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Whey protein isolate and prebiotic biopolymers to stabilize pink-pepper oleoresin microcapsules

Abstract – The objective of this work was to evaluate the use of whey protein isolate (WPI) associated with the prebiotic biopolymers inulin (IN) and oligofructose (OL) as a wall material on the physicochemical, morphological and thermal stability of pink-pepper oleoresin microcapsules. For spray drying in the treatments (WPI, WPI/IN, and WPI/OL), ultrasound-assisted emulsions were used, being prepared with 30% (w/w) wall material, 5% (w/w) oleoresin, and a 5:1 (w/w) replacement ratio for the WPI/IN and WPI/OL treatments. WPI increased the size of the oil droplets and of the reconstituted powder, which provided a greater encapsulation efficiency. The WPI and WPI/OL treatments showed a better antioxidant capacity, whereas WPI/IN and WPI/OL provided a better powder solubility. The microcapsules showed a spherical structure with some roughness, but no rupture, which favored the retention of oleoresin. Moreover, the microcapsule walls had no defined crystalline region. The addition of prebiotic biopolymers decreased the temperature at which the wall material started to degrade, decreasing its thermal stability. The use of WPI associated with inulin and oligofructose is suitable for the production of emulsions for the spray-drying microencapsulation of pink-pepper oleoresin.

Index terms: *Schinus molle*, adsorption isotherms, antioxidant capacity, biopolymers, prebiotic fibers, ultrasonication.

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Proteína de soro de leite e biopolímeros prebióticos para estabilizar microcápsulas de oleoresina de pimenta-rosa

Resumo – O objetivo deste trabalho foi avaliar o efeito da proteína isolada de soro de leite (PIS) associada aos biopolímeros prebióticos inulina (IN) e oligofrutose (OL) como material de parede na estabilidade físico-química, morfológica e térmica de microcápsulas de oleoresina de pimenta-rosa. Para a atomização nos tratamentos (PSI, PSI/IN e PSI/OL), foram utilizadas emulsões assistidas por ultrassom, preparadas com 30% (p/p) de material de parede, 5% (p/p) de oleoresina e proporção de substituição de 5:1 (p/p) para os tratamentos PSI/IN e PSI/OL. A PSI aumentou o tamanho das gotas de óleo e do pó reconstituído, o que proporcionou maior eficiência de encapsulação. Os tratamentos PSI e PSI/OL apresentaram maior capacidade antioxidante, enquanto PSI/IN e PSI/OL proporcionaram maior solubilidade do pó. As microcápsulas apresentaram estrutura esférica com alguma rugosidade, mas sem ruptura, o que favoreceu a retenção da oleoresina. Além disso, as paredes das microcápsulas não apresentaram nenhuma região cristalina definida. A adição de biopolímeros prebióticos reduziu a temperatura de início de degradação do material de parede, o que diminuiu sua estabilidade térmica. O uso de PIS associado à inulina e à oligofrutose é adequado para a produção de emulsões para a microencapsulação por atomização de oleoresina de pimenta-rosa.

Termos de indexação: *Schinus molle*, isotermas de adsorção, capacidade antioxidante, biopolímeros, fibras prebióticas, ultrassonicação.

Introduction

The microencapsulation process consists of imprisoning a product within a polymeric structure to protect and preserve the chemical, biological and sensory characteristics of food (Santos et al., 2006). Spray drying is the most widely used technique for the encapsulation of bioactive compounds, and has vast scientific dissemination. Oils are commonly studied in encapsulation processes due to their susceptibility to oxidative, thermal and luminous degradation, since microencapsulation promotes the retardation of these undesirable reactions (Campelo et al., 2017; Ferreira et al., 2019).

Numerous wall materials are studied as encapsulating material for the microencapsulation of oils. Whey protein isolate (WPI) is among the most used ones; it has great potential for emulsification and stabilization, due to its absorption in the oil–water interface, which favors the binding, entrapping or coating of the core material. Thus, a crust that provides thermal protection during the drying and storage processes is formed (Calva-Estrada et al., 2019; Alves et al., 2017).

