ENCAPSULATION OF OLEORESINS FOR SALT REDUCTION IN FOOD

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ABSTRACT

Background. In our study, oleoresins were explored for their ability to replace the original ground spice and herbs with a standardized taste and aroma, and mask the salt reduction. In order to protect taste and aroma oleoresins from high temperature, and to improve their solubility in food matrices, encapsulation with inulin and maltodextrin was carried out from two mixtures of oleoresins using two drying processes (spray and freeze drying), thus allowing it to be used as an additive to reduce salt for convenience in the food industry.
Materials and methods. The oleoresins experiment was conducted with two mixtures to apply to meat and fish. Oleoresins were obtained by solvent extraction, and the solvent was removed by evaporation, encapsulated into inulin and maltodextrin microcapsules, and powdered by spray and freeze drying. Physicochemical analyses were carried out using several methods (drying yields, water activity, solubility, hygroscopicity, color, encapsulation efficiency), and characterization of the microcapsules was done by scanning electron microscopy. The total phenolic compounds were quantified using the Folin-Ciocalteu method, and the chemical compounds present in the microcapsules were elucidated by high resolution mass spectrometry.
Results. Freeze and spray drying the microcapsules presented good quality products with high yields, high encapsulation efficiency and good solubility. The spray drying process can offer better applications for the food industry due to the more regular shape of the microcapsules. In addition, inulin microcapsules obtained by spray drying showed a more protective effect for flavonoid compounds in fish oleoresins, while maltodextrin microcapsules offered more protection for hydroxycinnamic acids in meat oleoresins.
Conclusions. The present study shows an attractive encapsulation system for non-volatile compounds from oleoresins, which results in standardized taste and aroma products that can reduce salt in food systems with different compositions.

Keywords: oleoresins, encapsulation, inulin, maltodextrin, morphology, spray and freeze drying, salt reduction

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INTRODUCTION

Excessive salt intake has a strong risk factor for cardiovascular diseases, with prevalence and a high impact around the world. The Member States of the World Health Organization agreed to reduce the world's salt consumption by 30% by 2025 (WHO, 2016). Therefore, the demand for products containing less salt is strong. In response, many food manufacturers aim to reduce salt in their products, and new applications have been developed (Yang et al., 2011). Among several taste-improving options are mineral salts; food acids, amino acids and salts; simple carbohydrates and sugar substitutes; food polymers; umami ingredients; spices, vegetables and flavors; miscellaneous taste improvers; and a plethora of their specific combinations. However, KCl-based salt substitute products have been reported to provide bitter, acrid, and metallic tastes (Cepanec et al., 2017). To avoid compromising on taste, other ingredients are used to mask the salt reduction. Oleoresins from aromatic plants and spices can offer this function.

Oleoresins are liquid or semisolid extracts obtained from plants by solvent extraction followed by removal of the solvent. Prepared oleoresins contain volatile and non-volatile (i.e. taste substances) flavoring components of spices as well as other non-volatile ingredients that include fixed oils, antioxidants, pigments, and other extractives soluble in the particular solvent used.

Moreover, oleoresins can replace the original ground spice and herbs with a standardized taste and aroma, providing easier quality control, a longer shelf-life, and no bacterial contamination (CBI, 2018). According to several studies (Hufnagel and Hofmann, 2008; Schmiech et al., 2008), a variety of the non-volatile oleoresin compounds strengthen sensory stimulation.

Salt reduction is another significant driver of the increase in the demand for oleoresins. However, in order to protect oleoresin flavorings from high temperatures, and to improve their solubility in food matrices, encapsulation processes are used (Pegg and Shahidi, 2007). Encapsulation has great importance and relevance in the food and flavor industries due its ability to protect sensitive food components against degradation reactions and loss of volatility. The encapsulation process allows the retention of sensitive compounds sealed inside another substance (carrier agents, coating material or capsules), and release of their content under specific and controlled conditions (Anandharamakrishnan and Ishwarya, 2015).

