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The porosity of carbohydrate-based spray-dried microparticles containing limonene stabilized by pea protein: Correlation between porosity and oxidative stability

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ABSTRACT

In this study, the effects of different concentrations of pea protein concentrate (PPC) in the physical properties, porosity features, and oxidative stability of maltodextrin-based spray-dried microparticles containing orange essential oil (OEO, rich in limonene) were evaluated. The use of PPC resulted in spray-dried microparticles with encapsulation efficiencies of about 99 wt%, without visible pores, and relatively high glass transition temperature (66,4 °C) at $A_w \sim 0.3$. The nitrogen adsorption and positron annihilation lifetime spectroscopy measurements showed that the increase of PPC concentration from 2.4 to 4.8 wt% (g of PPC/100 g of emulsion) did not affect the porosity features of the microparticles. These results were confirmed by the profiles of OEO retention and limonene oxide production, which were similar for both samples throughout four weeks of storage. Based on these results, we verified that the lower amount of PPC we tested can effectively protect the OEO during storage, showing that a relatively cheaper orange flavor powder can be produced using less protein.

1. Introduction

Citrus essential oils have been extensively used in food applications and non-food applications such as cosmetics and cleaning as flavoring agents due to their pleasant odor, similar to lemon, which is typical of a low molecular weight terpene called limonene (Asbahani et al., 2015; Ravichandran et al., 2018). Apart from its use as flavor, limonene presents several bioactive properties such as anti-inflammatory, anticancer, antioxidant, antidiabetic, and antimicrobial (Vieira et al., 2018). However, its non-polar character, high volatility, and high susceptibility to oxidation arise as limitations of handling limonene as an ingredient.

The encapsulation of limonene has been widely performed to reduce its volatilization and oxidation over time (Khoshakhlagh et al., 2018; Li and Lu, 2016; Sultana et al., 2018). Over the years, the spray-drying of food emulsions has been the most common approach to encapsulate flavor compounds such as limonene. The conversion of the liquid oil into a low moisture powder increases the oxidative stability of the encapsulated material and makes possible its transportation and storage (Reineccius, 2004). Blends of maltodextrin and proteins have been among the most used material combinations to encapsulate hydrophobic compounds in spray-dried matrices. Animal proteins such as whey protein isolate are commonly used as emulsifiers in these systems due to their good technological properties that result in highly stable emulsions, which are necessary to produce powders with high oil retention (Carmona et al., 2013; Karrar et al., 2020; Teo et al., 2021).

On the other hand, the continuous increase in the vegetarian and

Abbreviations: DE, dextrose equivalent; EE, encapsulation efficiency; DSC, differential scanning calorimetry; FID, flame ionization detector; GC, gas chromatography; OEO, orange essential oil; o-Ps, ortho-positrons; PALS, positron annihilation lifetime spectroscopy; PPC, pea protein concentrate; PPC2.4, orange essential oil microparticles containing 2.4 g of pea protein concentrate/100 g of emulsion; PPC4.8, orange essential oil microparticles containing 4.8 g of pea protein concentrate/100 g of emulsion; S_{BET}, specific surface area; SEM, scanning electron microscopy; T_g, glass transition temperature; $\rho_{apparent}$, apparent density; ρ_{true}, true density; $\tau_{o\text{-}Ps}$, ortho-positrons lifetime; $\sigma_{o\text{-}Ps}$, average pore diameter.

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vegan population has driven the food industry to study and apply novel natural alternative sources of food ingredients (Aydar et al., 2020; de Boer and Aiking, 2021; Sha and Xiong, 2020). In order to meet the needs of these restricted diets, plant proteins have been studied as substitutes for synthetic emulsifiers and animal proteins in the production of emulsion-based food systems (Sharif et al., 2018). In the case of the encapsulation of food flavors through emulsification followed by spray-drying, blends of maltodextrin and plant proteins can be used to produce vegetarian/vegan flavor powders with high oil retention (Francisco et al., 2020).

Despite the roles of carbohydrates and proteins in the matrix formation are now well understood, we may not say the same about the roles of these materials in the protection of the oil inside the spray-dried matrix (Moreau and Rosenberg, 1999; Rosenberg et al., 1988). While the function of carbohydrates in acting as an oxygen barrier in the vitreous state has already been established, the fundamental mechanisms that drive the functionality of proteins in such systems remain unclear. Proteins are mainly exploited for their ability to act as emulsifiers that allow the formation of stable emulsions with a desired oil droplet size. This ability is specifically essential for the retention of volatile compounds (Reineccius, 2004). However, they are also responsible for developing the three-dimensional architecture in the encapsulation matrix, which affects the porosity in the molecular and microscopic length scales and, consequently, the diffusion of oxygen and volatile compounds through the matrix (Reineccius and Yan, 2016).

