixent demanda per part dels consumidors per productes sans i sostenibles. L'innegable paper de les fruites com a part d'una dieta equilibrada i sana ha fet que variats organismes institucionals i no institucionals promocionen el seu consum al llarg dels últims anys. La indústria alimentària ha trobat en aquest sector un nínxol d'oportunitats per a elaborar nous productes a base de fruites com a manera d'atraure l'atenció dels consumidors. No obstant això, no és habitual la comercialització de fruita en forma de puré liofilitzat. La liofilització del puré suposa l'obtenció d'una coca que pot consumir-se directament com a snack, o pot triturar-se per a l'obtenció d'una pols que pot utilitzar-se per a la preparació de sucs, infusions, postres, ensalades, entre altres, i fins i tot per a l'enriquiment en compostos bioactius de quasi qualsevol aliment. La liofilització permet obtindre aliments deshidratats d'alta qualitat. No obstant això, malgrat la possibilitat que ofereix aquesta tècnica d'aprofitar tota la part comestible de la fruita i del seu molt elevat rendiment, es tracta d'un procés lent i energèticament costós. Una adequada optimització de les condicions del procés podria contribuir a reduir la duració del mateix sense afectar les característiques del producte final obtingut. D'altra banda, malgrat els avantatges que ofereix la fruita deshidratada (estabilitat microbiològica i menor volum), aquesta presenta problemes de col·lapse estructural relacionats amb la seua baixa temperatura de transició vítria. En aquest sentit, una tècnica freqüent per a l'estabilització dels productes deshidratats és la incorporació de biopolímers d'alt pes molecular. Així mateix, alguns d'aquests agents poden actuar com agents que encapsulen i prevenen la degradació dels compostos bioactius durant el processament, emmagatzematge dels aliments i de les dràstiques condicions del tracte gastrointestinal. No obstant això, els biopolímers també han de permetre l'alliberament controlat d'aquests compostos durant la digestió, perquè puguen ser bioaccessibles i per tant biodisponibles.

L'objectiu d'aquesta Tesi ha sigut el disseny del procés de liofilització per a l'obtenció d'un snack de taronja. Per a això s'ha estudiat la influència de diferents combinacions de biopolímers en l'estabilitat física del puré de taronja liofilitzat (snack de taronja) i en la bioaccessibilitat dels seus compostos bioactius per digestió in vitro. Així mateix, s'ha avaluat el seu efecte en les propietats de flux en aire i de rehidratació d'una pols de taronja obtingut després de la trituració del snack. En concret s'ha treballat amb diferents combinacions de goma Aràbiga, maltodextrina, midó substituït per grups octenil succínic, midó natiu de dacsa, fibra de pésol i fibra de bambú. Els resultats van mostrar la necessitat d'incorporar estos biopolímers per a augmentar l'activitat d'aigua crítica i el contingut d'aigua crític per a la transició vítria, l'inici de la qual s'ha relacionat amb la pèrdua de la textura del snack. En este sentit, el mapa d'estabilitat permet recomanar la condició temperatura-humitat relativa per a l'emmagatzemament del producte. Quant a les diferents mescles de biopolímers estudiades, que, si bé cap de les elles va ser millor que els altres en termes d'higroscopicitat, caràcter anti-plastificant, color i propietats mecàniques del snack, la mescla GA amb FB va ser la que va millorar la bioaccessibilitat de la vitamina C (VC) i dels compostos fenòlics totals (TP). A més, aquesta mateixa combinació va ser la que va conferir al producte en pols un dels temps de mullat més curts i una menor viscositat del producte rehidratat, desitjat per a un producte tipus suc. D'altra banda, s'ha estudiat l'impacte de les condicions de liofilització en el consum total

d'energia del procés i en la qualitat del snack formulat amb GA i FB. Les variables del procés considerades han sigut tant la velocitat de congelació (convencional i abatedor), com la temperatura de safata (30, 40, 50 °C) i pressió de treball (5 i 100 *Pa) durant l'assecat. Els resultats van mostrar com la menor pressió i la major temperatura van promoure un lleuger major assecat de les mostres, que va suposar l'obtenció d'un producte més cruixent, a més d'amb un color groc menys intens. No obstant això, a nivell sensorial, no va haver-hi preferència significativa per cap de les mostres processades sota les diferents condicions estudiades. A més, VC i β-caroté (BC) es van preservar millor en aquestes condicions. Al seu torn, treballar en aquestes condicions va suposar una reducció significativa, de fins a un 75%, en el consum d'energia total durant l'assecat, a causa de la reducció del temps del procés. La velocitat de congelació no va tindre impacte significatiu sobre cap de les propietats avaluades. Per tant, les condicions recomanades per a l'assecat per liofilització de manera que es maximitze la preservació de compostos bioactius, amb un menor consum d'energia, i que proporcionen un snack amb propietats estructurals interessants, percebut com un producte cruixent pels consumidors, són 5 *Pa de pressió i 50 °C com a temperatura de safata.

Finalment, en aquest treball s'ha avaluat l'estabilitat física (color, humitat, activitat de l'aigua, textura, higroscopicitat), dels compostos bioactius (VC, BC, TP) i de l'activitat antioxidant) del snack emmagatzemat en bosses zip, a 4 i 20 °C, durant sis mesos, simulant condicions domèstiques d'emmagatzematge. Com a resultat, es va observar un cert guany d'humitat de la mostra, amb la conseqüent pèrdua en porositat i caràcter cruixent a partir dels 2 mesos. Així mateix, la lluminositat del snack emmagatzemat a 20 °C va disminuir passats 2 mesos, probablement a causa de les reaccions de enfosquiment, que inclouen la degradació de la VC (20%). BC va patir una gran disminució, des del principi de l'emmagatzematge i més com més gran va ser la temperatura. Per tant, per a este producte es recomana un emmagatzematge en refrigeració per a una millor preservació dels compostos bioactius.

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LIST OF ACRONYMS

AA: ascorbic acid

ANOVA: analysis of variance

AOA: antioxidant activity

a_w: water activity

α°: angle of repose

b: compressibility

BC: β-carotene

BF: bamboo fiber

B_x: concentration of each bioactive compound (x) analysed

C: energy constant related to the sorption heat

C*: chroma

CWA: critical water activity

CWA^o: critical water activity for the onset of the glass transition temperature

CWA^e: critical water activity for the endpoint of the glass transition temperature

CWC: critical water content

DE: dextrose equivalent

DPPH: 2,2-diphenyl-1-picryl-hydrazyl-hydrate

E.U.: European Union

ε: Porosity

ΔE*: total differences in colour

F: fast freezing rate

FAO: Food and Agricultural Organization

FDP: freeze-dried purees

FOP: formulated orange purees with biopolymers

F_f: fracture force

Fmax: maximum force

FR: freezing rate

FR-F: fast freezing rate

FR-S: slow freezing rate

FRAP: ferric reduction antioxidant power

GA: gum Arabic

GAE: gallic acid equivalents

GD: gastric digestion

*h**: hue angle

HPLC: high-performance liquid chromatography

I_eD: external intestinal digest

I_iD: internal intestinal digest

K: consistency index

L*: Luminosity

MD: maltodextrin

n: flow behaviour index

η: apparent viscosity

NCS: native corn starch

O: orange puree

OD: oral digestion

OS: orange's own solutes

OSA: starch modified with octenylsuccinic anhydride

P_x: freeze-drying working pressure at x Pascal

P: purees with and without biopolymers before being freeze-dried

PF: pea fiber

PD: primary drying

PLS-R: Partial Least Squares Regression

PSD: particle size distribution

RH: relative humidity

S: slow freezing rate

SD: secondary drying

S_L: Slope

σ: Shear stress

t_x: x months of storage

Tx: shelf temperature at x ºC

Tg: glass transition temperature

$Tg_{(s)}$: glass transition temperature of the anhydrous solids

Tg^o: onset temperature of the glass transition

Tg^m: midpoint temperature of the glass transition

Tg^e: endpoint temperature of the glass transition

TP: total phenolic compounds

U.S.A.: United Stated of America

VC: vitamin C

W_o: the monolayer water content value

x_w: water content

$\dot{\gamma}$: Shear rate

I. INTRODUCTION

I.1 Importance of consumption of fruits. The orange.

Fruits are a rich source of vitamins and minerals, dietary fibres, and a whole host of beneficial non-nutrient compounds, such as phytosterols, flavonoids, and other bioactive compounds with antioxidant properties, among others (Martínez-Navarrete et al., 2008). These antioxidants seem to contribute to the prevention of a wide range of pathologies, such as cancer, cardiovascular diseases, and degenerative diseases connected to aging processes (Tavarini et al., 2008). The varied consumption of fruits and vegetables helps to ensure an adequate intake of many of these essential compounds (WHO, 2004). Fruits are foods with high water content (80-90%), generally null in fat and low in protein. With respect to carbohydrates, fruits usually contain a higher proportion of sugars (fructose and sucrose), so their taste is usually sweet but without having a high energy value (40-60 kcal/100 g edible part). They are an excellent source of vitamin A and the best alimentary source of vitamin C. They are also good sources of calcium, iron, phosphorus, magnesium and cooper, and fibre (Moreno-Rojas, 2000). In addition, there are studies indicating that the increase in the intake of fruits and vegetables may contribute to a lower risk of significant weight gain and obesity (He et al., 2004).

Epidemiological studies indicate that the regular consumption of fruits and vegetables provides health benefits and so institutions recommend a minimum daily intake of these foods (Tomás-Barberán et al., 2001). For that reason, the Spanish Community Nutrition Society set a daily intake of fruits of 400 g as a final objective for 2020 (AESAN, 2017). However, according to the last Food Consumption Report in Spain, in 2019 the population took only 62% of the recommended intake of fruits (MAPA, 2019a). Also, in all the European Region, investigations indicate an excessive consumption of calories, saturated and *trans*-fats, sugar, and salt, as well as a low consumption of vegetables, fruits, and cereals. This leads to an increase in the number of people with obesity, which not only lowers life expectancy, but also its quality (WHO, 2019).

I.1.1 General aspects of oranges

Citrus plants have their origin in the South-Eastern Asia from where were spread up to other regions following the paths of civilisation (Calabrese, 2002). The first varieties of citrus fruits were *Citrus maxima*, pummelo, *Citrus medica*, citron, *Citrus reticulata*, mandarin, *Citrus halimii*, that are the predecessor species of all the citrus known today (Scora & Kumamoto, 1983). In Spain, citrus fruits such as citron, sour orange, lemon, and pummelo were introduced by Arabs in 1150 A.D. The common or sweet orange was introduced in Spain by Portuguese and Italian merchants in the sixteenth century, and it was not until the eighteen century (1700 A.D.) when it started to be farmed in the Eastern coast, mainly around Valencia, because of the climate and soil conditions, which make orange the most profitable crop (Dugo & Di Giacomo, 2004; Scora & Kumamoto, 1983).

The common orange (*Citrus sinensis*) is considered a hybrid derived from cross-pollination between pummelo with some mandarin (Scora & Kumamoto, 1983). Citrus belong to the Rutaceae family and its fruit is a typical berry called hesperidium. The fruit is made up of the following parts: exocarp, mesocarp, and endocarp (Fig. 1.1). The

exocarp and mesocarp constitute the rind and the albedo of the fruit, respectively. Inside the endocarp locules and the seeds (Agustí et al., 2003).

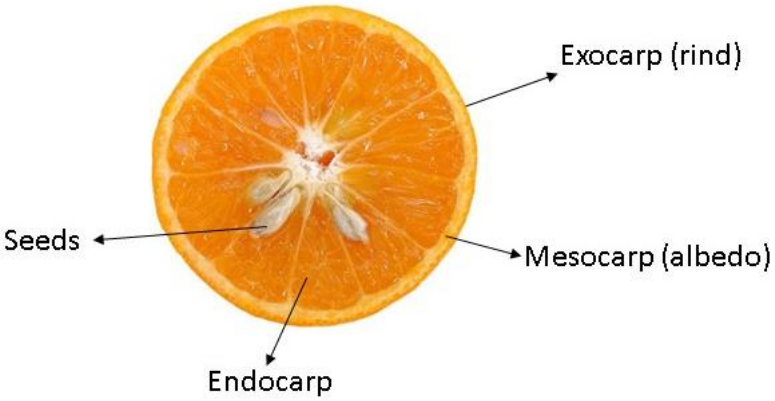

Figure 1.1. Parts of a citrus fruit.

Depending on the geographical location, the phenology of citrus varies from one country to another. This implies that the fruit needs to be imported to satisfy the internal demand in a region with the season outside the harvesting period. Imports involve transport, by land or sea, in which the type of storage of the materials is decisive in determining the quality of the product when it reaches the consumer. In Figure 1.2 the orange harvesting periods in Spain based on the variety used is shown. It can be observed that the production of both *early* and *late* varieties spans from October to June. During this long period of time, Spain not only produces, but also exports to other countries. The variety *Naveline, Navel, Navelate, Salustiane, Valencia*, and *Sanguinello* are the main orange varieties grown in Spain (USDA, 2019).

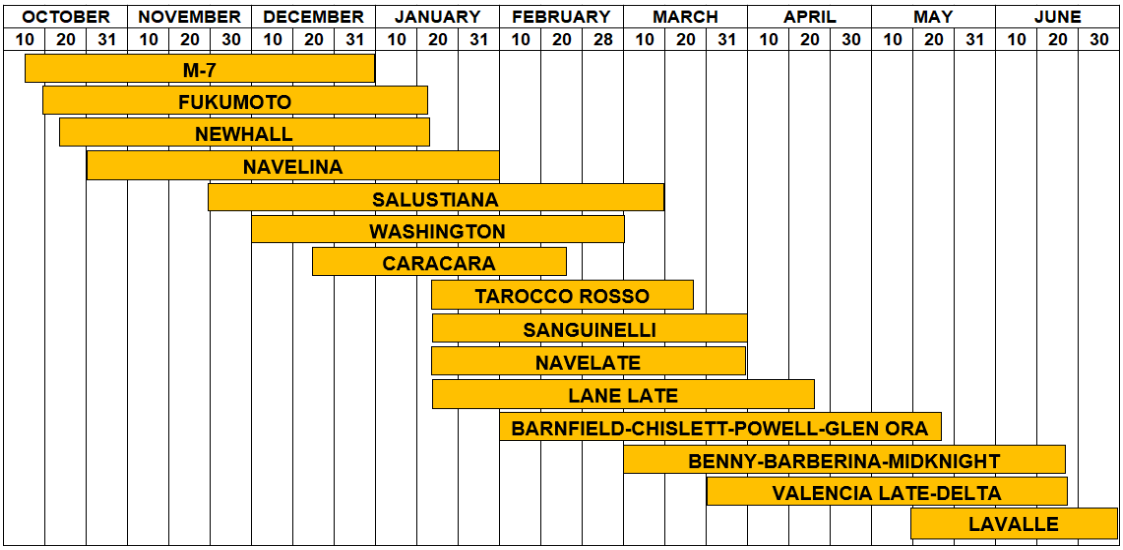

Figure 1.2. Periods of harvesting of oranges in Spain. Source: Adapted from Pardo and Buj, 2019.

The shelf life of refrigerated oranges ranges from 1 to 3.5 months (Table 1.1). During this short period of time, in case of exporting the product, the orange must be stored, conditioned, transported to the destination region, commercialized, and finally consumed by the user. The fruits present a series of problems after harvesting resulting from the lack of water and nutrient supply from the plant, therefore they depend only on their own metabolism. These results in a gradual loss of quality of the fruit and of its organoleptic characteristics (texture, flavour, and aroma) as its senescence progresses, finally determining physiological death (Salvador et al., 2003).

Table 1.1. Temperatures and time of preservation of oranges. Source: Martínez-Jávega, 2002.

Cultivar	Temperature (ºC)	Time (months)
Caracara	3-4	1.5-2
Lanelate	2-3	2-3
Navel Washing	2-3	2-3
Navelate	3-4	1.5-2.5
Navelina	2-3	2-3
Powell	4-5	1-1.5
Rhode Summer	4-5	1.5-2.5
Salustiana	2-3	2-3
Valencia Delta	4-5	1.5-2
Valencia late	2-3	2.5-3.5
Valencia Midknight	4-5	1.5-2
Verna	2-3	2.5-3.5

I.1.2 Production and consumption of oranges

Citrus is one of the most important commercial fruit crops, with a world production of oranges in 2018/2019 of 54,3 million tons, with Brazil, U.S.A. and the Mediterranean countries being the main producers. The citrus production in the European Union (E.U.) is concentrated in the Mediterranean regions. Spain is the first orange producer in the E.U., with 139,630 ha of planted area for oranges, and a production of 3,6 million tons in 2018/2019, which represents 55% of the whole production of the E.U. Furthermore, it is the sixth orange producer in the world after Brazil, China, U.S.A., Mexico, and Egypt (USDA, 2020).

Regarding the local production in Spain, Valencia and Andalucía are the regions accounting for 90% of the whole orange production, with Castellón, Valencia, Alicante, Murcia, Almería, Malaga, and Huelva being the main producer cities (Palacios, 2005; USDA, 2019).

In 2018/2019, the orange exports from the E.U. were 357 thousand tons to the rest of the world. Spain is the first global exporter of mainly oranges, mandarins, and lemons with 60% of its citrus production destined for exports (USDA, 2019). In the same period Spain exported 1.7 million tons of oranges, with 70% exported from the region of Valencia. Up to 85% of the global national export was destined to the E.U., mainly to Germany, France, Netherlands, Italy, and the United Kingdom (Figure 1.3). A significant increase of the export to non-European countries such as China and Canada has also been reported (USDA, 2019).

Regarding to the consumption, the global fresh domestic orange consumption was of 30.2 million tons in 2018/2019, while the E.U. is the second global consumer with 6 million tons, just behind China with 6.9 million tons (USDA, 2020). Twenty percent of Spain's citrus production is destined for domestic fresh consumption. Spain's per capita orange consumption is estimated at approximately 20 kg (USDA, 2019).

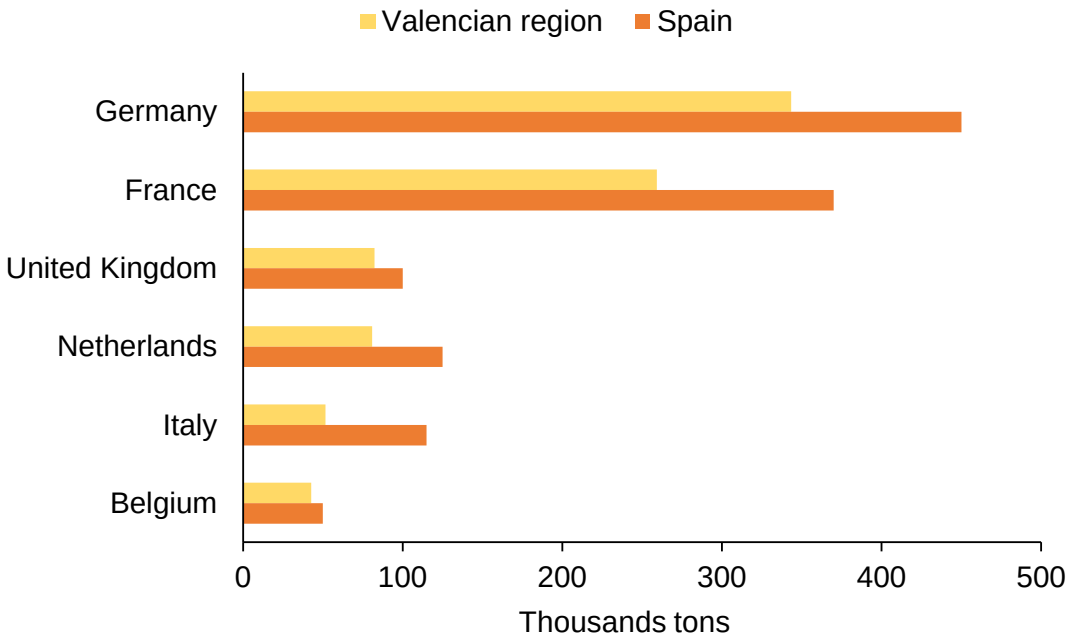

Figure 1.3 Main destination countries of orange exports from Spain and Valencian region. Export quantities expressed in thousands tons. Source: GVA, 2019; MAPA, 2019b.

Common oranges are frequently destined for fresh consumption, but in the United States and Brazil they are mainly for juice products. The global orange juice production was estimated to be 1.7 million tons in 2019 (USDA, 2019). Orange juice is the most popular juice within the E.U., with a consumption of 694 thousand tons in 2018/2019. Spain is the major processor in the E.U., and around 20 percent of Spanish orange production is used for processing, mainly into orange juice (USDA, 2019). The consumption per capita of juices and nectars in Spain was 17.6 L in 2017, similar to the E.U. mean value with 17.9 L per capita (AIJN, 2018). The juices and nectars market are

mainly concentrated in three flavours, which capture almost the 75% of total consumption: orange juice (35%), pineapple (20%), and peach (18%) (AIJN, 2018).

These data reflect the importance that the commerce of the oranges has worldwide, especially in Spain.

I.1.3 Nutritional aspects and bioactive compounds of oranges

Regarding the nutritional characterization, citrus fruits are mainly made up by simple carbohydrates (fructose, sucrose, and glucose) and by non-starchy polysaccharides (dietary fibres) (Table 1.2). In the oranges, the pectin is the major constituent of the fibre, accounting for around 65-70%. The rest of the fibre is represented by cellulose, hemicellulose, and some lignin. As regards to the bioactive compounds, oranges display high content of ascorbic acid, folates, and carotenoids with provitamin A activity. In addition, oranges are rich in flavonoids, such as hesperidin, neohesperidin, naringin, narirutin, tangeretin, and nobiletin (Peterson et al., 2006).

Table 1.*2*. Orange nutritional composition. Data refer to 100 g of edible portion. Source: Moreiras et al., 2013.

Energy (kcal)	42	Phosphor (mg)	28
Proteins (g)	0.8	Selenium (µg)	1
Carbohydrates (g)	8.6	Riboflavin (mg)	0.03
Fibre (g)	2	Vitamin B_6 (mg)	0.06
Water (g)	88.6	Folate (µg)	37
Calcium (mg)	36	Vitamin B_{12} (µg)	0
Iron (mg)	0.3	Vitamin C (mg)	50
Magnesium (mg)	12	Vitamin A (µg Retinol)	40
Sodium (mg)	3	Vitamin E (mg)	0.2
Potassium (mg)	200	Thiamine (mg)	0.1

The bioactive compounds of citrus fruits such as oranges, mandarins, and grapefruits and their potential health benefit effect currently is a research priority topic. In particular, vitamin C, phenolic compounds, and carotenoids seem to contribute in the prevention of illness such as cancer, cardiovascular, and eyes cataracts (Eggersdorfer & Wyssm 2018; Gorinstein et al., 2001). These health-promoting effects may be related to their antioxidant activity, which prevent the harmful effects of free radicals (Martínez-Navarrete et al., 2008).

The antioxidants are compounds which can inhibit or delay the oxidation of other molecules, blocking the initiation and/or propagation of free radical chain reactions (Sherwin, 1972). A free radical is a chemical compound that in its structure has one or more unpaired electrons, therefore, behaving as a highly reactive molecular specie.

These radicals are released during human metabolism, but also by environmental pollutants (Singh et al., 2004). Antioxidants are capable of trapping free radicals responsible for oxidative stress, as well as preventing diseases such as cardiovascular, circulatory, carcinogenic, and neurological diseases. Furthermore, they have anti-inflammatory, antiallergic, antithrombotic, and antineoplastic activity (Ross & Kasum, 2002). The antioxidant capacity of a mixture not only depends on the set of antioxidant capacity of the components, but the environment where the compound is found must also be taken into account, being able to show synergistic or inhibitory effects (Kuskoski et al., 2005).

The biological functions of vitamin C depend on its ability to act as an electron donor. Therefore, it acts as a cofactor for some enzymes with very important functions in the body. It is a very effective water-soluble antioxidant and seems to be involved in the regeneration of vitamin E (Bruno et al., 2006; Pearson et al., 2017). There are numerous beneficial effects provided by vitamin C, including improved endothelial vascular function, reduction of systemic inflammation, increased bioavailability of iron, inhibition of lipid oxidation, and a reduced likelihood of cardiovascular disease and some cancers (Block, 1991; Hallberg et al., 1989; Siti et al., 2015). A low intake of vitamin C can cause scurvy. Since the body is not able of synthesizing this vitamin, it is important to eat fruit and vegetables to obtain the required daily intake (Traber & Stevens, 2011). On the other hand, the side effects that can derive from an excessive intake of vitamin C must be considered, such as the presence of kidney or urinary tract stones (Holmes et al., 2016; Moe, 2016).

Phenolic compounds are the most extensive group of non-energy substances in foods of plant origin. A diet rich in plant polyphenols may contribute to improving health and reducing the incidence of cardiovascular diseases. Their beneficial effects are mainly due to its antioxidant properties, which seem to be related to their chelating capacity, lipoxygenase inhibition, and free radical capture. In addition, phenolic compounds have various biological effects such as antibacterial, antiviral, anti-inflammatory, antithrombotic, and vasodilatory actions (Quiñones et al., 2012).

The primary function of the carotenoids pigments in the plants is to capture the light energy to transfer it to chlorophylls during photosynthesis. Carotenoids can provide yellow, orange, and reddish colours to fruits and vegetables, which is possible due to the presence of a chromophore in its molecule. In fruits, the content of carotenoids increases with ripening (Meléndez et al., 2007). In addition to providing coloration, carotenoids are of great importance at the nutritional level. Some of them, such as α-carotene, β-carotene, and cryptoxanthin, have provitamin A activity (Simpson, 1983). Vitamin A is essential for high night vision and essential for the maintenance of healthy skin and superficial tissues (Meléndez et al., 2007). Furthermore, carotenoids without provitamin A activity such as lutein and zeaxanthin, which are also contained in the citrus, are present in the retina and lens of the eye, and they are inversely associated with the risk of cataracts and macular degeneration (Khachik et al., 1997). It has to be pointed out the positive effect of carotenoids on the immune response, a fact that has long been postulated (Bendich, 1989; Chew, 1993), and some studies demonstrate the antioxidant activity of carotenoids (Stahl & Sies, 2003). Although epidemiological studies show that

the intake of carotenoids rich foods is closely related to a lower risk of suffering chronic diseases, there is still insufficient evidence to conclusively establish the link (Meléndez et al., 2007).

I.2 Food waste: fruit waste valorisation strategy

One of the major problems related with fruit production is its waste. Food waste is essentially related to poor buying and consumption habits, as well as to inadequate management and handling. The definition provided by the Agricultural and Rural Commission of the European Parliament about "food waste" is the whole of the discarded products of the food supply chain which, for economic or aesthetic reasons, or for closeness to the expiry date, despite still being edible and therefore potentially intended for human consumption, in the absence of possible alternative use, are eliminated and disposed of, producing negative effects from the environmental point of view, economic costs and missed revenue for companies (European Parliament, 2011). FAO (2013) estimated that 28% of the world's agricultural area, corresponding to approximately 1,400 million ha cultivated, is used annually to produce food that is wasted. Also, it was estimated that more than 1.3 billion tons of food are wasted every year in the world, which is a third of world production.

The food shelf life is a concept related to waste, and it is necessary to understand better the reasons leading to food waste generated by the consumers. The shelf life of a food is the period of time during which it remains suitable for consumption from a sanitary perspective, keeping sensory, functional, and nutritional characteristics within the range of those previously established as acceptable (Hough & Wittig, 2005). It is important to differentiate the sanitary shelf life from the sensory shelf life, since the former relates to the aspect that the food is suitable for consumption at the microbiological level, while the latter refers to the time in which a product is attractive to the consumer. On many occasions, fruits do not present microbiological problems, but due to operations during transport, storage, or distribution, they suffer shocks or pressures, causing physical changes, such as progressive change of colour or bruises causing product rejection by the consumer. The food waste generated at the consumer level results from two factors: either the consumer does not buy the product in the store because it is not considered sensorially attractive or, once the product is at home, it is not consumed within the sensory shelf life and therefore oxidations and/or rot occur forcing the consumer to waste the product.

According to the Spanish Ministry of Agriculture, Fishing, and Food, fruits account for 32.7% of the total volume of food waste, being the main category of wasted products (Figure 1.4). Within this category, orange is the second most wasted fruit after the apple one (MAPA, 2017).

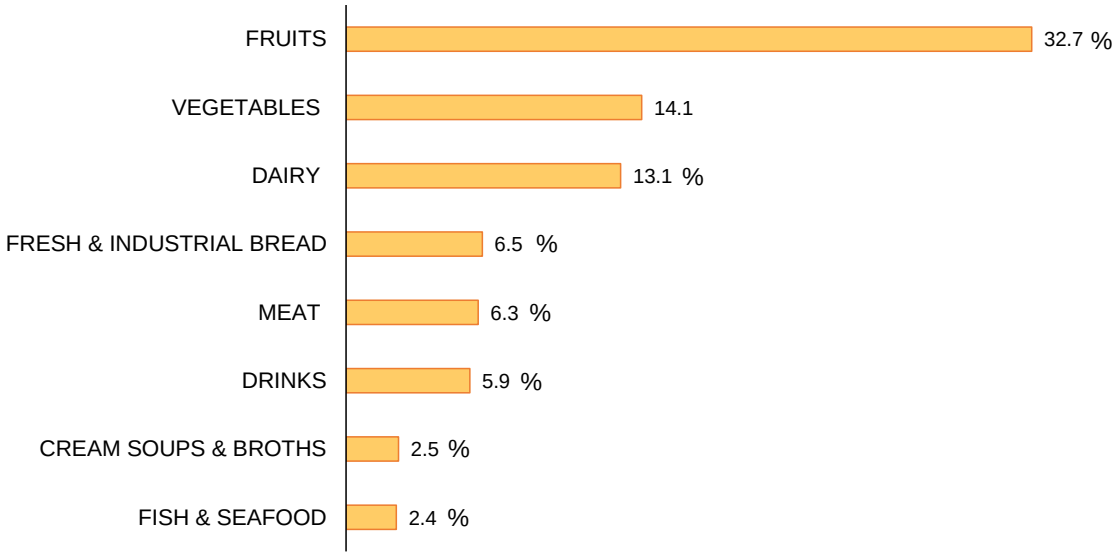

Figure 1.4. Percentage of unprocessed products wasted at the household level (MAPA, 2017). Fruits, vegetables, meat, and fish/seafood include fresh and frozen, and not canned. Dairy includes milk and dairy products (dairy products do not include ice cream). The category “bread” includes fresh, industrial, and frozen bread.

The intention of the work presented in this Thesis is the design and development of new, healthy, and orange-based innovative products which can satisfy the demand of the consumers. But also, the oranges that are not well accepted either by the distributors or by the consumers because of presence of physical damage but being microbiological adequate for the consumption could be used as a raw material to develop these products. This would increase the added value of the products while reducing the fruit waste.

I.3 Consumer trend

The consumer’s choice is characterised by the demand of safe, ethical, healthy, and sustainable products (Jodar, 2018). The Mintel research ‘Global Food & Drink Trends’ (2018) points to the texture as differential element and to personal well-being as the central axis. Also, the consumer demands for natural products, without additives or preservatives, and searches for a healthy diet low in sugar and/or fat and that also helps in fighting stress, reducing fatigue, and gaining energy. As for the sensations, the trend “eat with the eyes” will continue. The consumer searches for a complete sensory experience in food, hence the demand for products that involve the senses: the appearance, the flavour, and the texture (Mintel, 2018).

Snacks are widely consumed between the main meals. The growth trend of the snacks is expected to increase over the years, which offers the opportunity of formulating new food products (Jodar, 2018). The Global Survey “Snack Attack” reported by Nielsen about Snacking was carried out in 2014, surveying more than 30,000 consumers online in 60 countries in Asia, the Pacific, Europe, Latin America, the Middle East, Africa, and North America. Globally, consumers spent $347 billion on snacks per year between 2013 and 2014 (Nielsen, 2014). According to this study, the interviewees said that fresh fruit (18%) is the snack that would choose as a first option from a list of 47 different options,

followed by chocolates (15%). The snacks with all-natural ingredients are considered as very important by 45% of the interviewees and as moderately important for 32% of them (Figure 1.5). Attributes in snacks such as the absence of artificial colours (44%), not genetically modified (43%) and that do not contain artificial flavours (42%) are also very important for consumers. The study also indicates that roughly one-third of consumers are looking for beneficial ingredients, rating fibre as one of the very important attributes in the snacks they eat.

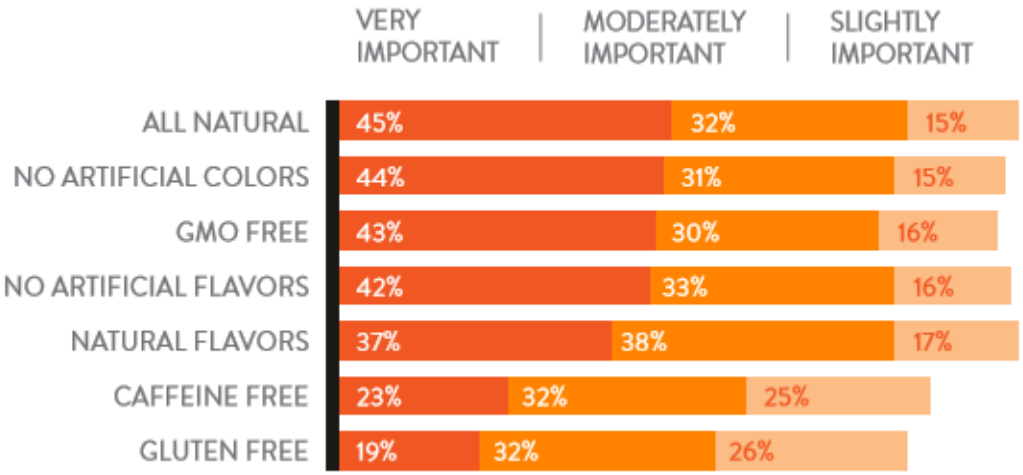

Figure 1.5. Percentage of interviewees ranking snack healthy attributes in order of importance (Nielsen, 2014).

Another type of format that has gained prominence is powdered food. In addition to the microbiological stability offered by powdered foods, this type of format offers great versatility and functionality, both in application and in consumption itself. In this way, both transformers and consumers may incorporate the powder into different matrices or be consumed directly. In particular, in recent years, the bakery industry has contemplated the incorporation of fruit powders to biscuits or cakes, due to the improvement they offer to the physical characteristics and the addition of fibre to the final product (Salehi, 2020; Salehi & Aghajanzadeh, 2020). Moreover, in recent years, the Food Industry has opened new lines of innovation in the production of powdered products from fruit waste, being the food waste one of the main challenges for this industry (Mercados, 2019, INTENSO, 2020).

Considering that oranges are a source of beneficial compounds for our health and the consumer demand for healthy and ready-to eat food products or ready-to use ingredients to formulate foods, the design of orange products such as snack or powder may be considered as sustainable approach to reduce orange waste along the food supply chain. Both formats may be a different way of offering fruit to the consumer, which would be natural, stable, and innovative, with high content in fruit, while reducing the orange waste.

Freeze-drying of the orange puree may be a suitable process to obtain both products (Section 1.4). The cake obtained after the process may be directly consumed as snack or may be crushed to obtain a powder. The powder could be used as an ingredient for the preparation of desserts, dairy products, salads, ice creams, among others, and even for the enrichment of bioactive compounds of almost any food. In addition, it could also be rehydrated for consumption as juice or infusion. Freeze-dried products are considered high value-added products (Ratti, 2013). These products may be aimed at all types of consumers, including those demanding higher nutritional value and ready to-eat products, such as athletes, children, or the elderly.

Fruits are mostly made by low-molar-mass sugars such as sucrose, glucose, and fructose and organic acids such as citric, malic, and tartaric acid. When the fruit is dehydrated, it is common to obtain a fruit matrix in an amorphous physical state which may exhibit problems related to the glass transition. The glass transition is a phase transformation from the glassy state (very stable) to the rubbery state (unstable). It occurs when the glass transition temperature (Tg) of a product is exceeded. As the Tg decreases with increasing water content, the glass transition of a material in the glassy state may result from an increase in its water content or temperature (Telis & Martínez-Navarrete, 2012). In the rubbery state, the problems shown by the product are related to the rate at which deterioration reactions occurs, the development of collapse phenomena, that includes the loss of crunchiness/cripsness or stickiness, caking problems, and colour changes (Telis & Martínez-Navarrete, 2012). Dehydrated fruit is usually in a rubbery state at room temperature due to its low Tg as related to its composition (Telis & Martínez-Navarrete, 2012). Since the Tg increases with the average molecular weight of the solutes, the addition of some biopolymers such as gums, maltodextrins, or starch is being used for the stabilization of dried products (Bhandari et al., 1993; Bormann et al., 2013; Roos & Karel, 1991; Telis & Navarrete, 2009; Truong et al., 2005).

I.4 Biopolymers used as carrier agents in drying of fruit products

The control of water content and temperature in dehydrated fruits is an important approach to prevent structural and undesired changes. However, another important approach is the addition of biopolymers to hygroscopic dried fruit to act such as carrier agents or drying aids (Adhikari et al., 2004; Sablani et al., 2008). The biopolymers exert their stability function based on the competition for water with the dried product components, acting as physical barrier between particles and on the surface of otherwise hygroscopic particles, and/or increasing the Tg (Aguilera et al., 1995; Sablani et al., 2008). Due to their different mode of action, often different types of biopolymers are combined. In this study six different biopolymers were used. Gum Arabic, maltodextrin dextrose equivalent 19, and chemically modified starch with octenylsuccinic groups were used to increase the Tg; native corn starch, pea fibre, and bamboo fibre were used as fillers with steric role to minimise the formation of interparticle bridges in the food matrix, thus also delaying structural collapse.

Gum Arabic is a mixture of heteropolysaccharide and glycoproteins complex with a branched structure, with a main chain consisting of D-galactopyranose units linked by β-

D glycosidic bonds. Gum Arabic is a highly efficient film-forming material for microencapsulation (Glicksman, 1983). It has been widely used as microencapsulation material by spray-drying, especially due to its good emulsification capacity and low viscosity in aqueous media. Also, it contributes to increase the Tg of the products, reducing the hygroscopicity and stickiness, determining better handling during processing and transporting (Gabas et al., 2017). Furthermore, it provides high retention of volatile compounds and protects against oxidation reaction (Rosenberg et al., 1990). However, it presents high cost (Kanakdande et al., 2007).