Consumer markets have been increasingly searching for healthier foods containing functional ingredients, such as natural bioactive compounds, that may somehow improve the functioning of the body and provide health benefits (Zhao et al., 2019). The addition of prebiotics as wall material in microencapsulation processes may be a good alternative. Inulin is a natural fructan that consists of a major chain of fructose units linked by β (2 → 1) glycosidic bonds and contains a terminal β-D- fructose or D-glucose. Its degree of polymerization (DP) ranges from 10 to 60 unit. The lengths of its molecular chains affect certain properties, are associated with its technological bonds, and may be used as a texture agent, because they increase the viscosity of the medium. This biopolymer and their derivatives, such as oligofructose, are also considered prebiotic nondigestible carbohydrates, and have a functional effect of stimulating the intestinal tract (Alvarez et al., 2013).

Ingredients like inulin are widely used as fat, sugar, thickener, or gelling agent replacements in the food industry (Mishra & Mishra, 2018). Some studies have reported good results on the retention of chemical and biological properties of the encapsulated material when using inulin in the microencapsulation process of bioactive compounds (Beirão-da-Costa et al., 2013; Fernandes et al., 2014; Silva et al., 2016).

The *Schinus molle* L. tree, also known in Brazil as Aroeira, is widely distributed in the Brazilian territory, and mostly found in Atlantic Forest regions. Its fruit (pink pepper) has important biological activity, such as antioxidant, antimicrobial, anti-inflammatory and antitumor potential, due to the polyphenols found in its plant extract, such as apigenin, ellagic acid, and naringin and ursolic acid (Wang et al., 2021). Extracts of *Schinus molle* ripe fruit also contain the main monoterpenes α-phellandrene, β-phellandrene, α-terpeneol, α-pinene and β-pinene. Due to its composition and sensorial characteristics, such as taste and aroma, pink-pepper oleoresin is a good alternative for the food industry (Dannenberg et al., 2016).

Considering the antioxidant potential and numerous therapeutic applications associated with compounds found in pink-pepper oleoresin (Andrade et al., 2017), adding value to and preserving this natural product becomes necessary. However, only studies about microencapsulation using solvent extraction technique in the encapsulation of pink-pepper extracts (Andrade et al., 2017) have been found in the literature so far.

The objective of this work was to evaluate the effect of whey protein isolate combined with the prebiotic biopolymers inulin and oligofructose as wall material on the physicochemical and morphological properties and thermal stability of pink-pepper oleoresin microcapsules.

Material and Methods

All experiments and analyses were performed at Universidade Federal de Lavras, Department of Food Science, located in the municipality of Lavras, in the state of Minas Gerais, Brazil. The pink-pepper (*S. molle*) oleoresin used as core material and the whey protein isolate (WPI) were obtained in specialized markets, as were inulin (IN) Frutafit DP = 2-60 and oligofructose (OL) Frutalose DP = 2-10 (Sensus Ingredients, Netherland). The experiments were performed in a completely randomized design and comprised three treatments and three replicates, in a total of nine experimental units. The proportion of solids (wall material) used in the feed solution was 30% (w/w) for all treatments; the oleoresin load employed was 5% (w/w), based on the final solution volume. Each

treatment was prepared using WPI and its combination with prebiotic biopolymers inulin or oligofructose (WPI/IN and WPI/OL) at a 5:1 (w/w) ratio (Table 1). The effects of the three wall material formulations were evaluated in terms of the characteristics of the microencapsulated pink-pepper oleoresin powders.

The biopolymers were dissolved in distilled water, and the solutions were prepared 24 h before being emulsified at room temperature, to ensure full saturation of the molecules. Pink-pepper oleoresin was then added to the biopolymer solution and subjected to ultrasonic homogenization at 240 W (Digital Sonifer Branson, S-450D, Branson Ultrasonics Corporation, Danbury, Connecticut, USA) and 20 kHz for 2 min, to ensure complete emulsification of the pink-pepper oleoresin. The contact between ultrasonic probe and emulsions was standardized at 30 mm. The emulsion was then used as feed liquid for the spray-drying process (Fernandes et al., 2014; Silva & Meireles, 2015).