Few studies have found that high concentrations of dairy proteins in carbohydrate-based spray-dried microparticles increase the porosity (free volume) of the matrix, leading to higher oxidation of the encapsulated bioactive compound. For example, measuring the true density by helium pycnometer, Moreau and Rosenberg (1999) verified that the microparticles containing a higher whey protein isolate amount presented a lower density indicating a higher porosity. Using positron annihilation lifetime spectroscopy (PALS), Drusch et al. (2012) verified that the increase of sodium caseinate content in glucose syrup spray-dried microparticles resulted in the increase of porosity (free volume) in the matrix and decreased the oxidative stability of the encapsulated fish oil, showing a possible correlation between the porosity of the matrix and the oxidative stability of the bioactive compound.

The most important parameter derived from PALS is the lifetime of an ortho-positronium (probe), which correlates with the size of the free volume elements in a polymer matrix, making it possible to understand how differences in free volume elements of the carrier matrix affect the stability of microencapsulated lipophilic bioactive compounds (Drusch et al., 2009). PALS has been mostly applied to assess the free volume and pore size of polymer-based matrices in the field of material science (Fan et al., 2018; Fukuzumi et al., 2011; Nuruddin et al., 2020). In the field of Food Science, the application of PALS is still limited to the study of porosity of food coatings, films and packaging been a reliable technique to differentiate the porosity features of distinct formulations in the molecular scale (Hughes et al., 2016; Szabó et al., 2012; Martini et al., 2020). Recently, PALS has shown great potential to differentiate the size of free volume elements of several glassy carbohydrate encapsulation systems that present similar bulk properties but significant differences in the stability of the microencapsulated oil, showing that the oxygen diffusivity through the matrix must be a key determinant in autoxidation of the encapsulated oil (Reineccius and Yan, 2016). In this sense, PALS is considered a powerful tool to assess the porosity features of carbohydrate-based encapsulation systems, being very useful for the rational design of microencapsulation systems to protect food-grade bioactive compounds.

Apart from the effort of some studies to understand the fundamental effects of animal proteins on the porosity of spray-dried microparticles consisting of blends of carbohydrates and proteins, there is still a lack of information about the effects of using plant proteins in the porosity features and oxidative stability of spray-dried microparticles containing volatile compounds such as limonene. In our understanding, the study of such effects in microparticles consisting of blends of plant proteins and carbohydrates is significant to the food industry. Considering this, the conceptualization of spray-dried microparticles with low amounts of plant proteins (the pricier ingredient) is mandatory to produce a commercially viable product.

In this context, the current study aimed at investigating the effects of using low amounts of pea protein as a co-wall material on the physical properties, porosity features, and oxidative stability of spray-dried maltodextrin-based microparticles containing orange essential oil. This work has focused mainly on evaluating the porosity features of the microparticles through density determination and PALS to understand the role of the carbohydrate, protein, and core conformation in the protection of limonene.

2. Material and methods

2.1. Material

Orange essential oil (OEO, 95 wt% of limonene) was kindly donated by Citrosuco (Matão, Brazil). Maltodextrin MOR-REX® 1910 with a dextrose equivalent (DE) of 10 was kindly donated by Ingredion (Mogi Guaçu, Brazil). Pea protein concentrate (PPC, 69 g of protein/100 g of powder on a wet basis) Pisane® C9 was kindly donated by R&S Blumos Industrial e Comercial Ltda (Cotia, Brazil). Deionized water was used in all assays. All other chemicals and solvents were of analytical grade and purchased from common suppliers.

2.2. Emulsion production

Emulsions (300 g) were produced with 6 g of OEO/100 g of emulsion and 24 g of wall material/100 g of emulsion. Two emulsions formulations were made using the amounts described in Table 1. Wall material consisted of PPC and MD, and PPC was employed in two different concentrations, 2.4 and 4.8 wt% (g of PPC/100 g of emulsion). The wall material was mixed with water one day before use to allow their complete hydration. Then, the mixture was kept under stirring at 450 rpm for 1 h. Finally OEO was added to the dispersion, and the homogenization was performed with an Ultra-Turrax IKA T18 basic (Campinas, Brazil) at 11,200 rpm for 3 min (Paulo et al., 2020). Samples were produced in duplicate.

2.3. Microparticles production

The process was performed in a Mini Spray Dryer Büchi B-290 (Flawil, Switzerland) with a double fluid nozzle atomizer of 0.7 mm internal diameter. Feeding was carried out with 13 g min^{-1} with inlet and outlet temperatures of 180 and 95 ± 1 °C, respectively, and an atomization airflow rate of 600 L h^{-1} (Francisco et al., 2020). Microparticle samples were identified as PPC2.4 and PPC4.8, referring to the concentration of 2.4 and 4.8 wt% of PPC on the emulsions, respectively. Samples were produced in duplicate. Microparticles without orange essential oil (unloaded) were also produced.

Table 1
Amount of each component required to produce pea protein concentrate (PPC) emulsions containing orange essential oil (OEO).