Maltodextrins consists of β-D glucose units mainly linked by α-1,4 glycosidic bonds and is usually classified according to its dextrose equivalency (DE). Maltodextrins are defined as products with DE <20. They are non-sweet solids that contribute as body or bulk agents in food systems. They are mainly used in products that are difficult to dry, such as juices of fruit, to reduce stickiness, thereby improving the stability of the product (Bhandari et al., 1993, Roos & Karel, 1991). Maltodextrins offer advantages such as good solubility, neutral aroma and taste, low viscosity at high solids concentrations and good protection of flavours against oxidation, in addition to its use being economically feasible (Righetto & Netto, 2005).

The native corn starch, as all the native starches, is widely used in the industry since it contributes to stabilize food, helps emulsification, and improves texture (Luallen, 1985). Furthermore, the starches when mixed with hydrocolloids modify and control the texture and improve moisture retention in food products (Appelqvist & Debet, 1997). The chemically modified starches with octenylsuccinic groups (OSA) have active surface properties, which gives stabilization capacity in food. Despite this modification, the OSA preserves its biodegradability (Järnström et al., 1995), which makes its use convenient for many foods, cosmetics, and pharmaceutical applications. As compared to native starches, OSA starch is soluble in cold water and provide relatively low viscosity even at high concentrations (Dokić et al., 2012).

There is growing interest by the food industry in increasing the fibre content in foods, as numerous studies have demonstrated the beneficial effects of fibre 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). Many of the fibres are flavour neutral, odourless, and calorie and fat free. Pea fibre has been used in the food industry to enrich breads and breakfast cereals (Vetter, 1984). Also, since bamboo fibre is a cheaper alternative compared to other fibres, many companies use it in their products. It is a common ingredient in breakfast cereals, pasta, cheeses, sauces, mustards, ketchup, beverages, fruit juices, snacks, frozen desserts, and pastries. These fibres have a water absorption capacity, which improves the texture and consistency of the products and are very useful in dehydrated products where anti-caking properties are necessary (Chongtham et al., 2011; Mackee & Latner, 2000).

However, the addition of biopolymers may have unintended effects on other properties, such as changes in the flavour, colour, or inadequate texture of the final product. Since the biopolymers differ as to their composition and nature, their interactions with the food matrix may vary, causing different water-solid interactions. Likewise,

biopolymers may act as encapsulation agents, helping to prevent the degradation of some bioactive compounds (Rascon et al., 2011). Microencapsulation consists of introducing biologically active compounds into the matrix of biopolymers to prevent their loss or their oxidation by light or by presence of oxygen (Gutiérrez & Álvarez, 2017). It is reported in the literature that the encapsulated compounds are bioavailable, and therefore, may keep their functional activities during the processing and storage of food (Pal et al., 2009). Also, one of the objectives of the encapsulation is to protect the bioactive compounds from the drastic environmental conditions occurring along the gastrointestinal tract (Hu et al., 2017). Nevertheless, the encapsulating agents should allow a controlled release of the bioactive compounds. Therefore, for bioactive compounds to be available, they must first be bioaccessible. However, the nature and functionality of different biopolymers can affect how these compounds are released from the food matrix to be absorbed by the organism.

I.5 Freezing-drying as a dehydration method yielding high-added value products

Dehydration is one of the most common techniques used to preserve food. In addition, it also entails a reduction in the volume and weight of the product, which facilitates its transport and handling (Fazaeli et al., 2012). Among the dehydration technologies, freeze-drying is known to yield dried products with high quality (Hammami et al., 1999). The freeze-drying is a process of dehydration based in the sublimation of the ice of the food, by applying lower temperature and pressure than the water triple point (T=0.01 ºC, P=4580 mTorr = 612 Pa; Figure 1.6). For that reason, following the arrows in Figure 1.6., the product which initially is at ambient conditions, needs to be cooled inducing freezing of the water. Once the product is frozen, low pressure is applied to induce the sublimation of the ice.

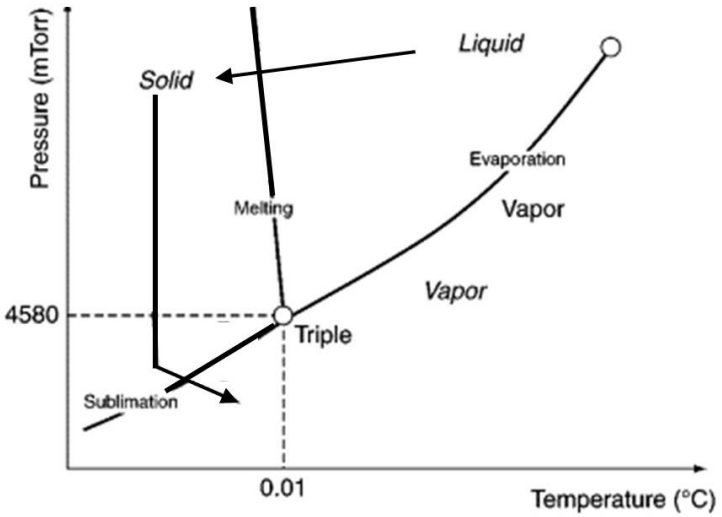

Figure 1.6. Phase diagram of water. Adapted from Ratti (2013).

The water of a food product can be distinguished in freezable and non freezable water, which play a role in different stages during freeze-drying. Freeze-drying consists of three stages: freezing, primary drying and secondary drying (Figure 1.7). The first stage consists in freezing the product, where all freezable water must be frozen. For that reason, the freezing stage usually takes several hours to occur. In the second stage, called primary drying, the frozen product is subjected to low pressure in the freeze-drier chamber to increase the rate of ice sublimation, applying mild temperatures. In this way, the water of the food in solid state is sublimated. The sublimation is the phase transition where water goes from a solid state to a vapour one without passing through a liquid state. For sublimation to occur, it is necessary to provide the sublimation heat of ice. The heat is mainly provided by conduction but also by radiation by the heating plate or shelf in the chamber of the freeze-dryer. Most of the water is removed in this stage. The third stage or secondary drying has the objective to reduce the residual water content to an optimal level for stability. The desorption of most of the unfrozen water retained in the solid food matrix is carried out (Pikal et al., 1990). The water vapour released from both steps is usually caught on the surface of a condenser (Berk, 2013).

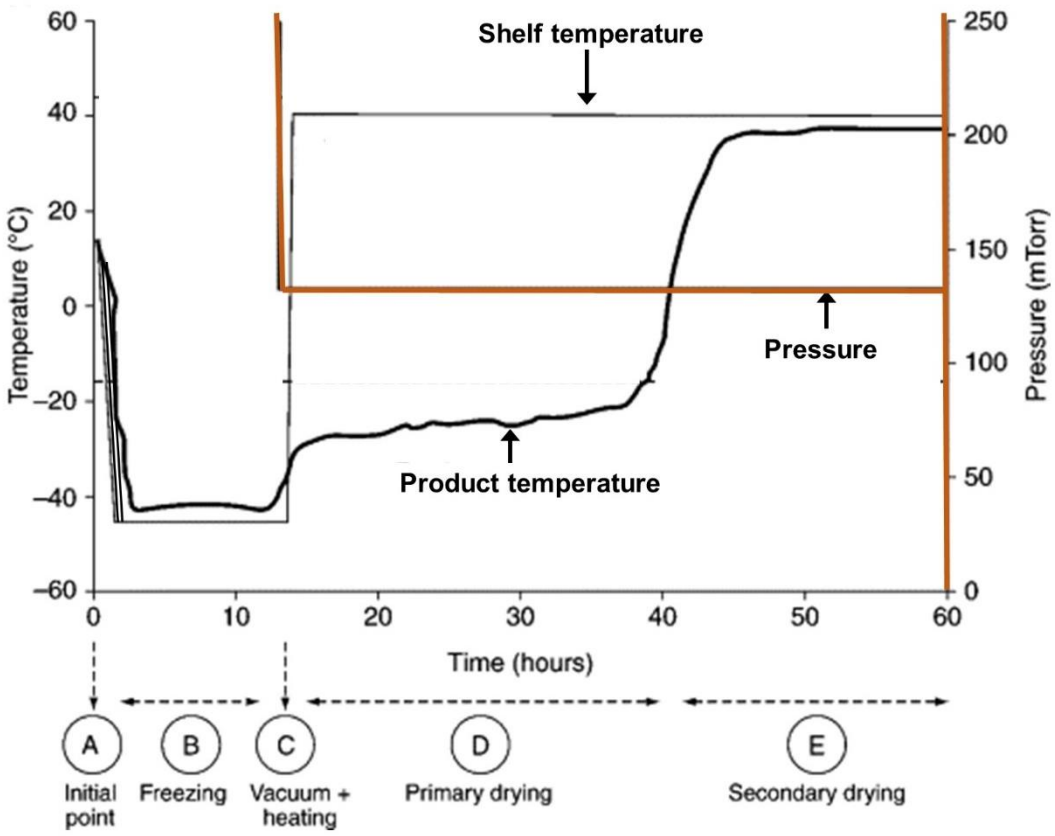

Figure 1.7. Product temperature profile during freeze-drying. Adapted from Ratti (2013).

The freeze-drying is not only used in the food industry, but its use stands out in the pharmaceutical industry to preserve thermolabile compounds from blood and strains of microorganisms, among other applications. Furthermore, it is used in the chemical

industry for the conservation of books and documents, as well as the drying of flowers and wood (Chen et al., 2000; Flink & Hoyer, 1971; Jones et al., 2009). Freeze-drying was employed by some ancient populations for food conservation. The origin of this technique in traced back to 200 B.C. in Perú, where local indigenous people were freezing tubers and potatoes atop the chilly Andes mountains, leaving them to be sublimated by the effects of the sun's radiation in the dry and low-pressure environment. Japanese monks on Mount Koya (1185-1333 A.D.) and Vikings in northern Europe also used this technique to preserve tofu and codfish, respectively. In Western countries, freeze-drying was substantially unknown until the early 1900s. The first application came up in 1881, when R. Altman discovered a similar system to the freeze-drying, which was used in the conservation of animal tissues. In 1933, Flosdorf and Mudd at the University of Pennsylvania performed freeze-drying of blood serum under full aseptic conditions. But it was in 1955 when it appeared again as innovation in food technology, and it was applied to obtain foods such as coffee, milk, eggs, soups, fruit juices, and yeast (Fissore et al., 2019). By freeze-drying, it is possible to obtain foods with higher quality than that achieved with conventional drying methods. The freeze-drying is usually used in high added value products, such as products for mountaineers, astronauts, babies, military, but also those foods in which it is especially important to preserve the organoleptic quality, such as coffee, tea, fruits, among many others (Stapley, 2008).

It is remarkable that freeze-drying is carried out at low temperatures and low pressure, so it facilitates the preservation of the flavour and colour of the food, and it also minimizes the thermal damage caused by high temperatures on the nutrients sensitive to the heat and the loss of volatile compounds (Barbosa-Cánovas et al., 2005, Berk, 2013). The high decrease in the water activity allows to slow down the deterioration processes to which a food is subjected and obtaining microbiologically stable products (Barbosa-Cánovas et al., 1996). Freeze-dried products have good capacity of rehydration, due to their porous structure formed during sublimation (Barbosa-Cánovas et al., 1996).

Despite its advantages, freeze-drying has a high cost, due to the long duration of the freezing and vacuum steps, which involve high energy cost as compared to another dehydration technology as spray-drying, known for being cheaper and leading to powdered products of similar quality. Flink (1977) reported that freeze-drying has 4-5 times higher costs than the spray-drying. However, a recent study for obtaining powdered fruit observed that even though the electrical cost associated with freeze-drying is 8.5 times higher than that reported for spray-drying, it is more cost-effective to use freeze-drying rather than spray-drying, the latter with a total cost 2.3 times higher than the former. This fact is due to the raw material cost for freeze-drying, being 3.5 times lower than that required by spray-drying, because there was no loss of dry solids during freeze-drying, as compared to spray-drying (Camacho et al., 2018). Furthermore, it has been reported that modifying the process variables such as freezing rate, shelf temperature, or working pressure may affect the duration of the freeze-drying process (Hammami et al., 1999). For this reason, in order to try to reduce freeze-drying cost, it is important to know how these process modifications affect its duration and therefore, the energy consumption.

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II. OBJECTIVES

The aim of this Ph.D. Thesis was the development of a high-quality orange snack obtained from the fruit puree by selecting the adequate formulation and appropriate freeze-drying conditions. The orange snack might be either consumed directly or crushed to obtain a powder which can be also an interesting format to offer to the consumers, as it can be used either as a functional ingredient or rehydrated to be consumed as a juice. In both cases adequate physicochemical properties and the highest content of bioactive compounds must be achieved.

To reach this purpose, the specific objectives were:

1. To select the best combination of biopolymers to add into the orange puree as to obtain an orange snack and its derived powder with the best physical characteristics while maximally preserving the fruit bioactive compounds.

2. To design an efficient freeze-drying process based on the best combination of freezing rate-pressure-shelf temperature to be applied during the freeze-drying process as to shorten the operation as much as possible without compromising the quality of the product.

3. To establish the storage temperature of the designed orange snack to preserve the content of bioactive compounds and to ensure adequate physicochemical properties of the orange snack under simulated consumer home storage conditions.

III. RESULTS

The results of this Thesis are organised in three Chapters, corresponding to each of the three specific objectives.

Chapter 1: Influence of different biopolymers on the properties of a freeze-dried orange snack and the corresponding powder product.

Chapter 1 covers three sections, two of which are the study of the impact of the different biopolymers on the physicochemical properties and on the *in vitro* bioaccessibility of bioactive compounds of the orange snack. The third section focuses on the influence of these biopolymers on the flowability in air and rehydration properties of the powdered orange snack. Likewise, two of these three sections correspond to two published articles and one to an article under review.

Chapter 2: Selection of freeze-drying conditions to obtain an orange snack.

Chapter 2 also covers three sections. The impact of the different studied freeze-drying variables on the physical-chemical properties and bioactive compounds (first section), on the sensory perception (second section) and on the energy consumption (third section) of a freeze-dried orange snack was evaluated. Likewise, two of the three sections correspond to two published articles and one to an article under review.

Chapter 3: Effect of storage temperature on the physical-chemical properties and bioactive compounds of an orange snack obtained by freeze-drying.

Chapter 3 is a unique section where the effect of usual home storage conditions on the properties of the freeze-dried orange snack was evaluated. This section corresponds to one published article.

Chapter 1: *Influence of different biopolymers on the properties of a freeze-dried orange snack and the corresponding powder product*

Chapter 1.1. Use of different biopolymers as carriers for purposes of obtaining a freeze-dried orange snack

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Abstract

In addition to colour, one of the most important qualities of a snack-type product is its crispy texture. A freeze-dried fruit snack is characterised by a low water content, susceptible to losing its crispness related to its low glass transition temperature (Tg). In this sense, a common technique implemented to increase the Tg of these types of products is to add different biopolymers. However, these compounds can, at the same time, affect the colour and texture of the product. In this study, different biopolymers have been tested in order to discover their similarities or differences in terms of hygroscopicity, antiplasticising character, colour, and impact on the mechanical properties of a freeze-dried orange snack formulated from their different mixtures. Gum Arabic, maltodextrin, starch modified with octenylsuccinic anhydride, pea fibre, bamboo fibre, and native corn starch have been selected as biopolymers. The impact of any of them on the studied properties can be confirmed, without any of them being more or less effective than the others.

Keywords: gum Arabic, maltodextrin, modified and native starch, pea and bamboo fibre, crunchiness.

1. Introduction

The consumption of fruit is part of a healthy diet, and it is recommended as a way of improving our health and well-being (WHO, 2018). With the intention of promoting the consumption of fruit among the population, there is continuous interest in offering consumers new products that stimulate this consumption (Telis & Martínez-Navarrete, 2012). To this end, there exists the possibility of obtaining a snack from freeze-dried orange puree.

The final water content of the freeze-dried fruit will condition its characteristics, especially its optical and textural properties, and the expected shelf life under given conditions (Telis & Martínez-Navarrete, 2012). During the processing of typical snacks, a low water content is determinant for their characteristic brittleness, crispness, or crunchiness. The plasticising effect of water on the mechanical properties of the food systems is well known (Roos, 1995). The primary mechanical effect of a plasticizer is that of weakening or breaking intermolecular bonds, thus decreasing its mechanical resistance and increasing its deformability, gumminess, or sogginess (Pittia & Sacchetti, 2008). The impact of water content change on the mechanical behaviour of a food has been related to its physical state (Roos, 1995). Dehydrated fruits obtained by freeze-drying are normally in an amorphous state. Above the glass transition temperature (Tg), the change from the glassy state, more stable, to the rubbery occurs. The much lower viscosity of the rubbery state determines the aforementioned product softening, so that the typical crispness is lost. For this reason, it is of great importance to ensure the glassy state of a crispy product. To this end, it should be processed and stored at temperatures below its Tg. Likewise, it is important to know, at a specified storage temperature, the critical water content (CWC) and the critical water activity (CWA) of the product in order to ensure the glassy state. As in the low water content range, a small increase in the food water content leads to a significant decrease in the corresponding Tg, the storage relative humidity (RH) has to be strictly controlled. The state diagram of a food is one tool with which to predict and control the phase transitions. According to different authors, it is the right tool for the purposes of finding out the relationships between Tg and the water content in order to design efficient processes to obtain high-quality products and also to optimize storage conditions (Fabra et al., 2009; Rahman, 2006; Roos & Karel, 1991; Roos, 1995).

Freeze-dried fruit pulps, such as sugar–rich foods, present structural problems of stickiness, caking, and collapse. The Tg of freeze-dried fruit products present very low values, between 25 ºC and -38 ºC for a water content of between 3.3 and 25% (Telis & Martínez-Navarrete, 2010). Therefore, it is fairly easy to find these products in a rubbery state under the usual storage conditions. One way to prevent this, is, for example, the addition of biopolymers of high molecular weight that contribute to increasing the value of Tg or that even play a steric role delaying the structural collapse. Biopolymers, such as gums (gum Arabic, xanthan), maltodextrins, proteins (whey protein concentrate), starches (octenyl succinic anhydride, waxy starch), and natural fibres (bamboo fibre), have been used as drying carriers to obtain stable dehydrated products (Agudelo et al., 2017; Bhusari et al., 2014; Cano-Chauca et al., 2005; Da Silva et al., 2013; Fongin et al., 2017; González et al., 2019; Martínez-Navarrete et al., 2019; Telis & Martínez-Navarrete,

2009). However, the addition of biopolymers may cause unintended effects in other properties, such as changes in the colour or texture of the final product. Non-enzymatic browning reactions, such as Maillard reactions and caramelization, may stem from heating or occur during the long-term storage of foods containing carbohydrates, especially reducing sugars (BeMilller & Whistler, 1996; Telis & Martínez-Navarrete, 2012). Since the biopolymers differ as to their composition and nature, their interactions with the food matrix may vary, causing different water-solid interactions. According to Acevedo et al. (2008), there is a correlation of the effects of water–solid interactions and water mobility on the non-enzymatic browning rates in freeze-dried potato. Some authors have studied the effect of different storage vapour pressure atmospheres on the stability of fruit powders containing biopolymers. They have found that the mechanical properties of the dried food are closely dependent on the a_w (Pérez-Alonso et al., 2006; Telis & Martínez-Navarrete, 2009).

In this study, different biopolymers, gum Arabic, maltodextrin, starch modified with octenylsuccinic anhydride, pea fibre, bamboo fibre, and native corn starch, have been tested to evaluate their possible advantages or disadvantages when added to an orange puree for the purposes of freeze-drying and obtaining a snack.

2. Materials and Methods

2.1. Raw materials

2.1.1. Fruit

The oranges (*Citrus x sinensis* var. Navel) used in this study were bought from a local supermarket in the city of Valencia (Spain). The fruit pieces were chosen by visual inspection based on the size, homogeneity, colour, and good physical integrity.

2.1.2. Biopolymers

The drying carriers used to obtain the dehydrated orange samples were gum Arabic (GA, Scharlab, Sentmenat, Spain), maltodextrin (MD, Roquette, France), starch modified with octenylsuccinic anhydride (OSA, Roquette, France), pea fibre (PF, Roquette, France), native corn starch (NCS, Roquette, France), and bamboo fibre (BF, VITACEL®, Germany). These biopolymers were selected to avoid structural collapse of the dehydrated product. GA, MD, and OSA for their ability to increase Tg and BF, NCS, and PF because of their steric role avoiding the formation of interparticle bridges.

2.2. Samples preparation

To obtain the orange puree, the fruit was washed, peeled, cut, and triturated in a bench top electrical food processor for 40 s at speed 4 (2000 rpm) followed by 40 s at speed 9 (91000 rpm) (Thermomix TM 21, Vorwerk, Spain). The orange puree was mixed (10 min at speed 3 (1000 rpm) with the biopolymers (Table 1) to obtain five different formulated samples to be freeze-dried, in addition to the orange puree with no biopolymers (O) that was also considered. The ratio orange puree:biopolymers was selected to ensure the physical stability of the dried product (Agudelo et al., 2017).

For freeze-drying purposes, each of the samples was distributed on two aluminium plates, 10.5 x 7.8 cm, 0.5 cm thickness, and immediately frozen at -45 ºC (Liebherr LGT 2325, Germany) and then dried (Telstar Lioalfa-6, Spain), at 0.05 mbar, -45 ºC on the

condenser and 40 ºC on the shelves for 20 hours, to obtain two cakes from each of the six different freeze-dried cakes (O, GA+BF, MD+PF, MD+NCS, OSA+PF, OSA+NCS). One cake from each sample was crushed manually with a mortar to obtain the corresponding powder.

Table 1. Sample code as a function of the corresponding formulation.

	Biopolymers added and concentration	
Sample	5g/100 g orange puree	1g/100 g orange puree
GA+BF	gum Arabic	bamboo fibre
MD+PF	maltodextrin	pea fibre
MD+NCS	maltodextrin	native corn starch
OSA+PF	starch modified with octenylsuccinic anhydride	pea fibre
OSA+NCS	starch modified with octenylsuccinic anhydride	native corn starch

2.3. Sorption experiments

The freeze-dried cake and the powder from each of the six samples were placed at 20 ºC in hermetic chambers containing saturated salt solutions (BrLi, ClLi, CH_3COOK, $MgCl_2$, K_2CO_3, $Mg(NO_3)_2$), the corresponding RH ranging between 6% and 53% (Greenspan, 1977). The samples were weighed every week in order to determine the equilibrium condition with the surroundings ($\Delta m \leq \pm 0.001$ g) (Kaymak-Ertekin & Gedik, 2004). In this moment, the water activity of each sample was assumed to be equal to the corresponding RH/100. The time needed to reach equilibrium was approximately two months.

2.4. Analytical determination

2.4.1. Water content (x_w)

The x_w was obtained by drying the equilibrated powdered samples in a vacuum oven (Selecta®, Vaciotem-T, J.P. Selecta S.A., Spain) at 60ºC ± 1ºC under p < 100 mm-Hg until constant weight (AOAC, 1990). Three replicates were carried out on each of the six equilibrated freeze-dried samples, and the mean value was considered as the corresponding x_w.

2.4.2. Glass transition temperature (Tg)

The Tg of the powdered samples conditioned at different water contents was determined by differential scanning calorimetry (DSC 220CU-SSC5200, Seiko instruments Inc., Japan). Approximately 15 mg of each sample were placed into DSC pans (P/N SSC000C008, Seiko Instruments Inc., Japan). The heating rate was set to 5 ºC/min and the temperature range varied between -80 ºC and 80 ºC, depending on the

water activity of each sample. The onset, midpoint, and endpoint of the glass transition were obtained from each thermogram.

2.4.3. Fitted models

In order to predict the water sorption behaviour of the samples, the linearized BET model (Eq. 1) was used to predict water sorption up to $a_w \approx 0.5$ (Brunauer, Emmett & Teller, 1938).

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$$\frac{a_w}{(1-a_w)\,W_e} = \frac{1}{W_0\,C} + \frac{C-1}{W_0\,C}.\,a_w \qquad \text{(Eq. 1)}$$

Where W_e is the equilibrium water content (g water/g dry solute), W_o is the monolayer water content value (g water/g dry solute), C is the energy constant related to the sorption heat, and a_w is the water activity.

In order to predict glass transition temperatures, experimental Tg - x_w data were fitted to the linearized Gordon & Taylor (1952) equation (Eq. 2) considering the onset, midpoint, and endpoint of the transition.

$$T_g = T_{g(s)} + k\frac{x_w .(T_{g(w)} - T_g)}{(1-x_w)} \qquad \text{(Eq. 2)}$$

Where Tg is the glass transition temperature (ºC), $Tg_{(s)}$ is the glass transition temperature of the anhydrous solids (ºC), k is the Gordon y Taylor constant model, x_w is the mass fraction of water (g water/g product), and $Tg_{(w)}$ is the glass transition temperature of amorphous water: -135 ºC (Roos, 1995).

The relationship between Tg - a_w is given by the linear regression proposed by Roos (1995) (Eq. 3), where Y and Z are the constants of the model.

$$T_g = Ya_w + Z \qquad \text{(Eq. 3)}$$

2.4.4. Mechanical properties

The mechanical behaviour of the equilibrated samples was registered using a texture analyser TA-XT2i (Stable Micro Systems, UK). Portions of 20 x 20 mm of the freeze-dried cakes were compressed using a cylindrical probe of 10 mm diameter applying a strain of 80% with a test speed of 1 mms^{-1}. Five replicates were performed per sample. The parameters analysed in the test were the maximum force (F), expressed in Newtons.

2.4.5. Colour measurements

CIE L*a*b* colour space was selected as a uniform and objective method with which to specify the colour of equilibrated freeze-dried cake samples. The L* coordinate denotes lightness on a 0–100 scale from black to white; a*, (+) red or (−) green; b*, (+) yellow or (−) blue. Colour coordinates (10° observer and D65 illuminant) were obtained from the reflectance spectrum using a spectrophotometer (Minolta, CM 3600D, Japan). The colour was measured at four different points of the whole cake, and the mean value was considered. From the colour coordinates, the hue angle (h*, Eq. 4), and chroma or saturation (C*, Eq. 5) were obtained. Measurements were taken with the specular component excluded.

$$h^* = \text{arctg}(b^*/a^*) \quad \text{(Eq. 4)}$$

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

3. Results and Discussion

3.1. Sorption behaviour

The obtained sorption isotherms predict the relationship between w_e and a_w of the different freeze-dried orange purees at 20 ºC (Fig. 1). As it can be observed, all the added biopolymers reduced the hygroscopicity of the orange cake over the whole a_w range, OSA seeming to be the most effective for this purpose.

The experimental data of each sample were fitted to the BET model (Table 2). The BET monolayer water content (W_o) was in the range of 0.0619 – 0.0742 g water/g dry solid, the highest value being that of the orange sample without biopolymers and the lowest that of the orange with OSA ones. The W_o indicates the amount of water that is tightly adsorbed in specific sites on food surfaces, which has been related with a security water content below which the product stability is guaranteed (Choudhury et al., 2011; Telis & Martínez-Navarrete, 2010; Wan et al., 2018).

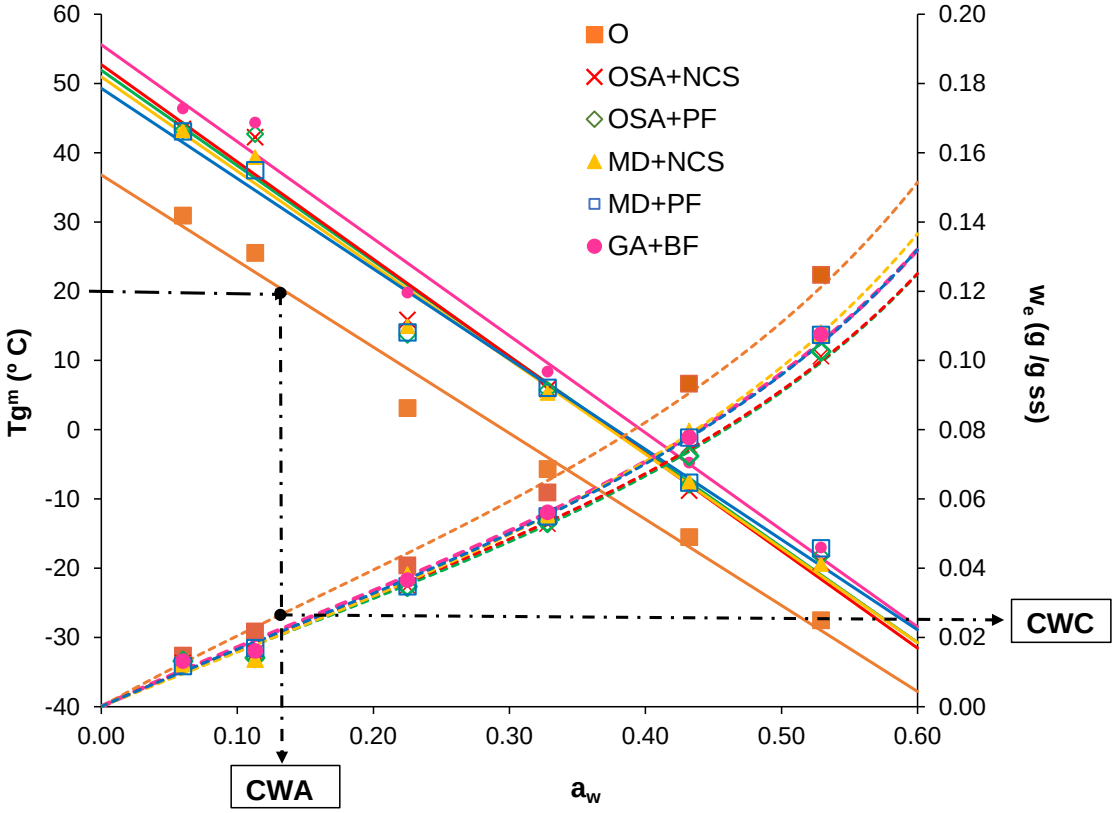

Figure 1. Water content (w_e) - water activity (a_w) – midpoint glass transition temperature (Tg^m) relationships of freeze-dried orange puree (O) and that formulated with: starch modified with octenylsuccinic anhydride and native corn starch (OSA+NCS) or pea fibre (OSA+PF); maltodextrin with native corn starch (MD+NCS) or pea fibre (MD+PF); gum Arabic with bamboo fibre (GA+BF). The continuous lines represent the Tg-a_w fitted model, the discontinuous lines predict x_w-a_w data obtained from the BET fitted model and the points correspond to experimental data. The black dashed lines show, for the O sample, how to obtain the critical water activity and water content (CWA and CWC, respectively) for glass transition at 20ºC.

The values of constant C, the other BET parameter, varied between 2.25 – 2.96, which allows these sorption isotherms to be classified as type II (Brunauer et al., 1940). In order to evaluate the significant differences in the sample's sorption behaviour, the BET equation fitted to each sample and that fitted to different groups of samples were statistically compared through the values of the statistic E, which was compared with tabulated F – Snedecor (Moraga et al., 2004). The obtained results permitted a twofold confirmation: that there were no differences between the formulated samples ($P>0.05$) and that all of them were different to the O sample ($P<0.05$). In this way, the capacity of any of the biopolymers used to reduce the hygroscopicity of the freeze-dried orange snack can be confirmed, without any of them being more or less effective than the others.

Table 2. Parameters of the BET (W_o, C) and linear (Y, Z) models fitted to experimental water content-water activity (Eq. 1) and midpoint glass transition temperature-water activity (Eq. 3) data, respectively.

	C	W_o (g water/g dry solids)	R^2	Y	Z	R^2
O	3.0	0.074	0.911	-124.4	36.8	0.981
OSA+PF	2.7	0.062	0.708	-137.8	51.9	0.969
OSA+NCS	2.8	0.062	0.837	-140.5	52.7	0.981
MD+PF	2.6	0.066	0.901	-130.4	49.3	0.982
MD+NCS	2.3	0.071	0.580	-136.2	50.9	0.985
GA+BF	2.8	0.066	0.827	-140.2	55.6	0.987

The sample codes can be identified in Table 1.

3.2. Glass transition temperature- water content-water activity relationships

The glass transition temperature of dried foods is extremely important as a means of predicting the conditions of a proper drying process and product storage (Roos & Karel, 1991). Nevertheless, glass transition is a state transition that is developed over a temperature range. That is why the onset (Tg^o), midpoint (Tg^m), and endpoint (Tg^e) of glass transition can be characterized (Fig. 2). The majority of studies published take Tg^m as the characteristic Tg value (Goula et al., 2008; Khallooufi & Ratti, 2003; Roos, 1995; Wu et al., 2019). However, the change in the food properties associated with glass transition, could start from the moment in which the transition begins or might not become patent until the entire amorphous matrix has changed to the rubbery state, at the end of the Tg. For this reason, it may be interesting to consider Tg^o and Tg^e as being related to the physical properties (Rahman, 2006; Wan et al., 2018). The samples considered in this study showed a Tg amplitude of about 5-15 ºC and, as expected, the Tg fell as the water content increased (Fig. 2). The orange sample without biopolymers presented lower Tg values than the samples with added biopolymers, especially in the lower water content range (approximately up to 0.07g water/g product). From this value, the added biopolymers do not seem to have so much influence on the Tg. The GA+BF sample seems to be the one that most increases the Tg over the entire water content range. Each one of the 3 characteristic values of the Tg of each sample was related to the corresponding water content by fitting the Gordon and Taylor model (Eq. 2).

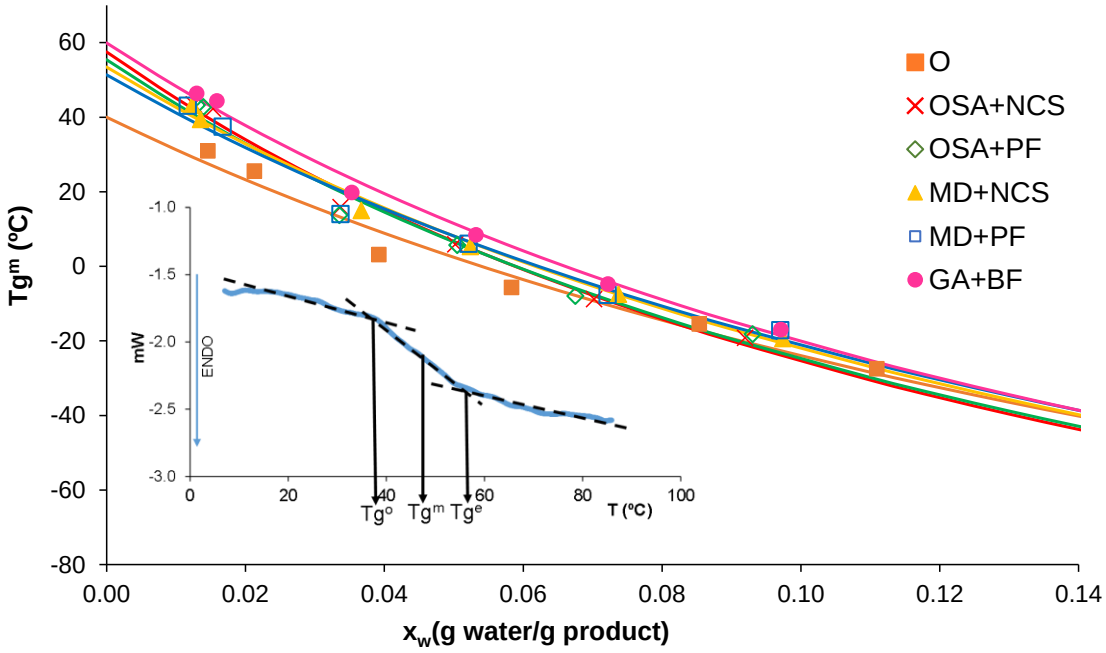

Figure 2. Mid-point glass transition temperature (Tg^m)-water content (x_w) relationship of freeze-dried orange puree (O) and that formulated with: starch modified with octenylsuccinic anhydride and native corn starch (OSA+NCS) or pea fibre (OSA+PF); maltodextrin with native corn starch (MD+NCS) or pea fibre (MD+PF); gum Arabic with bamboo fibre (GA+BF). The lines represent the Gordon and Taylor fitted model and the points correspond to experimental data. The inner graph shows an example of one of the DSC thermograms obtained with GA+BF sample with a_w = 0.060. The dashed lines and arrows indicate the way to obtain the temperature at the onset (Tg^0), midpoint (Tg^m), and endpoint (Tg^e) of glass transition.

Table 3 shows the corresponding k and $Tg_{(s)}$ parameters. The value of these parameters confirms the above comments. In this sense, the lowest $Tg_{(s)}$ was that of the O sample and the highest that of the GA+BF sample, the amplitude of the corresponding Tg ranging between 7.6 and 14.7 ºC, respectively. Again, the statistical comparison among the different Gordon and Taylor fittings, performed through comparing the values of statistic E with the tabulated F-Snedecor, showed that only the O sample was different as regards the water plasticization behaviour. No significant differences (P>0.05) were detected among the formulated samples.

Table 3. Gordon and Taylor parameters obtained when fitting the experimental onset, midpoint, and endpoint of the glass transition temperature (Tg^0, Tg^m, and Tg^e, respectively) - water content relationship (Eq. 2).

		O	OSA+PF	OSA+NCS	MD+PF	MD+NCS	GA+BF
	K	5.69	6.93	7.24	6.16	6.52	6.62
Tg^o	Tgs	37.2	48.6	50.6	45.1	47.7	52.6
	R^2	0.985	0.972	0.981	0.970	0.984	0.985
	K	5.19	6.56	6.81	5.74	6.02	6.28
Tg^m	Tgs	40.0	55.4	57.5	51.4	53.4	60.0
	R^2	0.958	0.963	0.982	0.964	0.989	0.988
	K	4.86	6.22	6.44	5.36	5.56	5.97
Tg^e	Tgs	44.8	62.3	64.4	57.8	59.2	67.3
	R^2	0.953	0.951	0.981	0.957	0.985	0.991

The sample codes can be identified in Table 1.