The appropriate encapsulating agent should be selected based on its physical-chemical properties (solubility, molecular mass, crystallinity, glass transition or melting temperature, and emulsifying properties etc.), in the compound to be encapsulated, in the industrial application, and in the selected encapsulation method (Gharsallaoui et al., 2007).

There are several commercially approved encapsulating agents that are used in encapsulation, such as natural gums (gum arabic, alginates, carrageenans), proteins (milk proteins, soy proteins, gelatin), polysaccharides (inulin, maltodextrins, and cellulose derivatives) and lipids (waxes and emulsifiers) (Corrêa-Filho et al., 2019; Mahdavi et al., 2014). Encapsulated flavorings are prepared from oleoresins mainly by spray drying with modified starch derivatives and/or gum acacia. Such products have an extended shelf life, and are more convenient to handle in dry applications.

Maltodextrin is composed of multiple glucose units linked by α: $1 \rightarrow 4$ glycosidic bonds (Gharsallaoui et al., 2007). The number of units of glucose is a variable that decides its equivalence to dextrose (DE) and plays an important role because high dextrose equivalence is needed to make the particle peel spray dry less permeable to oxygen.

It is highly soluble in water and exhibits low viscosity even with concentrated solutions. This makes it possible to increase the solids content of the emulsions, which is advantageous for retaining the core during spray drying (Özkan and Bilek, 2014).

Inulin is a polymer of β $(2 \rightarrow 1)$-linked D-fructose units, of different chain lengths, each of which has a terminal glucose unit. Having dietary fiber properties, inulin exhibits a bifidogenic effect (Roberfroid, 2007). Furthermore, the inclusion of prebiotics decreased the moisture content and water activity in encapsulates, which is positive for powder stability during storage (Tonon et al., 2009).

As most of the encapsulating compounds are applied in liquid form, most technologies are based on drying, allowing the development of powder products, offering flexibility for innovative formulations, good storage, and a longer shelf life due to the water activity reduction, and reduced transport costs. Drying technologies

have advantages and limitations, and the final product may differ in the physical-chemical, nutritional and morphological properties of the microstructures.

Spray drying, used in the production of commercial powders (Kha et al., 2010; Kim et al., 2009), presents a fast drying of the product, a high yield and continuous operation. During the drying process, the feed solution is sprayed in droplets in a stream of hot air (Gharsallaoui et al., 2007; Toledo, 2007). The final product can be manufactured in the form of powder, granules or agglomerates (Nindo and Tang, 2007). The size and shape of the particles can be optimized (Barbosa-Cánovas et al., 2005), however, due to the high drying temperatures, there may be losses of certain sensory and quality attributes (Desai and Park, 2005; Dziezak, 1988). To avoid the degradation of heat-sensitive compounds, freeze drying is another process in which the food is first frozen and then dried by direct sublimation of the ice under reduced pressure (Oetjen and Haseley, 2004), operating at low temperatures. However, the freeze-dried products are not easily rehydrated, as it has a high specific surface area (porous structure), but is expensive and slow, which means high investment, operation and maintenance costs (Ratti, 2001).

Thus the objective of the present work was to investigate the behavior of oleoresins extracted from aromatic plants and spices, encapsulated in microcapsules of inulin and maltodextrin using two drying processes (spray drying and freeze drying), in order to select powders with better characteristics for convenient use as additives in the food industry, which allow the retention of the main flavorings compounds, thus permitting salt reduction in food.

MATERIALS AND METHODS

Chemicals and reagents
Ethanol p.a, hydrochloric acid (370 g·L⁻¹), were purchased from Merck (Darmstadt, Germany). Folin-Ciocalteu reagent, gallic acid was from Sigma-Aldrich (990 g·L⁻¹). Sodium carbonate anhydrous was obtained from BDH (Poole, UK), maltodextrin from Glucidex (19 DE 18–20, F. Duarte, Lda, Portugal), and inulin from Alfa Aesar (Kandel, Germany). All other unlabeled chemicals and reagents were of analytical grade.