Material	PPC2.4	PPC4.8
Water (g/100 g of emulsion)	70	70
Maltodextrin (g/100 g of emulsion)	21.6	19.2
Pea protein concentrate (g/100 g of emulsion)	2.4	4.8
Orange essential oil (g/100 g of emulsion)	6	6

2.4. Microparticles characterization

2.4.1. Encapsulation efficiency (EE)

The encapsulation efficiency was determined according to Jafari et al. (2007) with slight modifications using the Eq. (1).

$$EE\ (\%) = \left[\frac{Total\ oil\ in\ the\ microparticles - Surface\ oil}{Total\ oil\ in\ the\ microparticles}\right] x100 \quad (1)$$

For the oil quantification at the surface of the microparticles (surface oil), 1 g of freshly prepared microparticles was stirred with 10 mL of hexane for 30 s at room temperature. This mixture was centrifuged at 3000 rpm for 10 min. The supernatant was then filtered with a syringe filter, and 1 mL of the filtrate was added to a vial containing 3 μL of n-decane (Sigma-Aldrich, San Luis, USA), which was used as an internal standard. The oil content in the organic phase was then quantified by gas chromatography using a GC 7890 A Agilent Technologies equipment (Santa Clara, USA). The separation was performed in a HP-5 column (30 m × 0.250 mm x 0.25 μm) J & W Scientific (Agilent Technologies, Santa Clara, USA). The chromatographic conditions were injector at 250 °C; flame ionization detector (FID) at 200 °C; split injection (1:50); an initial temperature of 60 °C with an initial time of 2 min; program rate of 8 °C/min to 100 °C; and 10 °C/min to 200 °C. The external standard calibration method was used for limonene quantification. For this purpose, a calibration curve of (R)-(+)-Limonene (Sigma-Aldrich, San Luis, USA) in hexane (0.0123–16.8 mg/mL) was produced. The mass of surface oil was obtained by the conversion of the mass of limonene into the mass of orange essential oil.

The total oil was determined by hydrodistillation using a Clevenger apparatus. For this, 5 g of microparticles were dissolved in 500 mL of deionized water, and distillation was performed for 1 h. The distilled oil volume was read at the Clevenger graduated scale and multiplied by the density of the orange essential oil (0.843 g/cm^3) to calculate the mass of recovered oil.

2.4.2. Glass transition temperature (T_g)

Samples (5–7 mg) of microparticles (A_w of ~0.3) were submitted to Differential Scanning Calorimetry (DSC) using a 2920 Modulated DSC (TA Instruments, USA) in isothermal and non-isothermal modes for the determination of the glass transition temperature (T_g) of the microparticles. The following program was set: heating up from 25 °C to 160 °C at 10 °C.min^{-1}; maintenance at 160 °C for 5 min; cooling down from 160 °C to 25 °C at 10 °C.min^{-1}; maintenance at 25 °C for 5 min. In all tests, samples were placed in aluminum sample pans that were hermetically closed, N_2 was used as an inert atmosphere. An empty aluminum sample pan was used as a reference in all assays. The equipment was calibrated according to the ASTM E967 standards.

2.4.3. Scanning electron microscopy (SEM)

The microstructure of the microparticles was evaluated using scanning electron microscopy (SEM). The powders were attached to SEM stubs using double-sided adhesive tape and coated with gold under vacuum in a Sputter Coater K450 (EMITECH, Kent, UK), resulting in a coat of 200 Å. The coated samples were observed with a LEO440i scanning electron microscope (LEICA Electron Microscopy Ltd., United Kingdom). The SEM operated at 15 kV and 50 pA with magnifications of 1500 and 6000 x.

2.5. Evaluation of the barrier properties of the microparticles

2.5.1. Apparent density, true density, and porosity of the microparticles

The apparent density ($\rho_{apparent}$) was determined in a pycnometer, using toluene as an immiscible liquid. The true density (ρ_{true}) was measured by gas pycnometry using a Helium pycnometer AccuPyc 1330 (Micromeritics Inc., USA). The particle porosity was calculated using Eq. (2) (Boukouvalas et al., 2006). In these assays, only the microparticles containing OEO were analyzed.

$$Particle\ porosity = \left(1 - \frac{\rho_{apparent}}{\rho_{true}}\right) x100 \quad (2)$$

2.5.2. Nitrogen sorption measurements

Measurements of the specific surface area (S_{BET}) of the unloaded microparticles were taken using an ASAP 2010 unit (Micromeritics Inc., USA). Analysis of sorption was performed in duplicate. The specific surface area measurement was performed using the BET method (Brunauer et al., 1938). For this measurement, the area of a nitrogen molecule of 16.2 × 10^{-20} m^2 was used.