Emulsion droplet size was measured using dynamic light scattering (Zetasizer Nano ZS, Malvern Instruments, Malvern, U.K.) immediately after the homogenization process and the reconstitution of powder particles using 1.5 mL emulsion samples diluted 1,000× with purified water (Milli-Q, Merck, Germany) to avoid multiple light scattering effects. The droplet size data are reported as Z-average mean diameter at 25°C.

The emulsions were dried using a spray-dryer (model MSD 1.0; Labmaq do Brasil, Ribeirão Preto, Brazil) equipped with a two-fluid nozzle atomizer (nozzle diameter: 3 mm; drying chamber dimensions: 670 × 200 mm). The following operational conditions were used, as described in previous studies: 170°C inlet temperature and 0.9 L h^{-1} feed rate, 40 L min^{-1} airflow and 400 kPa air pressure. The microcapsules obtained were sealed in aluminum packing and stored under refrigeration (4–7°C), protected from temperature changes, light penetration and gas permeation until further analysis (Fernandes et al., 2014).

Twenty milliliters of petroleum ether were added to 1 g of powder in a sealed glass bottle. To extract the surface oleoresin (SO), the mixtures were shaken for 2 min at room temperature using a vortex shaker. The solvent mixture was then passed through a no. 1 paper filter (Whatman GmbH, Germany). The powder trapped in the filter was washed three times using 20 mL of petroleum ether. The solvent was evaporated at 60°C until constant weight. The percentage of surface oleoresin in the microcapsules was calculated based on the ratio between the extracted oleoresin and the initial mass of the microcapsules (Silva et al., 2016). Total oleoresin (TO) was considered equal to the initial oleoresin rate, since preliminary tests revealed that all the initial oleoresin was retained, which was expected, since pink-pepper oleoresin is not volatile. Encapsulation efficiency (EE) is defined as the ratio between the oleoresin content inside the microcapsules and the total oleoresin in the microcapsules (Tonon et al., 2012) as in Equation 1:

$$EE(\%) = [(TO - SO) / TO] \times 100 \quad (1)$$

Antioxidant activity (AA) was evaluated using the DPPH radical (1,1-diphenyl-2-picrylhydrazyl) (Fu et al., 2015). To extract oleoresin, 0.1 g of microcapsules was added to 50 mL of ethanol solution (1:3 v/v). The mixture was homogenized using an ultrasonic homogenizer (Branson, USA) for 1 min at 250 W. The same procedures used to homogenize the microcapsules were used for the pure oleoresin (0.01 mL). Then, 2.4 mL of absolute ethanol, 1 mL of the DPPH solution (2 mg 50^{-1} mL) and 0.1 mL of the sample after extraction were added to the tubes. To correct possible contributions of staining samples, a blank test sample volume (0.1 mL) and 3.4 mL of absolute ethanol were used in parallel. The control

Table 1. Composition of the wall materials for each treatment used as a feed solution in the spray-drying process for the microencapsulation of pink-pepper oleoresin (*Schinus molle* L.).

Treatment	Wall material (g $100g^{-1}$ of solution)			Core material (g $100g^{-1}$ of solution)
	Whey protein isolate (WPI)	Inulin (IN)	Oligofructose (OL)	Pink-pepper oleoresin
WPI	25	-	-	5
WPI/IN	20	5	-	5
WPI/OL	20	-	5	5

treatment was prepared by mixing 1.0 mL of the DPPH solution with 2.5 mL of absolute ethanol. After 1 h of incubation in the dark at room temperature, the absorbances at 517 nm were recorded. Tests were performed in triplicate and the inhibition of free radical DPPH was calculated using Equation 2:

$$AA(\%) = 100 - [(A_A - A_B / A_C) \times 100] \quad (2)$$

in which A_A is sample absorbance, A_B is blank absorbance, and A_C is control absorbance.