As shown in Fig. 1, Tg-a_w-w_e relationships may be plotted together to construct the stability map, which is a tool that permits an easy identification of the critical processing or storage conditions (temperature and relative humidity) for glass transition (Fabra et al., 2009). Table 4 shows the critical water content (CWC) and critical water activity (CWA) values for the onset and endpoint of the glass transition for each sample, both at 20 and 4 ºC, common room and refrigeration temperatures. The freeze-dried orange cake with added biopolymers showed higher values of CWC and CWA than the O sample, related to the greater capacity of these samples to maintain the more stable glassy state. It is remarkable that, despite the great microbiological and chemical stability of the dehydrated products, decreasing the storage temperature appreciably increases the CWC and CWA of all the samples. In this regard, at 20 ºC for instance, the glass transition of the GA+BF sample will take place, at a surrounding RH in the range of 19.0-30.2%, this increasing to 31.1-42.5% at 4 ºC. This increase in the critical values may be related to the physical stability of the products in terms of aspects such as the texture or colour of the snack.

On the other hand, in order to obtain a tool that makes it easy to relate both temperature and RH storage conditions for glass transition, Tg and a_w were related using the linear relationship (Eq. 3) proposed by Roos (1995). Table 2 shows the results of this fit, where a very good and useful linear relationship can be observed.

Table 4. Values of the critical water content (CWC) and critical water activity (CWA) for the onset (^{o}) and end (^{e}) of the glass transition at 20 and 4ºC.

		O	OSA+PF	MD+PF	OSA+NCS	MD+NCS	GA+BF
20 ºC	CWA^{o}	0.095	0.170	0.160	0.165	0.169	0.190
	CWA^{e}	0.165	0.279	0.275	0.275	0.275	0.302
	CWC^{o}	0.019	0.026	0.026	0.026	0.026	0.031
	CWC^{e}	0.032	0.042	0.044	0.042	0.043	0.049
4 ºC	CWA^{o}	0.213	0.293	0.289	0.285	0.295	0.311
	CWA^{e}	0.305	0.404	0.409	0.400	0.405	0.425
	CWC^{o}	0.040	0.044	0.046	0.044	0.046	0.050
	CWC^{e}	0.057	0.063	0.067	0.063	0.067	0.071

The sample codes can be identified in Table 1.

3.3. Colour and mechanical properties

The addition of biopolymers led to an increase in L* and a decrease in a* and b*, so that the chroma of the samples decreased and the hue angle increased (Fig. 3). This colour change is related to the colour of the biopolymers themselves and to the dilution of the orange pigments in the formulated samples (Telis & Martínez-Navarrete, 2009). The whitish colour of the biopolymers contributes to the increase in lightness, the colour becoming yellower and less pure. The most significant colour change in every sample was observed at water activities between 0.328 and 0.432. At this a_w, the amorphous matrices of all the samples are already in a totally rubbery state (Table 4), and the water content is enough for enzymatic and non-enzymatic oxidation and browning reactions to occur. At this water activity, the greater amount of water present in the samples exerts a dilution effect of the components responsible for the colour, which means that the change in colour is no longer so marked.

The variation in the mechanical properties of the studied freeze-dried orange snacks was evaluated from the force–deformation curves. Fig. 4 shows the typical shape of the force–distance curves obtained from the different studied samples with several a_w. For the purposes of clarity, only one of the replicates at each a_w was shown in the Figure. In every case, the increase in the water content dramatically affected the shape of the curve, so that a change from a jagged to a smooth and regular trend of the force–deformation relationship was observed. This change is related to the material transformation from hard and brittle (or crunchy and crispy) when dried to soft and ductile at a higher water content (Martínez-Navarrete et al., 2019). The loss of crispness in the orange sample without added biopolymers was evident at water activities between 0.113 and 0.225, while in the samples with added biopolymers it was in the range of 0.225-0.328 (Fig. 4). In every case, the number of fracture peaks observed at the lower a_w decreased when the water content rose. These results point to the fact that any of the studied biopolymers may be used to protect the mechanical properties of the snack over

a wider water content range, although there is still a limit to the gain of water if the quality of the product is to be ensured.

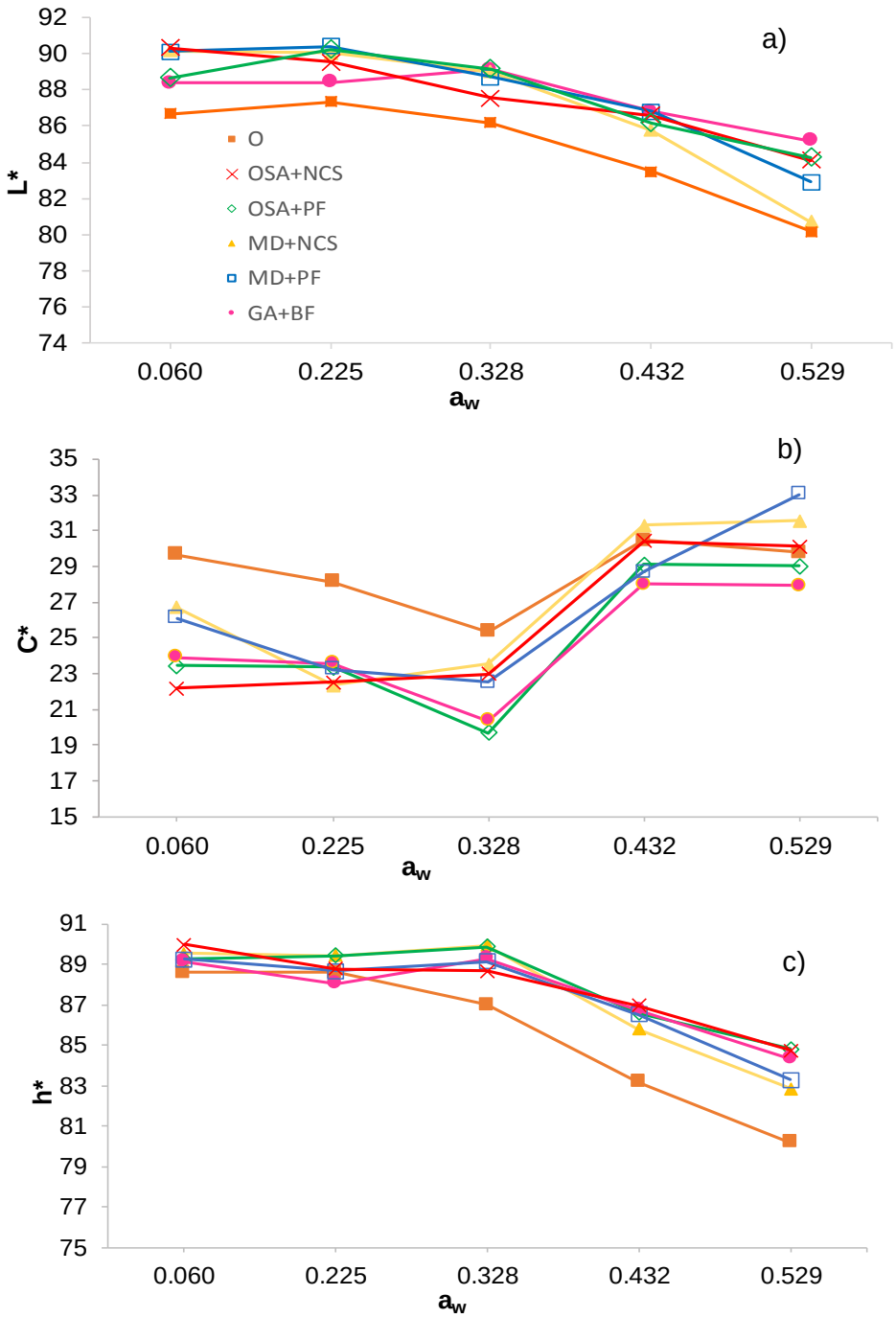

Figure 3. Values of a) luminosity (L*), b) chroma (C*) and c) hue angle (h*) of freeze-dried snack obtained from orange puree only (O) and formulated with: starch modified with octenylsuccinic anhydride and native corn starch (OSA+NCS) or pea fibre (OSA+PF); maltodextrin with native corn starch (MD+NCS) or pea fibre (MD+PF); gum Arabic with bamboo fibre (GA+BF), conditioned at different water activity (a_w).

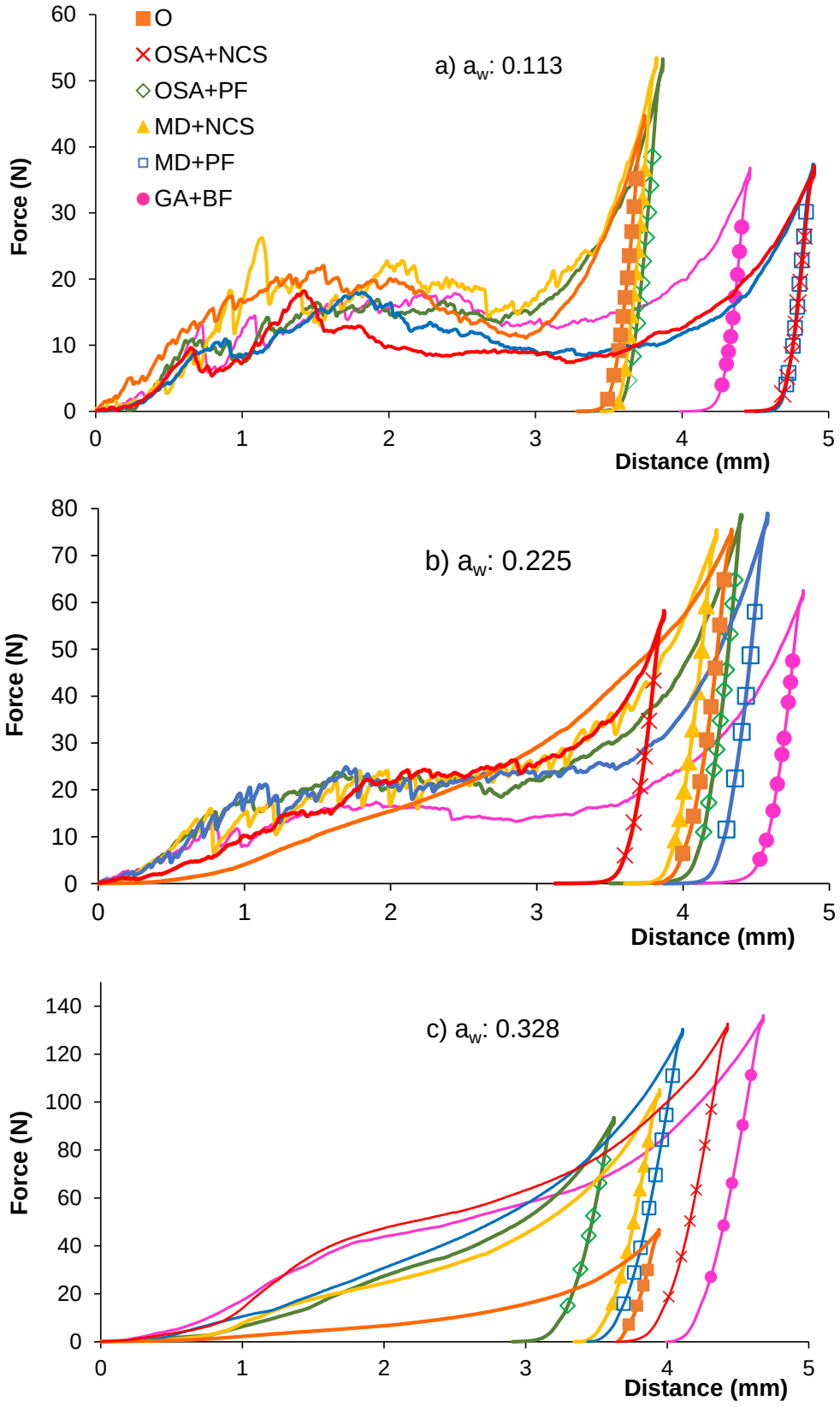

Figure 4. Force (F) – distance (d) curves obtained with freeze-dried snack obtained from orange puree (O) and that formulated with: starch modified with octenylsuccinic anhydride and native corn starch (OSA+NCS) or pea fibre (OSA+PF); maltodextrin with native corn starch (MD+NCS) or pea fibre (MD+PF); gum Arabic with bamboo fibre (GA+BF), conditioned at different water activity (a_w).

As a measurement of the strength of the samples with different a_w and so different water contents, the maximum force achieved in the mechanical test (Fmax) was characterized from the force–distance curves (Fig. 5). Significant differences ($p<0.05$) were observed between samples and at different a_w. In order to confirm the impact of the physical state of the snack matrix on the measured mechanical response, the CWA for the glass transition of every sample (Table 4) was related with Fmax. Fig. 5 shows CWA for the onset and endpoint of the Tg (CWA^o and CWA^e, respectively) of the O and GA+BF samples, and also those of the OSA+PF sample, whose values were close to the rest of the samples. As may be observed, the values of Fmax remain low below CWA^o, while all the product matrix is in the glassy state. Fmax sharply increases while the transition from the glassy to the rubbery state occurs (between CWA^o and CWA^e) and sharply decreases again when the rubbery state is fully achieved, above CWA^e. The small differences observed in the Tg-x_w-a_w relationships of the different samples containing biopolymers, despite not being significant ($P>0.05$), were enough to ensure a significantly ($P<0.05$) higher Fmax value of the GA+BF sample at $a_w = 0.328$ compared to the other samples, MD+PF having intermediate values.

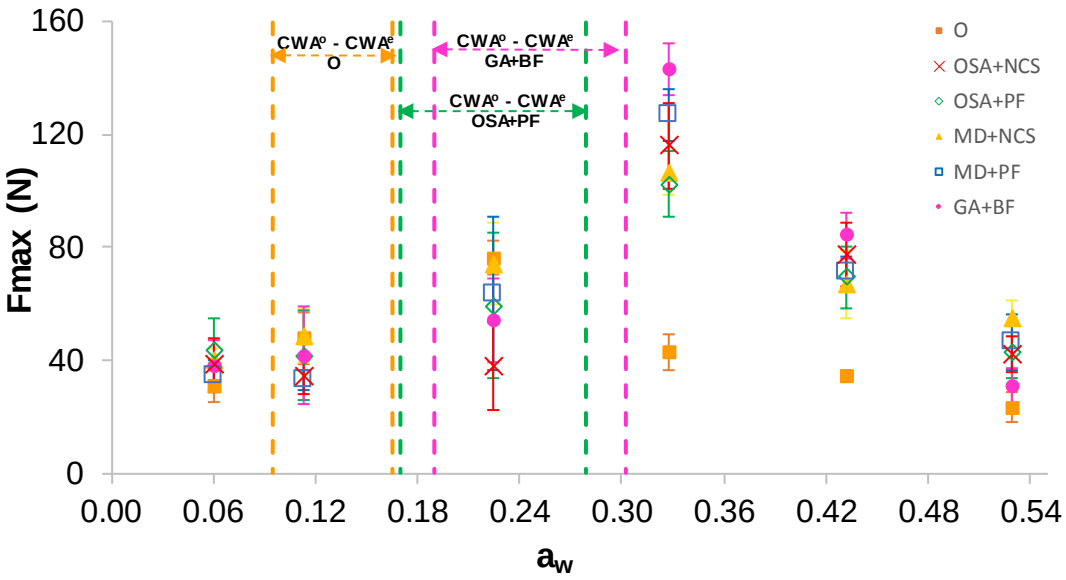

Figure 5. Values of maximum force (Fmax) obtained in the mechanical test carried out with the freeze-dried snack obtained from orange puree (O) and that formulated with: starch modified with octenylsuccinic anhydride and native corn starch (OSA+NCS) or pea fibre (OSA+PF); maltodextrin with native corn starch (MD+NCS) or pea fibre (MD+PF); gum Arabic with bamboo fibre (GA+BF), conditioned at different water activity (a_w).

The decrease in Fmax is related with the plasticizing effect of water in the fully rubbery state. In this state, the amount of water in the sample is enough to ensure all the primary interactions with the matrix and there is still water available to contribute to the softening of the sample by weakening intermolecular bonds. The increase in Fmax at

the lowest a_w, also observed in other studies (Chang et al., 2000; Moraga et al., 2011; Sogabe et al., 2018), has been called the anti-plasticization effect of water and it has been related with an increase in the cohesiveness of the glassy food matrix that increases its rigidity and firmness (Pittia & Sacchetti, 2008). This offers a greater resistance upon force application and more energy is needed to compress the sample. For these authors, the anti-plasticization effect could be considered as a mere hardening or 'toughening' effect. This hardening limit the number of fractures when a_w increases over this low a_w range. In fact, it seems that the loss in the number of observed multiple fracture peaks occurs at the same a_w from which Fmax starts to decrease (Figs 4-c and 5), when the whole matrix is in the rubbery state.

The above results indicate the need to keep the orange snack in a completely glassy state to ensure a crispy texture and adequate colour. Despite the fact that the W_o value has frequently been considered as a secure water content below which product stability is guaranteed (Choudhury et al., 2011; Telis & Martínez-Navarrete, 2010; Wan et al., 2018), in this study, the CWC that ensured the glassy state of the studied snack was lower than W_o (Tables 2 and 4). In this sense, regardless of whether W_o may be considered an optimum value for the purposes of preventing processes such as oxidative deterioration (Goula et al., 2008), it is not only a_w but also Tg data that may be considered as complementary tools with which to ensure the stability of some products.

4. Conclusions

Any of the biopolymers studied permit a reduction in hygroscopicity and an increase in the glass transition temperature of the freeze-dried orange snack, without any of them being more or less effective than the others. The typical crispy characteristics of the snack are lost at the onset of glass transition, while the colour changes are evident at its end. This indicates the need to keep the snack at temperatures below its Tg, depending on its water content. In this sense, it is recommended that the orange puree be formulated with any of the biopolymers studied and, although unnecessary from the perspective of chemical and microbiological stability, stored in refrigeration.

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Chapter 1.2. 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 these 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 bioaccessibility. This study analysed the impact of gum Arabic (GA), bamboo fibre (BF), native corn starch (NCS), starch substituted with octenylsuccinic groups (OSA), pea fibre (PF), and maltodextrin (MD) 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: orange, gum Arabic, fibre, maltodextrin, starch, *in vitro* digestion.

Abbreviations

AA, ascorbic acid; ANOVA, analysis of variance; AOA, antioxidant activity; BC, β-carotene; BF, bamboo fibre; B_x, concentration of each bioactive compound (x) analysed; 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 fibre; 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 fibre). 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 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 flavour, colour 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 study, gum Arabic (GA), maltodextrin (MD), starch modified with octenylsuccinic groups (OSA), bamboo fibre (BF), pea fibre (PF), and native corn starch (NCS) were 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 of these biopolymers are widely used in the food industry. GA, MD, and OSA have been applied as thickeners, stabilizers, emulsifiers, and flavour encapsulants of confectionary products and in various beverages (Agama-Acevedo & Bello-Perez, 2017; Kennedy et al., 2012; Williams & Phillips, 2021). The selection of fibres responds to the growing interest of the food industry in increasing the fibre 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, colour, and good physical integrity (no external damage). The biopolymers used as carriers were gum Arabic (GA, Scharlab, Sentmenat, Spain), pea fibre (PF, Roquette, Lestrem, France), bamboo fibre (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 analysing 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 aluminium 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 p < 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).

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

	Biopolymers concentration		Water content (g water/ 100 g sample)	
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 fibre	83.37	2.35
MD+PF	Maltodextrin	Pea fibre	81.97	2.00
MD+NCS	Maltodextrin	Native corn starch	82.16	2.17
OSA+PF	Starch modified with octenylsuccinic anhydride	Pea fibre	82.26	2.05

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 completed, 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. Samples analysed 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. Samples analysed after gastric digestion were coded as GD.

Finally, a dialysis membrane with a pore size of 14000 Da, 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 analysed. The external and internal intestinal digests were coded as I_eD and I_iD, respectively. The *in vitro* bioaccessibility of the different bioactive compounds was calculated from the internal intestinal digest (I_iD) using Eq. 1 (Rodríguez-Roque et al., 2013a).

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

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.

$$\text{Ratio} = \frac{[\text{B}_\text{x}]\,(\text{OD,GD,I}_\text{e}\text{D})}{[\text{B}_\text{x}]\,(\text{P or FDP})} \qquad \text{(Eq. 2)}$$

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 of P and FDP, were analysed, as well as after each different step of *in vitro* digestion. The analysis was 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 analysed 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 for the calibration curve.

The AOA was determined in the extracts obtained for the determination of TP. 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.

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

Statgraphics Centurion XVII software was employed to perform a one-way analysis of variance (ANOVA) using Tukey's HSD test 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 analysed (Table 1). Data compiled in Table 2 allow to quantify the effect of freeze-drying on each analysed bioactive compound and on the antioxidant activity of each sample. 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 the higher porosity of the orange puree structure after the process of freeze-drying (Michalczyk et al., 2009).

Table 2. Values (mean ± standard deviation) of each bioactive compound and the antioxidant activity of both the orange puree (O) and the orange puree formulated with gum Arabic and bamboo fibre (GA+BF); maltodextrin and pea fibre (MD+PF); maltodextrin and native corn starch (MD+NCS) and starch modified with octenylsuccinic anhydride and pea fibre (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.

As regards to 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 fibres (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 links 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 at low working 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 $DPPH^{\cdot}$ 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 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 $DPPH^{\cdot}$ 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 $DPPH^{\cdot}$ 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 close to 1. These results are in agreement 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 favour 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.

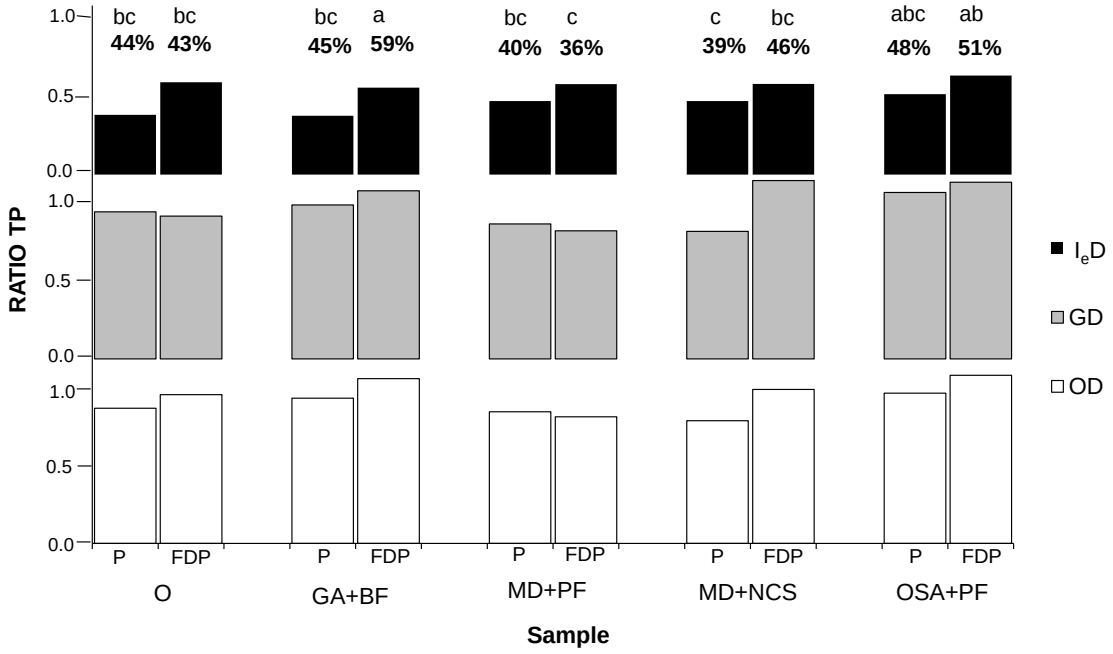

Figure 1. Ratio value (Eq. 2) between total phenolic content after the oral (OD), gastric (GD) and external intestinal (I_eD) digestion and that of the non-digested sample, for each formulation before (P) and after being freeze-dried (FDP) (nomenclature according to Table 1). Percentages indicate the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

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, favouring 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 favour 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 reactivity of oxygen.

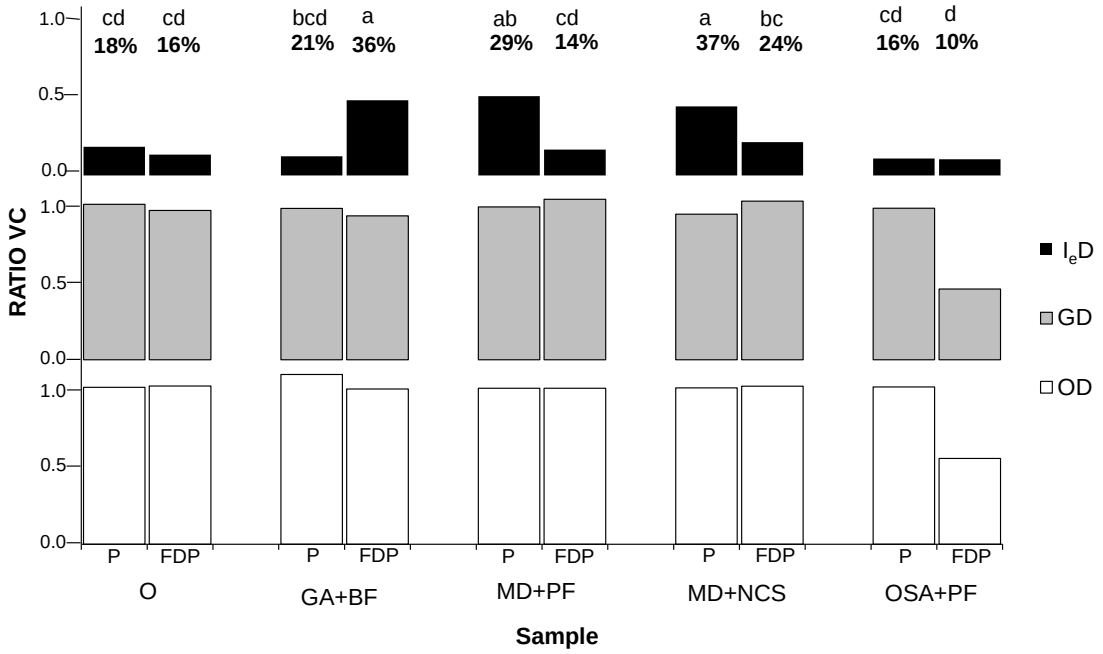

Figure 2. Ratio value (Eq. 2) between vitamin C content after the oral (OD), gastric (GD) and intestinal (I_eD) digestion and that of the non-digested sample, for each formulation before (P) and after being freeze-dried (FDP) (nomenclature according to Table 1). Percentages indicate the bioaccessibility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

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 behaviour 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 behaviour, 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 evaluation 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, reducing 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 their 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 discussed 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 to 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 fibre present in plant-based foods may decrease the carotenoids bioaccessibility by interacting with the bile and thus reducing 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 analysed 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 suggested

the protective effect of biopolymers on the compounds responsible for the DPPH free-radical scavenging activity when the digestion is carried out on the dried matrix. In this case, biopolymers would not have that favourable response in the FRAP reducing test.

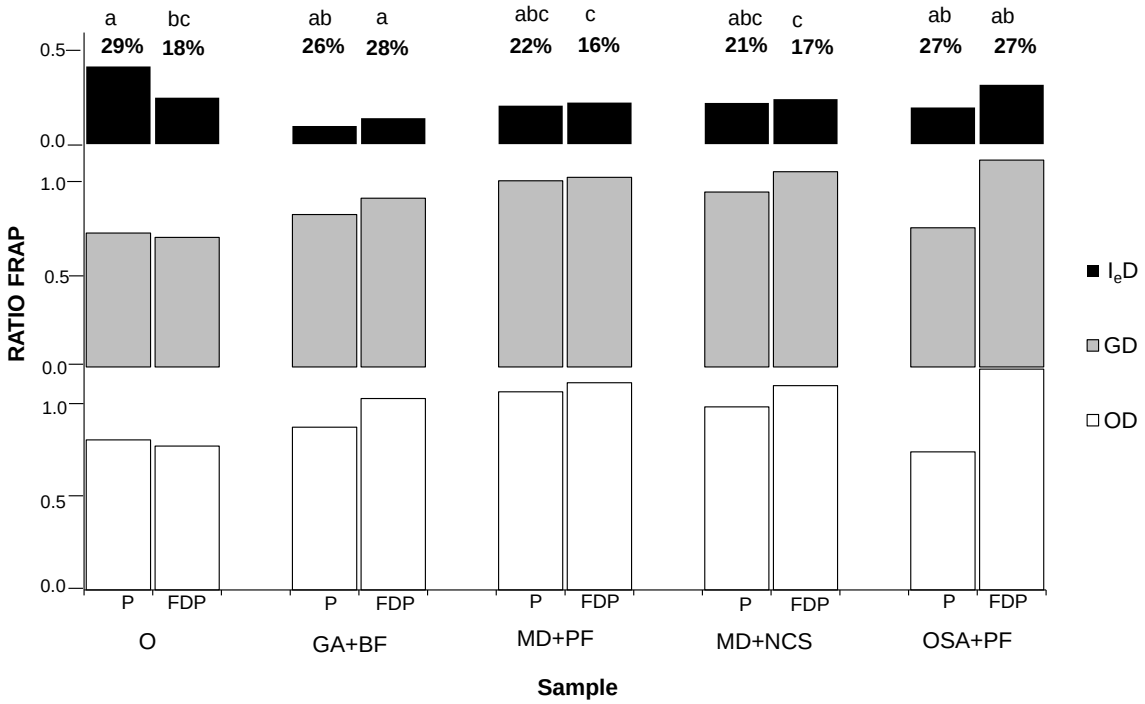

Figure 3. Ratio value (Eq. 2) between FRAP values after the oral (OD), gastric (GD) and external (I_eD) digestion and that of the non-digested sample, for each formulation before (P) and after being freeze-dried (FDP) (nomenclature according to Table 1). Estimation of % of antioxidant activity preserved 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.

The general decrease in the AOA in the intestinal step may be due to the action 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. The 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 for 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 analysed in this study, could also contribute to this result.

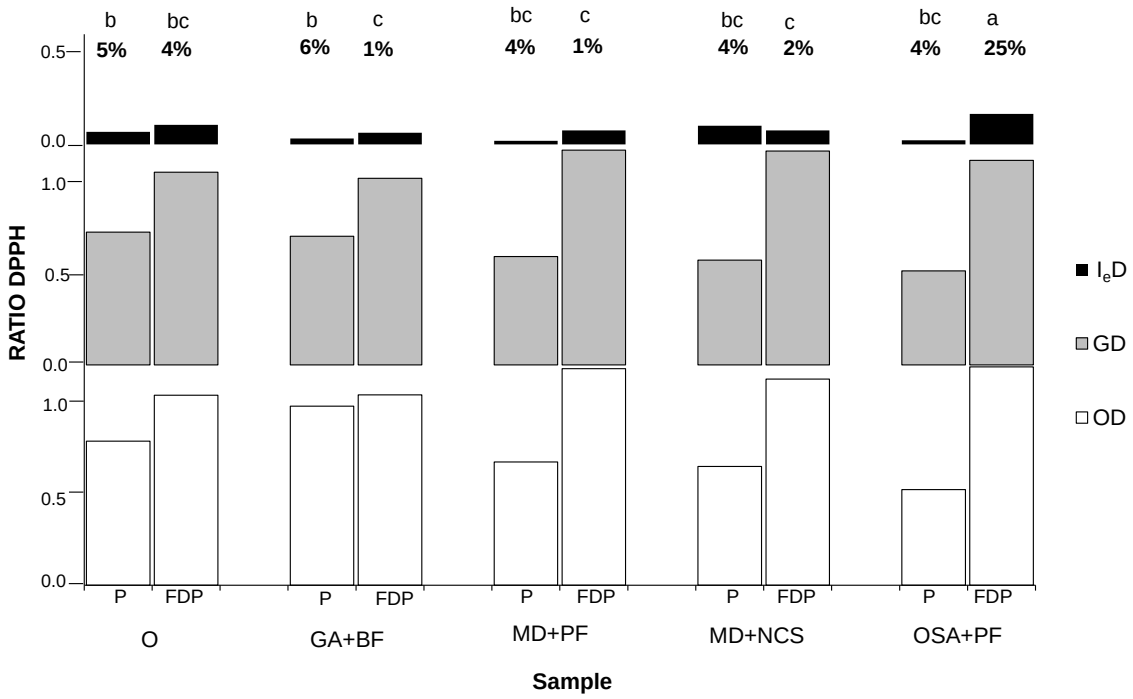

Figure 4. Ratio value (Eq. 2) between DPPH values after the oral (OD), gastric (GD) and external (I_eD) digestion and that of the non-digested sample, for each formulation before (P) and after being freeze-dried (FDP) (nomenclature according to Table 1). Estimation of % of antioxidant activity preserved 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.

4. Conclusions

All 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, with the sample containing modified starch and pea fibre being 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 fibre provided a clear protective effect of vitamin C and total phenolic compounds in the freeze-dried sample.

The increase of this indicator to 36 and 59 %, respectively, 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 fruit-based products with added nutritional value.

Supplementary Material

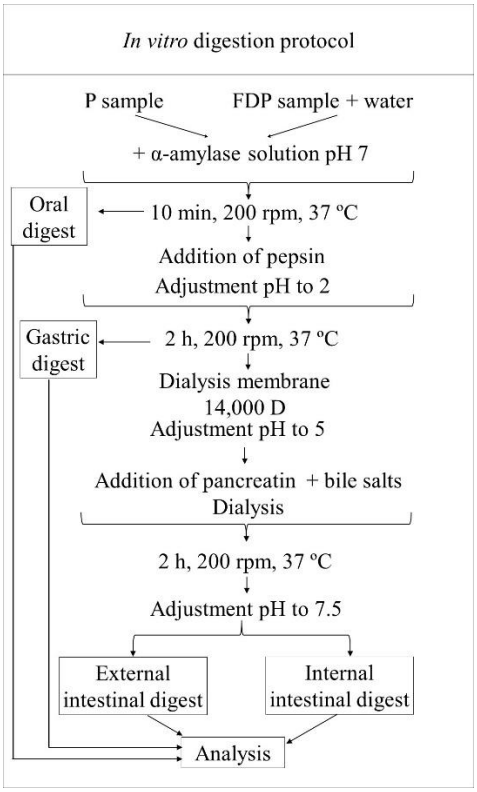

Figure S1. *In vitro* digestion protocol of the samples.

Acknowledgments

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Chapter 1.3. Impact of maltodextrin, gum Arabic, different fibres, and starches on the properties of freeze-dried orange puree powder

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Abstract

Fruits are essential components of a healthy diet contributing to the prevention of different diseases. Nevertheless, their consumption is limited by the population for reasons of convenience, among others. High-quality products are obtained by freeze-drying; however, dehydrated fruit presents a physical stability problem associated with the glass transition of its amorphous matrix. A common technique to prevent the rubbery state is the incorporation of biopolymers that contribute to increase the glass transition temperature or that may exert a steric role. Nevertheless, the chemical composition and physical properties of these biopolymers may affect the quality of the dehydrated product, which could compromise its use for specific applications. This work studies the impact of gum Arabic, bamboo fibre, maltodextrin, pea fibre, starch substituted with octenyl succinic groups and native corn starch added to an orange puree, on powder flowability and rehydration behaviour of the freeze-dried fruit powder. As regards the flowability, according to the angle of repose values (37-42º), all powder formulations were considered in the range of 'acceptable' powders. However, both samples containing maltodextrin showed significantly lower angle of repose value (37-38º), and therefore a better flowability. Samples containing gum Arabic, bamboo fibre and maltodextrin showed the lower wetting time (175-570 s), which is desired for rehydration, compared to those formulated with OSA (914-1887 s). Moreover, sample with gum Arabic showed the lowest viscosity after rehydration (0.199 Pas), desired to be consumed as a juice. According to the obtained results, if an orange puree powder with a good flowability is desired, the use of maltodextrin may be recommended. However, if rehydration is preferred, the use of gum Arabic is preferable.

Keywords: powder flowability, rehydration properties, gum Arabic, maltodextrin, fibre.

1. Introduction

Fruits and vegetables are a rich source of vitamins, minerals, dietary fibre, and other beneficial non-nutrient bioactive compounds which seem to be able to prevent a wide range of pathologies, such as cancer, cardiovascular disease and degenerative diseases connected to aging processes (Tavarini, Degl'Innocenti, Remorini, Massai, & Guidi, 2008). According to FAO, a minimum of 400 g of fruits and vegetables per day should be taken to prevent chronic diseases and mitigate several micronutrient deficiencies. However, according to the last Food Consumption Report in Spain, the average daily intake of fruits and vegetables was 248 g and 233 g, respectively, in 2018 (MAPA, 2017). It is thought that the population does not consume fruit and vegetables for various reasons: cost, convenience, and taste, among others (FAO, 2003).

As a result of the composition, seasonality, and the geographic distribution of the fruits, transformation is required to extend their accessibility while preserving the nutritional benefits. Freeze-drying is a dehydration technique that operates at low temperatures and under vacuum, so oxidation reactions and the degradation of thermolabile compounds are minimized, many of those compounds being responsible for the aromas and nutritional value of the fruits (Ratti, 2013). Since the freeze-drying is known to yield high added value products (Hammami et al., 1999), in this study this technology is proposed to obtain powdered fruit as a different way to offer fruit to the consumer. The powdered fruit may have a target audience among those people who are demanding in convenient foods with high nutritional value, spanning athletes, children, or the elderly.

Due to the increasing amount and variety of powdered products produced in the food industry and their complexity, there is a need for information on their handling and processing characteristics. Powdered fruits could be consumed as an ingredient to complete some other foods or after rehydration as a juice. In this sense, the powdered product must achieve different characteristics according to the format of use. Therefore, its flow behaviour both in air and in water, its compressibility, or its rehydration capability are important properties to be known. Despite the microbiological stability of powdered foods, due to their low water activity, in the case of fruits, they present physical problems associated with the development of stickiness (Telis & Martínez-Navarrete, 2012). This is due to their high content of sugars and organic acids, which makes their glass transition temperature (Tg) being very low, so that they are often in a rubbery state at usual consumption and storage temperatures. A common technique to increase the Tg is the addition of biopolymers of high molecular weight, such as gums, starches, maltodextrins, among others (Fongin et al., 2019, Pacheco et al., 2020, Silva-Espinoza et al., 2020, Telis & Martínez-Navarrete, 2012). Furthermore, Agudelo et al. (2017) observed that the functional quality of grapefruit powder was improved when a filler like a fibre was also added. Additionally, rehydrated powder quality can be improved also by using fillers as dispersants. If these fillers can serve to provide added fibre, then a nutritionally rounded product could be produced. In this study, gum Arabic (GA), maltodextrin 19DE (MD) and starch modified with octenylsuccinic anhydride (OSA) have been used as Tg modifiers, and bamboo fibre (BF), native corn starch (NCS) and pea fibre (PF) as fillers.