2.5.3. Positron annihilation lifetime spectroscopy (PALS)

Microparticles without OEO (unloaded) were equilibrated at 25 °C and water activity of 0.33 before analysis. Samples for PALS experiments were prepared by compaction of about 0.3 g of water activity equilibrated powder into disks with a diameter of 10 mm and thickness of ~4 mm using a Carver laboratory tableting press at 1000 psi. The measurements were performed as described by Hohman (2017). The ortho-positrons lifetime ($\tau_{o\text{-}Ps}$) generated by each sample (measured in nanoseconds) is directly proportional to the size of the molecular voids of the sample. Only unloaded microparticles were analyzed by PALS since, according to Drusch et al. (2009), the oil affects the average lifetime leading to wrong values of free volume. Thus, 'duplicate' samples were prepared, one with OEO for studying oxidation during storage and one without the OEO (to determine ortho-positrons (o-Ps) data), assuming that the presence of the encapsulated material does not change the structure of the microparticles (pore volume in a molecular scale) (Reineccius and Yan, 2016).

2.5.4. Orange essential oil retention and limonene oxidation in the spray-dried microparticles by gas chromatography (GC)

The orange essential oil retention and the limonene oxidation progress in the microparticles were evaluated for four weeks. Microparticles were maintained at 45 °C and water activity of 0.33 throughout the experiment. These conditions were chosen in order to accelerate the oxidation process (Carneiro et al., 2013). Aliquots were taken at 0, 1, 2, and 4 weeks of storage, and limonene and limonene oxides were quantified by gas chromatography as described by Anantharamkrishnan and Reineccius (2018), using a 5890 A chromatograph Hewlett Packard equipment (Santa Clara, USA). The separation was performed in a HP-5 column (30 m × 0.250 mm x 0.25 μm) J & W Scientific (Agilent Technologies, Santa Clara, USA). The chromatographic conditions were injector at 200 °C; flame ionization detector (FID) at 250 °C; an initial temperature of 40 °C; program rate of 7 °C/min to 150 °C; and 15 °C/min to 250 °C. The total oil was determined as described in section 2.4.1. The OEO retention was expressed as the percentage of retained oil (%, w/w), and the oxidative stability of limonene was reported as mg of limonene oxide formed per g of limonene.

2.6. Statistical analysis

The *t*-test for the difference between means was performed for treatments at a significance level of 95%. The software Minitab® 18.1 (Minitab Inc., State College, PA, USA) was used for data analysis.

3. Results and discussion

3.1. Microparticles characterization

The use of stable emulsions is essential to produce spray-dried microparticles with high oil encapsulation efficiency. Nevertheless, other properties such as emulsion viscosity and operational conditions such as inlet and outlet temperatures and emulsion feed rate can impact the encapsulation of volatile oils (Reineccius, 2004). In our previous work

(Francisco et al., 2020), we evaluated the kinetic stability of emulsions containing OEO stabilized by different concentrations of PPC, and we verified that 2.4 wt% of PPC (g of PPC/g of emulsion) was the minimal concentration required to keep the emulsion stable throughout the spray-drying process. Higher concentrations of PPC were tested; however, we have not verified relevant improvement in the stability of the emulsions. We also observed that the increase of PPC concentration up to 4.8 wt% (g of PPC/g of emulsion) resulted in microparticles with a similar OEO retention. Considering the use of these microparticles as an ingredient in the food industry, concentrations of PPC higher than 4.8 wt % cannot be economically justified because, compared to maltodextrin, PPC is more expensive, which could substantially increase the cost of these microparticles as an ingredient. Once we had two formulations with similar initial OEO content but considerably different PPC concentrations, we selected those systems to be studied in the present work to verify if using a higher concentration of PPC could result in the modulation of the OEO oxidative stability. A control without PPC was not produced since this system would not represent an efficient encapsulation system as maltodextrins do not have surface activity and have been unable to produce stable oil-in-water emulsions by themselves (Ré, 1998). The encapsulation efficiency values of orange essential oil spray-dried microparticles are presented in Table 2. Both samples showed encapsulation efficiency values around 99 wt%. These high encapsulation efficiencies are related to a low amount of oil on the surface of the microparticles. The small amount of oil on the surface indicates that the emulsions were stable throughout the drying process, which resulted in small oil loss (Reineccius, 2004). The OEO mainly was entrapped inside the microparticles, once only about 10–15 wt% of the total OEO added initially were lost during the atomization process (Francisco et al., 2020); thus, the oil will be less vulnerable to oxidation since the wall is a physical barrier that limits the oil volatilization and the access of oxygen to the oil. A similar value of encapsulation efficiency (98.9 wt%) was found by de Melo Ramos et al. (2019) on the microencapsulation of orange essential oil using maltodextrin and modified starch by emulsification followed by spray-drying.

Scanning Electron Microscopy (SEM) micrographs of the microparticles are shown in Fig. 1. The particles presented an irregular shape and a great extent of surface dentations, typical of spray-dried material containing maltodextrin. These dentations are related to fast water loss and shrinkage at the initial drying stage (Ré, 1998). Apart from that, the microparticles presented a wall without cracks or apparent pores, which potentially promotes the preservation of the orange essential oil by limiting the oxygen entrance and the release of the volatile compounds. The increase in protein concentration did not seem to have changed the structural characteristics of the microparticles.