Particle morphology was assessed using a scanning electron microscope (SEM). The particles were immobilized on double-sided adhesive tape and mounted on 1-cm diameter and 1-cm height microscope stubs. The microcapsules were covered with gold in a vacuum chamber and examined using a SEM (1430 VP-SEM LEO Electron Microscopy Ltd. Cambrige, UK) operated at 20 kV using magnifications from 900 to 1200×.

The adsorption isotherms for the microcapsules were determined by applying the static method using seven saturated salt solutions (LiCl, MgCl, K_2CO_3, $NaNO_3$, $Mg(NO_3)_2$, NaCl, and KCl) at 25°C; water activity ranged from 0.113 to 0.843. Moisture sorption isotherm data were correlated to water activity data (relative humidity) using the following mathematical models: Guggenheim, Anderson, and de Boer (GAB – Equation 3), Halsey (Equation 4), Smith (Equation 5), and Oswin (Equation 6).

$$X_{eq} = X_0 CKA_w / (1 - KA_w)(1 - KA_w + CKA_w) \quad (3)$$

$$X_{eq} = \left[a / \ln(A_w)\right]^{\frac{1}{b}} \quad (4)$$

$$X_{eq} = a + b\log(1 - A_w) \quad (5)$$

$$X_{eq} = a\left[Aw / (1 - Aw)\right]^b \quad (6)$$

The parameters were estimated by correlating the mathematical models to the experimental data via a quasi-Newton nonlinear regression, at a 5% significance level. The model considered most suitable was the one featuring the lowest relative mean error (E%) (Equation 7):

$$E = 100 / N \sum_{i=1}^{N} \left| m_i - m_{pi} \right| / m_i \quad (7)$$

in which X_{eq} is the equilibrium moisture content (g g^{-1} dry powder); X_0 is the monolayer moisture content (g g^{-1} dry powder); C and K are the model constants related to monolayer and monolayer properties; Aw is the water activity; a, b are the parameters for the empirical models, in which the temperature dependence was assumed to be linear; E is the mean relative deviation modulus (%); m_i is the experimental value; m_{pi} is the predicted value; and N is the experimental data population.

The thermal stability of the microcapsules was evaluated by thermogravimetric analysis using a Thermogravimetry-Differential Thermal Analysis (TG-DTA) analyzer (H Shimadzu 60, Shimadzu Corporation, Japan). Analyses were performed using synthetic air to reproduce the real conditions in the food industry. The synthetic air flow was of 10 mL min^{-1}, heated from 25°C to 600°C at 10°C min^{-1}.

Analyses of variance were carried out using the R package software to verify the effects of the wall materials on the characteristics of the pink-pepper oleoresin microcapsules. Differences in the mean values obtained were examined by Duncan's test, at 5% probability. Rheology, thermal analysis, and X-ray powder diffraction (XRD) were conducted using the XRD-6000 X-ray diffractometer (Shimadzu, Kyoto, Japan), and the obtained data were analyzed descriptively.

Results and Discussion

The droplet size values showed significant difference (p-value = 10^{-4}); the highest values were detected for WPI emulsions (Figure 1). Despite their high emulsifying capacity and the formation of small droplets in emulsions, the WPI proteins may have undergone a change in their structure during the ultrasonic homogenization process and formed larger droplets, as reported in other works (Kuhn & Cunha, 2012). The degree of inulin polymerization significantly influenced droplet size, since larger molecular chains tend to form solutions of higher viscosities and consequently larger droplets. Silva & Meireles (2015) observed an increase in droplet size for annatto seed oil along with increasing degrees of inulin polymerization. At low homogenization power (160 W), other authors observed droplet sizes from 2.09 to 7.1 μm in WPI emulsions (Silva et al., 2016).

An increase in the size of reconstituted microcapsules is noted when compared to the size of the oil droplets immediately after the homogenization process. Tang & Li (2013) observed a droplet size increase in reconstitution when compared to the droplet size after the homogenization process in soybean and linseed oil emulsions, respectively. In the reconstitution process, the droplets may undergo coalescence, and increase their average size. Also, their reconstitution occurred via homogenization using lower energetic density when compared to ultrasonic homogenization, which may also explain the results.