Plant material
The aromatic plants were provided by local producers of aromatic plants in Portugal, and the spices were purchased in a commercial shop. The aerial parts of the aromatic plants were frozen in liquid nitrogen and lyophilized for 48 hours in a Labogene lyophilizer (Scanvac Cool Safe model). After drying, the plant material was powered in a mill (Mikro-Feinmühle Culati (Janke & Kunkel Ika – Werk) using a 1.0 mm thick sieve, stored under vacuum in a packaging film polymer (LDPE 60 µm / PA 30 µm) (Amcor Flexibles, Portugal) and placed in desiccators until further analysis.

Oleoresin extracts
A mixture of dry aromatic plants and spices composed of *Allium schoenoprasum* L. (2 g), *Anethum graveolens* L. (2 g), *Capsicum frutescens* (4 g) and *Mentha pulegium* L. (2 g), selected from herbal seasoning used in culinary preparations of fish and *Allium schoenoprasum* L. (2 g), *Satureja montana* L. (2 g), *Capsicum annuum* L. (4 g) and *Origanum vulgare* L. (2 g) selected to be applied to culinary preparations of meat, were used to obtain two oleoresin mixtures.

Oleoresins were extracted from dry aromatic plants and spices using the Soxhlet apparatus method. Briefly, the powered samples, weighing about 10 g, were packaged in filter paper, tied, and distillated in 70 mL of ethanol at 80°C for 2 h. The ethanol extracts were filtered under a vacuum through a Buckner funnel with filter paper (Whatman #4, Maidstone, UK), and evaporated by a rotary evaporator under vacuum (40°C, 178 mbar). Each weighed dry oleoresin sample was then reconstituted in 10 ml of ethanol and stored in the dark at a low temperature (4°C) until testing.

Encapsulation
The oleoresin extracts were encapsulated in two different carrier agents (maltodextrin and inulin). The maltodextrin carrier agent was used in a maltodextrin: oleoresin 8:2 (v/v) ratio then oleoresin extract was added 1:20 (v/v). The second carrier agent, inulin:oleoresin was used at the same ratio of 8:2 (v/v) with the same oleoresin extract added 1:20 (v/v). The concentrations of the carrier agents were selected in a preliminary study as those having the lowest concentration without excessive powder stickiness on the chamber wall.

Below this concentration, the high powder stickiness resulted in an insignificant process yield.

This mixture was homogenized in a high-speed homogenizer (ultraturrax) at 4000 rpm for 5 min. Each of the carrier solutions with the oleoresins was split in two and half of the solutions were spray dried and half were freeze dried. The oleoresin extracts without carrier agents were frozen at –80°C until further analysis.

Spray drying (SD)

The SD process was performed in a laboratory scale spray dryer (Buchi B-290, Labortechnik AG, Flawil, Switzerland), with a 0.7 mm diameter nozzle, a main spray chamber of 500 mm × 215 mm, and a standard cyclone. The gas flow (nitrogen) of the drying air was about 35 $m^3 \cdot h^{-1}$.

The feed flow rate used for red meat mixtures was 0.54 and 1.34 $g \cdot min^{-1}$, and 0.58 and 1.34 $g \cdot min^{-1}$ for fish mixtures, for maltodextrin and inulin, respectively. The following parameters were fixed: pump (10%), aspirator (100%), inlet temperature (150°C) and outlet temperature (80°C). These latter conditions were selected in a previous work based on an experimental design. Briefly, the samples were atomized with a hot air stream in the drying chamber. The experiments were performed at constant process conditions.

The material obtained was collected and stored in glass flasks (Schott 250 mL) in desiccators containing silica gel in the dark at room temperature.

Freeze drying (FD)

The samples were frozen with liquid nitrogen, poured into stainless steel pans, and freeze dried using a laboratory vacuum pressure freeze dryer (Scanvac Cool Safe, Labogene Scadinavian by Design). The vacuum pressure of the freeze drier was set at 0.2 hPa, the plate temperature was 20°C, and the condenser was at –50°C for 24 h. The powders obtained were stored in glass flasks (Schott 250 mL) in a desiccator containing silica gel in the dark at room temperature.