The PPC2.4 and PPC4.8 samples presented similar glass transition temperatures (T_g) around 66,4 °C (Table 2). Since the composition of the wall material (maltodextrin and PPC) consisted mainly of maltodextrin (90 and 80 wt% of maltodextrin in PPC2.4 and PPC4.8, respectively), it seemed reasonable that the increase of PPC concentration in the formulations would not affect the T_g of the microparticles and the T_g of the microparticles would reflect the T_g of pure maltodextrin. Roos and Karel (1991) found a T_g of 84 °C for maltodextrin with a DE of 10 at a 23% relative humidity. Carolina et al. (2007) found a T_g value of 50 °C for spray-dried orange flavor produced with maltodextrin (DE of 12) at a 33% relative humidity. The microparticles must be kept at a lower temperature than the T_g to limit the molecular mobility of the amorphous structure and the diffusion of the low molecular weight compounds from the OEO through the dried matrix (Reineccius and Yan, 2016). Therefore, at a water activity of about 0.3, the OEO spray-dried microparticles must be kept at temperatures lower than 66 °C, a relatively high temperature, to maintain their glassy state, contributing to the OEO stability. In addition, the fact that there was no difference in T_g between the samples, we probably can assume that the vitreous state of both samples is similar, and the molecular mobility of volatile compounds retained in the dried matrices would also be equivalent.

Table 2
Encapsulation efficiency (EE) and glass transition temperature (T_g) of the orange essential oil microparticles containing 2.4 (PPC2.4) and 4.8 wt% (PPC4.8) of pea protein concentrate (PPC) (g of PPC/100 g of emulsion).

Physicochemical properties	PPC2.4	PPC4.8
EE (%)	98.83 ± 0.53	99.23 ± 0.58
T_g (°C)	66.7	66.1

Values are expressed as mean ± standard deviation.

3.2. Evaluation of the barrier properties of the microparticles

3.2.1. Porosity features

The barrier properties of the microparticles were evaluated by assessing their porosity using different approaches: at first, by the calculus of the particle porosity using the Eq. (2), secondly by nitrogen adsorption, and finally by PALS. The porosity calculated indirectly using the apparent and true densities is commonly used to express the porosity of spray-dried microparticles. Although they can give some insights into the particle porosity, there are other techniques with higher accuracy to identify minor differences between microparticle samples and quantify the porosity of the whole powder or a single particle. The nitrogen adsorption technique quantifies the porosity of the entire powder through the determination of the specific surface area. PALS is a technique that measures the free volume between the polymer chains of a single microparticle on a molecular scale and gives more reliable information about the free path to oxygen entry and volatiles release through the microparticles wall (Reineccius and Yan, 2016).

The results of the porosity features of the microparticles assessed by all three methods are presented in Table 3. PPC2.4 showed a significant ($p < 0.05$) lower value of particle porosity than PPC4.8. This result could indicate that the higher amount of PPC on the PPC4.8 sample probably affected the porosity of the microparticles on a microscale, probably due to the formation of a less compacted structure. The PPC presents a high insoluble fraction (~80 wt%) that may not be packed at the dried matrix, and it been present in a higher amount in the PPC4.8 sample, it could produce a more porous matrix. The particle porosity quantifies the air occluded in the microparticles wall, which is closer to the oil droplets dispersed in the dried matrix; therefore, low values of particles porosity are preferable.

The nitrogen adsorption measurements are based on the permeation of a small probe (N_2) into the microparticles structure. It directly gives the extent of the surface area of the microparticles being a more precise technique to trace a correlation between the porosity of the microparticles and the oxidative stability of the encapsulated oil. We expected this technique to show some difference between the porosities of the microparticles samples due to the increase of PPC content; however, the porosity features of the unloaded microparticles were similar (Table 3). The S_{BET} values of both samples were around 0.8 m^2/g, indicating that the increase of the PPC concentration has not affected the porosity of the dried matrix when assessed by this technique. In both formulations, the amount of protein used was small compared to the amount of maltodextrin; thus, this result seems reasonable, indicating that the two microparticles samples (PPC2.4 and PPC4.8) probably would present the same resistance to oxygen entry.