The microcapsules showed spherical shapes, and some particles exhibited roughness (Figure 2). Rough surfaces are commonly found in polymers, but are undesirable in powdered products (Lim & Siow, 2017). It is noteworthy that there is no fracture or rupture of the wall material in any of the treatments, which is interesting for microencapsulation processes, as it reduces the degradation or volatilization of the encapsulated material. Similar results were observed in studies of fish oil microcapsules using WPI and inulin (Botrel et al., 2014), and bifidobacteria using inulin and oligofructose (Fritzen-Freire et al., 2012).

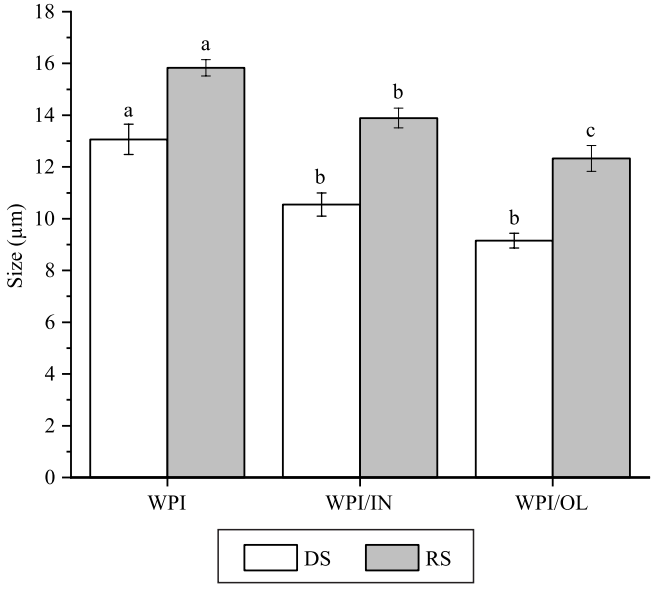

Figure 1. Droplet size of pink-pepper (*Schinus molle L.*) oleoresin particles. The same lowercase letters above the bars indicate no difference between means by the Duncan's test ($p<0.05$) for each analyzed parameter. DS, Droplet size after homogenization; RS, Reconstitution droplet size. WPI, Whey protein isolate; IN, Inulin; OL, Oligofructose.

Surface oleoresin content is often used as a parameter to predict biopolymer effectiveness in retaining oleoresin in microencapsulation processes. The addition of prebiotic biopolymers significantly