The biopolymers studied and their combination were selected based on previous studies. In a first study, GA and FB were selected and the ratio GA:FB:grapefruit puree was optimized in order to obtain a freeze-dried powder with the highest content of bioactive compounds and antioxidant activity, the lowest water content, hygroscopicity and porosity, together with an appropriate colour (Agudelo et al., 2017). Although the results obtained were highly satisfactory from the point of view of the product quality, the great structural heterogeneity of both GA and FB together with the high cost of the former, led us to look for possible alternatives to these biopolymers suitable to be added to the orange powder. MD is a cheaper biopolymer that has been widely used in spray-drying for its effect on properties such as wetting, tackiness, performance and hygroscopicity that it imparts to powdered products (Fang & Bhandari, 2012). Modified starches with improved properties, high solubility, and relatively low viscosity are becoming increasingly important, not only because of their low cost but also because of their numerous industrial applications (Dokić et al., 2012). In this sense, OSA has already been used as a substitute for some food components such as gum Arabic, fats, and proteins and is characterized as an amphiphilic polymer, obtained by chemical modifications to improve the chemical, physicochemical, and functional properties of native starch. All the native starches are also widely used in the industry since it contributes to stabilize food, helps emulsification, and improves texture (Luallen, 1985). In particular, the insolubility of NCS may suggest its use as filler. As regards to pea fibre, it was selected based on the popularity of pea within the organic and health food markets, as it is a good source of dietary fibre and other important nutrients (Powers et al., 2020), and from the sustainable point of view of pulse crops (Stagnari et al., 2017). In any case, despite the benefits of using different biopolymers as to increase the stability of the dried products, all of them will affect the properties of the raw and powdered fruit so that the best combination of them must be selected considering its final use.

As to ensure the effectiveness of the biopolymers selected for the study, the Tg-water content-water activity relationships of the orange product obtained by the freeze-drying of different mixes of 100:5:1 puree:Tg modifier:filler were studied, in relation to the stability of its texture and colour (Silva-Espinoza et al., 2020). As reported by Silva-Espinoza et al. (2020), any of the mixtures of the biopolymers considered in this study permitted a reduction in hygroscopicity and an increase in the Tg of the freeze-dried orange snack, without any of them being more or less effective than the others. What remained unclear was whether any of these mixtures provided a powdered product with improved flowability and rehydration behaviour, which was the objective of this study. Besides the particle size distribution, the powder flowability was characterized by means of its angle of repose, porosity, and compressibility. The rehydration behaviour was also studied throughout the powder wettability and the rheological properties of the rehydrated powder to produce a juice, the latter compared with four commercial orange juices.

2. Materials and methods

2.1. Raw materials

2.1.1. Fruit

Orange (*Citrus sinensis* cultivar Delta seedless*)* used in this study were bought from a local supermarket in the city of Valencia. The selection of fruit pieces was made by visual inspection based on the size homogeneity, colour, and good physical integrity.

2.1.2. Biopolymers

Carriers used to obtain the dehydrated orange samples were GA (Scharlab, Sentmenat, Spain), MD DE 19 (Roquette, Lestrem, France), OSA (Roquette, Lestrem, France), PF (Roquette, Lestrem, France), NC (Roquette, Lestrem, France), and BF (VITACEL®, Rosenberg, Germany). Some relevant physical properties and composition of each biopolymer according to the suppliers are shown in Table 1.

Table 1. Physical properties and composition of bamboo fibre (BF), starch modified with octenylsuccinic anhydride (OSA), gum Arabic (GA), native corn starch (NCS), pea fibre (PF), and maltodextrin (MD).

Biopolymer	pH	Solubility (20 ºC)	Appearance[4]	Concentration
BF	5-8[1]	Insoluble	White	95%
OSA	3.8[2]	>500 g/L	White to off-white	≥94%
GA	<7[2]	500 g/L	Slightly yellow	≥85%
NCS	5.1[2]	Insoluble	Off white	≥87%
PF	7.1[1]	Not specified	Light brown	Fibre: 50% Protein: 10% Starch: 35%
MD	4.7[3]	~600 g/L	White	≥96%

[1] at 10%; [2]at 20%;[3]at 50%; [4] all fine powders.

2.1.3. Commercial juices

Four commercial orange juices were purchased in a local supermarket in the city of Valencia, which will be called A, B, C, and D. The compositional information according to its label is: A: orange juice and vitamin C; B: orange juice, pulp, and vitamin C; C: orange juice; and D: orange juice from concentrated and vitamin C.

2.2. Obtaining the different samples

To obtain orange puree, fruit pieces were washed, peeled, cut, and triturated in a bench top electrical food processor for 40 s at 2000 rpm followed by 40 s at 9200 rpm (Thermomix TM 21, Vorwerk, Spain). Different biopolymers were added to the orange puree and mixed for 10 min at 1000 rpm to obtain a homogeneous blend. Five different samples were formulated according to Table 2.

2.3. Obtaining and characterization of the powdered products

2.3.1. Freeze-drying

The formulated orange purees (FOP) (Table 2) were placed in aluminium plates of 250 mm diameter, with a thickness of 0.5 cm per plate, and were immediately frozen at -45 ºC (Liebherr LGT 2325, Baden-Wurtemberg, Germany) for at least 48 hours. The frozen samples were dried (Telstar Lioalfa-6, Spain), at 0.05 mbar, -45 ºC in condenser and 40 ºC in the shelves for 20 hours, obtaining the corresponding freeze-dried puree (FDP).

Table 2. Sample codes assigned to the orange puree samples formulated with different amounts of biopolymers added.

Formulated orange puree (FOP)	5g/100 g orange puree	1g/100 g orange puree
GA+BF	Gum Arabic	Bamboo fibre
MD+PF	Maltodextrin	Pea fibre
MD+NCS	Maltodextrin	Native corn starch
OSA+PF	Starch modified with octenylsuccinic anhydride	Pea fibre
OSA+NCS	Starch modified with octenylsuccinic anhydride	Native corn starch

2.3.2. Crushing and sieving

The obtained FDP were crushed in the Thermomix (Section 2.2) at 3700 rpm for 20 s to obtain the corresponding powders. Before sieving, the water content was analysed (Section 2.3.3). Different sieves (CISA 200/50, Spain) were used, with the corresponding top and bottom placed, and a vibrating drum (CISA, AMP0.40, Spain) working at 50 Hz for 5 min. The powder obtained after crushing each of the five formulations was passed through a sieve of 800 µm mesh as to discard not clearly enough crushed parts of the FDP. A part of the powder with smaller size to 800 µm was characterized in all the properties related to its flow behaviour (sections 2.3.5-2.3.7) and the rehydration behaviour (Section 2.4.). In order to investigate a possible impact of the different biopolymers used in the mechanical resistance to crushing of the samples, another part of the powder < 800 µm was destined for the determination of the particle size distribution (Section 2.3.4) using sieves of 500, 300, 200, 150, and 100 µm mesh.

2.3.3. Water content

The water content (x_w, g water/100 g product) of all the FOP and of their corresponding powders was obtained by drying in a vacuum oven (Vaciotem, J.P. Selecta) at 60ºC ± 1ºC under p < 100 mm Hg until constant weight (AOAC 2000, method 934.06). To calculate the water content, weight difference before and after the drying to

the initial sample weight (XS204 DeltaRange®, Mettler Toledo, Switzerland) was obtained. Three replicates were carried out on each of the five samples.

2.3.4. Particle size distribution (PSD)

The PSD was carried out by the sieving method (Ahmed et al., 2016; Barbosa-Cánovas et al., 2012). About 90 g of the powder obtained after crushing the FDP, was sieved in batches of 30 g. The sample retained on each sieve and on the bottom was weighed and both the particle size distribution, according to the relative frequency of each size (Eq. 1), and the average particle size (Eq. 2) was calculated.

$$Relative\ frequency\ \ (\%) = \frac{w_i}{w} * 100 \qquad \text{(Eq. 1)}$$

$$Average\ particle\ size = \frac{\sum_i (AD_i * w_i)}{w} \qquad \text{(Eq. 2)}$$

Being w_i the weight of powder retained on each sieve (g), w the sum of the weight of powder retained on the different sieves and on the bottom (g), and AD_i the average mesh diameter of the sieve in which the powder is retained and the previous one (μm).

2.3.5. Angle of repose (α°)

This is the angle shaped between the slope of the cone of product formed when the powder is dropped down onto a horizontal surface and the latter. Based on the method proposed by Gallo et al. (2011), to determine α° 15 g of powdered product in a funnel (top diameter= 80 mm, stem = 11 mm, steam length = 29 mm, approx. overall height = 85 mm), placed 5 cm height from the horizontal surface covered with 80 g/m^2 DIN-A4 (Apli Paper S.A.U., Barcelona, Spain), and measuring the diameter and height of the product cone formed (Eq. 3). Four replicates were carried out to each of the five formulated samples.

$$\alpha^{\circ} = \arctan * (\frac{2h}{d}) \qquad \text{(Eq. 3)}$$

Where h = height from the top of the formed product cone to the horizontal surface (cm); d = maximum cone product diameter (cm), taken as an average of at least 6 values.

2.3.6. Density and porosity

True and apparent densities were characterized. The former excludes the air present in the sample and the latter considers the air inter and intra (open and closed pores) powder particles. True density (ρ, g/cm^3) was calculated based on the sample composition (Eq. 4). As apparent density, the density of the compacted powder (tapped density) was considered. To obtain the tapped density (ρ_T, g/ cm^3), the powder was poured inside a graduated tube to a volume of 10 mL and submitted to a vibration process in a vortex (Advanced Vortex Mixer, ZX3, VELP® SCIENTIFICA, Italy, 1200 rpm, 10 s). The density was calculated from the weight of the powder and the volume that it occupied. From the above data, the porosity (ε, Eq. 5), was calculated. Four replicates were carried out to each of the five powders.

$$\rho = \frac{1}{\frac{X_w}{\rho_w} + \frac{X_{CH}}{\rho_{CH}}} \quad \text{(Eq. 4)}$$

Where x_w and x_{CH} are the mass fractions of the two main components of each sample (water and carbohydrates), respectively; x_w determined following section 2.3.3 and x_{CH} by difference; ρ_w and ρ_{CH} are their densities (ρ_{CH} = 1,4246 g/cm^3, ρ_w = 0,9976 g/cm^3, Okos, 1986).

$$\varepsilon(\%) = 100\,\frac{\rho - \rho_T}{\rho} \quad \text{(Eq. 5)}$$

2.3.7. Compressibility

The compressibility of a product (b, Pa^{-1}) may be obtained from the variation of its apparent density when applying low stress levels (σ<9.807*10^4 Pa) (Eq. 6, Peleg, 1977).

$$\frac{\rho_\sigma - \rho_0}{\rho_0} = \frac{v_0 - v_\sigma}{v_\sigma} = a + b\,log\sigma \quad \text{(Eq. 6)}$$

Where ρ_0 and v_0 are the apparent density and the volume of the initial poured sample, ρ_σ and v_σ are the apparent density and the volume of the sample under the normal stress applied at each moment (σ, Pa) and a and b are constants. Constant b represents, specifically, the compressibility of a powder (Pa^{-1}).

The variation of the apparent density was obtained from a mechanical compression test using a texture analyser TA-XT (Stable Micro Systems, Surrey, UK), using a cylindrical probe of 10 mm diameter. The powder was placed and filled up in a circular aluminium sample holder of 11 mm diameter and 4.5 mm height. The sample holder was placed on the corresponding support. The test was carried out at constant speed of 0.05 mm/s and total deformation of 3 mm. Force (F, N), distance (h', m) and time (s) values were recorded. Five replicates of each sample were carried out. From the results, data of σ<9.807*10^4 Pa were selected and related to the volume of the sample at each moment (v_σ, m^3) to calculate the compressibility (Peleg, 1977). This volume was calculated considering the dimensions of the sample holder and the distance travelled by the cylindrical probe. The first part of this curve considered until the limit value of stress was linearized. Equations 7, 8 and 9 were used. This process was made with all replication of each formulation. Fig. 1 shows, as an example, the procedure used for one of the replicates (compressibility was 0.1311 Pa^{-1}).

$$\sigma = \frac{F}{A} \quad \text{(Eq. 7)}$$

$$h = H - h' \quad \text{(Eq. 8)}$$

$$V_\sigma = \left[\pi * \left(\frac{Di}{2}\right)^2\right] * h_\sigma \quad \text{(Eq. 9)}$$

Being σ applied stress (Pa); F: force (N); A: area of the cylindrical probe (m^2); h_σ: powder height at each σ (m); H: sample holder height (m); h': distance travelled by the cylindrical probe (m), V_σ: powder volume at each σ (m^3); Di: sample holder diameter (m).

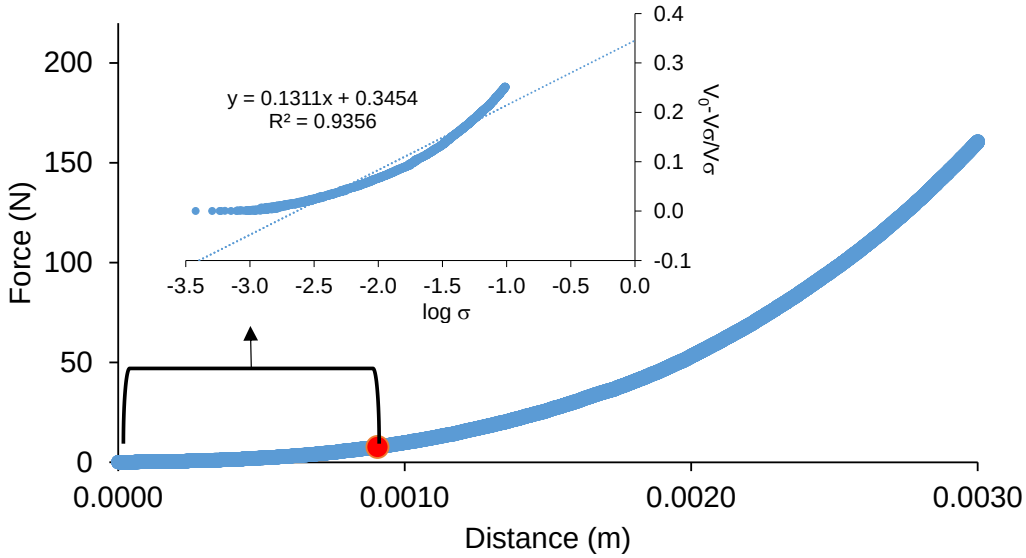

Figure 1. Example of force-distance curve corresponding to the compression test of the powder obtained from orange puree added with gum Arabic and bamboo fibre. Red point indicates the limit value of applied stress (σ) of $9.807*10^4$ Pa for linearizing to get the relation between the variation of volume of the powder and the stress applied during the compression test.

2.4. Rehydration behaviour

2.4.1. Rehydration

Powder rehydration was carried out in order to obtain products with the same water content than the corresponding FOP. A mass balance, taking into account the water content of both the FOP and the powder, was applied to calculate the water content to be added to the powder (Eq. 10 and 11). Rehydration was carried out in jacketed beakers, connected to a thermostatic bath (Poly Science, Refrigerated Circulator 901, USA) at 20 ºC, with constant magnetic stirring (800 rpm) during 5 min (Multi-Channer Stirrer MS-51M, JEIO TECH Lab Companion, Korea). Rehydrated product was left resting for 24 hours at 8 ºC. Analysis of the rehydrated products was carried out in triplicate for the rheological behaviour.

$$m^{rp} = m^{p} + m^{w} \quad \text{(Eq. 10)}$$

$$m^{p} \times \left(1 - x_w^{p}\right) = m^{rp} \times \left(1 - x_w^{FOP}\right) \quad \text{(Eq. 11)}$$

Being m^{rp}: final mass of the rehydrated product (g); m^{w}: water mass (g); m^{p}: powder mass (g); x_w^{FOP}: water content of the formulated orange puree (g water/g product); x_w^{p}: water content of the powder product (g water/g product).

2.4.2. Wettability

The wettability considers the time required for wetting all the particles of the powder when poured over water. This time is inversely related to the wettability. It was evaluated by the standard method 34-849-86 (UNE, 1986). Four replicates were made for each sample.

2.4.3. Rheological behaviour

The flow behaviour of the rehydrated products and the commercial samples was obtained at 8 °C by means of a controlled shear stress rheometer (Haake RheoStress 1, Thermo Scientific, Karlsruhe, Germany) with a coaxial cylinder sensor system (Z34 DIN), coupled to a thermostatic bath (Viscotherm VT 10, Physica). A relaxation time of 300 s was selected for the sample before running the test. Shear rate ($\dot{\gamma}$) was increased from 0 to 120 s^{-1} and shear stress (σ, Pa) was recorded. Results were fitted to the Ostwald de Waele model (Eq. 12), to obtain the flow behaviour index (n) and the consistency index (K, Pa·s^n). Instead to calculate the corresponding apparent viscosity (η, Pa·s) at a defined $\dot{\gamma}$, η was calculated applying the mean value theorem (Eq. 13, Mosquera, 2010). Taking into account that, for the samples considered in this study, η followed Eq. (14), Eq. (13) can be expressed as Eq. (15) to give the referred representative value.

$$\sigma = K(\dot{\gamma})^n \quad \text{(Eq. 12)}$$

$$\eta = \frac{1}{\dot{\gamma}max} \int_0^{\dot{\gamma}max} \eta\,(\dot{\gamma})\, d(\dot{\gamma}) \quad \dot{\gamma} \in [0, \dot{\gamma}\max] \quad \text{(Eq. 13)}$$

$$\eta = K(\dot{\gamma})^{(n-1)} \quad \text{(Eq. 14)}$$

$$\eta = \frac{K}{n}\, \dot{\gamma}\max^{(n-1)} \quad \text{(Eq. 15)}$$

Where σ: shear stress (Pa), $\dot{\gamma}$: shear rate (s^{-1}), n: flow behaviour index, K: consistency index (Pa·s^n), $\dot{\gamma}$max: maximum rate (s^{-1}) = 120 s^{-1}, η: apparent viscosity (Pa·s).

2.4.4. Particle morphology

Micrographs of rehydrated samples were acquired using an AMG EVO XL digital inverted microscope (ThermoFisher Scientific, USA).

2.5. Statistical analysis

An analysis of variance (ANOVA) using Tukey's HSD test was performed to establish the significant differences among the studied samples, which were considered when $p<0.05$. Pearson correlation coefficient (r) between the different studied properties was obtained. Statistical analysis was conducted using Statgraphics Centurion XVI.II.

3. Results and discussion

3.1. Characterization of the powder

3.1.1. Particle size distribution

Particle size distribution is directly related to the physical properties of a powdered product. Properties such as the bulk density, compressibility and flowability are highly dependent on the particle size and its distribution (Barbosa-Cánovas et al., 1987). But in the case of a powdered product obtained by freeze-drying, the particle size distribution may also be indicative of the mechanical resistance of the freeze-dried product to crushing, which may be of interest from a technological point of view. With this aim, the dry sieving size method was used as to characterize the particle size distribution of the different formulations (Section 2.3.2). PSD of each formulation is represented in Figure 2. The mode for all formulations was the particle size smaller than 100 µm. The cumulative relative frequency allows knowing the distribution median, this being 150-200 µm.

The average particle size of all the powders ranged between 201 and 221 µm. A larger particle size may be related to a greater mechanical resistance of FDP when crushed to obtain the powder, as promoted by the different biopolymers used in the formulation. In this sense, GA combined with BF, with a value of 221 µm, seems to promote a certain greater resistance while OSA+PF, with a value of 201 µm, leads to the more fragile FDP. As it can be observed in Fig. 2, these differences are mainly due to the higher frequency of particle size between 500 and 800 µm as opposed to the lower frequency of particle size between 100 and 150 µm found in the GA+BF sample. On the other hand, the combined use of PF instead of NCS contributed to the lower mechanical resistance. Nevertheless, significant differences ($p<0.05$) were found only between GA+BF and the sample OSA+PF. From this point of view, differences among the powder properties described in the following sections may be attributed to the different composition of the samples and not to a different particle size resulting from the crushing process to which they have been subjected.

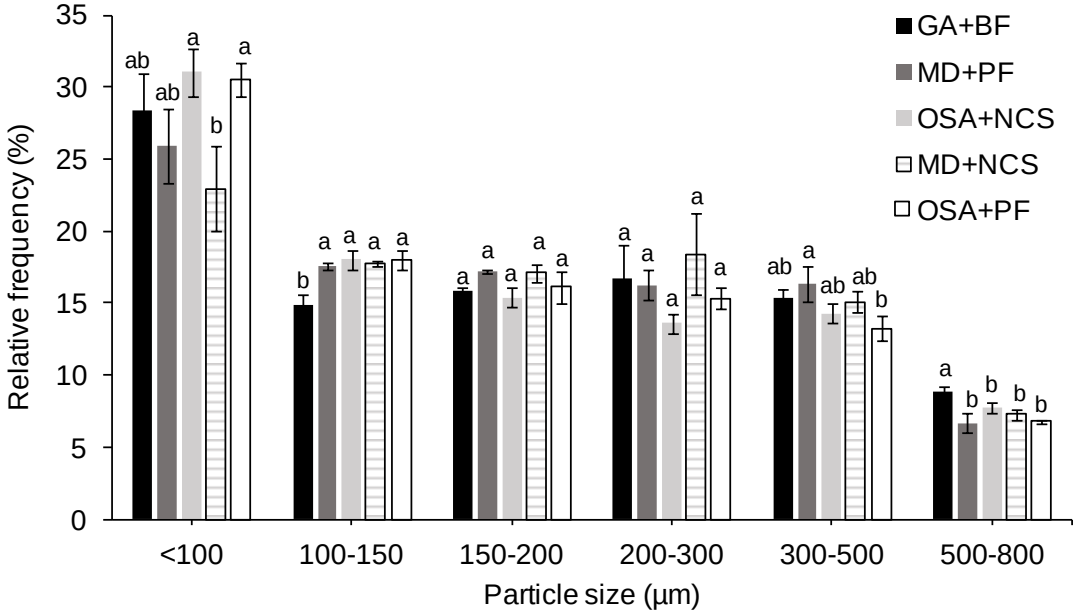

Figure 2. Particle size distribution according to the ratio weight of the retained sample in each sieve to total weight (relative frequency, %). Samples formulated with GA: gum Arabic, BF: bamboo fibre, MD: maltodextrin, NCS: native corn starch, PF: pea fibre, OSA: starch modified with octenylsuccinic anhydride.

3.1.2. Powder flowability

In this study, α°, ε and *b* were selected to evaluate the powder flowability. The α° depends on many variables, the fine particle content, density homogeneity, shape irregularities, shape of the particles themselves, particle-particle and particle-wall frictions being some of the most important (Ilari & Mekkaoui, 2005). In any case, the smaller the α°, the more easily the powder flows (Choi, Ryu, Kwak, & Ko, 2010). Regarding to *b* high values of this property are related with a worse flow behaviour (Schubert, 1987). The ε of the powder depends on the particles morphology and how this property is related to the powder flow depends on each product (Barbosa-Cánovas & Juliano, 2005; Ilari & Mekkaoui, 2005; Shenoy et al., 2015).

Three different homogeneous groups were observed (Table 3), the samples containing MD with the lower values of the three properties and GA+BF that with the highest ones ($p<0.05$). According to the Royal Spanish Pharmacopoeia (RFE, 2015) powders with α° values between 31-35º are considered with good flowability, 36-40º with regular flowability and 41-45º with acceptable flowability. In this case, samples formulated with MD, with smaller α° ($p<0.05$), can be considered as "regular" in terms of flow, while the GA+FB, with the greatest α°, is classified as "acceptable" flow powder. The results indicated the enhanced flowability provided by MD to the powders, related to a low α° and *b* and also, in the case of these samples, with a low ε. OSA samples showed an intermediate behaviour; OSA+PF could also be grouped with MD formulations while OSA+NCS could be with GA+BF.

Table 3. Values (mean ± standard deviation) of the different studied properties of each powder formulation.

Sample[a]	Angle of repose (º)	Porosity (%)	Compressibility (Pa^{-1})	Wetting time (s)
GA+BF	42.0 ± 2.0 [a]	76.9 ± 0.6 [a]	0.138 ± 0.013 [a]	570 ± 54 [cd]
MD+PF	37.0 ± 1.7 [c]	66.5 ± 0.4 [c]	0.0809 ± 0.0102 [c]	367 ± 56 [de]
MD+NCS	38.0 ± 0.7 [c]	67.0 ± 0.3 [c]	0.082 ± 0.009 [c]	175 ± 19 [e]
OSA+PF	39.4 ± 0.9 [bc]	72.4 ± 0.4 [b]	0.088 ± 0.009 [bc]	1887 ± 284 [a]
OSA+NCS	41.5 ± 0.8 [ab]	73.1 ± 1.8 [b]	0.108 ± 0.004 [b]	914 ± 87 [b]

[a]GA: gum Arabic, BF: bamboo fibre, MD: maltodextrin, NCS: native corn starch, PF: pea fibre, OSA: starch modified with octenylsuccinic anhydride. The same lowercase letter within columns indicates homogeneous groups established by Tukey HSD ANOVA.

The surface composition of the powder particles is relevant in the flow behaviour as the possible interactions between the molecules that make up the powder would affect its flowability (Fitzpatrick, Iqbal, Delaney, Twomey, & Keogh, 2004). In the cases of OSA and GA, they both have some hydrophobic groups in their structure, providing an emulsifying character, which may cause the increase of cohesiveness between particles, and therefore decrease the flowability as compared with the MD. The existence of

significant and positive linear correlation ($r=0.74$, $p<0.05$) between the compressibility and the angle of repose was verified. Furthermore, as observed by Ilari & Mekkaoui (2005), a significant and positive correlation ($r=0.79$, $p<0.05$) between angle of repose and the porosity of the powder was obtained, which indicates for this product the usefulness of porosity as a measurement of the flowability. The highest porosity of GA+BF may be due to the irregular shape of bamboo fibre (Fig 4a), that could be hindering the flowability of the powder. Similar results about the relation between the irregular shapes and porosity were found by Shenoy et al. (2015) and Barbosa-Canovas and Juliano (2005) who related the particle shape with the flowability. On the other hand, the PF used in this study is a blend of pea fibre (50%), starch (35%) and protein (10%), where the latter is also used as a drying aid. It has been reported that the surface morphology of the powders got indented and wrinkled when protein is used as a drying carrier. This fact helped to reduce the cohesiveness, and therefore improved the flowability of the powder (Muzaffar & Kumar, 2015).

3.2. Characterization of the rehydrated products

The water content of the orange puree was of 88.00 ± 0.07 g water/ 100 g product, which decreased to between 82.8 and 83.1 g water/ 100 g product when biopolymers were added. The powders obtained after crushing FDP had values between 0.3 and 1.3 g water / 100 g product.

3.2.1. Wettability

Rehydratable powders require high wettability, which means a short wetting time. The powders with the lower wetting time were those containing maltodextrin (Table 3). Sample GA+BF was statistically grouped with MD+PF, showing an intermediate behaviour. Finally, OSA+NCS and specially OSA+PF presented a significant greater wetting time ($p<0.05$, Table 3). The worst wettability of samples containing OSA may be attributed to its physicochemical properties. As a result of the chemical modification with OSA, the hydrophobicity of modified starch is greatly increased. When dissolved in water, such macromolecules preferentially migrate to the air/water interface forming a boundary layer whereby hydrophobic groups are oriented toward the air and starch extending to the water. When the interface is saturated, amphiphilic macromolecules start to aggregate (Sweedman et al., 2013).

3.2.2. Rheological behaviour

The flow behaviour of each rehydrated formulation was compared with four commercial juices (Fig. 3). The flow curves were well fitted to the Ostwald-de Waele model (Table 4), with coefficient of determination (R^2) $\geq$ 0.85 for the commercial juices and $\geq$ 0.93 for the different rehydrated products. All the samples showed a typical pseudoplastic behaviour, except the commercial juice D which showed a Newtonian behaviour. Although what characterises pseudoplastic products is the decrease in viscosity as the shear rate increases, in this case η was calculated applying the mean value theorem (Eq. 15) to give a representative value of this variable in all the shear rate range considered in the study (Table 4). All the rehydrated powders had much higher values of η than the commercial juices A and D ($p<0.05$), which did not contain visible pulp and are the least viscous.

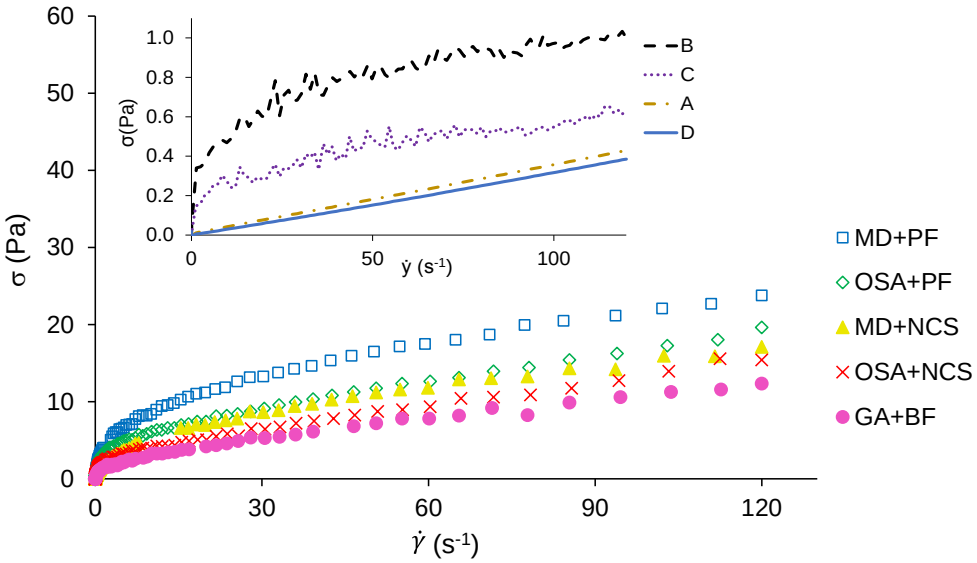

Figure 3. Flow shear stress (σ) vs. shear rate (γ̇) curves of one of the replicates of the rehydrated products and the commercial juices. GA: gum Arabic, BF: bamboo fibre, MD: maltodextrin, NCS: native corn starch, PF: pea fibre, OSA: starch modified with octenylsuccinic anhydride. Commercial juices: A: orange juice with vitamin C; B: orange juice with pulp and vitamin C; C: orange juice; D: orange juice from concentrated with vitamin C.

Table 4. Mean values (and standard deviation) of flow behaviour index (n), consistency index (K, Pa.s^n) and apparent viscosity calculated throughout the mean value theorem (η, Pa.s) for the rehydrated products of each formulation and commercial juices.

Sample[a]	n	K (Pa·s^n)	η (Pa·s)
GA+BF	0.521 ± 0.008 ^{bc}	1.027 ± 0.008 ^{c}	0.199 ± 0.003 ^{d}
MD+PF	0.589 ± 0.016 ^{bc}	2.09 ± 0.15 ^{a}	0.496 ± 0.016 ^{a}
MD+NCS	0.6046 ± 0.0102 ^{b}	1.20 ± 0.03 ^{bc}	0.299 ± 0.002 ^{b}
OSA+PF	0.47 ± 0.02 ^{c}	1.97 ± 0.09 ^{a}	0.334 ± 0.028 ^{b}
OSA+NCS	0.49 ± 0.02 ^{bc}	1.42 ± 0.08 ^{b}	0.249 ± 0.019 ^{c}
A	0.93 ± 0.05 ^{a}	0.0047 ± 0.0012 ^{e}	0.0035 ± 0.0003 ^{e}
B	0.23 ± 0.04 ^{d}	0.37 ± 0.09 ^{d}	0.041 ± 0.009 ^{e}
C	0.25 ± 0.12 ^{d}	0.22 ± 0.13 ^{de}	0.025 ± 0.013 ^{e}
D	1.00 ± 0.03 ^{a}	0.0032 ± 0.0006 ^{e}	0.0032 ± 0.0002 ^{e}

[a]GA: gum Arabic, BF: bamboo fibre, MD: maltodextrin, NCS: native corn starch, PF: pea fibre, OSA: starch modified with octenylsuccinic anhydride. Commercial juices: A: orange juice with vitamin C; B: orange juice with pulp and vitamin C; C: orange juice; D: orange juice from concentrated with vitamin C. The same lowercase letter within columns indicates homogeneous groups established by Tukey HSD ANOVA.

Among the rehydrated products, formulation GA+BF showed the closest viscosity to the commercial juices, especially to B and C, even though there were significant

differences between them ($p<0.05$). Despite these commercial juices having added pulp, not all the pulp provided by the fruit puree used in the study is present. On the other hand, the rehydrated products containing PF showed the highest η. As commented above, the PF used in this study was composed for a blend of pea fibre, pea starch and pea protein where the pea starch corresponded to 35%. In this sense, a study carried out in extruded feeds showed that pea starch also offers a higher value of viscosity than other starches like wheat starch (Sorensen, Morken, Kosanovic, & Overland, 2011). Those samples formulated with NCS showed lower values of η than those with PF. Also, it was observed that those rehydrated products containing maltodextrin showed higher values of η.

The structure of some of the rehydrated formulations are shown in Figure 4. It can be clearly observed the presence of bamboo fibre in the rehydrated powder product (Figure 4a), which indicates the low solubility of this fibre in aqueous system. Native starches generally have limited solubility in water (Sweedman et al., 2013). In this sense, NCS was reported to be insoluble in water at 20 ºC, where the insoluble starch granules can be observed in Figure 4b. However, with regards to the presence of pea fibre in the rehydrated products, this observation was not appreciated (Figure 4c and 4d).

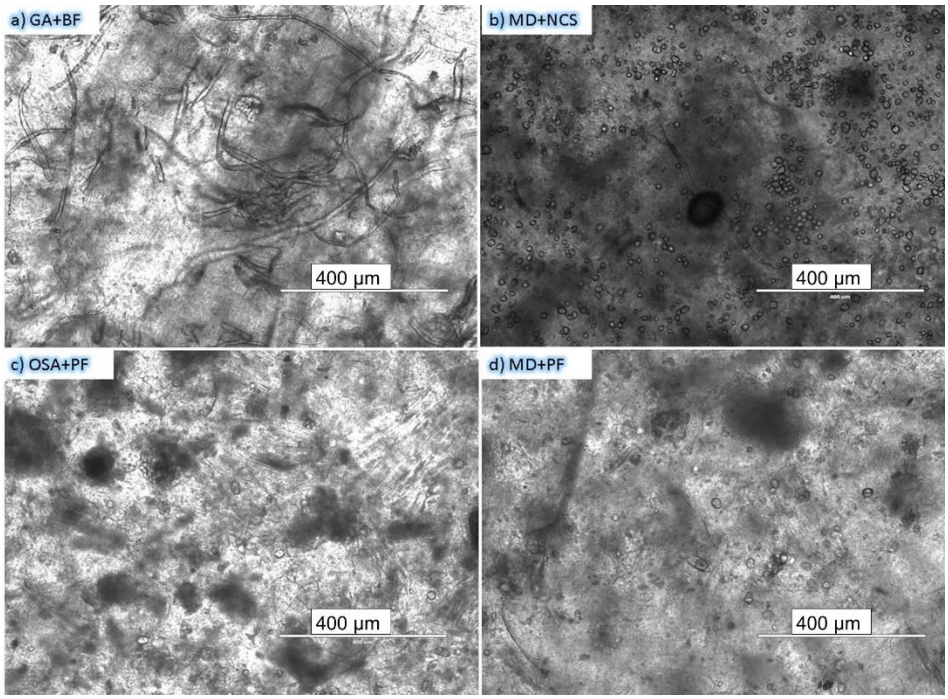

Figure 4. EVO's micrographs of the rehydrated products. Rehydrated and formulated with: a) GA: gum Arabic and BF: bamboo fibre; b) MD: maltodextrin and NCS: native corn starch; c) OSA: starch modified with octenylsuccinic anhydride and PF: pea fibre; d) MD: maltodextrin and PF: pea fibre.

This may be due to the lower amount of fibre in the samples formulated with PF than that with BF, because the previously mentioned PF composition or it is having a greater solubility. Both hypotheses may cause the increase of viscosity in those rehydrated products containing PF, while the presence of particles non-soluble, such as the BF and NCS may be causing the lower viscosities.

Regarding the correlation with the other studied properties, the apparent viscosity showed a significant and negative linear correlation ($p<0.05$) with angle of repose ($r=-0.67$), compressibility ($r=-0.75$) and porosity ($r=-0.77$). It seems to be that the worst product regards to flowability, GA+BF, gives a rehydrated product with the lowest viscosity, closest to those of the commercial juices.

4. Conclusions

The results confirm the influence of the different biopolymers studied on the characteristics of the freeze-dried orange puree powder. If the objective is to obtain a powder with good flow behaviour in air, it is preferable to formulate the puree with maltodextrin, either with pea fibre or with native corn starch. If the use of the powdered product is intended to be for rehydration and consumption as a juice, it is preferable those biopolymers that promote both a shorter wetting time and lower viscosities of the rehydrated products. In this sense, gum Arabic and bamboo fibre or those with maltodextrin should be selected. However, maltodextrin provides products of much higher viscosity after their rehydration. Then, the gum Arabic seems to be the biopolymer more adequate for this purpose, thereby demonstrating that adding key biopolymers improve the physicochemical properties of the freeze-dried fruit powder.