PALS technique gives information about the average microscopic size of free volume entities of the spray-dried matrices (Drusch et al., 2009). According to Reineccius and Yan (2016), the PALS technique has an advantage compared to other experimental probe methods like nitrogen adsorption since PALS can measure hole sizes in the range of oxygen, e.g., around 0.1 nm. Based on this, we expected that PALS could show any difference in porosity between the PPC2.4 and PPC4.8 microparticles. The evaluation of the free volume of the PPC2.4 and PPC4.8 samples concerning o-Ps lifetime ($\tau_{o\text{-}Ps}$) and the average pore diameter ($\sigma_{o\text{-}Ps}$) are shown in Table 3. There was a small difference

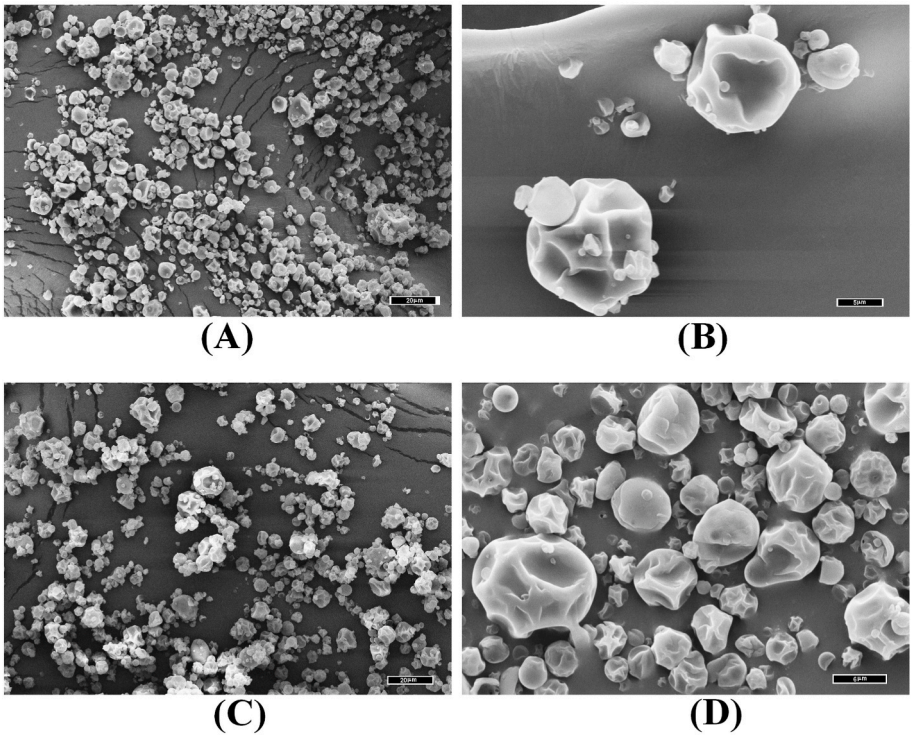

Fig. 1. Scanning electron microscopy of the orange essential oil microparticles containing 2.4 (PPC2.4) and 4.8 wt% (PPC4.8) of pea protein concentrate (PPC) (g of PPC/100 g of emulsion). A (magnification of 1500× and scale bar = 20 μm) and B (magnification of 6,000× and scale bar = 5 μm): PPC2.4; C (magnification of 1500× and scale bar = 20 μm) and D (magnification of 6000× and scale bar = 6 μm): PPC4.8.

Table 3
Physical properties of the orange essential oil microparticles containing 2.4 (PPC2.4) and 4.8 wt% (PPC4.8) of pea protein concentrate (PPC) (g of PPC/100 g of emulsion) assessed by the calculus of porosity, nitrogen adsorption, and positron annihilation lifetime spectroscopy (PALS).

Technique	Physical properties	PPC2.4	PPC4.8
Calculated porosity	Particle porosity (%)	$8.04^{b} \pm 0.01$	$15.14^{a} \pm 2.24$
Nitrogen adsorption	S_{BET} (m^2/g)	0.83 ± 0.02	0.78 ± 0.03
PALS	$\tau_{o\text{-}Ps}$ (ns)	1.41	1.39
	$\sigma_{o\text{-}Ps}$ (nm)	0.22	0.23

S_{BET}: specific surface area; $\tau_{o\text{-}Ps}$: ortho-positrons lifetime; $\sigma_{o\text{-}Ps}$: average pore diameter. Values are expressed as mean ± standard deviation. In each column, different letters correspond to values statistically different ($p < 0.05$).