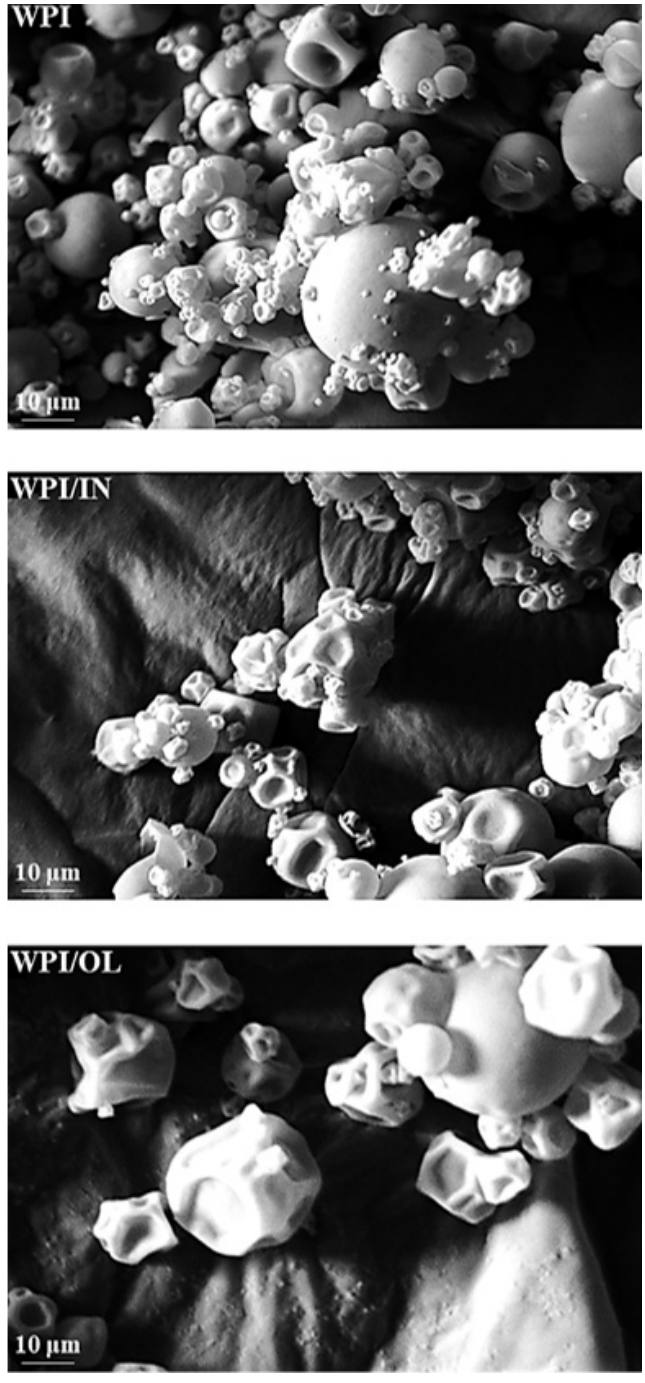

Figure 2. Microscopy images of pink-pepper (*Schinus molle L.*) oleoresin microcapsules. WPI, Whey protein isolate; IN, Inulin; OL, Oligofructose.