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Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Conflicts of interest

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

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Chapter 2. *Selection of freeze-drying conditions to obtain an orange snack*

Chapter 2.1. The impact of freeze-drying conditions on the physico-chemical properties and bioactive compounds of a freeze-dried orange puree

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Abstract

Fruits are essential for a healthy diet, as they contribute to the prevention of cardiovascular diseases and some cancers, which is attributed to their high bioactive compound content contributing to their antioxidant capacity. Nevertheless, fruits have a short shelf life due to their high-water content, and freeze-drying is a well-known technique to preserve their nutritive quality. However, it is an expensive technology, both due to the use of low pressure and long processing time. Therefore, an optimisation of variables such as the freezing rate, working pressure, and shelf temperature during freeze-drying may preserve fruit quality while reducing the time and costs. The impact of these variables on colour, porosity, mechanical properties, water content, vitamin C, total phenols, β-carotene, and antioxidant activity of a freeze-dried orange puree was evaluated. The results showed a great impact of pressure and shelf temperature on luminosity, chroma and water content. Vitamin C and β-carotene were more preserved with higher shelf temperatures (shorter times of processing) and lower pressure, respectively. The optimum freeze-drying conditions preserving the nutrients, and with an interesting structural property, perceived as a crunchy product by consumers, are low pressure (5 Pa) and high shelf temperature (50 °C).

Keywords: Vitamin C; total phenols; total carotenoids; antioxidant activity; colour; mechanical properties; pressure; shelf temperature; freezing rate

1. Introduction

It is known that there is growing interest in the consumption of fruits, as they are recommended as components of a healthy diet due to their contribution to the prevention of some diseases when they are consumed in adequate quantities (Kader, 2008; Polouse et al., 2005). This effect is attributed to their high content of bioactive compounds such as phytochemicals, some vitamins and fibres (De Ancos et al., 2000). In particular, the orange and its derived products are a rich source of flavonoids (mainly hesperidin), carotenes, and vitamin C, with concentrations in the range of 15–238.8 mg, 182–198 µg and 43.5–50 mg/100 g edible fruit, respectively (Aschoff et al., 2015; Gökmen et al., 2000; Goulas & Manganaris, 2012; Moreiras et al., 2018; Peterson et al., 2006; Ramful et al., 200). In fact, an average orange would contribute 80% of the RDA (recommended daily allowance) of vitamin C (Institute of Medicine, 2000). However, fruits have two main problems that affect their continuous availability, which are seasonality and short shelf life. Dehydration is one of the most common techniques used to preserve food. In addition, it also entails a reduction in the volume and weight of the product, which facilitates its transport and handling (Fazaeli et al., 2012).

Freeze-drying is a dehydration technique based on the sublimation of the water present in a product, which results in the reduction of its water activity and therefore the related deterioration processes to which a food is subjected (Barbosa-Cánovas et al., 2005). The product is frozen prior to be subjected to vacuum pressure with the consequent sublimation and desorption of the water. Freeze-drying operates at low temperatures, which contributes to preserve characteristics such as taste, colour, or appearance and to minimize the degradation of thermolabile compounds, many of them responsible for the aromas and nutritional value of the fruits. Thus, the final freeze-dried product is high quality as compared with other techniques of dehydration (Karam et al., 2016).

Despite the improved microbiological stability of the final product, the chemical and physical attributes may sometimes be compromised. On the one hand, the high porosity and the low water content of the freeze-dried products make the interaction between the solutes and the oxygen at the end of the process more accessible. In this way, the oxidation of bioactive compounds, such as vitamin C, phenols, or carotenoids may be promoted. On the other hand, the physical problems are related to the glass transition of the amorphous matrix, which is usually developed during the freeze-drying process. Above the glass transition temperature (Tg), the change from the more stable glassy state to the rubbery state occurs (Roos, 1995). Freeze-dried fruit pulps, as sugar-rich foods, have a low Tg value in the range of 5–15 °C (Conceição et al., 2016; Silva et al., 2006). For this reason, they present collapse and other structural problems related to stickiness and caking, which begin to be developed about 20 °C above the Tg (Roos, 1995). A usual way to delay these problems is the incorporation of high molecular weight biopolymers that contribute to an increase in Tg, or that exert a steric role (Martínez-Navarrete et al., 2019; Telis & Martínez-Navarrete, 2012).

The disadvantage of freeze-drying is its high cost, due to the long process times and the energy cost related to the vacuum stage. For this reason, it has only been widely used to obtain products with high value added, as occurs in the pharmaceutical industries

as well as in some specific food industries, such as rehydratable coffee. However, given the high sensory and functional value of fruits, associated with their high content of bioactive compounds, freeze-drying can provide a niche opportunity in this case. In this sense, the technique can provide different food formats, among them, a crunchy fruit product with good consumer acceptance as a snack (Martínez-Navarrete et al., 2019). Despite adequate optimization of the process conditions contributing to reduce the duration of the process, several reports have indicated that both the freezing and the drying variables, such as the freezing rate or the working pressure and shelf temperature during the drying step, may affect the quality of the obtained product (Ceballos et al., 2012; Genin & Rene, 1996; Hammami & René, 1997; Karel & Flink, 1973; Lombraña, 1997; Martínez-Navarrete et al., 2019; Ratti, 2001). As regards the impact of increasing the shelf temperature, a study carried out with grapefruit puree indicated a decrease of more than 50% in drying time when increasing the temperature up to 40 °C, without a great impact on aspects such as colour, texture, or vitamin C content (Egas-Astudillo et al., 2018), nor there was an effect observed on the vitamin C content when a mandarin juice was freeze-dried at 40 °C compared to that processed at room temperature (Martínez-Navarrete et al., 2019). Nevertheless, the shelf temperature should not exceed either the collapse temperature or that which could cause damage to the thermolabile compounds of interest.

In this study, the impact of freeze-drying conditions on the quality of a freeze-dried orange puree with added gum Arabic and bamboo fibre was evaluated. Two freezing rates (conventional and blast freezer), three different shelf temperatures (30, 40, 50 °C) and two working pressures (5 and 100 Pa) were combined. The quality indices measured were the water content, colour, porosity, mechanical properties, vitamin C, carotenoids, and phenolic content, as well as the total antioxidant capacity.

2. Materials and Methods

2.1. Raw Materials

Oranges (*Citrus x sinensis* cultivar Navel) used in this study were selected by subjective visual inspection based on a similar weight and size colour homogeneity and good physical integrity (absence of external physical damage). They were bought in October 2019 from a local supermarket in the city of Valencia (Spain) and immediately processed. Carriers used to obtain the dehydrated orange samples were gum Arabic (GA, Scharlab, Sentmenat, Spain) and bamboo fibre (BF, VITACEL®, Rosenberg, Germany).

2.2. Freeze-Drying Processing

Oranges were washed, peeled, cut, and triturated in a bench top electrical food processor for 40 s at speed 4 (2000 rpm) followed by 40 s at speed 9 (9100 rpm) (Thermomix TM 21, Vorwerk, Spain). The orange puree was mixed for 10 min at speed 3 (1000 rpm) with (5 g GA + 1 g BF)/100 g orange puree as to ensure the physical stability of the dried product (Agudelo et al., 2016). The formulated orange puree (FOP) was distributed in 10.5 × 7.8 cm aluminum plates of 0.5 cm thickness. Samples were immediately frozen at two different freezing rates (FR): slow freezing (FR-S) in a conventional freezer (Liebherr Mediline LGT 2325, Liebherr, Baden-Wurtemberg,

Germany) for 48 h and fast freezing (FR-F), where the samples were frozen for 3 h at −38 °C in a blast freezer (Hiber RDM051S, Hiber, Cernusco sul Naviglio, Italy), and then stored at −45 °C in the conventional freezer for at least 24 h. Frozen samples were dried in a freeze-drier (Telstar Lyoalfa-6, Telstar, Terrassa, Spain) at different pressures (P) in the chamber and shelf temperatures (T). Twelve different conditions were studied (Table 1). The shelf temperature conditioned the drying time, this being 25 h at 30 °C, 7 h at 40 °C, and 6 h at 50 °C. The time was selected based on preliminary experiments to be enough to achieve a water content lower than 4%. At these conditions, the physical stability of the formulated puree was known to be guaranteed, as no structural collapse was observed.

Table 1. Sample and conditions code according the twelve different freeze-drying conditions studied.

Sample Code	Shelf Temperature (T)			Pressure		Freezing Rate (FR)	
	30 °C	40 °C	50 °C	5 Pa (P_5)	100 Pa (P_{100})	Slow (S)	Fast (F)
S_30_P_5	X			X		X	
F_30_P_5	X			X			X
S_30_P_{100}	X				X	X	
F_30_P_{100}	X				X		X
S_40_P_5		X		X		X	
F_40_P_5		X		X			X
S_40_P_{100}		X			X	X	
F_40_P_{100}		X			X		X
S_50_P_5			X	X		X	
F_50_P_5			X	X			X
S_50_P_{100}			X		X	X	
F_50_P_{100}			X		X		X

2.3. Water Content

The water content (x_w, g water/100 g product) of FOP was determined using the AOAC method (AOAC, 1990). The sample was dried in a vacuum oven (Selecta®, Vaciotem-T, J.P. Selecta S.A., Barcelona, Spain) at 60 ± 1 °C under P < 100 mm Hg until constant weight (XS204 DeltaRange®, Mettler Toledo, Switzerland). For the freeze-dried puree, an automatic Karl Fisher titrator (Mettler Toledo, Compact Coulometric Titrator C10S, Worthington, OH, USA) was used to obtain the water content. Triplicates were performed in each case.

2.4. Mechanical Properties

The mechanical behaviour of the freeze-dried puree was registered using a texture analyser (TA-XT2i, Stable Micro Systems, Godalming, UK). Portions of 20 × 20 mm of the freeze-dried puree were compressed using a cylindrical probe of 10 mm diameter, applying a strain of 80% with a test speed of 1 mms^{-1}. Six replicates were performed per sample. The parameters analysed in the test were the force required to fracture the sample (Fracture force, F_f), expressed in Newtons, and the slope (S_L, N/mm) of the curve in the linear zone prior to fracture point, related to the sample resistance to deformation (rigidity) (Contreras et al., 2008).

2.5. Colour Measurements

The CIE L*a*b colorimetric space was considered to characterize the colour (Hutchings, 1999). A colorimeter (Minolta, CM 3600D, Japan) was used to measure the colour of the surface of the freeze-dried puree, taking the system observer 10° and illuminant D65 as reference. Colour coordinates, L*, a*, b*, were obtained for each freeze-dried puree. From them, the hue angle (h*, equation 1) and chroma or saturation (C*, equation 2) were obtained. When total colour differences (ΔE*) were calculated, Equation (3) was used. Measurements were carried out with the specular component excluded. Six replicates were performed per sample.

$$h^* = \arctan(b^*/a^*) \quad \text{(Eq. 1)}$$

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

$$\Delta E^* = ((\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2)^{1/2} \quad \text{(Eq. 3)}$$

2.6. Porosity

True density (ρ) and apparent density (ρ_a) were obtained in order to obtain the porosity (ε, %). True density was calculated based on the sample composition (Equation 4). Portions of the cakes were obtained with a punch of 22 mm diameter and were exactly measured in height and diameter with a calliper. Apparent density of each portion was calculated from the weight (m, g; XS204 DeltaRange®, Mettler Toledo, Switzerland) and corresponding volume (V, cm_3) (Equation 5). The porosity was calculated from Equation 6.

$$\varrho = \frac{1}{\frac{X_w}{\varrho_w} + \frac{X_{CH}}{\varrho_{CH}}} \quad \text{(Eq. 4)}$$

where x_w and x_{CH} are the mass fractions of the two main components of each sample (water and carbohydrates, respectively, x_w was determined as described in Section 2.3, and x_{CH} by difference); ρ_w and ρ_{CH} are their densities (ρ_{CH} = 1.4246 g/cm^3, ρ_w = 0.9976 g/cm^3, Okos, 1986).

$$\rho_a = \frac{m}{v} \quad \text{(Eq. 5)}$$

$$\varepsilon(\%) = 100\frac{\rho - \rho_a}{\rho} \quad \text{(Eq. 6)}$$

2.7. Total Polyphenolic Compounds

The extraction of total phenolic compunds (TP) was carried out according to Tomás-Barberán et al. (2001) with minor modifications. FOP (2.5 g) or freeze-dried puree (0.5 g) were mixed with 9 mL of methanol:water (70:30) using a magnetic multi-stirrer at 200 rpm (JEIO TECH Lab Companion MS-51M, JEIO TECH Lab Companion, Seoul, Korea) under darkness and at room temperature for 30 min. The homogenates were centrifuged at 11,515× *g* at 4 °C for 10 min (GYROZEN Co., 1236R, GYROZEN, Daejeon, Korea). The supernatant was collected and analysed as to TP using the Folin–Ciocalteu method, which was adapted from Benzie et al. (1999) with some modifications as described by Selvendran et al. (1990). The TP content was calculated as mg of gallic acid equivalents (GAE)/100 g dry basis (db) sample, using a standard curve in the range of 0–1000 ppm of gallic acid (Sigma-Aldrich, Saint Louis, MO, USA). In this study, all bioactive compounds were referred to the percentage (%) of the corresponding bioactive compound preserved in the freeze-dried puree (FDP) in reference to the FOP, calculated based on Equation 7. This test was done in triplicate for each sample.

$$PBc\ (\%) = \frac{Bc_{FDP}}{Bc_{FOP}} \times 100 \quad \text{(Eq. 7)}$$

where PBc (%) is the percentage of the corresponding bioactive compound preserved; Bc_{FDP} is the bioactive compound content in the freeze-dried puree (mg/100 db); and Bc_{FOP} is the bioactive compound content in the formulated orange puree (mg/100 g db).

2.8. Antioxidant Activity

The antioxidant activity (AOA) was determined with the DPPH and FRAP tests. The methanolic extract obtained for the quantification of TP was used to this end. DPPH was carried out according to Brand-Williams et al. (1995) with minor modification. For these samples, the steady state of the reaction was reached at 15 min, when the absorbance at 515 nm was measured again. The FRAP test was carried out according to Benzie et al. (1999). The results for both methods were converted to mmol Trolox equivalents/100 g db freeze-dried puree. The AOA was also expressed as the percentage (%) of this activity preserved in the FDP compared to the FOP (Equation 7). Three replicates were performed per sample.

2.9. Vitamin C

Total vitamin C content (VC) was determined by the reduction of dehydroascorbic acid to ascorbic acid (AA) using high-performance liquid chromatography (HPLC) (Jasco, Italy). The reduction was carried out by mixing 0.5 g of FOP or 0.075 g of each of the 12 freeze-dried puree samples with 2 mL of a 20 g/L DL-dithiothreitol solution (Scharlab, Spain) for 2 h at room temperature and under darkness (Sánchez-Mata et al., 2000; Sánchez-Moreno et al., 2003). The extraction of the mixture was carried out according to Xu et al. (2007). The HPLC conditions were: Kromaphase100-C18, 5 mm (4.6 × 250 mm) column (Scharlab SL); 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. A standard solution of L (+) ascorbic acid (Scharlab SL, Sentmenat, Spain) in the range of 5–200 ppm was prepared. The VC content was calculated as mg AA/100g db sample and the percentage (%) of this bioactive compound preserved in the FDP in reference to the FOP was calculated (Equation 7). Three replicates were performed per sample.

2.10. β-Carotene

The extraction of β-carotene (BC) was performed using the method of Olives et al. (2006) with some modifications. FOP (0.8 g) or freeze-dried puree (0.2 g) were mixed with 9 mL of hexane/ethanol/acetone (50:25:25, *v*/*v*/*v*) using a magnetic multi-stirrer at 200 rpm (JEIO TECH Lab Companion MS-51M, Korea), under darkness and at room temperature for 30 min. The homogenates were centrifuged at 11,515× *g* at 4 °C for 10 min (GYROZEN Co., 1236R, Daejeon, Korea). Distilled water was added to the supernatant (10 mL distilled water/100 mL supernatant) and was manually stirred for 2 min. The absorbance of the upper layer was measured at 446 nm (spectrophotometer V-1200 VWR, VWR, Radnor, PA, USA) (Olives et al., 2006). The BC was calculated as mg BC/100 g db sample using a β-carotene (Dr. Ehrenstorfer, Augsburg, Germany) calibration curve in the range of 0.5–7 ppm. The BC was referred to the percentage (%) of this bioactive compound preserved in the FDP in reference to the FOP (Equation 7). Three replicates were performed per sample.

2.11. Statistical Analysis

Data were subjected to Partial Least Squares Regression (PLS-R) and a three-way analysis of variance (ANOVA) using Tukey's HSD test to establish the significant effect of shelf temperature, pressure and freezing rate on the parameters studied, with 95% confidence interval, by using XLSTAT statistical and data analysis solution (Addinsoft, 2019, Long Island, NY, USA). F-Values obtained with the ANOVA were also considered to identify the most important factors. Furthermore, a Pearson's correlation analysis between antioxidant capacity and the bioactive compounds content was carried out.

3. Results and Discussion

All the results obtained are detailed in the supplementary material (Tables S1, S2, and S3). The most relevant aspects are described below.

3.1. Physicochemical Characterization

The experimental results of the colour characterization, mechanical properties, porosity, and water content of the freeze-dried purees obtained under each of the 12 studied conditions were processed by PLS-R (Figure 1 and Table S4). Axis 1 (t1) mainly represents the impact of pressure on the qualitative explanatory variables (Y), while axis 2 (t2) represents the impact of shelf temperature on Y. The vectors of freezing rate are in the inner circle, which indicates that in general, these factors are not significantly correlated with the different studied properties of the samples. The variables Y that are significantly affected by a specific independent variable (X) are circled in red, blue, orange, and green.

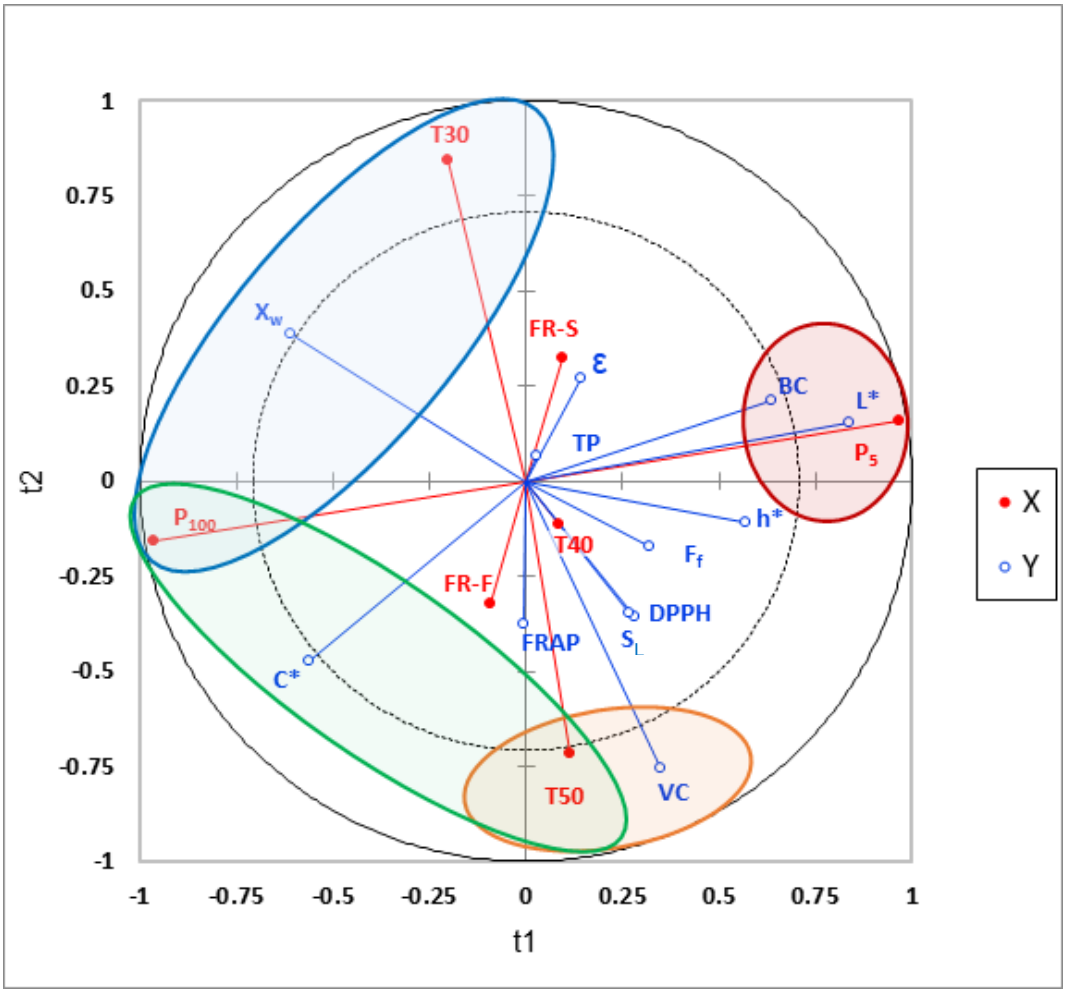

Figure 1. PLS-R projections of freeze-dried orange puree at a range of pressure, temperature, and rate of freezing conditions. The independent variables (X) are projected in red and the qualitative explanatory variables (Y) are projected in blue. The variables Y significantly correlated with a specific independent variable are circled in red, blue, orange, and green. Variables X: T30, T40, T50: shelf temperature applied during freeze-drying at 30, 40 and 50 °C, respectively; P_5 and P_{100}: working pressures of 5 Pa and 100 Pa respectively; FR-S: slow freezing rate and FR-F: fast freezing rate. Variables Y: percentage (Equation 7) of TP: total phenols, VC: vitamin C, BC: beta carotene, antioxidant activity (FRAP and DPPH); F_f: fracture force (N); S_L: slope related to rigidity (N/mm); Xw: water content (g water/100 g sample); ε: porosity (%).

3.1.1. Colour

The PLS–R revealed a good correlation between pressure and L*, C*, h* values. They can be observed according to axis 1: P_5 projected on the positive side while P_{100} is projected on the negative side. The external and near position of P_5 to L* and P_{100} to C* denotes a significant effect of pressure on colour attributes, so that low pressure leads to a high L* value (see red circle in Figure 1) while high pressure leads to a high C* value. Despite h* also being positively projected on axis 1 and so affected by pressure, its projection stays in the inner circle of the PLS-R although near the external limit. This indicates a lowered impact of the pressure on the hue angle when compared with that

observed for C* and L*. It can also be notice that the projection of C*on the PLS-R, in the negative part of axis 1 and axis 2, is higher when the interaction between P_{100} and T50 is considered (see green circle in Figure 1). The shorter freeze-drying process carried out at 50 °C contributes to promoting freeze-dried products with a higher value of chroma. In addition, C* is projected in the same side of the fast freezing rate (FR-F), the last one being in the inner circle (poorly correlated according to axis 2).

These observations were confirmed by the ANOVA, as values of L*, C*, and h* of the samples were significantly affected by working pressure ($p<0.05$). Further, C* was also affected by the interaction between shelf temperature and freezing rate ($p < 0.05$), but with a low F-Value (7.86). Taking into account the significances shown by the PLS-R analysis and the F-Values of the ANOVA, Figure 2 was constructed, showing L* and C* values of the samples obtained at the different pressure and shelf temperature and considering the mean value at both freezing rates. When working with the highest pressure during freeze-drying (P_{100}), the samples showed lower values of L* and higher C*, which means a darker and saturated colour. In this case, the chroma is specially enhanced at a higher temperature in the freeze-drier shelves, either 40 °C or 50 °C (Figure 2, $p<0.05$). The hue angle, with values between 80.3 and 82.6, showed a lower value when working with high pressure and freeze-drier shelves temperature below 50 °C ($p<0.05$).

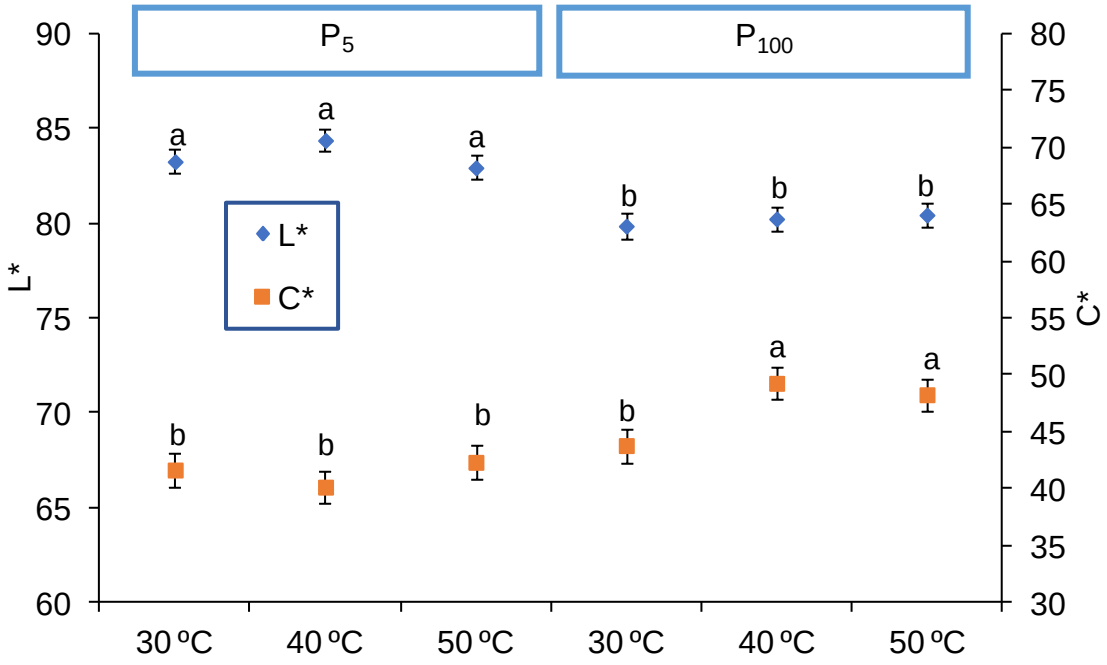

Figure 2. Values (mean and Tukey's HSD) of L* in left axis and C* in right axis of the freeze-dried purees according to the interaction between shelf temperature (30, 40, or 50 °C) and pressure (P_5: 5 Pa and P_{100}: 100 Pa) factors. Different letters for each attribute indicate different homogeneous groups for the Temperature*Pressure interaction ($p < 0.05$). Data of both freezing rates are considered in the mean values.

Similar results have been reported by Hammami and René (1997), who noted a slight L* decrease when working at higher pressures (pressure > 108 Pa) for strawberries pieces, which was related to the pronounced shrinkage observed under these conditions. Different authors found that the operating working pressure should be lower than 50 Pa to avoid shrinkage for strawberries pieces and banana slides (Hammami & René, 1997). With regards to shelf temperature, increasing temperature may cause some slight sugar browning reactions such as non-enzymatic or Maillard reactions, which means a reinforcement of the colour shown by the increase in C*. Nevertheless, this is more easily detected in shrunken samples due to its different optical light reflection capacity (Hammami & René, 1997).

According to the PLS-R and the ANOVA, pressure and shelf temperature were the factors that had a significant impact on the colour. The total differences in colour were calculated to evaluate the impact of both factors on the colour. Regarding the shelf temperature, values of ΔE* between 1.11 and 5.51 were obtained, while the range was 4.45–9.98 for the pressure influence. According to Bodart et al. (2008), total differences in colour are not obvious to the human eyes when $\Delta E^* < 1$, minor colour differences could be appreciated by the human eye depending on the hue when $1 < \Delta E^* < 3$ and visually obvious changes for human eyes occur when $\Delta E^* > 3$. Therefore, it seems that pressure has a greater effect than the other factors on the colour.

3.1.2. Structure

Freeze-dried purees showed high porosity, typical of freeze-dried products, with values ranging from 86.4–87.4%. The PLS–R indicated a correlation between FR-S and porosity (mainly projected in the positive side of axis 2, Figure 1), which means that a slow rate of freezing leads to larger ice crystals and so a bigger expansion of gas cells in the structure (higher porosity) during the freeze-drying process. Nevertheless, they are in the inner circle, which means the effect is not significant. In fact, according to ANOVA, porosity of the samples was not significantly affected by any of the factors evaluated ($p<0.05$).

As a result of the mechanical analysis, the force versus distance curves were obtained for each sample freeze-dried under each of the 12 conditions (Figure 3). The linear regression of the first part of the curve was taken to calculate the slope before F_f, related to the rigidity of the sample. As can be observed in the PLS-R (Figure 1), factors T50 and rigidity are both partially correlated and projected on the negative side of axis 2, which means that a higher temperature leads to an increase in the rigidity of the freeze-dried samples. However, because the rigidity is projected in the inner circle, it indicates a moderate positive correlation. In regards to the ANOVA results, the shelf temperature had a significant effect on the rigidity (slope) of the freeze-dried samples ($p<0.05$); however, its low F-Value (8.65) makes it possible to confirm its low significance.

Values of the slope 18–26 N/mm were obtained when heating the freeze-drier shelves to 50 °C, as compared to 11–18 N/mm values obtained at 30 and 40 °C. As a greater slope indicates less deformation of the sample by exerting an effort on it, it can be concluded that freeze-drying heating the shelves to 50 °C promotes the mechanical rigidity of the freeze-dried samples before they fracture. This can be considered desirable

as it would be related to a higher mechanical resistance of the sample during its handling before consumption.

Fracture force of all the samples varied between 12.1 ± 1.6 N and 19 ± 5 N. The PLS-R revealed a moderate positive correlation between fracture force and both lower pressure (P_5) (both projected in the positive side of axis 1) and T50 (both projected in the negative side of axis 2). This means that working at lower pressure and higher temperature seems to promote a freeze-dried puree that is more resistant to fracture. According to the ANOVA, neither the temperature nor the freezing rate showed a significant effect on the fracture force ($p>0.05$). The pressure did show $p<0.05$, but again, with a very low *F*-Value (7.17).

The water content of the samples ranged between 2.2 ± 0.3 and 4.2 ± 0.2 g water/100 g sample. The PLS-R indicated a high correlation between water content (%), high pressure (P_{100}) and low temperature (T30), circled in blue colour in Figure 1. In fact, this statement was confirmed by the ANOVA that indicated a significant effect on the final water content of the freeze-dried samples by the shelf temperature and the pressure ($p < 0.05$). In this way, drying at the highest pressure (100 Pa) and the lowest shelf temperature (30 °C) promoted samples with the highest water content. On the other hand, Figure 1 shows a clear negative correlation between water content and fracture force, which means the smaller the water content of the freeze-dried samples, the greater the mechanical resistance to fracture. In this case, the final water content of the samples was lower than 4.2%. However, it seems that small changes outside this range, related to working conditions, will have an important impact on the mechanical properties of the sample.

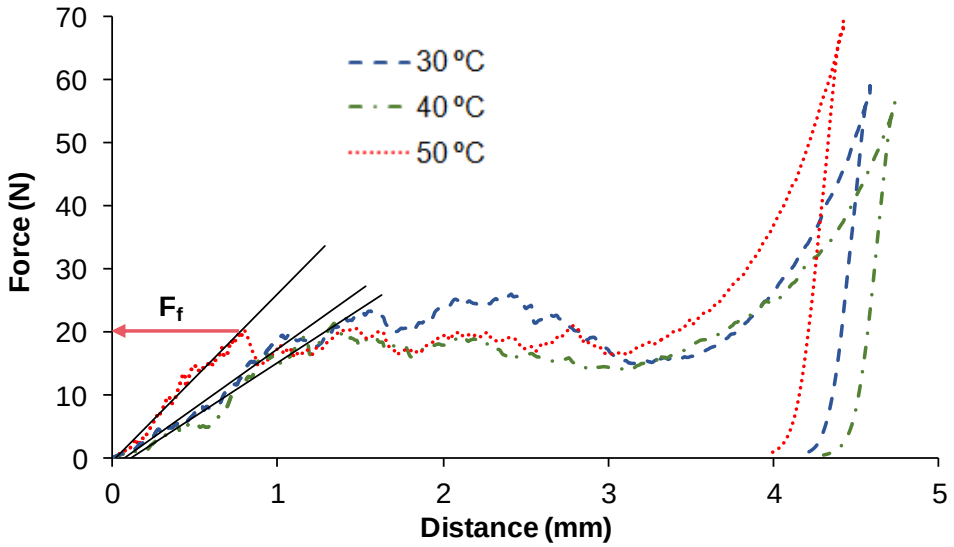

Figure 3. Examples of force–distance curves obtained from the freeze-dried purees frozen at a slow rate and freeze-dried at 5 Pa and with different shelf temperatures (30, 40 and 50 °C). F_f: fracture force.

3.2. Bioactive Compounds

The quantification of total phenols, vitamin C, β-carotene, and antioxidant activity evaluated by two methods (FRAP and DPPH), was carried out in the FOP and after being freeze-dried for each of the 12 conditions evaluated.

The PLS-R indicated that TP is not projected on the bi-plot (Figure 1), which means that it is relatively less impacted than the other bioactive compounds (TP vector very close to the origin 0.0). In fact, according to the ANOVA, the TP was not affected by working pressure and freezing rate ($p>0.05$) and was better preserved when the freeze-drying was carried out at 30 or 50 °C as compared to 40 °C ($p < 0.05$), despite the temperature factor also having a low F-Value (8.07). In fact, the most marked difference was observed with the factor temperature, which was 5% of preservation between 30 and 40 °C (Figure 4). No significant interactions between the factors were observed ($p>0.05$).

Figure 4 shows TP preservation by shelf temperature, considering both freezing rates and pressures in the mean values. It seems that TP may be affected by the ratio of time and temperature of processing, as reported by other authors (Obied et al., 2008). In this case, mild 40 °C heating for more than 7 h seems to compromise TP preservation.

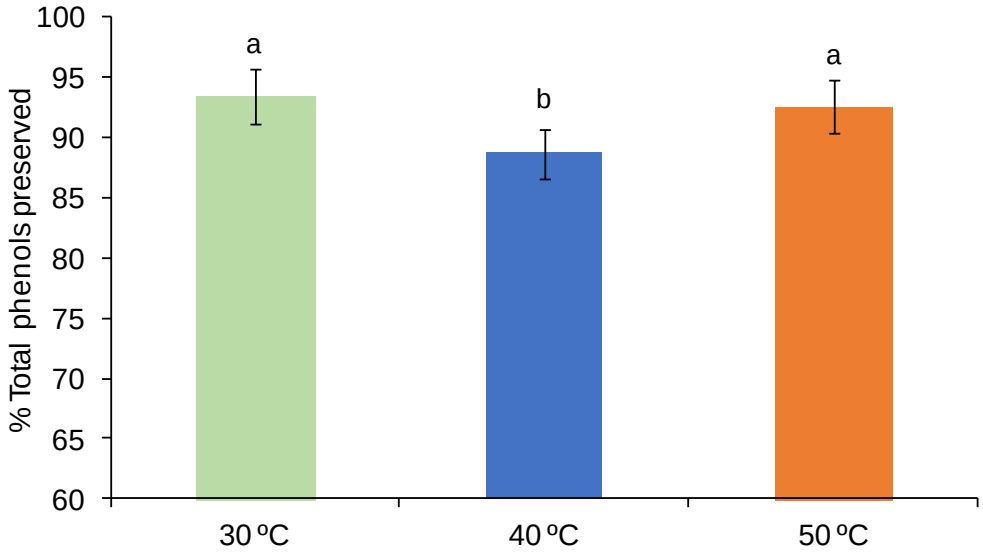

Figure 4. Percentage (%) of preserved total phenols (mean and Tukey's HSD) of samples according to the shelf temperature factor (30, 40, or 50 °C). Different letters indicate different homogeneous groups for the shelf temperature factor ($p < 0.05$). Data of both freezing rates and both pressures are considered in the mean values.

The presence of VC in the final product is used as a reference of high nutritional quality for the different industrial processes, due to its relative instability to heat, oxygen, and light (Igual et al., 2010; Lin et al., 1998). The impact of temperature on VC can be clearly observed on the PLS-R according to axis 2 (Figure 1). Vector T50 and T40 are projected in the same direction (negative side of axis 2), while T30 is anti-correlated to them (projected on the positive side of axis 2). This confirms that a higher shelf

temperature along the process preserved the vitamin C of the samples. According to the ANOVA, it can be confirmed that VC was affected by the shelf temperature and the pressure during freeze-drying (Figure 5, $p<0.05$). A significant interaction between both factors indicated that heating the freeze-drier shelves to 40 or 50 °C promoted samples with higher vitamin C content than those freeze-dried at 30 °C. Despite VC being reported to have thermal stability (Igual et al., 2010), the length of time required when the freeze-drying is carried out at 30 °C (25 h) may cause VC loss. However, the lower content of VC of samples freeze-dried at 30 °C was even lower when higher pressure was applied (Figure 5). This means that for a long expected process time, oxygen presence should be maximally avoided.

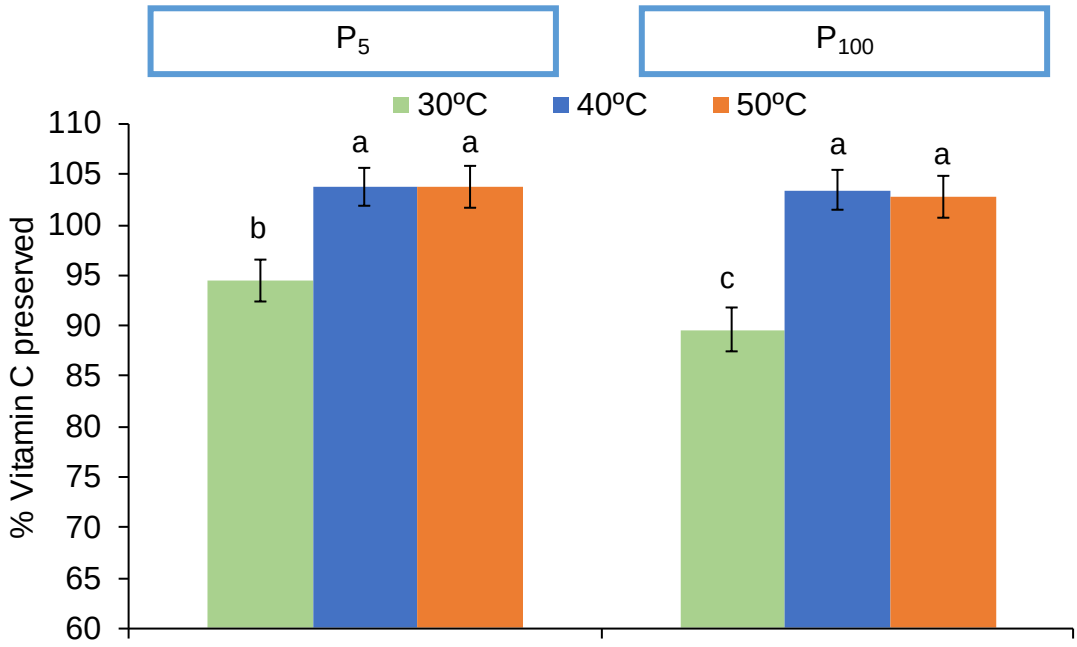

Figure 5. Percentage (%) of preserved vitamin C (mean and Tukey's HSD) according to the interaction between shelf temperature (30, 40, or 50 °C) and pressure (P_5: 5 Pa and P_{100}: 100 Pa) factors. Different letters indicate different homogeneous groups for the temperature*pressure interaction ($p < 0.05$). Data of both freezing rates are considered in the mean values.