between the $\tau_{o\text{-}Ps}$ and the $\sigma_{o\text{-}Ps}$ of the two microparticles samples, indicating that, in the range studied, the use of a higher concentration of PPC probably has not changed the free volume of the matrices. We also compared those values of $\tau_{o\text{-}Ps}$ and $\sigma_{o\text{-}Ps}$ of the microparticles containing PPC to the respective values we obtained for similar microparticles formulations containing whey protein isolate or gelatin we produced, which were quite similar (Paulo et al., 2021). Similar values were obtained for microparticles containing pectin and glucose syrup in a 1:40 ratio ($\tau_{o\text{-}Ps} = 1.36$ ns) (Serfert et al., 2013a), maltodextrin with DE of 18 ($\tau_{o\text{-}Ps} = 1.33$ ns) (Drusch et al., 2009), and a mixture of chitosan and glucose syrup 1:40 ($\tau_{o\text{-}Ps} = 1.38$ ns) (Serfert et al., 2013b). Studies have shown that the increase of protein concentration in spray-dried matrices resulted in increased free volume due to the large volume of the protein molecule and its structure, making its packing difficult. Drusch et al. (2012) observed that by increasing the concentration of sodium caseinate from 1.25% to 5% in a mixture with glucose syrup (DE of 38), the value of $\tau_{o\text{-}Ps}$ increased from 1.33 to 1.47 ns. This effect of protein concentration in the polymeric matrices was not verified in our study, probably due to the differences between the animal and plant protein sizes and the difference between the DE of glucose syrup and the DE of the maltodextrin we used. Probably, the difference between the molecular weights of maltodextrin with a DE of 10 and pea protein main fractions (vicilin, convicilin, and legumin) is smaller than the difference between the molecular weights of sodium caseinate and glucose syrup (DE of 38) (Koyoro and Powers, 1987). Based on this, the addition of pea protein to the maltodextrin matrix may not have affected its free volume on the same scale that sodium caseinate would affect the free volume of a glucose syrup matrix. It is also likely that the increase in pea protein concentration we tested was not high enough to promote some change in the microparticles matrix. Overall, based on all the three approaches we used to characterize the porosity features of the two microparticles samples, the increase of PPC amount seems to have not affected the porosity of the microparticles; thus, it was expected that both PPC2.4 and PPC4.8 microparticles would present a similar resistance to oxygen and OEO diffusion through the microparticles wall.

3.2.2. Orange essential oil retention and limonene oxidation in the spray-dried microparticles by gas chromatography (GC)

The oxidative stability of the orange essential oil encapsulated in the spray-dried microparticles was evaluated over four weeks of storage by quantifying orange essential oil retention and limonene oxidation using gas chromatography. The obtained results were used to correlate the porosity features of the microparticles and the oxidative stability of the encapsulated material. Fig. 2A shows the retention of OEO in the microparticles during four weeks of storage at 45 °C and water activity of 0.33. The two microparticles formulation generally showed similar retention profiles and high oil retention at day 0, with values around 76 and 81 wt% for PPC2.4 and PPC4.8, respectively. After a week, the microparticles preserved a high amount of OEO since the retention values slightly decreased to 70 and 74 wt% for PPC2.4 and PPC4.8, respectively. In the following week, there was a significant decrease in OEO retention in both samples to about 53 wt%. After two more weeks (fourth week), the amount of OEO in the microparticles was maintained with no significant difference between the second and fourth weeks. At

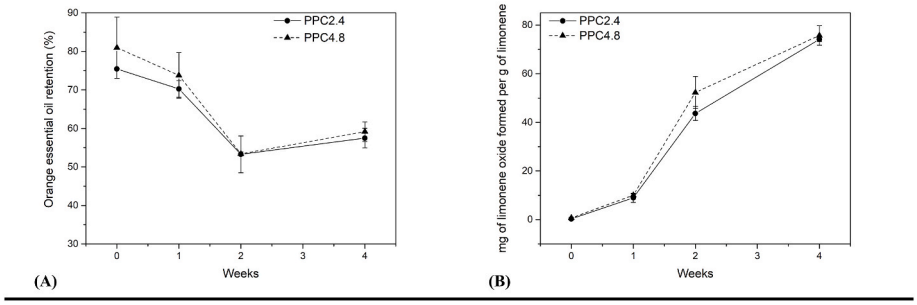

Fig. 2. Profiles of orange essential oil retention (A) and limonene oxide production (B) in the orange essential oil microparticles containing 2.4 (PPC2.4) and 4.8 wt% (PPC4.8) of pea protein concentrate (PPC) (g of PPC/100 g of emulsion) during four weeks of storage at 45 °C and water activity of 0.33.

the end of the test, both samples preserved about 58 wt% of the oil initially added. These retention profiles probably result from the polydispersity of the emulsions oil droplets (Francisco et al., 2020) that could have resulted in a non-constant oil release rate from the microparticles over time. In the current study, the microparticles produced with PPC showed an inferior ability to retain the OEO compared to the microparticles produced by Soottitantawat et al. (2005) using mixtures of Arabic gum and maltodextrin or soluble soy polysaccharide and maltodextrin, that retained about 90% of the limonene added to the formulation, after 30 days of storage at 23% of relative humidity.