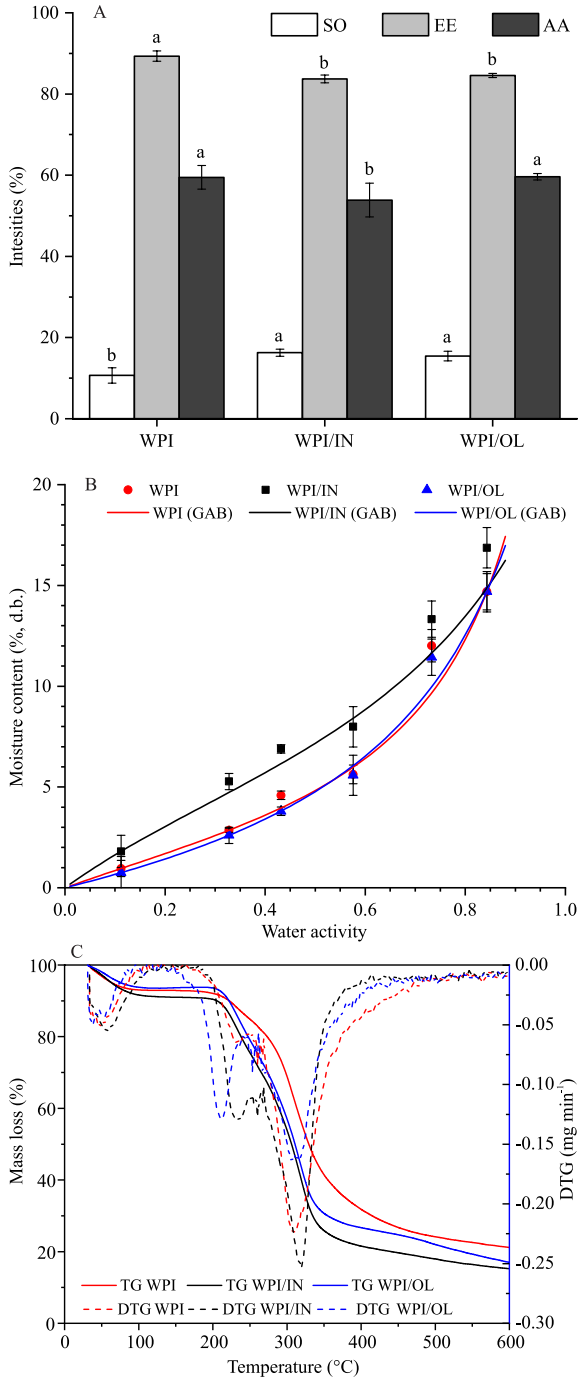

Figure 3. Physicochemical properties, sorption isotherms at 25°C, and thermal stabilities of pink-pepper (*S. molle*) oleoresin microcapsules. Bars followed by equal lowercase letters on top do not differ by the Duncan's test at 5% probability for each analyzed parameter. SO, surface oleoresin; EE, encapsulation efficiency; AA, antioxidant activity. GAB, Guggenheim, Anderson, and de Boer equation; WPI, whey protein isolate; IN, inulin; OL, oligofructose. TG, thermogravimetric analysis; DTG, derivative thermogravimetry curves.

increased the unencapsulated oil (surface oil) content of the pink-pepper oleoresin microcapsules (Figure 3). Increased surface oil values have been observed in other studies of WPI/MD microcapsules (Bae & Lee, 2008), and corroborate the result obtained in in this work, since maltodextrins and inulin show low emulsifying capacity and increase the unencapsulated oil content. Proteins are good wall materials for bioactive compound protection, since they form a rigid barrier during the drying process and do not allow volatilization through the pores nor the penetration of compounds that accelerate oxidation.

The encapsulation efficiency was significant for all treatments, and reached values greater than 80%. The treatments using prebiotic biopolymers showed lower encapsulation efficiency values when compared to the microcapsules containing only WPI. Inulin's low emulsifying capacity may be a factor for the reduced encapsulation efficiency of the pink-pepper essential oil microcapsules. Although the addition of prebiotic fibers decreased encapsulation efficiency, the values found are higher than those detected by Andrade et al. (2017) in studies on microencapsulation of pink-pepper extracts using solvent extraction technique (from 34% to 74%).

Antioxidant activity values were significantly different for pink-pepper oleoresin. The lowest value was detected in the WPI/IN treatment. The oleoresin's antioxidant activity did not differ significantly among the WPI and WPI/OL treatments. The low encapsulation efficiency value and the high oil content of the inulin treatment are related to the low antioxidant activity, since unencapsulated oil is more susceptible to degradation and volatilization. Antioxidant activity for oregano essential oil was lower under inulin when compared to other materials (Beirão-da-Costa et al., 2013). Proteins contained in WPI, such as β-lactoglobulin, have physicochemical mechanisms that eliminate free radicals, thus reducing the oxidation of microencapsulated oils (Waraho et al., 2011).

The GAB, Oswin, and Smith models produced good adjustments. The GAB model was chosen to represent the isotherms because, in addition to its lower average values for E (%), it featured an important parameter, the monolayer moisture content (X_0) (Table 2).

The moisture value of the monolayer may be defined as the critical moisture content at which water

is strongly bound to the molecular structure of a food. The addition of prebiotic biopolymers increased the moisture content of the monolayer in comparison to the treatment containing only protein. This may be because carbohydrates have a greater amount of hydrophilic functional groups which facilitate the connection with water molecules. X_0 values of 6.5% and 7.4% were found in inulin and WPI treatments of fish oil microencapsulation (Botrel et al., 2014). In comparison to WPI, inulin has a stronger tendency towards water absorption as the storage relative humidity increases. Water facilitates the binding among carbohydrate molecules, favoring agglomeration (Lim & Siow, 2017). Due to the large number of OH^- and H^+ radicals in carbohydrates, homogenization processes by ultrasound may result in the breakdown of their polysaccharide molecular structure and increase of their water absorption capacity (Tiwari et al., 2010).