Certain carotenoids are highly coloured compounds that also exhibit provitamin A activity. BC has the highest vitamin A activity (Fisher & Rouseff, 1986). The PLS-R (Figure 1) underlined, in particular, the BC preservation by low pressure, as it is projected on the positive side of axis 1 and highly correlated with vector P_5. In this case, the ANOVA indicated a significant effect of the three factors considered ($p<0.05$, Figure 6), the higher the pressure, the higher the temperature, and the slower the freezing rate, the greater the loss of BC. Nevertheless, the F-Values were 88, 6, and 6 for pressure, shelf temperature and freezing rate, respectively. Again, a low F-Value in the ANOVA is correlated with no significant effect detected by the PLS-R analysis. However, the ANOVA also revealed a significant interaction between the pressure and both the shelf

temperature and the freezing rate ($p < 0.05$). Figure 6 shows the interaction of pressure and shelf temperature for each FR.

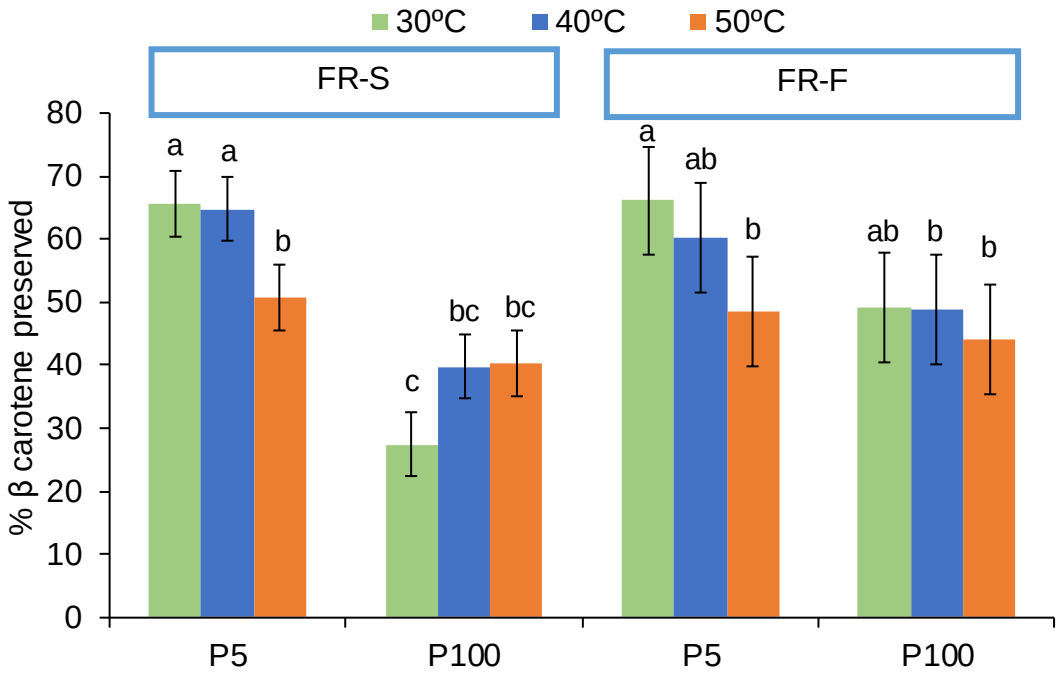

Figure 6. Percentage (%) of preserved β-carotene (mean and Tukey's HSD) according to the interaction between shelf temperature (30, 40, or 50 °C) and pressure (P_5: 5 Pa and P_{100}: 100 Pa) factors for each freezing-rate (FR-S and FR-F: slow and fast freezing rates, respectively). Different letters indicate different homogeneous groups for the temperature*pressure interaction for both freezing-rate independently ($p < 0.05$).

According to these interactions, the pressure effect is no longer significant at 50 °C, when a significant part of BC has already been degraded by the effect of the shelf temperature. In addition, the effect of the shelf temperature was not significant at higher pressure. Furthermore, when almost no oxygen is present (P_5), BC is conserved quite well, regardless of freezing rate. It is in the greater presence of oxygen (P_{100}) when the effect of the freezing rate is significant in relation to the better preservation of BC at the FR-F. From the ANOVA results, the PLS-R analysis can be nuanced in the sense that the most recommendable way to keep the maximum carotenoids present in the orange puree during freeze-drying is when the drying stage is carried out at the lowest pressure studied and heating the freeze-dryer shelves to 30 or 40 °C, without the freezing-rate being relevant in this case.

As regards antioxidant activity, values of DPPH between 86.5 ± 1.8% and 94.3 ± 1.5% were observed. The PLS-R revealed that the vector DPPH is projected on the lower right corner of the graph, which means a moderate positive correlation with both the lowest pressure and the highest temperature. According to the ANOVA analysis, no significant effect of pressure was detected ($p>0.05$). Although freeze-drying carried out at 30 °C leads to samples with lower DPPH than those processed at 40 °C or 50 °C

($p<0.05$), once again, with a low F-Value (9.51) for shelf temperature factor. On the other hand, FRAP values between 91 ± 2% and 103 ± 6% of preservation for all the conditions studied were analysed. Neither the PLS-R nor the ANOVA analysis showed a significant effect of any of the freeze-drying process variables on FRAP ($p>0.05$).

From the Pearson correlation, only a significant and positive correlation (0.5774, $p<0.05$) was obtained between values of DPPH and vitamin C. Despite AOA being correlated in a positive way with the total phenolic, vitamin C content, and carotenoids, it has been suggested that VC contributes to antioxidant capacity more than others antioxidant constituents, such as phenols or carotenoid in fruits with high VC content (Igual et al., 2010; Tavarini et al., 2008). This can also be observed on the PLS-R projection as VC, DPPH, and FRAP are projected on the same direction.

4. Conclusions

In conclusion, the optimum freeze-drying conditions for preserving the nutrients considered in this study and with interesting structural properties of the obtained product, as to be perceived as crunchy by the consumers, are low pressure (5 Pa) and high shelf temperature (50 °C). These conditions also promote freeze-dried puree with a clear, yellowish, and less saturated colour. The fact that a lower nutrients degradation was observed at higher temperatures may be explained by the great reduction (75%) of the duration of freeze-drying process at 50 °C, and the mild temperatures used. The shorter exposure of nutrients to a minimal presence of oxygen in a high porous matrix is less favourable to oxidation/degradation reactions and contributes to the preservation of nutrients. As regards to the statistical analysis of the data obtained in this study, PLS-R projection may be recommended against ANOVA as an easier tool to detect the most important factors and interactions to be considered for freeze-drying process optimization. ANOVA allows a more precise analysis, though less practical.

Author Contributions

Conceptualization: M.A.S.-E., M.d.M.C. and N.M.-N.; Data curation: M.A.S.-E. and M.d.M.C.; Formal analysis: M.A.S.-E., C.A., and N.M.-N.; Funding acquisition: M.A.S.-E., M.d.M.C. and N.M.-N.; Investigation: M.A.S.-E., M.d.M.C. and N.M.-N.; Methodology: M.A.S.-E., C.A., M.d.M.C. and N.M.-N.; Project Administration: M.d.M.C. and N.M.-N.; Supervision, N.M.-N.; Writing-original draft: M.A.S.-E., C.A., and N.M.-N.; Writing-review and editing: M.A.S.-E., C.A., T.F., and N.M.-N.

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Conflicts of Interest

The authors declare no conflict of interest.

Supplementary Materials:

Table S1. Values (mean ± SD) of the different physicochemical properties evaluated. All acronyms used are described in the main text.

Sample	L*	C*	h*	Porosity (%)	S_L (N/mm)	F_f (N)	x_w (%)
S_30_P_5	83.1±0.7	40.1±0.9	81.4±0.6	87.4±0.5	15±6	15±4	3.18±0.09
F_30_P_5	83.44±1.58	43±3	82.5±0.9	86.6±0.6	17±6	20±5	3.4±0.2
S_30_P_{100}	80.45±1.12	44±2	80.5±0.6	87.4±0.8	11±2	12.0±1.6	3.96±0.12
F_30_P_{100}	79.28±1.18	44±3	80.5±0.7	87.6±0.9	12±7	14±4	3.98±0.13
S_40_P_5	84.9±0.5	38.7±1.8	81.9±0.4	87.8±0.4	16±5	19±6	2.9±0.6
F_40_P_5	83.8±0.8	42±2	81.4±0.5	87.2±0.6	18±5	17±3	3.3±0.7
S_40_P_{100}	80.7±0.6	46.6±1.6	80.5±0.4	87.0±0.6	17±4	15.8±1.2	4.2±0.2
F_40_P_{100}	79.8±0.9	51.9±1.9	81.2±0.4	86.7±0.6	16±4	17±2	3.88±0.08
S_50_P_5	83.51±1.05	38±2	81.5±0.4	87.0±0.7	26±10	19±4	2.2±0.3
F_50_P_5	82.4±0.7	47±3	82.1±0.5	87.3±0.8	18±8	16±4	2.5±0.4
S_50_P_{100}	80.9±0.6	45.4±1.9	81.3±0.4	86.4±0.4	18±5	17.1±1.9	2.93±0.09
F_50_P_{100}	79.9±0.8	51.2±1.9	81.4±0.4	86.6±0.6	22±7	17±3	2.68±0.13

Table S2. Percentage (mean ± SD) of the bioactive compounds preserved in the FDP for each condition evaluated. All acronyms used are described in the main text.

Sample	TP*	VC*	BC*	DPPH*	FRAP*
S_30_P_5	90±5	93±6	65.5±1.8	87.9±0.5	99±6
F_30_P_5	97.48±0.05	96.7±1.6	66±3	86.81±1.03	85±11
S_30_P_{100}	91.6±1.2	90.0±0.3	27±6	84.1±0.8	93±3
F_30_P_{100}	96±3	89.8±0.1	49.2±1.9	90.5±0.8	93±3
S_40_P_5	87±3	103.8±1.5	65±8	92±5	94±3
F_40_P_5	88±4	104.7±0.9	60±2	93±7	99±4
S_40_P_{100}	89±2	103.1±0.9	39.8±1.9	93±2	97±4
F_40_P_{100}	90.4±1.8	104.09±0.12	49±10	94.3±1.5	94±2
S_50_P_5	93±6	104.1±0.7	50.73±1.14	93±4	96±7
F_50_P_5	98.05±0.17	103.8±0.4	49±3	93±4	97.5±0.8
S_50_P_{100}	92±3	103.3±0.2	40±6	89±2	99±10
F_50_P_{100}	89.0±1.9	102.4±0.9	44±10	86.5±1.8	104±6

Table S3. Values (mean and Tukey'HSD classification) of the different physicochemical properties and bioactive compounds evaluated for: each individual factor (a–c), the interaction between two different factors (d–f) and for the interaction between the three factors studied (g). All acronyms used are described in the main text.

a) Summary (LS means) - Shelf temperature:

	L*	C*	h*	F_f (N)	S_L (N/mm)	ε (%)	X_w (%)	TP (%)	DPPH (%)	FRAP (%)	BC (%)	VC (%)
30	81.568 b	42.730 b	81.238 a	15.414 a	13.706 b	87.256 a	3.618 a	93.855 a	87.345 b	92.436 b	52.064 a	92.305 b
40	82.317 a	44.776 a	81.243 a	16.984 a	16.644 b	87.178 a	3.571 a	88.660 b	93.307 a	95.976 ab	53.420 a	103.933 a
50	81.700 ab	45.306 a	81.570 a	17.487 a	20.859 a	86.843 a	2.575 b	93.021 a	90.510 ab	99.373 a	45.916 b	103.399 a
p-value	0.039	0.001	0.095	0.124	0.000	0.073	< 0.0001	0.003	0.001	0.034	0.008	< 0.0001

b) Summary (LS means) - Pressure:

	L*	C*	h*	F_f (N)	S_L (N/mm)	ε (%)	X_w (%)	TP (%)	DPPH (%)	FRAP (%)	BC (%)	VC (%)
P_5	83.534 a	41.418 b	81.792 a	17.761 a	18.444 a	87.227 a	2.900 b	92.364 a	91.127 a	95.173 a	59.313 a	100.977 a
P_{100}	80.189 b	47.123 a	80.908 b	15.496 b	15.695 a	86.958 a	3.609 a	91.326 a	89.648 a	96.683 a	41.620 b	98.781 b
p-value	< 0.0001	< 0.0001	< 0.0001	0.010	0.055	0.093	< 0.0001	0.385	0.195	0.459	< 0.0001	0.003

c) Summary (LS means) - Freezing-rate:

	L*	C*	h*	F_f (N)	S_L (N/mm)	ε (%)	X_w (%)	TP (%)	DPPH (%)	FRAP (%)	BC (%)	VC (%)
F	81.453 b	46.527 a	81.509 a	16.769 a	17.099 a	87.022 a	3.278 a	93.233 a	90.718 a	95.473 a	52.848 a	100.249 a
S	82.270 a	42.014 b	81.191 b	16.489 a	17.040 a	87.162 a	3.231 a	90.457 b	90.056 a	96.383 a	48.085 b	99.510 a
p-value	0.002	< 0.0001	0.025	0.741	0.967	0.376	0.638	0.027	0.556	0.655	0.018	0.275

d) Summary (LS means) - Shelf temperature*Pressure:

	L*	C*	h*	F_f (N)	S_L (N/mm)	ε (%)	X_w (%)	TP (%)	DPPH (%)	FRAP (%)	BC (%)	VC (%)
30_P_5	83.268 ab	41.655 bc	81.962 a	17.800 a	16.338 ab	87.004 ab	3.268 b	93.731 ab	87.383 b	91.885 a	65.807 a	94.716 b
30_P_{100}	79.867 c	43.806 b	80.514 c	13.029 b	11.074 b	87.507 a	3.968 a	93.978 ab	87.307 b	92.986 a	38.321 c	89.893 c
40_P_5	84.388 a	40.266 c	81.635 a	17.777 a	16.908 ab	87.498 a	3.083 b	87.768 b	92.763 ab	96.590 a	62.499 a	104.254 a
40_P_{100}	80.246 c	49.286 a	80.850 bc	16.191 ab	16.379 ab	86.859 ab	4.060 a	89.551 ab	93.851 a	95.362 a	44.341 bc	103.613 a
50_P_5	82.946 b	42.332 bc	81.780 a	17.706 a	22.086 a	87.178 ab	2.350 c	95.591 a	93.235 a	97.044 a	49.632 b	103.960 a
50_P_{100}	80.453 c	48.279 a	81.359 ab	17.269 ab	19.632 a	86.508 b	2.800 bc	90.451 ab	87.785 ab	101.702 a	42.199 bc	102.838 a
p-value	0.038	< 0.0001	0.011	0.106	0.387	0.003	0.105	0.052	0.054	0.503	0.001	0.032

e) Summary (LS means) - Freezing-rate*Shelf temperature:

	L*	C*	h*	F_f (N)	S_L (N/mm)	ε (%)	X_w (%)	TP (%)	DPPH (%)	FRAP (%)	BC (%)	VC (%)
S_30	81.773 ab	41.798 b	80.956 b	13.893 a	12.866 b	87.376 a	3.568 a	90.811 ab	86.052 b	95.805 ab	46.470 b	91.371 b
F_30	81.363 b	43.663 b	81.520 ab	16.936 a	14.546 b	87.136 a	3.668 a	96.898 a	88.638 ab	89.066 b	57.658 a	93.239 b
S_40	82.795 a	42.655 b	81.224 ab	17.377 a	16.442 ab	87.397 a	3.563 a	88.022 b	92.842 a	95.344 ab	52.260 ab	103.479 a
F_40	81.839 ab	46.897 a	81.261 ab	16.592 a	16.845 ab	86.959 a	3.580 a	89.297 b	93.772 a	96.609 ab	54.580 ab	104.388 a
S_50	82.242 ab	41.588 b	81.392 ab	18.195 a	21.811 a	86.714 a	2.563 b	92.536 ab	91.275 ab	97.999 ab	45.526 b	103.680 a
F_50	81.157 b	49.023 a	81.747 a	16.780 a	19.907 ab	86.972 a	2.588 b	93.506 ab	89.745 ab	100.746 a	46.305 b	103.119 a
p-value	0.484	0.001	0.292	0.076	0.579	0.186	0.932	0.184	0.316	0.136	0.071	0.337

f) Summary (LS means) - Freezing-rate*Pressure:

	L*	C*	h*	F_f (N)	S_L (N/mm)	ε (%)	X_w (%)	TP (%)	DPPH (%)	FRAP (%)	BC (%)	VC (%)
S_P_5	83.843 a	38.857 c	81.590 a	17.967 a	19.062 a	87.388 a	2.754 b	90.167 a	91.221 a	96.417 a	60.329 a	100.220 ab
F_P_5	83.225 a	43.978 b	81.994 a	17.555 a	17.826 a	87.066 a	3.047 b	94.560 a	91.033 a	93.929 a	58.297 a	101.733 a
S_P_{100}	80.697 b	45.170 b	80.791 b	15.010 a	15.018 a	86.937 a	3.708 a	90.746 a	88.892 a	96.349 a	35.842 c	98.799 b
F_P_{100}	79.680 c	49.077 a	81.025 b	15.983 a	16.372 a	86.978 a	3.510 a	91.907 a	90.404 a	97.018 a	47.399 b	98.764 b
p-value	0.425	0.302	0.541	0.416	0.360	0.253	0.018	0.182	0.451	0.439	0.001	0.254

g) Summary (LS means) - Freezing-rate*Shelf temperature*Pressure:

	L*	C*	h*	F_f (N)	S_L (N/mm)	ε (%)	X_w (%)	TP (%)	DPPH (%)	FRAP (%)	BC (%)	VC (%)
$S\_30\_P_5$	83.095 ab	40.070 def	81.403 bc	15.708 ab	15.214 ab	87.369 ab	3.175 bcd	89.979 a	87.959 ab	99.022 ab	65.524 ab	92.777 cd
$F\_30\_P_5$	83.442 ab	43.240 cde	82.520 a	19.892 a	17.462 ab	86.639 ab	3.360 bc	97.483 a	86.807 ab	84.749 b	66.091 a	96.655 bc
$S\_30\_P_{100}$	80.450 cd	43.527 cd	80.508 c	12.079 b	10.519 b	87.382 ab	3.960 ab	91.643 a	84.145 b	92.589 ab	27.416 f	89.964 d
$F\_30\_P_{100}$	79.283 d	44.085 cd	80.520 c	13.979 ab	11.629 b	87.632 ab	3.975 ab	96.312 a	90.469 ab	93.383 ab	49.225 bcde	89.822 d
$S\_40\_P_5$	84.928 a	38.696 ef	81.912 ab	18.911 ab	16.019 ab	87.788 a	2.886 cde	87.390 a	92.254 ab	93.684 ab	64.734 abc	103.816 a
$F\_40\_P_5$	83.848 ab	41.836 cdef	81.358 bc	16.644 ab	17.797 ab	87.209 ab	3.280 bcd	88.147 a	93.273 ab	99.496 ab	60.264 abcd	104.691 a
$S\_40\_P_{100}$	80.662 cd	46.613 bc	80.537 c	15.844 ab	16.865 ab	87.007 ab	4.240 a	88.655 a	93.431 ab	97.004 ab	39.785 ef	103.141 a
$F\_40\_P_{100}$	79.830 d	51.958 a	81.164 bc	16.539 ab	15.893 ab	86.710 ab	3.880 ab	90.447 a	94.271 a	93.721 ab	48.897 bcde	104.085 a
$S\_50\_P_5$	83.506 ab	37.806 f	81.456 bc	19.283 a	25.953 a	87.006 ab	2.200 e	93.133 a	93.449 ab	96.545 ab	50.728abcde	104.068 a
$F\_50\_P_5$	82.386 bc	46.858 bc	82.104 ab	16.129 ab	18.218 ab	87.349 ab	2.500 de	98.049 a	93.020 ab	97.543 ab	48.536 cde	103.853 a
$S\_50\_P_{100}$	80.978 cd	45.370 c	81.328 bc	17.108 ab	17.669 ab	86.422 b	2.925 cde	91.939 a	89.100 ab	99.454 ab	40.323 ef	103.291 a
$F\_50\_P_{100}$	79.928 d	51.188 ab	81.390 bc	17.430 ab	21.595 ab	86.594 ab	2.675 cde	88.962 a	86.471 ab	103.949 a	44.074 de	102.385 ab
p-value	0.268	0.133	0.004	0.316	0.080	0.305	0.468	0.284	0.184	0.062	0.272	0.420

Table S4. Variable importance in the PLS-R Projection (VIP). VIP > 1 are considered as the most important variables for the model. All acronyms used are described in the main text.

Variable	Component 1				Component 2			
	VIP	Standard deviation	Lower bound (95%)	Upper bound (95%)	VIP	Standard deviation	Lower bound (95%)	Upper bound (95%)
Pressure-P_{100}	1.808	0.110	1.588	2.027	1.444	0.089	1.267	1.620
Pressure-P_5	1.808	0.110	1.588	2.027	1.444	0.089	1.267	1.620
Shelf T°C-30	0.488	0.583	-0.673	1.649	1.218	0.243	0.734	1.702
Shelf T°C-50	0.378	0.459	-0.536	1.292	1.008	0.252	0.506	1.511
Shelf T°C-40	0.102	0.377	-0.649	0.853	0.193	0.364	-0.533	0.918
Freez-rate-S	0.191	0.354	-0.515	0.896	0.382	0.279	-0.174	0.938
Freez-rate-F	0.191	0.354	-0.515	0.896	0.382	0.279	-0.174	0.938

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Chapter 2.2. Impact of freeze-drying conditions on the sensory perception of a freeze-dried orange snack

Running Title: Sensory perception of an orange snack as affected by freeze-drying conditions

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Abstract

BACKGROUND: The health benefits provided by fruit mean that there is continuous interest in offering consumers new products to stimulate its consumption. To this end, dehydrated fruit snacks may be an interesting option. In this study, the impact of the freezing rate (slow and high), shelf temperature (40 and 50 ºC) and working pressure (5 and 100 Pa) on the perception and acceptability of a freeze-dried orange snack obtained from an orange puree was evaluated. RESULTS: Of the different freeze-drying conditions studied, the working pressure was the variable with the greatest effect. The lowest working pressure (5 Pa) leads to samples being obtained with a slightly lower water content, which are perceived with higher citrus flavor and crispiest. The highest pressure (100 Pa) leads to samples with a greater water content, perceived with a more yellow intense colour. Nevertheless, there is no significant consumer preference for any of the different processed samples. The number of force peaks, which is positively correlated with the crispness, shows a significant and negative correlation (r=-0.91) with the water content of the sample. CONCLUSION: The study revealed that considerations other than the sensory can determine the best conditions of the freeze-drying process with which to obtain an orange snack. The number of force peaks obtained from a penetration test may be proposed as an instrumental analysis of the snack's crispness tool which supplies information that closely resembles customer perception of this attribute.

Keywords: free choice profile, hedonic comparison, texture, freeze-drying pressure, freeze-drying shelf temperature.

1. Introduction

In the last few years, an interest in following a healthy diet has led to people consuming greater amounts of fruit, since it seems to contribute to human well-being. This contribution is related to the fruit's bioactive compounds, mainly related to antioxidant capacity, which can help in preventing some pathologies (WHO, 2004). Citrus fruit is one of the most important commercial crops in a large number of countries. The global production of oranges in 2018/19, for example, reached 54.3 million tons (USDA, 2020), such a high figure due to their sensory and nutritional attributes.

Oranges are composed mainly of fibres, simple sugars, and a wide variety of bioactive compounds that possess antioxidant properties and have a positive impact on health. Vitamin C, phenolic compounds such as the flavanones hesperidin and narirutin, and carotenoids are some of the characteristic compounds of this fruit, which are related to the suppression of oxidative stress (Chanet et al., 2012; Du et al., 2012; Zou et al., 2016) and prevention and/or protection against cancer, cardiovascular disease, heart disease, and macular degeneration, among other illnesses (Meléndez-Martínez et al., 2007; Nowak et al., 2018; Roohbakhsh et al., 2015).

From a commercial point of view, oranges are widely consumed fresh or in processed forms, such as juice or jam, which are responsible for their economic importance. However, consumers are continually demanding new products and formats which can satisfy their needs, including ease of handling. Snacks are widely consumed between the main meals. The growth trend of the snacks is expected to increase over the years, which offers the opportunity of formulating new food products (AINIA, 2018). Nowadays, the promotion of a healthier lifestyle has made the population aware of the food they eat. Despite an increasing snack frequency has been associated with unhealthier dietary behaviour, in recent years 50% of the consumers declare to take healthy snacks (AINIA, 2018; Hartmann et al., 2012). In this scenario, snacks obtained from dehydrated fruit puree may offer a market opportunity.

Currently, the market offers dehydrated fruit products, with apples being the most common dehydrated fruit found in many supermarkets, but pineapple, strawberry, kiwi, mango, and banana are also available. One of the most common and classical dehydration techniques for getting this kind of products is air drying, osmotic dehydration, or freeze-drying. The air dryers use hot air that circulates around the food pieces. In general, air dryers are simple and versatile in comparison to other types of dryers, and food pieces of any shape and size can be handled (Mujumdar, 2014). However, they are not recommended to be used for drying thermolabile compounds due to the high temperature applied during the process. The osmotic dehydration requires low temperature and energy, and it leads to good quality final product, however, besides the fact that it can vary its characteristic taste, it does not allow to obtain very dry products.

Freeze-drying is a dehydration technique based on the sublimation of the water present in a product using low temperatures and pressure, which presents advantages and disadvantages. On the one hand, it permits the preservation of thermolabile compounds and contributes to the obtaining of a higher quality product as compared with other dehydration techniques (Karam et al., 2016). On the other hand, the characteristics of the freeze-drying process and the long process time involved make freeze-drying an

expensive process. One way to shorten the process time and, therefore, reduce the cost, is the adequate fit of the conditions of the freezing and drying steps. However, the variables involved may affect the quality of the obtained product (Ceballos et al., 2012; Egas-Astudillo et al., 2018; Genin & René, 1996; Hammami & René, 1997; Martínez-Navarrete et al., 2019; Silva-Espinoza et al., 2020a).

With the above considerations, offering a high added value orange snack obtained from freeze-dried orange puree may not only be an interesting option for consumption to the general public, but also to those groups with special needs, such as children, athletes, the elderly, etc. A prior study, recently carried out on freeze-dried orange puree obtained under different conditions (freezing rate, shelf temperature and, working pressure), concluded that the colour and texture of the obtained products, measured by instrumental analysis, were affected by these process conditions. Bioactive compound preservation, however, was not significantly affected, retaining more than 80% of the content of vitamin C, total phenols, and antioxidant activity after freeze-drying (Silva-Espinoza et al., 2020a). However, it is also important to know the consumer perception of this product, its global acceptability, and the role the process conditions can play in consumer opinion. One important factor contributing to the choice of a snack is that its consumption should be a pleasure from the sensory point of view. The sensory attributes of a food item tend to be perceived in the following order: appearance, odour/aroma, consistency/texture, and flavour (Meilgaard et al., 1999). As regards snacks, despite 44% of the consumers looking for aspects related to nutrition and well-being, flavour is the most important general attribute for 71% of the consumers (AINIA, 2018).

In addition, the texture of a snack is another factor to consider, since the crunchiness/crispness of a snack is considered a very good indicator of its acceptability (Luyten et al., 2004; Martínez-Navarrete et al., 2019). A consensus meaning for crisp would be “desirably firm and brittle and easily crumbled” and for crunch “chew with a crushing noise” (Tunick et al., 2013). However, different definitions of crunchy and crispy have been reported, even the differences between them are not so clear because they vary between different studies, countries, and languages (Luyten et al., 2004; Tunick et al., 2013) and some researchers consider the terms interchangeable (Chen et al., 2005). Nevertheless, Alonzo-Macías et al. (2014) analysed different studies and compiled practical examples of crunchy/crispy foods, indicating raw carrot and apple or, pickled ginger as crunchy food, and breakfast cereals, biscuits, and freeze-dried and swell-dried products as crispy food. According to this classification, the orange snacks evaluated in this study would be in the crispy food group.

In this study, the impact of the freezing rate, shelf temperature, and working pressure throughout freeze-drying on the sensory perception of an orange snack was evaluated. The objective was to identify if any of the samples was preferred by consumers as a snack type food, with a view to a better selection of the process conditions in order to obtain a rounded product. Complementary to this aim, some instrumental textural properties were measured in order to evaluate the crispness of the samples.

2. Material and Methods

2.1. Raw material and formulation

The oranges (Citrus x sinensis cultivar Navelina) used in this study were purchased in October 2019 from a local supermarket in the city of Valencia (Spain). They were selected after a visual inspection based on a similar weight and size, colour homogeneity, and good physical integrity. The carriers used to obtain a stable dehydrated orange puree were gum Arabic (GA, Scharlab, Sentmenat, Spain) and bamboo fibre (BF, VITACEL®, Rosenberg, Germany). The orange puree was triturated and mixed with GA and BF using a bench top electrical food processor (Thermomix TM 21, Vorwerk, Spain) under the speed and time conditions described by Silva-Espinoza et al. (2020a).

2.2. Freeze-drying conditions

Samples were frozen in a conventional freezer (S, Liebherr Mediline LGT 2325, Liebherr, Baden-Wurtemberg, Germany) for 48 h, at a supposed slow freezing rate (S) and dried at two different pressures, 5 and 100 Pa (P_5 and P_{100}, respectively) in the chamber and two different shelf temperatures (T), 40 and 50 ºC (Telstar Lyo Quest-55-, Telstar, Terrassa, Spain). In addition to these four conditions, another one including a faster freezing-rate (F) was considered in the study. This sample was frozen in a blast freezer (Hiber RDM051S, Hiber, Cernusco sul Naviglio, Italy) and dried at P5 and 50 ºC. The shelf temperature conditioned the drying time, this being 7 h at 40 °C and 6 h at 50 °C. This time was selected based on preliminary experiments as being enough to achieve a water content lower than 5 %; this is the critical water content for the glass transition of this product at the usual handling temperature, thus ensuring a crispy product (Silva-Espinoza et al., 2020b). In this way, five different conditions were studied, and the obtained samples were coded as S_40_P_5, S_40_P_{100}, S_50_P_5, S_50_P_{100} and F_50_P_5.

2.3. Water content

The water content of the freeze-dried samples was measured with an automatic Karl Fisher titrator (Mettler Toledo, Compact Coulometric Titrator C10S, Worthington, OH, USA). Three replicates were made for each sample.

2.4. Sensory analysis

2.4.1. Free choice profile

The Free Choice Profile (FCP) methodology (Williams & Langron, 1984) was used to describe the sensory profile of the orange snack obtained under the five different freeze-drying conditions. A total of 20 untrained assessors (80% women, 20% men), of ages ranging from 23 to 50 years old, participated in the study. The FCP consisted of two sessions. Portions of 20 x 20 x 5 mm of each freeze-dried orange puree were tested. In the first session, the assessors were given an explanation about the procedure and asked to evaluate the similarities and differences as regards the appearance, taste, aroma, and texture of the two most different samples. They were instructed to describe the samples using their own terms to point to the intensity of the attributes, avoiding the use of hedonic terms to specify their acceptability. Of the five samples obtained when

applying the different freeze-drying conditions, the results of Silva-Espinoza et al. (2020a) were considered to select the two most different ones as regards the water content and the instrumental colour and texture, these being S_40_P_{100} and F_50_P_5. In the second session, the assessors were asked to rate their own list of descriptors for each of the five samples using a 10 cm unstructured line scale with the anchors “Not perceived” and “Very intense”. In both sessions, the samples were presented with random three-digit codes for each one and were served at room temperature. Bottled water was provided to cleanse the palate between samples.

2.4.2. Hedonic pair comparison

A hedonic pair comparison test was carried out with a total of 60 consumers aged between 20 and 60 years old. Considering the results of FCP, the pair of samples S_50_P_5 and S_50_P_{100} was selected for evaluation. Each sample was identified by a random three-digit code and presented to consumers randomly in a standardized test room with separate booths (ISO, 1998). Each panel member evaluated the pair of samples and was asked to identify the sample that they preferred. The results were analysed using the corresponding table following a bilateral hypothesis (Roessler et al., 1978).

2.5. Textural properties

A penetration test (Texture analyzer TA-XT2i, Stable Micro Systems, Godalming, UK) was carried out to evaluate some instrumental textural properties of each freeze-dried orange puree. Portions of 20 x 20 x 5 mm of each orange snack were compressed using a cylindrical probe of 10 mm diameter, applying a strain of 80% at a test speed of 1 mms−1. Five replicates were performed per sample. The force-distance curve was registered and the total number of force peaks (force threshold 0.05 N), the maximum force (Fmax, N), and the slope of the first part of the curve up to 2 mm (N/mm), related to the rigidity or resistance of the sample to be deformed, were selected as the parameters to characterize the instrumental texture.

2.6. Statistical analysis

A Generalized Procrustes Analysis (GPA) was applied to the FCP data using XLSTAT statistical software 2010.5.02 (Addinsoft, Barcelona, Spain). An analysis of variance (ANOVA) using Tukey’s HSD test was performed to establish the significant differences of the textural properties among the studied samples, which were considered when $p<0.05$. The Pearson correlation coefficient values (r) were obtained using Statgraphics Centurion XVI.II.

3. Results and Discussion

Despite the fact that the water content of all the snacks was around the expected value, the samples that were freeze-dried at a lower pressure had a lower water content (3.63±0.15 g water/100 g sample, $p<0.05$) than that of samples S_50_P_{100} (4.70±0.14 g water/100 g sample, $p<0.05$) and S_40_P_{100} (5.300±0.001 g water/100 g sample, $p<0.05$). These results confirm the impact of both the shelf temperature and the working pressure on the duration of the freeze-drying process (Silva-Espinoza et al., 2020a). However, it is important to emphasize at this point the difficulty of obtaining an exact

water content in a freeze-dried product, especially in the low-moisture zone (Genin et al., 1996; Tang & Pikal, 2004).

3.1. Free choice profile

The FCP is a quantitative descriptive analysis which was developed for the purposes of finding out the perception of the consumers using their own terminology, avoiding a technical description of the products (Murray et al., 2001a). This analysis was selected to determine the attributes that describe the orange snacks. The consumers generated different terms, grouped by appearance, taste, aroma, and textural attributes. The results from the FCP analysis are shown in Fig. 1, which shows the two dimensions of the GPA graph. In this figure, the most commonly-mentioned attributes and their frequency of mention are summarized. The total amount of variance explained by the two dimensions was 72.93%: 48.63% accounted for by dimension 1 and 24.30% by dimension 2. On the left-hand side of the plot, the attributes related to texture and flavour, such as light texture, acid taste, and citrus flavour, were placed; these characterized orange snacks dried at P_5. On the right hand-side of the plot, the terms related to appearance (bright and yellow intensity) were related to orange snacks dried at P_{100}. As regards dimension 2, with the exception of sample F_50_P_5 which is related to the crispy texture, this does not explain the variability of the rest of the samples.

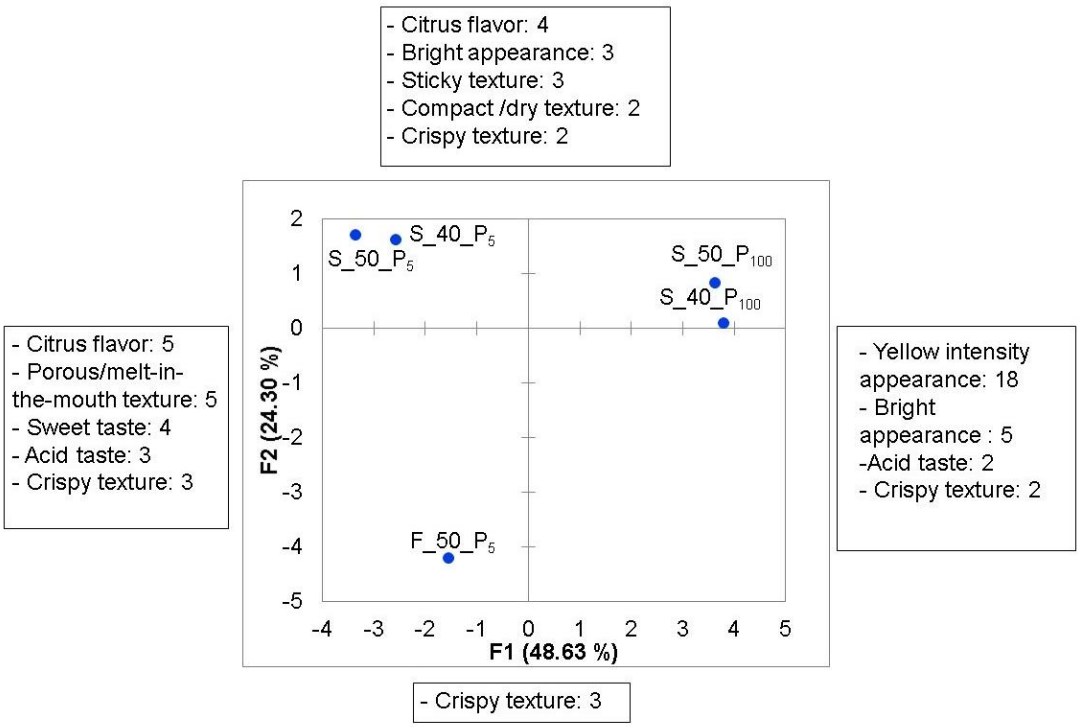

Figure 1. Two dimension Generalized Procrustes Analysis plot of the differences among snacks freeze-dried under different process conditions. The main descriptors correlated with the first two dimensions are listed together on the boxes with the times that the descriptor was mentioned.