Fig. 2B shows the production of limonene oxide in the microparticles during the four weeks of storage. Overall, the samples presented similar profiles. At the beginning of the study (day 0), both PPC2.4 and PPC4.8 have not presented oxidation products, indicating that the drying process did not cause limonene degradation. After one week of storage, the samples showed a small production of limonene oxide, about 10 mg of limonene oxide/g of limonene, in both formulations. During the second week of storage, there was a considerable increase in limonene oxidation, showing that besides the release of limonene, as seen in the limonene retention profile (Fig. 2A), part of the limonene retained in the microparticles was partially degraded (43 and 52 mg of limonene oxide/g of limonene for PPC2.4 and PPC4.8, respectively). Limonene oxidation took place at a slower rate during the two following weeks of storage; in the fourth week, the two samples presented about 76 mg of limonene oxide/g of limonene. The amount of limonene oxide produced in our microparticles after four weeks of storage was 76 mg of limonene oxide/g of limonene, which is higher than those observed by Soottitantawat et al. (2004) (12 mg of limonene oxide/g of limonene produced in Arabic gum and maltodextrin (20 DE) spray-dried microparticles after 30 days of storage at a relative humidity of 23% and 50 °C) and Finney et al. (2002) (5 mg of 1,2-epoxy/g of limonene produced in modified starch spray-dried microparticles after 28 days of storage at a water activity of 0.11 and 37 °C).

Compared to those studies mentioned above, the lower protection performance of the microparticles produced using PPC and maltodextrin is probably related to the higher relative humidity and the lower degree of hydrolysis of the maltodextrin we used. It is well known that the storage of spray-dried microparticles at higher relative humidity leads to a lower T_g and higher volatiles release and oxygen permeation. Additionally, according to Ré (1998), high molecular weight material results in less packed dried matrices, which allows a higher oxygen permeation into the microparticles, as seen in the study performed by Anandaraman and Reineccius (1986), where the maltodextrin with a DE of 10 offered poorer protection to the limonene encapsulated compared to the maltodextrins with a higher DE. Moreover, the low adsorption of PPC at the oil-water interface of the emulsions, as verified in our previous work (Francisco et al., 2020), may also have affected the oxidative stability of the OEO since there was not a thick protein layer around the oil droplets that could reduce the contact between oxygen and the OEO. These results are consistent with what was verified regarding the T_g and porosity features of the two microparticles samples, assessed by PALS and nitrogen adsorption techniques. Since the increase of PPC concentration was not enough to promote changes in the barrier properties of the microparticles as there was no difference in the T_g, size of pores, and the specific surface area of the samples, both matrices had similar barrier properties and capacity of retaining and protecting the OEO encapsulated. Drusch et al. (2009) also observed that two carbohydrate-based microparticles samples with values of $\tau_{o\text{-}Ps}$ of 1.15 and 1.17 (a difference of 0.02 units as seen in our samples), respectively, did not show a statistically significant difference production of oxidation products in the fish oil encapsulated.

We also evaluated the orange essential oil retention and the limonene oxidation in the microparticles containing whey protein isolate or gelatin as stabilizers that we mentioned before. Surprisingly those microparticles presented almost half of the limonene retention and more than the double amount of the limonene oxides compared to the microparticles containing PPC after four weeks of storage (data not shown). Since the porosity features of all those microparticles were similar when assessed by PALS (section 3.2.1), this higher protection of limonene performed by PPC compared to those animal proteins could be related to a superior antioxidant activity of PPC (Paulo et al., 2021).

Based on these results, we conclude that, unlike the previously mentioned studies that used other kinds of proteins, the increase of pea protein concentration at the range we tested did not affect the stability of the encapsulated bioactive compound. In summary, we verified that a lower amount of PPC could provide similar protection to OEO during storage, showing that a relatively cheaper orange flavor powder can be produced using less protein.

4. Conclusion

The effects of using different amounts of pea protein concentrate (PPC) on the physical properties, porosity features, and oxidative stability of spray-dried maltodextrin-based microparticles containing orange essential oil (rich in limonene) were evaluated in the current study. Both microparticles samples presented high encapsulation efficiency (>99 wt%), relatively high glass transition temperature, and morphology without pores and cracks. The increase of protein concentration from 2.4 to 4.8 wt% (g of PPC/g of emulsion) in the microparticles formulation did not affect the overall porosity features of the spray-dried matrices; the nitrogen adsorption and the positron annihilation lifetime spectroscopy results showed that microparticles samples have similar specific superficial area and molecular free volumes. The oxidative stability study confirmed these results since PPC2.4 and PPC4.8 samples presented similar orange essential retention and limonene oxide production profiles, ensuring that these samples had similar free paths for orange essential oil release and oxygen entry. Based on these observations, we conclude that, in our work, using a lower PPC amount (2.4 wt%), we were able to effectively encapsulate OEO and produce an orange flavor powder with the same oxidative stability as a formulation containing twice as much protein (4.8 wt%), which is a great result that contributes to the development of a cheaper food

ingredient. Overall, the encapsulation of OEO using PPC as a stabilizer reduced limonene loss, not to a great extent, as other encapsulation systems reported in the literature. Still, PPC showed better performance in protecting limonene from oxidation than two animal proteins commonly used in the food industry. These results contribute to the advancement in the use of vegan/vegetarian carrier systems used to protect and deliver flavors and help meet the food industry's demand for plant-based ingredients.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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