In the water activity range up to 0.8 the powders containing inulin showed higher humidity values when compared with the other treatments. Due to inulin's higher polymerization degree compared to oligofructose, the number of active sites for water binding is higher, thus favoring water absorption. When studying the hygroscopic behavior of inulin at different polymerization degrees, Schaller-Povolny et al. (2000) observed a higher water absorption trend under higher polymerization degrees. By absorbing a lot of moisture, inulin's amorphous structure may become crystalline, due to the easy mobility of the biopolymer's molecules in the presence of water, thus favoring structural rearrangement (thermodynamically more stable) and increasing crystallinity (Saavedra-Leos et al., 2014). Inulin's high moisture uptake may be associated with increased surface oil content and reduced encapsulation efficiency when compared to WPI-only microcapsules.

Table 2. Sorption isotherm coefficient models for pink-pepper (*Schinus molle L.*) oleoresin microcapsules at 25°C.

Model	Coefficient	WPI	WPI/IN	WPI/OL
GAB	X_0	3.93	6.70	5.80
	C	2.47	3.69	1.28
	K	0.90	0.72	0.83
	E (%)	6.61	8.43	3.64
Halsey	a	1.21	2.27	0.95
	b	0.94	1.12	0.80
	E (%)	15.87	14.74	17.33
Smith	a	0.002	0.67	0.68
	b	18.48	21.98	1.20
	E (%)	6.88	6.74	10.24
Oswin	a	4.47	6.59	4.30
	b	0.79	0.63	0.84
	E (%)	8.02	8.33	7.50

WPI, whey protein isolate; IN, inulin; OL, oligofructose; X_0, monolayer moisture content (g g^{-1} dry powder); C and K, model constants related to monolayer and monolayer properties, respectively; a and b, parameters for the empirical models in which the temperature dependence was assumed to be linear; E (%), mean relative deviation modulus.

For all three treatments there are three mass stages; the DTG (Derivative Thermogravimetry) curves show the beginning and the end of these stages (Figure 3). Three degradation stages are often observed in food microencapsulation processes.

First, a degradation peak at 25 to 110°C is observed for all three treatments. Loss of moisture and volatile compounds from the microcapsules' surface is a characteristic of this stage (Silva et al., 2016). These results corroborate the higher moisture content and nonencapsulated oleoresin results observed for protein–prebiotic blends compared with the only-protein treatment.

The second stage corresponds to a mass loss caused by wall material degradation. At this stage, the average mass losses were of 58, 63, and 62%, and there was no difference among the treatments. The initial wall material degradation temperatures were 216, 205, and 208°C, and the maximum degradation temperatures were 310, 322, and 312°C for WPI, WPI/IN, and WPI/OL respectively. The addition of prebiotic biopolymers reduced the microcapsules' wall material degradation onset temperature, reducing its stability. As Leyva-Porras et al. (2017) observed a correlation between inulin polymerization degree and thermal stability, they concluded that the smaller the chain size, the lower the melt temperature and consequently the lower the resistance to temperature rise. These authors observed maximum degradation values of inulin at various polymerization degrees in the 200–210°C range, which may have influenced the reduction in degradation temperature for the microcapsules. The low thermal stability of inulins is due to the rupture of the fructose chains in their structure, which increases degradation (Fritzen-Freire et al., 2012). Maximum peak degradation values at 302°C were observed for pure WPI (Azevedo et al., 2015). Cinnamon essential oil microcapsules with

WPI were more resistant to degradation when compared to other carbohydrate blends (Felix et al., 2017). Silva & Meireles (2015) observed a 247°C –270°C degradation temperature range for inulins at different degrees of polymerization.

The third stage (T > 400°C) is related to the final degradation of the microcapsules. Thus, the presence of WPI and prebiotic biopolymers favored the formation of thermally resistant pink-pepper oleoresin microcapsules.

Conclusion

1. The use of whey protein isolate associated with the prebiotic biopolymers inulin and oligofructose is suitable for the production of emulsions for microencapsulation of pink-pepper oleoresin by spray-drying.

2. The whey protein isolate increases the size of the oil droplets and reconstituted powder, and provides greater encapsulation efficiency.

3.The whey protein isolate used alone or associated with oligofructose has better antioxidant capacity.

4. The addition of prebiotic biopolymers decreases pink-pepper oleoresin retention, increases water adsorption onto the microcapsule surface and improves powder solubility.

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