As can be observed in Fig. 1, the crispy texture attribute appears on all 4 sides of the graph, which means that although the assessors perceived the samples as difficult

to deform, they break relatively easily producing a sharp sound (Luyten et al., 2004). However, the assessors also perceived another textural aspect. The orange snacks freeze-dried at P_5, were related with a crispy product after the first bit, although with a porosity that causes it to melt in the mouth when it comes into contact with saliva. This sensation was defined by the assessors as porous/melt-in-the-mouth texture. Another aspect to be pointed out is the water content of the samples, which can be related to the perception of the textural attributes. In fact, some studies have obtained a negative relationship (r2>-0.9) between the sensory score for crispness in crackers, extruded snacks, bread crust and potato chips, and their water content (Katz & Labuza, 1981; Primo-Martín et al., 2006; Srisawas & Jindal, 2003). Although the water content difference between the samples freeze-dried at P_5 and P_{100} was small (~1.5%), it seems that the lower water content of those samples freeze-dried at P_5 was what leads the assessors to qualify the texture of these samples also as porous/melt-in-the-mouth.

With respect to the appearance, the samples that were freeze-dried at P_{100} were widely perceived as samples of a more intense yellow (Fig. 1). This relationship was also obtained by Silva-Espinoza et al. (2020a) using an instrumental analysis, where the samples freeze-dried at P100 showed a higher chroma, which is directly related to the intensity of the colour. As regards the flavour properties, the samples freeze-dried at P5 were perceived as sweet, acid, and with citrus notes. Therefore, it can be suggested that the non-volatile and volatile compounds that characterize the orange flavour are better preserved at lower working pressures during freeze-drying. A study about the effect of different freeze-drying conditions on the retention of the principal mushroom aroma (1-octen 3-ol) showed its better retention when working at a lower pressure (5 Pa) (Kompany & René, 1993). This pressure effect has been related to the freeze-drying phenomenon known as “collapse” or shrinkage, promoted at higher pressures. The shrinkage leads to a decrease in the retention of volatile compounds and a loss of flavour due to changes in the structure of the freeze-dried matrix (Petersen & Lorentzen, 1973). However, we must also bear in mind that the samples freeze-dried at P_5 were also those with a lower water content. The higher concentration of compounds responsible for the aroma and flavour in this case could also contribute to this effect. Therefore, despite the FCP analysis showing the working pressure as being the only process variable with a significant effect on the different sensory perceived attributes, whether it is indeed the working pressure or the water content of the samples should be confirmed by further studies.

3.2. Hedonic pair comparison

A hedonic pair comparison was carried out so as to identify the sample preferred by consumers as a snack type product. The results of FCP indicate the main differences among samples as being due to the working pressure during the freeze-drying process. As no differences were found between either studied temperature at the same pressure, a shelf temperature of 50 ºC was selected due to the shorter freeze-drying time. In addition, Silva-Espinoza et al. (2020a) found that the bioactive compounds were slightly better preserved when freeze-dried at 50 ºC vs. 40 or 30 ºC due to the shorter process time. Therefore, the samples selected for the hedonic par comparison were S_50_P_5 and S_50_P_{100}.

From the 60 responses, sample S_50_P_5 was preferred by 34, while S_50_P_{100} was selected by 26. The tabulated (60, 0.05) value points to 39 as being the critical minimum number of times a sample must be preferred in a Two-Sided Directional Difference test in order to consider significant differences among samples (Meilgaard et al., 1999). As this value is higher than the 34 obtained in our experiment, this means that consumers do not significantly prefer one sample over another.

3.3. Textural properties

As an example, Figure 2 shows one of the replicates of the force versus distance curves, obtained from the penetration test, for each of the samples freeze-dried under the different process conditions. The number of peaks and the Fmax obtained from the curves are shown in Figure 3. These parameters are useful mechanical indicators to evaluate the level of crunchiness/crispness of a solid food.

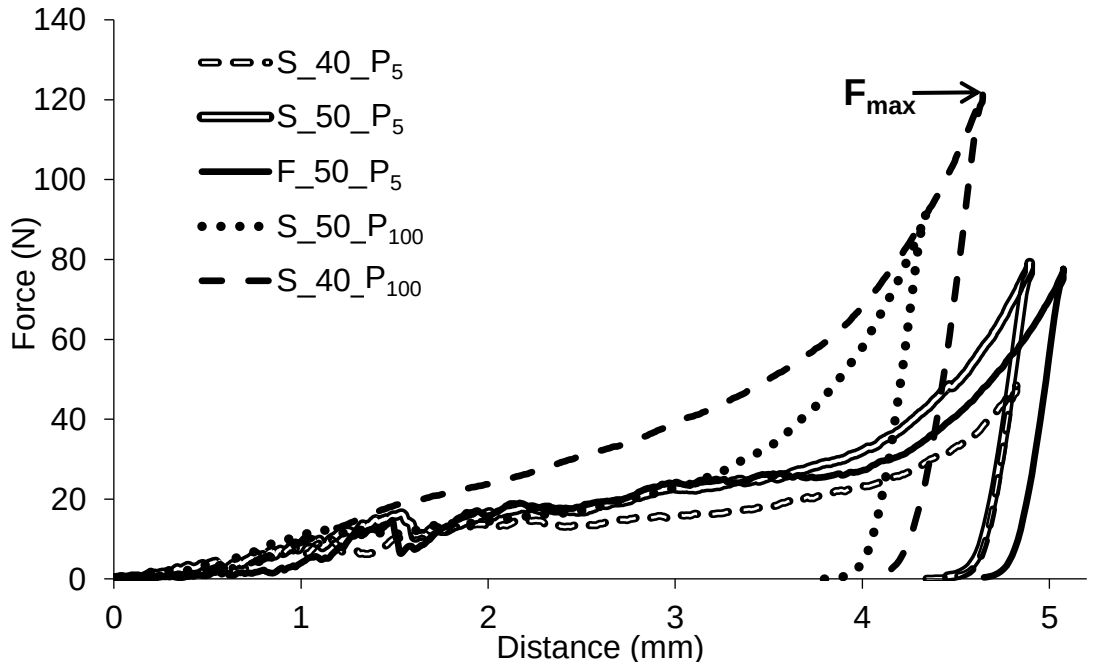

Figure 2. Examples of force–distance curves obtained from the freeze-dried orange purees frozen at slow (S) and fast (F) freezing rate, dried at 5 Pa (P_5) and 100 Pa (P_{100}) with 50 ºC and 40 ºC as shelf temperatures.

The crispness, an indicator of the firmness and the freshness of a snack, is positively related with the number of force peaks (Alonzo-Macías et al., 2014). In this study, the number of force peaks of the five samples allowed three different significant groups to be obtained (Fig. 3). Samples that were freeze-dried at the lowest pressure (P_5) exhibited a higher number of peaks ($p<0.05$) and so a crispier texture. The lower number of force peaks shown by the samples freeze-dried at P_{100}, even more marked at the lowest temperature (Fig. 3), is related to their less crispy nature ($p<0.05$). Nevertheless, a significant negative correlation ($r= -0.9125$) was obtained between the water content and the number of force peaks, which indicates that the higher the water content, the less

crispy the product. As in this case the samples obtained at P_{100} had higher water content than those at P_5, the differences observed in texture could be the result of either different pressure or different water content. It means, the different pressure could lead to a different structure development, for instance more or less porous, that affects the textural perception of the snack. Or maybe the structure is the same and the differences are simply a consequence of the different water content of the samples. In the latter case, the texture of two samples with the same water content obtained under different pressure should no show differences in texture. To confirm this statement, another set of samples was freeze-dried under the different process conditions in an attempt to obtain freeze-dried products with no significant differences in the water content ($p>0.05$). This was finally achieved in 3 cases: S_50_P_{100} (2.9±0.09 g water/100 g sample), S_40_P_5 (2.8±0.6 g water/100 g sample) and S_50_P_5 (2.2±0.3 g water/100 g sample). The crispness of these samples was evaluated through the number of force peaks and the obtained results (44±5, 46±6, and 50±6, respectively) showed no significant differences ($p>0.05$). These results permit the confirmation that it was the water content and not the different working pressure during freeze-drying that affected the texture of the samples, as was also observed by other authors (Hammami et al., 2001). Another important fact that has been observed is the impact that small differences in the water content of the snack, such as those obtained in this study (between 3.63 and 5.30 %), have on its crispy texture.

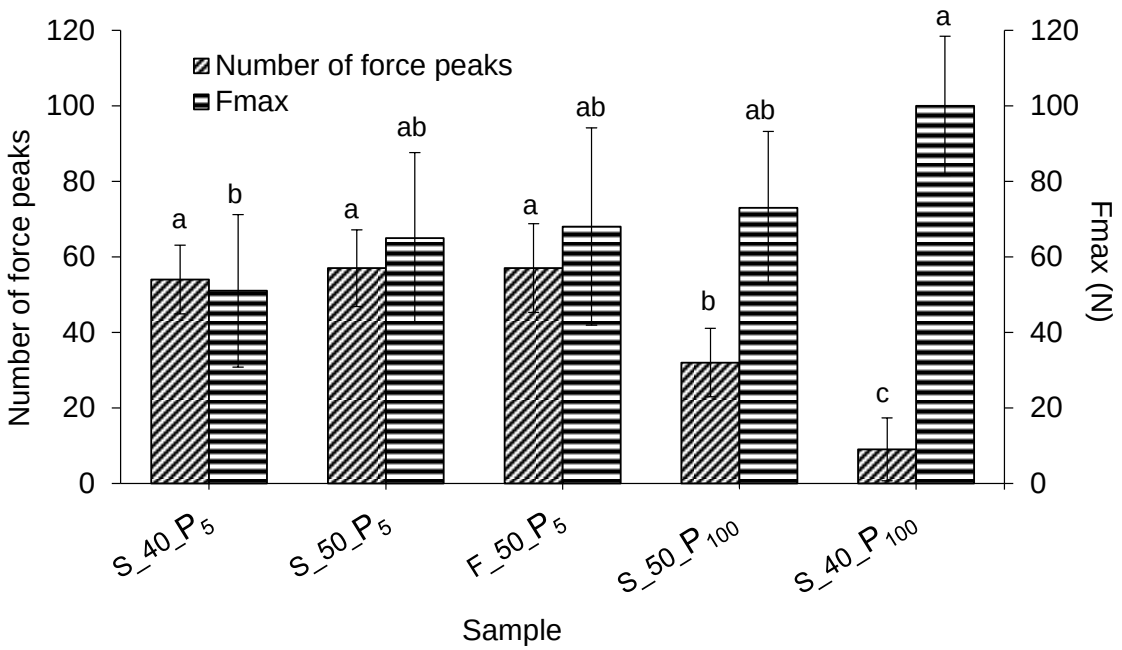

Figure 3. Textural parameters obtained from the mechanical penetration test. Mean values and Tukey's HSD of number of peaks in the left axis and F_{max} in the right axis of each of the freeze-dried orange purees frozen at slow (S) and fast (F) freezing rates, freeze-dried at 5 Pa (P_5) and 100 Pa (P_{100}) with 50 ºC and 40 ºC as shelf temperatures. Different letters for each parameter indicate different homogeneous groups ($p<0.05$).

As regards Fmax, this parameter was shown to be insufficiently sensitive to differentiate between samples, as only sample S_40_P_5 presented a significantly lower value than S_40_P_{100} ($p<0.05$, Fig. 3). However, a significant negative correlation ($r= -0.7109$) between the number of peaks and Fmax was observed. This correlation indicates a trend in which a snack with a greater number of force peaks, meaning a crispier product that breaks more easily when eaten, shows a lower Fmax value. As for the rigidity of the samples, a steeper slope indicates more resistance to deformation which means greater rigidity. The values of slope of the different samples obtained were between 8±4 and 11±4 (N/mm), with no significant differences ($p>0.05$). Therefore, it does not seem to be a suitable parameter for assessing the different texture of these samples either.

On the basis of the results of the textural properties measured instrumentally and those perceived by consumers (Section 3.1), the crispness of the snacks correlates well with the number of force peaks. However, when the number of peaks is high, the consumers perceive a crispy texture that was loosed when the snack is exposed to saliva and “melt in the mouth”. This is characteristic of crispy products based on dry foams (Lillford, 2017), extruded snack samples (Murray et al., 2001b) or, in our case, to highly porous products. Thus, the suggestion exists that there is a relationship between the perception of the samples as porous/melt-in-the-mouth and the fact that they are crispier, which may be due to their greater brittleness before they melt.

4. Conclusion

The consumers perceived and highlighted different attributes for the freeze-dried orange puree offered as an orange snack, related to their flavour, texture, and colour. Crispness was closely related to the water content, which must be precisely defined and controlled. Of the different freeze-drying conditions studied, the working pressure was the variable that affected the different perceived quality attributes of the samples the most. A lower working pressure during freeze-drying leads to samples with a lower water content, perceived with higher citrus notes (citrus flavour, acid taste, and sweet taste) and as the crispiest. A higher working pressure leads to samples perceived with a more intense yellow colour. Consumers do not significantly prefer any sample. Considering these results, aspects other than the sensory may be managed to select the best freeze-drying conditions under which to obtain a consumer-popular orange snack. As regards the instrumental analysis of crispness, the number of force peaks obtained from a penetration test may be proposed as an adequate tool with which to obtain results that closely resemble the texture perceived by the consumers.

Acknowledgments

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Conflict of interest: none.

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Chapter 2.3. Impact of the freeze-drying conditions applied to obtain an orange snack on energy consumption

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Abstract

Nowadays, the consumer is looking for healthier, more attractive and ready-to-eat, and safer foodstuffs than fresh products. This is possible through freeze drying known for providing high added value products. Despite its advantages, freeze-drying is a slow process which is conducted at low pressures, so, in terms of energy consumption, it turns out to be quite costly for the food industry. In this study, the impact of the variation in pressure and temperature throughout drying on the energy consumption of the process has been considered. The purpose was to obtain a freeze-dried orange puree, previously formulated with gum Arabic and bamboo fibre, which can be offered to consumers as a snack. The energy consumption was registered during the drying of frozen samples at different combinations of chamber pressures (5 and 100 Pa) and shelf temperatures (30, 40 and 50 ºC). In every case, the time processing was adapted in order to obtain a product with a water content of under 5 g water/ 100 g product. The results obtained led to the conclusion that the shorter duration of the process when working at 50 ºC results in significant energy saving. Working at lower pressure also contributes to shortening the drying time, thus reducing the energy consumption: the lower the temperature, the more marked the effect of the pressure.

Practical application

Freeze-drying is a well-known drying technology frequently used to obtain high added value products. With this study, solutions could be offered to food companies for the purposes of reducing the operating costs of their installations related to the freeze-drying process while keeping a high final quality of the product.

Keywords: shelf temperature, pressure, water content, primary drying, secondary drying

1. Introduction

In recent years, the incentive to consume greater quantities of fruit has been promoted by both governmental and non-governmental organizations, since fruit is the main component of a healthy daily diet. Its contribution lies in having a set of beneficial nutritional and non-nutritional substances, such as vitamin C, carotenoids, flavonoids, phytosterols, among other bioactive compounds, able to provide antioxidant activity (Martínez-Navarrete et al., 2008). The activity of the antioxidant compounds has been related to the prevention of a wide range of pathologies, such as cancer, cardiovascular diseases and degenerative diseases, associated with aging processes (Tavarini et al., 2007). However, the worldwide average intake of fruit is below that recommended by the health authorities. It is thought that the population does not consume fruit and vegetables for various reasons: cost, convenience, and taste, among others (FAO, 2003).

For this reason, the food manufacturing sector has seen an opportunity to develop new healthy fruit-based products in order to encourage its consumption. In addition, as consumers are more aware of the benefits of healthy food, they are demanding more and more new healthy products, which, at the same time, must be innovative and ready-to-eat (Crofton & Scanell, 2020). In this sense, the commercialization of dried fruit-based products can offer two things: solutions to problems related with the short shelf life of fruits and their seasonality and a means of providing microbiologically stable products as a consequence of their low water activity. Furthermore, dried fruit both facilitates the shipping operations and makes them more profitable due to its lower volume and weight and easier handling. In addition, dehydration can be used to provide different food formats, such as a fruit snack, or powdered fruit destined to be consumed rehydrated as a juice or infusion, or to be added to desserts, dairy products, salads, ice cream, among other things, and even for enriching almost any food in bioactive compounds.

Of the dehydration techniques, freeze-drying is one of the drying methods that provides the highest retention of chemical profile and antioxidant activity in foods, attributed to its less intense heating (An et al., 2016). In fact, several studies have successfully obtained a vegetable/fruit snack with good physical, chemical, and functional properties by using freeze-drying (Ciurzyńska et al., 2020; Egas-Astudillo et al., 2020; Leiton-Ramírez et al., 2020; Martínez-Navarrete et al., 2019; Silva-Espinoza et al., 2021; Uscanga, et al., 2020). Despite its advantages, freeze-drying is one of the techniques with the highest energy consumption as it requires a long drying time (An et al., 2016; Barresi et al., 2009). If compared with another dehydration technique, such as spray-drying, known for being cheaper and leading to products of similar quality, Flink (1977) reported that the costs of freeze-drying are 4-5 times higher. However, a recent study on the obtaining of grapefruit powder points out that it is much more profitable to use freeze-drying rather than spray-drying, the latter costing 2.3 times more than the former (Camacho, et al., 2018). This study indicated that although the electrical cost was 8.5 times higher in the case of freeze-drying, the costs of other items that intervene in the total cost of production, such as the raw material, is 3.5 times higher in the case of spray-drying; this represents 82% of its total cost, compared to 54% for freeze-drying. This is mainly due to the poor yield of spray-drying in the case of fruit processing, caused by the loss of the powder that remains adhered to the walls and nozzle of the spray-dryer (Camacho et al., 2018). This is a consequence of the structural collapse that the

powdered fruit suffers at temperatures above its glass transition temperature (Tg) which, among other things, implies the development of stickiness phenomena. In this sense, certain biopolymers that contribute to an increase in the Tg, stabilizing the dehydrated product (Telis & Martínez-Navarrete, 2012), are frequently incorporated.

It has been reported that modifying the freeze-drying variables, such as shelf temperature or working pressure, may affect the duration of the process (Hammami et al., 1999). In this way, selecting the most adequate variables for a product may further reduce time and, therefore, cost. Furthermore, Silva-Espinoza et al. (2020a) pointed out that, in general, these variables had a low impact on the physical-chemical quality and bioactive compounds of the final freeze-dried product. In fact, taking vitamin C as a reference of high nutritional quality for foods under different industrial processes, due to its relative instability to heat, oxygen, and light (Igual et al., 2010), a whole preservation after freeze-drying was observed, even when applying mild shelf temperatures such as 40 or 50 ºC (Silva-Espinoza et al., 2020a). The above-mentioned variables can be modified independently although they are intimately related. For this reason, it is of interest to know how these modifications of the process conditions affect its duration and, therefore, the energy consumption, in order to try to reduce its cost.

In this study, the effect that modifying the drying conditions (working pressure and shelf temperature) of the frozen sample in order to obtain a freeze-dried orange snack had on total energy consumption was evaluated. The final aim was to select the best combination of these variables so as to achieve an appropriate time-saving, energy-saving, economical process that ensures a high quality product.

2. Material and Methods

2.1. Raw material and formulation

The oranges (*Citrus x sinensis* cultivar Navelina) used in this study were purchased from a local supermarket in the city of Valencia (Spain). They were selected by means of a visual inspection based on a similar weight and size, colour homogeneity, and good physical integrity. The carriers used to obtain a stable dehydrated orange puree were gum Arabic (GA, Scharlab, Sentmenat, Spain) and bamboo fibre (BF, VITACEL®, Rosenberg, Germany). In order to obtain the orange puree, the fruit was washed and peeled. The pulp was cut and triturated in a bench top electrical food processor (Thermomix TM 21, Vorwerk, Spain) and mixed with (5 g GA + 1 g BF)/100 g orange puree with the same food processor. The conditions of trituration and mixing employed by Silva-Espinoza et al. (2020a) were followed.

2.2. Freeze-drying conditions

The formulated orange puree was distributed in 10.5 × 7.8 cm aluminium plates of 0.5 cm thickness and immediately frozen. A PT100-type thermocouple (Termya, Spain) was placed in the geometric centre of the sample, which was frozen in a conventional freezer (Liebherr Mediline LGT 2325, Liebherr, Baden-Wurtemberg, Germany) for 48 h. The drying step was carried out in a freeze-dryer (Telstar Lyo Quest-55, Telstar, Terrassa, Spain) at two different chamber pressures, 5 and 100 Pa (P_5 and P_{100}, respectively) and at three different shelf temperatures, 30, 40, and 50 ºC. In this way, six different conditions were studied and the obtained samples were coded as 30_P_5,

30_P_{100}, 40_P_5, 40_P_{100}, 50_P_5, 50_P_{100}. In each case, the drying time was adjusted so that it was enough to obtain a freeze-dried product with the same water content, lower than 5%, which guarantees the typical crispness of a snack (Silva-Espinoza et al., 2020b). Based on preliminary experiments, this time was 11 h for 30_P_5, 27h for 30_P_{100}, 7 h 20 min for 40_P_5, 8 h 30 min for 40_P_{100}, 5 h 50 min for 50_P_5, and 6 h for 50_P_{100}. The same amount of sample (about 160 g, 4 plates) was loaded into the freeze-dryer for each of the six process conditions.

2.3. Water content

The water content of the freeze-dried samples was measured with an automatic Karl Fisher titrator (Mettler Toledo, Compact Coulometric Titrator C10S, Worthington, OH, USA). Three replicates were made for each sample.

2.4. Power consumption and temperature recordings

The energy consumption during the drying step was recorded with a real time data logger (Lutron Electronic Enterprise Co., LTD, Taipey City, Taiwan) that was connected to the freeze-dryer. The power consumed (kWh) was registered every 15 s.

The evolution of the temperature of the sample during the drying step was recorded every 30 s using a wireless system consisting of a data transmitter (Datanet Logger DNL910A, Fourtec Technologies Ltd, Rosh Ha'ayin, Israel), coupled to the thermocouple described in Section 2.2., and a data receiver (Datanet Repeater DNR900, Fourtec Technologies Ltd, Rosh Ha'ayin, Israel) connected to a computer using DataSuite software (Fourtec Technologies Ltd, Rosh Ha'ayin, Israel).

2.5. Statistical analysis

An analysis of variance (ANOVA) was performed in order to obtain the significant differences between the various studied conditions according to Tukey 's HSD test, which were considered when $p<0.05$, using Statgraphics Centurion XVI.II.

3. Results and Discussion

Data in Table 1 shows the water content of the different orange snacks, all of which were crispy products with a water content of around 4% and no significant differences ($p>0.05$). The temperature evolution of the orange snack in the different drying conditions was registered. Figure 1 shows the progress of the product temperature during the drying at 30, 40, and 50 ºC. A similar trend was observed when varying the working pressure. The temperature evolution was similar to that obtained with other products (Raharitsifa & Ratti, 2010), and both the primary and secondary drying stages can be observed. The primary drying (PD) consists mainly of the transformation of the ice into vapour by sublimation while the secondary drying (SD) refers to the desorption of the unfrozen water.

Table 1. Values of the water content of the orange snacks and total power consumed during the drying (mean ± standard deviation) according to each process condition.

Shelf temperature (ºC)	Pressure (Pa)	Water content (g water / 100 g orange snack)	Total power consumed (kWh)
30 ºC	P_5	3.7 ± 0.2 [a]	6.83 ± 0.06 [b]
	P_{100}	3.8 ± 0.13 [a]	16.49 ± 0.12 [a]
40 ºC	P_5	3.6 ± 0.5 [a]	5.03 ± 0.12 [d]
	P_{100}	3.8 ± 0.4 [a]	5.72 ± 0.06 [c]
50 ºC	P_5	3.6 ± 0.3 [a]	4.14 ± 0.05 [e]
	P_{100}	3.80 ± 0.12 [a]	4.23 ± 0.09 [e]

The precise end of PD is difficult to establish as SD starts in some regions of the sample at the same time as primary drying ends in some others. Ratti (2013) identified the start of SD as the moment when the product temperature begins to increase markedly. Other authors indicated that SD mostly occurs when the product temperature approaches the shelf temperature, and so they assumed that point as the SD starting point (Barbosa & Vega, 2000, Koganti et al., 2011, Pikal et al., 1990, Tang et al., 2004). In this study, we were able to observe that the samples dried at 100 Pa and 50 or 40 ºC for 4 and 5 h, respectively, showed the presence of ice. As these times correspond to the moment when the product temperature truly approaches that of the shelf (Fig. 1), it was considered that the SD started when the product reached the shelf temperature.

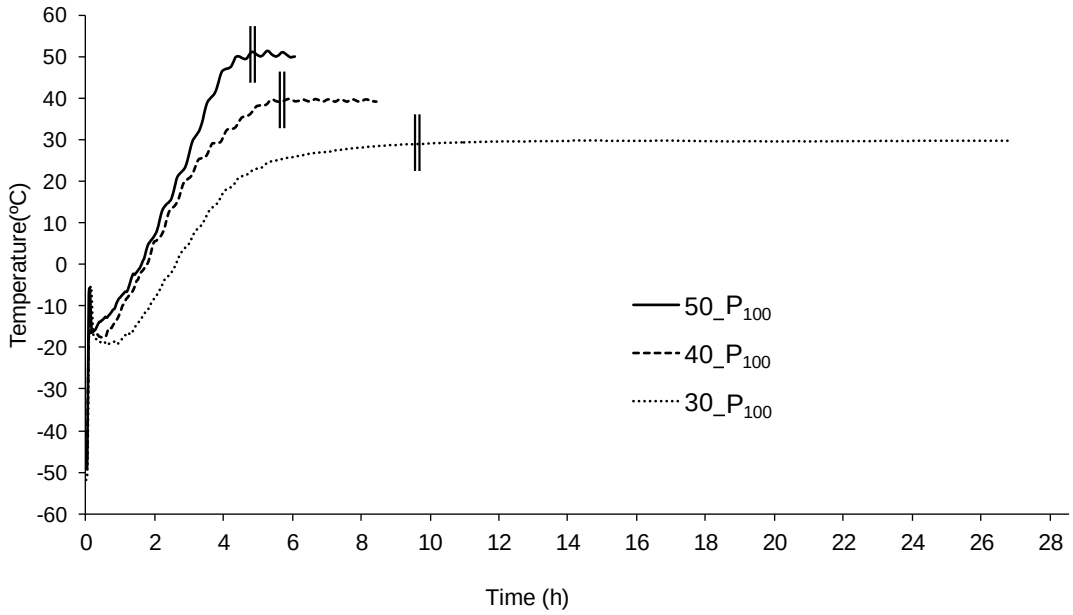

Figure 1. Evolution of the product temperature during the drying of the frozen orange puree at a working pressure of 100 Pa (P_{100}) and a shelf temperature of 30, 40, or 50 ºC

A double vertical line indicates the end of primary drying and the beginning of secondary drying.

From this point of view, as shown in Fig. 1, the first period of PD is characterized by a low and constant product temperature where the sublimation is mainly taking place, using the heat provided by the shelf to supply the needed phase transition latent heat. In the second period of PD, in which less ice is left, less heat is needed for the sublimation and the product temperature rises. The duration of these two periods of PD was conditioned by the process variables (Fig. 1). When comparing different shelf temperatures, the first period can be clearly observed to be shorter, and a steeper slope of the product temperature increase can be seen in the second period when working at the higher temperatures (40 and 50 ºC). It seems that the heat provided by the shelves when heated at these temperatures is more than that necessary for the sublimation of the ice to occur, so that almost from the beginning, the temperature of the product begins to increase. On the other hand, during SD, the removal of the residual water content takes place until reaching the target value, and the product heating rate greatly decreases until the shelf temperature is reached.

Taking into account the aforementioned criterion that the SD started when the product reached the shelf temperature, Fig. 2 shows the duration of PD and SD for each of the process conditions studied. According to the ANOVA, both the shelf temperature and pressure significantly affected ($p<0.05$) the duration of PD and SD (Fig. 2).

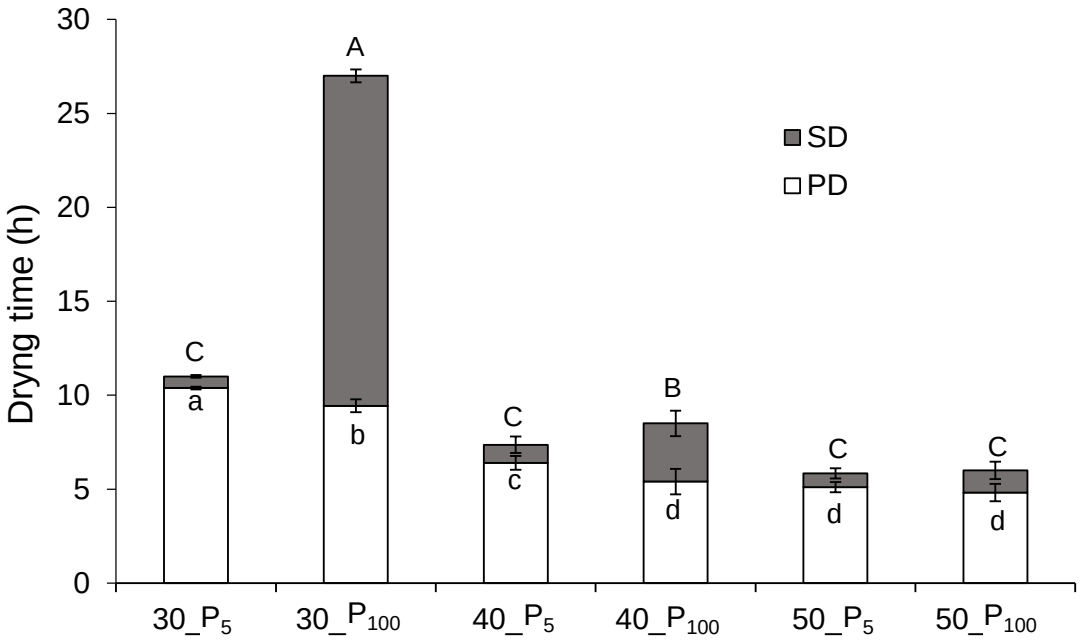

Figure 2. Mean value and standard deviation of the duration of primary and secondary drying (PD and SD, respectively) for the frozen orange snacks dried at 5 Pa (P_5) or 100 Pa (P_{100}) and a shelf temperature of 30, 40, or 50 ºC. Different lowercase or capital letters for PD and SD, respectively indicate different homogeneous groups ($p<0.05$) according to Tukey's HSD test.

Both a higher pressure and a higher temperature mean a shorter PD. In this study, this stage was significantly longer ($p<0.05$) when the drying was done at 30 ºC, at any pressure, compared to the other 2 temperatures studied. In the case of 40 ºC, PD was significantly longer ($p<0.05$) compared to 50 ºC, when the former was carried out at the lowest pressure only. Ratti (2013) explains that a higher pressure in the chamber enhances the heat transfer rate and, therefore, increases the sublimation rate, shortening the PD time. As regards SD, at P_{100} it was shorter as the shelf temperature increased but this effect of temperature was not observed at P_5 ($p<0.05$, Fig. 2). In addition, at 30 and 40 ºC, the SD lengthened as the pressure increased. This effect of a higher pressure during the SD is related with the higher partial pressure of water vapour in the chamber (Searles et al., 2017) and the decrease in the diffusivity of water vapour in air (Saravacos & Stinchield, 1965), which delays the desorption of the unfrozen water. As regards the duration of each drying step, it can be said that it depends on the freeze-drying conditions. Some authors indicated that primary drying normally consumes the largest fraction of the freeze-drying cycle time (Tang et al., 2005). However, it has also been observed that the time required to remove the water during the secondary stage may be equivalent to or even longer than in the primary stage (Egas-Astudillo et al., 2020; Jiang et al., 2011). In this study, SD was longer than PD only when the drying was carried out at 30 ºC and P_{100} (Fig. 2). Broadly speaking, when the drying was carried out at the lowest pressure, drying time reductions of 59, 14, and 3% at 30, 40, and 50 ºC, respectively, were observed. On the other hand, time reductions of 33 and 47% at P_5 and 68 and 78% at P_{100} were achieved when drying at 40 and 50 ºC, respectively, as compared with the process carried out at 30 ºC.

Fig. 3 shows, as an example, the evolution of the power consumed throughout the drying step of three of the samples when applying the same pressure but different shelf temperature, 30, 40, and 50 ºC.

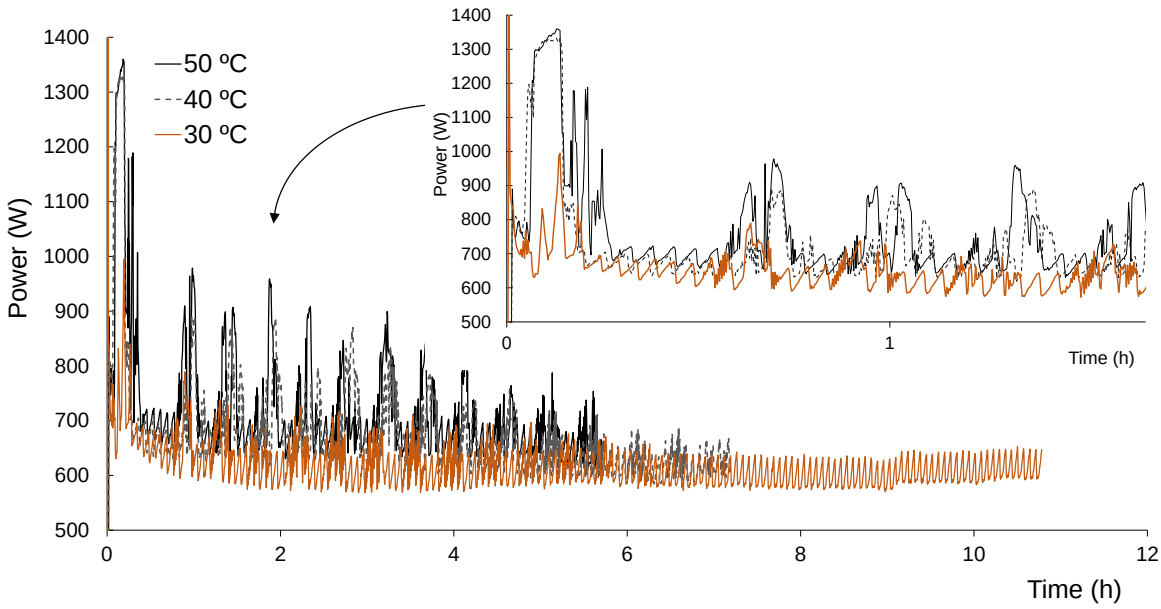

Figure 3. Evolution of the power consumed (W) over time (h) during the drying of the frozen orange puree at a working pressure of 5 Pa and a shelf temperature of 30, 40 and 50 ºC.

Each peak of power observed throughout the process is related to the operation mode of the freeze-dryer that has to supply heat for two purposes: so that the shelf temperature reaches and remains at the set value and in order to remove the water from the sample. As expected, a greater power consumption was observed at the beginning of the processes at 40 and 50 ºC, necessary to reach the higher shelf temperature set point. On the other hand, more intense peaks are observed at any temperature, at the beginning of the primary drying, while the sublimation of the ice is clearly taking place (Fig. 3). Since less ice is left as the sublimation progresses and the product temperature starts to rise, the shelves are allowed to preserve the temperature set point much better, so that the intensity of the power peaks decreases as the drying progresses (Fig. 4).

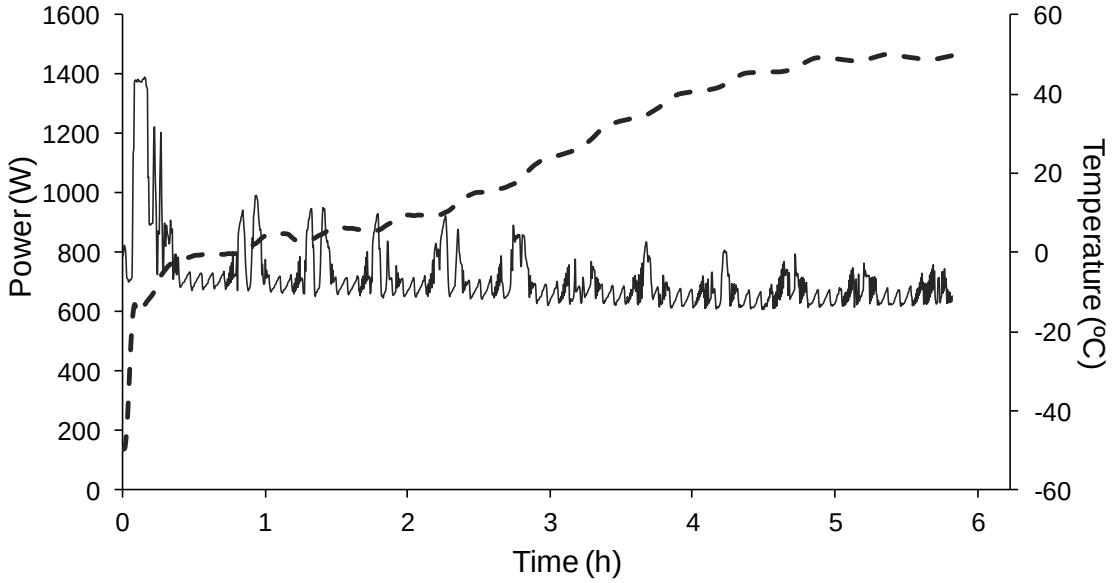

Figure 4. Evolution of the power consumed (continuous line) and of the product temperature (dashed line) over time (h) during the drying of the frozen orange puree at a working pressure of 5 Pa and a shelf temperature of 50 ºC.

The total power consumed by each of the different process conditions was obtained from the registered power consumption data, calculated from the enclosed area under the curve using the midpoint rectangular method (Table 1). Both shelf temperature and working pressure had a significant effect on the total power consumption ($p<0.05$). On the one hand, although at no given moment was the required energy input higher when the drying was carried out at 30 ºC, a greater amount of total power was consumed due to the much longer process (Table 1, $p<0.05$). On the other hand, although significant, the difference in the energy consumption of the processes carried out at 40 or 50 ºC was very small. This behaviour is related with the heat required for the drying of the product. Drying at 30 ºC does not provide enough heat to remove the water content from the product in a reasonable time, which is achieved at 40 ºC. From then on, 50 ºC also allows the process to be shortened a little more, although it is not a great deal more effective. Nevertheless, taking into account that the quality of the product was not observed to be affected by heating the shelves to 50 ºC (Silva-Espinoza et al., 2020a), this would be the

recommended temperature with which to obtain the orange snack. Higher pressure increased the total power consumption ($p<0.05$), except for the freeze-drying carried out at 50 ºC (Table 1). This increase at P_{100} is also related to the longer duration of the process needed to achieve the target water content.

4. Conclusion

Even though heating the freeze-dryer shelves during the drying stage implies an increase in the energy consumed at specific moments, this allows for a shorter process time, so that less total power is consumed. A lower working pressure also permits a shorter drying time, leading to a significant decrease in energy consumption at the lower temperatures. From this point of view, 50 ºC and P_5 may be recommended as the freeze-drying conditions with which to obtain a more economical orange snack.

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Declaration of interest: None

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Chapter 3. *Effect of conventional home storage on the physicochemical properties and bioactive compounds of an orange snack obtained by freeze-drying*

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

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Abstract

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

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

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

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

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

1. Introduction

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

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

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

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

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

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

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

2. Material and Methods

2.1. Raw material

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

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

2.2. Sample preparation and storage

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

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

2.3. Analytical determinations

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

2.3.1. Water content

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

2.3.2. Water activity

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

2.3.3. Porosity

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

$$\varepsilon(\%) = 100\,\frac{\rho-\rho_a}{\rho} \qquad \text{(Eq. 1)}$$

$$\rho_a = \frac{w}{\pi\left(\frac{d}{2}\right)^2 h} \qquad \text{(Eq. 2)}$$

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

$$\rho = \frac{1}{\frac{x_w}{\rho_w} + \frac{x_{CH}}{\rho_{CH}}} \qquad \text{(Eq. 3)}$$

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

2.3.4. Hygroscopicity

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

2.3.5. Colour

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

$$h^* = \arctan(b^*/a^*) \quad \text{(Eq. 4)}$$

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

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

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

2.3.6. Crispness

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

2.3.7. Vitamin C

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

2.3.8. Total phenolic compounds

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

2.3.9. Antioxidant activity

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

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

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

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

2.3.10. β-carotene

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

2.3.11. Statistical analysis

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

3. Results and Discussion

3.1. Physicochemical properties

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

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

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

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

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

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

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

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

3.2. Bioactive compounds and antioxidant activity

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

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

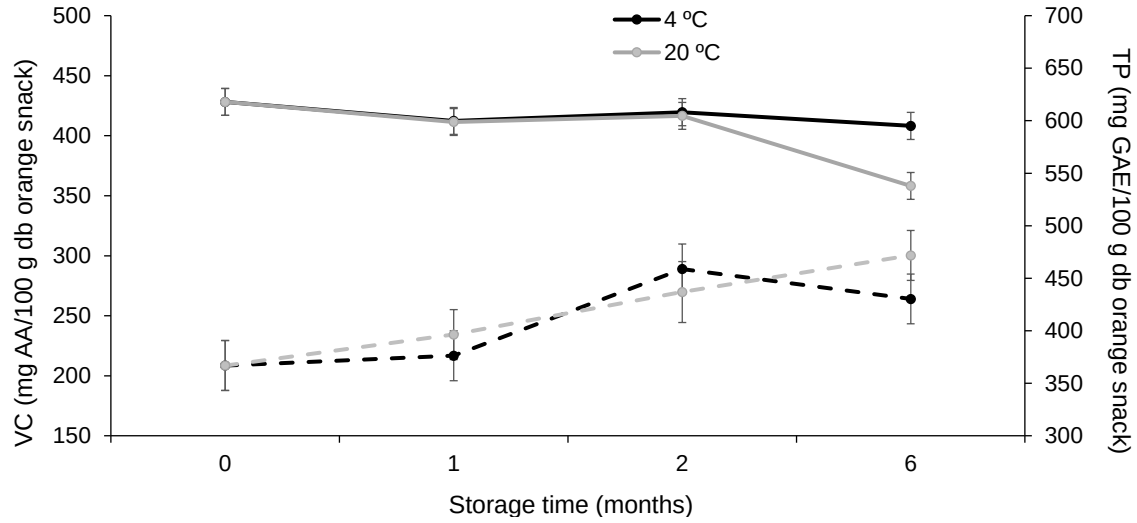

Figure 1. Evolution of vitamin C content (VC, left axis, solid lines) and total phenolic compounds (TP, right axis, dashed lines) throughout the storage period (storage time=0: newly-obtained orange snack) at 4 and 20 ºC. Error bars represent Tukey's HSD statistical significance bars.

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

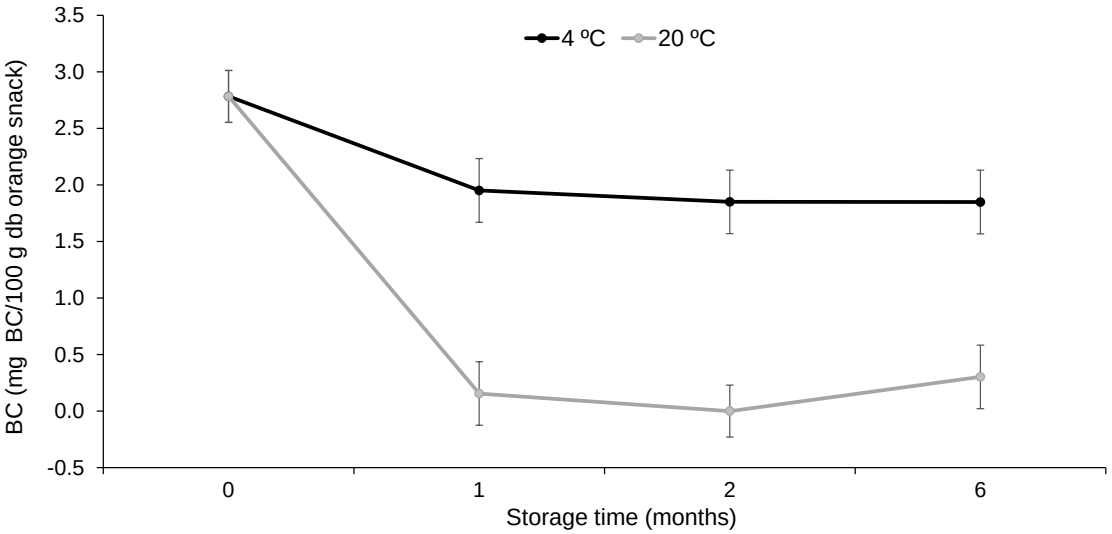

Figure 2. Evolution of β-carotene content (BC) throughout the storage period (storage time=0: newly-obtained orange snack) at 4 and 20 ºC. Error bars represent Tukey's HSD statistical significance bars.

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

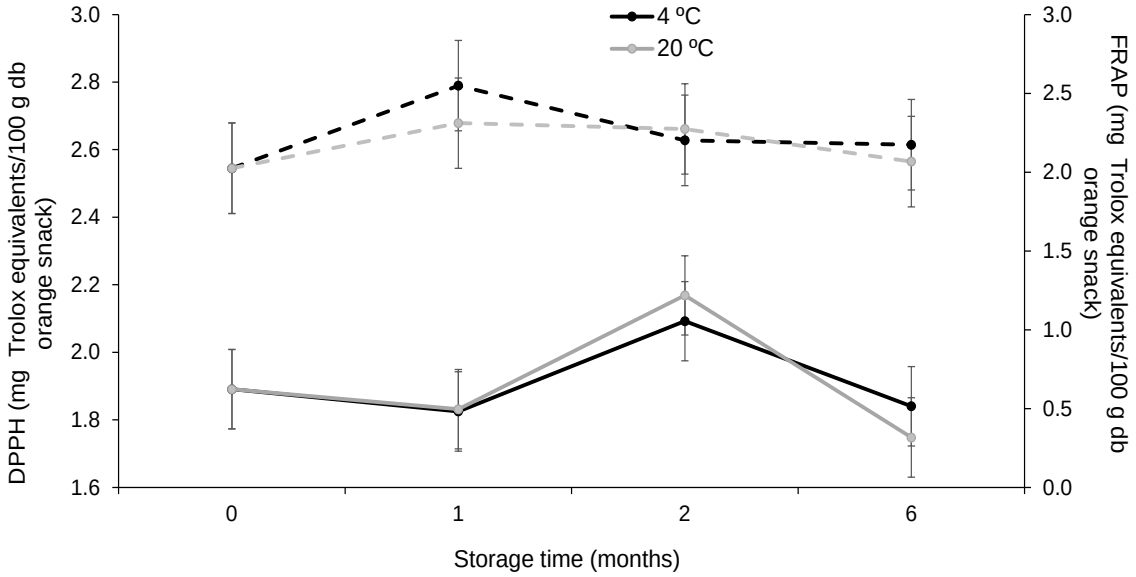

Figure 3. Evolution of the antioxidant activity measured by DPPH test (left axis, solid lines) and FRAP assay (right axis, dashed lines) throughout the storage period (storage time=0: newly-obtained orange snack) at 4 and 20 ºC. Error bars represent Tukey's HSD statistical significance bars.

4. Conclusion

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

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Conflicts of interest

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

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IV. GENERAL DISCUSSION

In this PhD Thesis, the process of obtaining a freeze-dried orange puree to be consumed as snack or to be used as powder has been designed. The aspects investigated in the study were the formulation of the puree by the addition of different biopolymers (Chapter 1), the processing conditions of the freeze-drying process (Chapter 2) and the optimal storage conditions of the obtained product (Chapter 3). The aim of this study was to identify the best conditions to achieve an orange product with the best physical and sensory characteristics and the highest retention of bioactive compounds obtained with the shortest time of freeze-drying, and therefore with a reduction in the costs of the process. This study is of direct industrial application use, so the information collected in this Thesis can serve as support for the different agri-food industries dedicated to the innovation of fruit-based processed products.

IV.1. Influence of different biopolymers on the properties of a freeze-dried orange snack and the corresponding powdered product

A common technique to prevent undesired structural changes in hygroscopic dehydrated fruit is the addition of biopolymers. An antiplasticising effect of different biopolymers related to their high Tg has been described in literature (Braga & Cunha, 2004; Ruiz et al., 2017). Some others can act like physical barriers between the low molecular weight molecules, minimising the stickiness. In Chapter 1.1 the influence of adding different biopolymers on the physicochemical properties and the bioaccesibility of some bioactive compounds of the freeze-dried orange puree **snack** was studied. The influence of the biopolymers on the properties of the freeze-dried orange puree **powder** obtained by crushing the snack also was studied (Chapter 1.2). Gum Arabic, bamboo fibre, maltodextrin, native corn starch, pea fibre, and chemically modified starch with octenylsuccinic groups, were used as drying carriers. Five different formulations were prepared: GA+BF; MD+PF; MD+NCS; OSA+PF; OSA+PF at a ratio 5+1 per 100 g of orange puree. In previous studies from the research group in which I have developed this project, Agudelo et al. (2017) worked with GA and BF and optimised the ratio puree:GA:BF to obtain freeze-dried grapefruit with high retention of bioactive compounds and high physical quality. Egas-Astudillo (2019) despite GA+BF, also considered OSA+ whey protein isolate as biopolymers, with no differences between them on the final product quality. In this study, some other biopolymers were wanted to be tested.

Regarding the freeze-dried **orange puree snack**, the water content-water activity-glass transition temperature relationships were studied to evaluate the influence of the different biopolymers on the hygroscopicity and anti-plasticizing behaviour of the snack (Silva-Espinoza et al., 2020a). On the one hand, all formulated samples containing biopolymers showed significant lower hygroscopicity than the one without biopolymers ($p<0.05$) over the whole water activity range (Chapter 1.1 – Fig. 1), confirming their physical stability role for this dehydrated product. No significant difference among the isotherms ($p>0.05$) was obtained between the different formulations considered. With respect to the glass transition temperature-water content relationships, the Tg decreased as the water content of the orange snack increased for all the formulations (Chapter 1.1 – Fig. 2). The snack without biopolymers showed significant lower Tg ($p<0.05$) than those ones with biopolymers. Despite GA+BF was the sample that showed the highest Tg over the entire water content range, there was no significant difference ($p>0.05$) among the

formulated samples. The increasing of Tg allows orange snack to tolerate higher temperature and relative humidity at storage environment, maintaining the more stable glassy state without showing structural collapse. The stability map built from the Tg-a_w-x_w relationship allowed to identify the critical water content and critical water activity values for the glass transition at specific storage temperature (Chapter 1.1 – Fig. 1). Higher values of CWC and CWA, or greater capacity to maintain the glassy state, were obtained when the samples when any of the biopolymers were added to the orange puree, with no significant differences among them (Chapter 1.1 – Table 4).

On the other hand, the relationship between the glass transition of this product and the changes in colour and texture was evaluated. All these changes may occur at any time while the glass transition occurs, from its onset to the endpoint. Despite the addition of biopolymers affected the colour of the orange snack, becoming yellower, with less colour intensity, and lither, a significant colour change of all the samples occurred at water activities between 0.328 and 0.432, when the glass transition is already finished, corresponding to a complete rubbery state of the amorphous matrix. At this moment, the water content is high enough for enzymatic and non-enzymatic oxidation and browning reactions to occur. The snack without biopolymers began to darken and became less yellow at lower a_w (Chapter 1.1 – Fig. 3). Regarding the textural properties, the softening process or plasticising effect on the orange snacks was observed as the water content increased (Chapter 1.1 – Fig. 4). The loss of crispness occurred when the a_w was between 0.113-0.225 for the orange snack without biopolymers and between 0.225-0.328 for the formulated snacks (Chapter 1.1 – Fig. 4). According to the CWA for the onset and endpoint of the glass transition to occur at 20 ºC, the loss of crispness in the orange snack occurred while the glass transition occurred (Chapter 1.1. – Fig. 5). So, to ensure the orange snack's crispness, its amorphous state should be in a complete glassy state.

Another aspect to consider for the selection of the best formulation of the freeze-dried orange puree snack is its ability to allow bioactive compounds such as vitamin C, total phenolic compounds, and β-carotene to be accessible for being absorbed by the organism (Chapter 1.2). It has to be pointed out that significant losses of TP and VC ($p<0.05$) due to freeze-drying were observed in the freeze-dried orange puree without biopolymers. Nevertheless, about 103% of VC and 111% of TP present in the sample before drying were recovered in the formulated samples, thanks to the protective effect of the biopolymers on the bioactive compounds and their enhanced extractability in the highly porous orange snack (Chapter 1.2 – Table 2). In particular, VC and TP showed higher percentages of preservation in the orange snack when the puree was formulated with the fibres used in this study, either BF or PF (Chapter 1.2 – Table 2). The high stability of TP and VC may have been responsible for the high values of AOA determined by FRAP assay of all formulations after freeze-drying.

In the case of BC, it suffered losses in the range of 18-58% in all the samples upon freeze-drying, indicating the high sensitivity of this compound to this process. The lowest lost was achieved with the formulation containing OSA, whose hydrophobic groups could have created lipidic links with BC, forming a protective barrier against its degradation. In contrast to the AOA measured by FRAP, the AOA determined by DPPH was significantly affected ($p<0.05$) by the freeze-drying with losses between 25 and 50% for every sample.

The decrease in the DPPH free radical scavenging activity after freeze-drying may be related with the loss of BC since some carotenoids show DPPH activity. GA+BF sample preserved the highest percentage of AOA determined by the DPPH assay. GA also has hydrophobic groups that can contribute to create lipidic links with BC and other lipidic compounds with AOA not considered in this Thesis.

The bioaccessibility of the different bioactive compounds studied was calculated based on *in vitro* digestion assays. Among the bioactive compounds studied, BC was not detected either in the oral, gastric, or internal intestinal digestion. It has been reported that the α-amylase and the pepsin affect to the structure of oil droplets, resulting in a greater aggregation (Mun & McClements, 2017; Nik et al., 2010). Also, the presence of some components of plant-based foods like the soluble fibre may inhibit the absorption of carotenoids. Usually, a very low proportion (<5%) of the carotenoids are bioaccessible (Hornero-Méndez & Mínguez-Mosquera, 2007). BC was only detected in the external intestinal digest, which simulated the pass to the large intestine. The enzymes involved in this step (pancreatic and bile) helped to emulsify fat-soluble substances like BC, facilitating their solubilisation and subsequent absorption in the large intestine.

As for TP and VC, the oral and gastric steps had low effect on them (Chapter 1.2 – Fig. 1 and 2). Regarding the intestinal step, TP, VC, and the AOA showed higher degradation and instability due to the conditions of this step such as the alkaline pH and the action of the enzymes, causing their chemical structural changes and formation of complexes with other compounds. The orange snack containing GA+BF was the only sample that showed higher bioaccessibility of TP (59%) and VC (36%) after freeze-drying ($p<0.05$, Chapter 1.2 – Fig. 1 and 2). This indicates an effective encapsulating power of these biopolymers, both protecting the TP and VC from the digestion conditions and allowing them to be released from the food matrix to be absorbed by the small intestine. The high bioaccessibility of TP of this product makes this orange snack a good potential source of polyphenols intake. Also, this formulation offered one of the highest percentages (27%, $p<0.05$) of AOA that could exert its effect at body level (Chapter 1.2 – Fig. 3).

With respect to the powdered product, to be used either as an ingredient to be added to any type of food or to be rehydrated and consumed as a juice, the properties of the **orange powder** obtained by crushing the freeze-dried orange snack were studied (Chapter 1.3). The impact of the biopolymers on the particle size distribution, flowability, and rehydration properties was analysed. It should be noted that the powder obtained from all the formulations was evaluated but not for the powder without biopolymers due to its high stickiness, which made the powder unfeasible to handle. Considering the average particle size, only that of GA+BF (221 μm) was significantly different ($p>0.05$) to OSA+PF (201 μm), the other powders having an intermediate (206-218 μm) particle size. This suggests that all the formulations offered comparable mechanical resistance to crushing and the differences observed in the powder formulations properties may be attributed to their chemical composition and not to differences in the average particle size. As for the flow behaviour, all powdered formulations were classified at least as 'acceptable' according to the angle of repose (α°) values (Chapter 1.3 - Table 3). Samples formulated with MD showed a slight better flowability in air due to their lowest α° value ($p<0.05$, Chapter 1.3 - Table 3), but also higher values of viscosity when

rehydrated ($p<0.05$, Chapter 1.3 - Table 4). GA+BF showed an intermediate wetting time and the lowest viscosity when rehydrated, being the closest viscosity to the commercial orange juices. PF also contributed to improve air flow properties, however it significantly increased ($p<0.05$) the viscosity of the rehydrated product, which is not appropriate for a formulation destined to be consumed as a juice. Powders formulated with OSA, with an intermediate flow behaviour, showed the longest wetting time. The hydrophobic groups in the OSA structure may contribute to its emulsifier character, causing stickiness, cohesiveness, and promoting particles forming aggregates, worsening the flowability and the wettability. Therefore, GA+BF was the most adequate formulation to be used for rehydration purposes to be consumed, for instance, as a juice.

From the work described in Chapter 1 it can be concluded that all the biopolymers used in this study are necessary and effective as drying carriers. All biopolymer combinations equally led to reduce the hygroscopicity and increase the Tg of the orange snack, delaying the colour changes and keeping the crispy texture at higher water content and water activity. Nevertheless, GA+BF provides a powder with one of the shortest wetting time and the lowest viscosity when rehydrated, which makes it more adequate to be consumed as a juice. The formulation with GA and BF promotes the bioaccessibility of the phenolic compounds and of the vitamin C. All these results allow proposing the combination GA+BF as the most suitable to obtain a freeze-dried orange puree intended for a more versatile consumption mode.

IV.2. Selection of freeze-drying conditions to obtain an orange snack

From the results shown in Chapter 1, GA+BF was selected as the most suitable biopolymer mix to be added to the orange puree due to its ability to reduce hygroscopicity, increase the Tg, and enhance the bioaccessibility of the bioactive compounds the most. At the same time, this formulation allows to obtain a powdered product more suitable for rehydration. Therefore, in Chapter 2, GA+BF formulation was used to carry out the selection of the adequate variables of the freeze-drying operation. The objective of this chapter was to adjust the process time to minimize its cost while ensuring the quality of the product. To this end, the impact of freeze-drying conditions on both the physicochemical and sensory properties of the orange snack and on the total power consumed was evaluated. Three variables were studied: the freezing rate (slower and faster) of the product, together with the shelf temperature (30, 40, 50 ºC) and the working pressure (5 and 100 Pa) during the drying step. All the combinations among the three variables were considered. The drying time for each condition was assigned based on previous experiments considering a final water content of the product being lower than 5%, which corresponds to the CWC for the glass transition of this product at common room and refrigeration temperatures (Chapter 1.1 – Table 4). The selected drying time was 6, 7, and 25 hours at 50, 40, and 30 ºC, respectively, at any pressure or freezing rate. The results indicated that the shelf temperature and the working pressure had a significant effect ($p<0.05$) on the different studied properties.

Despite the fact that the water content of all the snacks ranged between 2.2 ± 0.3 and 4.2 ± 0.2 g water/100 g sample, the statistical analysis indicated a significant effect

of the shelf temperature and the pressure ($p<0.05$) on the final water content of the freeze-dried samples (Silva-Espinoza et al., 2020b). It is important to emphasize at this point the challenge of obtaining an exact water content in a freeze-dried product, especially in the low-moisture zone (Tang & Pikal, 2004). This challenge is based on the limited availability of technical solutions to specifically monitor the water content of the product in a non-invasive way. Invasive measurements procedures are used, which are generally not representative for the entire batch since small variations in water content could be due to the product position in the shelf of the chamber (Schneid et al., 2011). Regarding the processing time selected for each freeze-drying condition, working at the highest pressure (100 Pa) and the lowest shelf temperature (30 ºC) promoted samples with the highest water content.

The colour of the snack was mainly affected by the working pressure. When the freeze-drying was carried out at higher pressure, luminosity and chroma decreased and increased ($p<0.05$), respectively (Chapter 2.1 - Fig. 2). This effect may be related to the shrinkage phenomenon reported to occur when freeze-drying is carried out at higher pressure (Hammami et al., 1999). That increase in C* was also perceived by the consumers, who related higher working pressure with a more intense yellow colour of the orange snack ($p<0.05$, Silva-Espinoza et al., 2021a). During freeze-drying, the wall of the pores created by the ice sublimation are exposed to shrink due to the surface forces or gravity (Krokida et al., 1998). Higher pressure accelerates the sublimation during the primary drying (Chapter 2.3), and the pores are exposed longer time under pressure during the secondary drying, which may promote the shrinkage. Although the orange snacks obtained by all the studied conditions didn't show significant differences in porosity (ε values between 86.4-87.79%, $p>0.05$), very slightly variations and minimal shrinkage reported to take place during freeze-drying (Ratti, 2001) could cause the more intense yellow colour of the orange snacks.

Regarding the structure, the number of force peaks, which is positively related to the crispness of a product (Alonzo-Macías et al., 2014), was significantly affected ($p<0.05$) by the working pressure and shelf temperature. Lower pressure and higher temperatures promoted samples with greater number of force peaks (Chapter 2.2 – Fig. 3), and therefore crispier, which is desirable for products such as snacks. In fact, consumers also perceived a significant influence of the working pressure ($p<0.05$) on the texture of the orange snacks. In this case, consumers perceived the orange snacks freeze-dried at any condition as crispy, but those samples freeze-dried at lower pressure were also perceived with a porous/melt-in-the-mouth texture (Silva-Espinoza et al., 2021a). Since a relationship between the texture and the water content has been reported (Katz and Labuza, 1981; Primo-Martín et al.; 2006; Srisawas et al., 2003) and in this study the samples obtained at lower working pressure and higher shelf temperature showed a lower water content, the perceived porous/melt-in-the-mouth texture of these samples could be related to the lowest water content promoted by lower pressure and not to the low pressure per se.

To confirm this hypothesis, it was compared the number of force peaks of the orange snacks freeze-dried at different process conditions with no significant differences in the water content ($p>0.05$). This comparison was made with 3 samples frozen at the slower freezing rate and dried at: 50 ºC and 100 Pa, 50 ºC and 5 Pa, and 40 ºC and 5 Pa, with

water content values of 2.9±0.09, 2.2±0.3, and 2.8±0.6 g water/100 g sample, respectively (Chapter 2.2). In this case, the crispness of the product evaluated through the number of force peaks showed no significant differences ($p>0.05$) among them with values of 44±5, 50±6, and 46±6, respectively. So, it can be confirmed that the texture is affected by the water content, rather than the process conditions. In addition, it is worth mentioning that the impact of the water content on the texture is important even when the difference among the water content of the samples is small, as is the case in the different snacks obtained for the studies presented in Chapter 2, this being between 2.2 ± 0.3 and 5.300 ± 0.001 g water/100 g sample.

The sensory study also revealed the orange snacks freeze-dried at lower pressure were perceived as sweet, acid, and with citrus notes, typical and expected characteristics for an orange-based product. This suggests a better retention of the volatile and non-volatile compounds when working at lower pressure, due to a reduced or to the absence of shrinkage in this case (Kompany & Rene, 1996; Petersen & Lorentzen, 1973). The effect of pressure seems to have a significant effect on flavour; however, it also promotes lower water content. So, it would have to be confirmed in further studies if this effect is related to the higher concentration of compounds responsible for flavour, or it is actually caused by pressure.

Concerning the bioactive compounds, the shelf temperature and working pressure were the variables with significant effect on them. Vitamin C showed a great stability at higher temperatures due to the shorter processing times (40 ºC-7h and 50 ºC-6h). The significant losses of VC (5-10 %, $p<0.05$) occurred at 30 ºC carried out for 25 h (Chapter 2.1 – Fig. 5), corresponding to a freeze-drying between 3 and 4 times longer than the other two temperatures studied. The loss at 30 ºC was more accentuated when working with higher pressure ($p<0.05$), which may be related to the contact with the low oxygen contained in the chamber of freeze-drying during long process times. So, if long process is carried out, the oxygen content should be maximally minimized. BC showed also better preservation at lowest pressure during the drying process, when almost no oxygen is present (Chapter 2.1 – Fig. 6), despite temperatures higher than 40 ºC should be avoided for greater BC preservation. This result indicates the high sensitivity of BC to the process conditions, especially to the heat and the oxygen. Total phenolic compounds and antioxidant activity were not significant affected by any of the process conditions ($p>0.05$, Chapter 2.1, Fig. 6)

All these results indicate an effect of the shelf temperature and working pressure on the final water content, and therefore, on the required drying time. Therefore, it was checked whether the different duration of freeze-drying, with each set of processing conditions, had a significant impact on the energy consumption of the process. For the study of the energy consumption another batch of snacks with a better adjustment of the processing time was prepared. Considering that the freezing rate does not affect the quality of the snacks, the combination working pressure/shelf temperature were adjusted to fit the drying time as to achieve the same final water content of the product. Based on a new set of preliminary experiences, the drying time for 30, 40, and 50 ºC was set in 11h, 7h 20 min, and 5h 50 min, respectively, at 5 Pa, and 27h, 8h 30 min, and 6h, respectively, at 100 Pa. Higher pressure and lower temperatures led to increase the drying time, which also affected the total power consumed ($p<0.05$). Although heat

provided by the freeze-dryer during the drying was greater when it was carried out at higher temperatures, the longer process at the lower temperatures made the power consumption significantly higher ($p<0.05$) as the temperature decreased (Chapter 2.3. – Table 1). Higher pressure for temperatures below to 50 ºC increased the drying time, and therefore the total power consumed. This increase on the total power consumed was 2.4 and 1.1 times higher at 30 and 40 ºC. Although higher pressure accelerated the sublimation during the primary drying, it delayed the secondary drying (Chapter 2.3 – Fig. 2). The worst diffusivity of water vapour in air (pores) and the higher partial pressure of water vapour in the chamber caused by the higher pressure during the secondary drying resulted in a longer total drying time (Saravacos & Stinchield, 1965; Searles et al., 2017).

The results of Chapter 2 permit concluding that the freeze-drying at higher temperature and lower pressure (5 Pa and 50 ºC) significantly reduces the total power consumed, since the drying times was shorter, and allows to obtain a clear, yellowish, and less saturated colour orange snack with crispy and melt-in-the-mouth texture, higher citrus notes, and high preservation of bioactive compounds, specially of VC and TP and therefore, of AOA.

IV.3. Effect of conventional home storage on the physicochemical properties and bioactive compounds of an orange snack obtained by freeze-drying

It is also relevant that the product may keep adequate physicochemical properties and highest retention of bioactive compounds as long as possible during storage. On the one hand, from Chapter 1.1 it can be suggested the maximum level of relative humidity in the environment which would preserve the colour and the crispy texture of the orange snacks. That RH may be ensured by the commercial package during marketing for long-time storage. On the other hand, it is also important to evaluate the stability of the product once the package is opened and stored under conventional home conditions.

From the results of Chapters 1 and 2, the orange snack obtained from the puree formulated with GA+BF, freeze-dried at 5 Pa as working pressure and 50 ºC as shelf temperature, was selected for the storage study. The slow freezing-rate was selected for experimental convenience. The water content, water activity, crispness, colour, and bioactive compounds of the orange snack were evaluated throughout the 6 months of storage packed inside zipper bags at both 4 and 20 ºC, trying to simulate habitual home storage conditions (Chapter 3, Silva-Espinoza et al., 2021b). The x_w and a_w significantly increased ($p<0.05$) as the storage period progressed independently of the storage temperature (Chapter 3 – Table 1). As expected, as the x_w increased, the crispness (number of force peaks) decreased ($p<0.05$, Chapter 3 – Table 1). The ε was also significantly decreased ($p<0.05$) but only after six months of storage, when the x_w was greater than 8.4% and no force peaks were obtained. The presence of force peaks and the porosity remained acceptable up to two months of storage, corresponding to a_w of 0.301 and 0.287 at 4 and 20 ºC, respectively (Chapter 3 – Table 1). Both values are lower than the CWC (Chapter 1.1 – Table 4), confirming that the crispy behaviour is maintained when the orange snack is in a glassy state. From these results, the maximum

storage time to ensure a proper texture of the formulated orange snack is between 2 and 6 months, both at 4 and 20 ºC, when packed in zipper bags.

The colour remained almost stable throughout all the storage period, with only a slight decrease in L* and h* for the sample stored at 20 ºC for 6 months ($p<0.05$, Chapter 3 – Table 1). The slightly darker and less yellowish orange colour could result from the gradual non-enzymatic browning. Nevertheless, when the storage was carried out at 4 ºC, no significant differences of L* and h* after six months were obtained ($p>0.05$), indicating a reduction of degradation rates at refrigeration temperature, even with the same a_w.

Concerning the bioactive compounds, VC remained stable throughout the storage period, demonstrating a high stability under the selected storage conditions, with the sole exception of the sample stored for 6 months at 20°C which showed a loss of 20% ($p<0.05$). The highest water content at that time and the higher storage temperature favour the ascorbic acid degradation as a result of non-enzymatic browning reactions (Kaanane et al., 1998; Pavlovska & Tanevska, 2013). This fact can also explain the darker and yellowish orange colour of the orange snack at this time and temperature. Independently of the storage temperature, the TP content increased as the storage period progressed. It also was observed by other authors that some polyphenolic compounds may be synthetized from other compounds like organic acid (Igual et al., 2010; Kalt et al., 1999). It was already observed in Chapter 2.1 that BC is sensitive to the oxygen and higher temperatures, which was confirmed in this Section. A significant decrease in BC content was observed just after one month ($p<0.05$), and the loss was even more pronounced at 20 ºC (Chapter 3 – Fig. 2). The AOA determined by both DPPH and FRAP methods remained stable during the storage period at both temperatures.

Considering colour changes and the higher sensitivity of VC and BC to higher temperatures, it is recommended to store the orange snack under refrigeration.

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V. CONCLUSIONS

The effect of the addition of biopolymers, freeze-drying conditions and storage conditions on the physicochemical properties and bioactive compounds of an orange snack obtained by freeze-drying was evaluated.

To avoid undesired changes in colour and texture, the glassy state of the orange snack obtained by freeze-drying needs to be assured. The addition of any of the biopolymers considered in this study into the orange puree improves similarly the physical quality of the orange snack obtained. Nevertheless, the orange snack with gum Arabic and bamboo fibre was the one that offered the highest bioaccessibility of the studied biocompounds of the orange snack and showed good rehydration properties of its corresponding powder to be consumed as a juice. For these reasons, it is considered the best formulation among the studied ones. Regardless the freezing rate, the optimum freeze-drying conditions to obtain an orange snack are lower chamber pressure of 5 Pa and shelf temperature of 50 ºC. These conditions preserve the highest bioactive compounds content and antioxidant activity in the orange snack. Also, they promote a brighter, yellowish, with less intensity of colour, porous, and crispier texture in a shorter time. The great reduction of drying time at these conditions also involved a reduction of the total energy consumption of up to 75%. The commercial packaging of the designed orange snack should ensure that RH is maintained below 30 or 20% during marketing at 4 or 20 ºC, respectively, for long-time storage to maintain the crispy texture and its colour. Once the package is opened and considering the storage temperatures usually available at home (room 20 ºC and refrigeration 4 ºC), the orange snack will maintain an adequate texture and colour for at least 2 months. Nevertheless, the refrigerated storage is recommended for a better preservation of vitamin C and β-carotene.

This study offers solutions to food companies to expand the offer of healthy and high-quality dehydrated fruit-based products. To this end, the use of freeze- drying in the conditions proposed in this study ensures the bioactive compounds preservation and reduces energy consumption. Moreover, with this process it is possible to valorise wasted fruits, developing a more cost-effective sustainable process and product by reducing the shipping cost, which also contributes to reduce the carbon footprint. This methodology can be easily transposed to other fruits and it worth to develop further studies to provide adapted methods for each fruit.

VI. DISSEMINATION OF RESULTS AND PREDOCTORAL STAY

VI.1 Dissemination of results

- Research Articles in International Journals JCR

Published

Silva-Espinoza, M. A., Ayed, C., Foster, T., Camacho, M. M, & Martínez-Navarrete, N. (2020). The impact of freeze-drying conditions on the physico-chemical properties and bioactive compounds of a freeze-dried orange puree. *Foods*, *9*(1). https://doi.org/10.3390/foods9010032

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Silva-Espinoza, M. A.., Camacho, M. M., & Martínez-Navarrete, N. (2021). Effect of storage temperature on the crispness, colour and bioactive compounds of an orange snack obtained by freeze-drying. *British Food Journal*. In press. https://doi.org/10.1108/BFJ-11-2020-1061

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Submitted

Silva-Espinoza, M.A., Camacho, M. M., Martínez-Monzó, J., & Martínez-Navarrete, N. Impact of the freeze-drying conditions applied to obtain an orange snack on energy consumption. *Journal of Food Science.*

- Communications in International Congresses

Silva-Espinoza, M. A., Martínez-Monzó, J., Camacho, M. M., & Martínez Navarrete, N. (2019, June 12). *Effect of orange puree freeze-drying conditions on the process energy efficiency* [poster]. Third Nordic Baltic Drying Conference, Saint-Petersburg, Russia.

Uscanga, M., Silva-Espinoza, M. A., Egas, L., Camacho, M. M., & Martínez-Navarrete, N. (2018, September 11). *Influence of freeze-drying conditions on orange powder flowability* [poster]. Proceedings of 21st International Drying Symposium, Valencia, Spain.

Silva-Espinoza, M. A., Algarra, E., Uscanga, M, Camacho, M. M., & Martínez-Navarrete, N. (2017, September 6). *Orange juice obtained from powdered freeze-dried puree. Powder particle size and juice viscosity relationship* [poster]. Iberian Meeting on Rheology (IBEREO). The multidisciplinary science of rheology, Valencia, Spain.

- Communications in National Congresses

Silva-Espinoza, M.A., Camacho, M. M., & Martínez-Navarrete, N. (2019, May 15). *Estudio de la relación entre la velocidad de congelación y la temperatura de liofilización de un puré de naranja* [poster]. X Congreso Nacional Ciencia y Tecnología de Alimentos (CyTA), León, Spain.

Silva-Espinoza, M.A., Uscanga, M, Camacho, M.M., & Martínez-Navarrete, N. (2017, May 16). *Efecto de diferentes solutos sobre los valores críticos de humedad y actividad del agua para la transición vítrea de un puré de naranja liofilizado* [poster]. IX Congreso Nacional Ciencia y tecnología de Alimentos CyTA-CESIA. Ayer, Hoy y Mañana de la Ciencia y Tecnología de los alimentos, Madrid, Spain.

- Publications related with this Thesis

International Congresses

Egas, L, Silva-Espinoza, M.A., Uscanga, M., Camacho, M.M., & Martínez-Navarrete, N. (2018, September 11). *Influence of freeze-drying conditions on orange powder flowability* [poster]. Proceedings of 21st International Drying Symposium, Valencia, Spain.

Uscanga, M, Silva-Espinoza, M.A., Camacho, M.M., & Martínez-Navarrete, N. (2017, September 6). *Viscosity of the juice obtained after the rehydration of a freeze-dried orange puree as affected by the initial water content* [poster]. Iberian Meeting on Rheology (IBEREO). The multidisciplinary science of rheology, Valencia, Spain

Silva-Espinoza, M.A., Agudelo, C., Camacho, M. M., & Martínez-Navarrete, N. (2015, September 16). *Rheological behaviour of fruit juice obtained from freeze-dried grapefruit* [poster]. 5th MoniQA International Conference "Food and Health – Risks and Benefits, Porto, Portugal.

National Congresses

Silva-Espinoza, M.A., Uscanga, M, Camacho, M.M., & Martínez-Navarrete, N. (2017, May 16). *Influencia de la humedad de la muestra y velocidad de congelación en el proceso de liofilización* [poster]. IX Congreso Nacional Ciencia y tecnología de Alimentos CyTA-CESIA. Ayer, Hoy y Mañana de la Ciencia y Tecnología de los alimentos, Madrid, Spain.

VI.2 Predoctoral stay

Stay at Division of Food, Nutrition and Dietetics, School of Biosciences, Faculty of Science, University of Nottingham (United Kingdom) from May to July 2019. It was supervised by Dr. Tim Foster. Study: Impact of biopolymers on the physical properties of the powder obtained by crushing a freeze-dried orange puree.