Microcapsules powder analysis

Drying yield (DY). After spray and freeze drying, the samples DY were calculated according to the following formula:

$$DY, \% = x\ 100$$

Water activity (a_w). The water activity of the powder samples was measured using an electronic water activity meter (Rotronic-HigroPalm a_{w1}). Duplicate samples were measured at 25°C.

Solubility. Solubility was determined according to the method used by Cano-Chauca, Stringheta, Ramos and Cal-Vidal (2005), where 100 mL of distilled water was transferred into a beaker and the powder sample (1 g) was carefully added with high velocity magnetic agitation up to 5 min. The solution was centrifuged at 3000 × g for 5 min, and an aliquot of 25 mL of the supernatant was transferred to Petri dishes and oven-dried at 105°C for 5 h. Solubility [%] was calculated by the weight difference.

Hygroscopicity. Hygroscopicity was determined according to Cai and Corke (2000), with some modifications. Samples of each powder were weighed (approximately 1 g) in Petri dishes, placed at 25°C, put in a glass desiccator containing a saturated NaCl solution (75.29% humidity), and stored at 25°C for 7 days. Hygroscopicity was expressed as grams of absorbed moisture per 100 g dry solids ($g \cdot 100\ g^{-1}$), and calculated using the following equation:

$$Hygroscopicity = [Dm / (M + M_i) / (1 + Dm / M)]$$

where:

Dm – the increase in weight of the powder after equilibrium, g,

M – initial mass of the powder,

M_i – the free water contents of the powder before exposure to the humid air environment, % wb.

The samples were classified in accordance with GEA Niro Research Laboratory (2010).

Color measurement. The powders were poured into Petri dishes, slightly shaken to form a layer of 10 mm thickness and covered with plastic transparent film. The color was measured by reflectance with a Minolta Chroma Meter CR 200b color meter (Minolta Co., Osaka, Japan) equipped with an 8 mm aperture, and evaluated according to the international CIE system (L^*, a^*, b^*) at a temperature of 20°C. The results were expressed in lightness (L^*), redness to greenness (a^*), and yellowness to blueness (b^*). Chroma (C^*),

indicating color intensity, was calculated using the formula: $C* = (a^2 + b^2)^{1/2}$, and the hue angle ($H°$), indicating the tonality, using the formula: $H° = \tan^{-1}(b / a)$.

Encapsulation efficiency (EE). The effectiveness of encapsulation is the relationship between the content of non-encapsulated phenolic compounds present on the surface of the micro particles and the total phenolic compounds encapsulated.

The total phenolic compounds content that was on the surfaces of micro particles was measured according to the methodology described by Nori et al. (2011): 0.2 g of the powder was dissolved in 2.0 ml of ethanol, stirred, added to a tube shaker, and centrifuged at 4000 × g for 2 minutes. The phenolic compounds content of the supernatant was quantified in UV spectrophotometer according to the methodology described in 2.9.1.

Micro particle rupture was performed as follows: 2.0 mL 10% (w/v) sodium citrate was added to 0.2 g powder. The pH was raised to 8.0 with 0.1 mol·L^{-1} NaOH solution. This mixture was stirred in a vortex tube mixer for 2 minutes. Then 5.0 mL of 99.5% (v/v) ethanol was added to the mixture and stirred for 2 minutes. The mixture was centrifuged at 4000 × g for 20 minutes, and the total phenolic compounds content of the supernatant was quantified in UV spectrophotometer according to the methodology described in 2.9.1. Encapsulation efficiency – EE was calculated using the following equation:

$$EE, \% = (TP - SP) / TP \times 100$$

and calculated according to Selamat et al. (2009), where TP is the content of the total phenolic compounds from a known amount of powder particles after rupture, and SP is the content of total phenol compounds contained in the surface of the same amount of powder particles.

Morphology and particle size distribution

The morphology of the particles was observed by scanning electronic microscopy (SEM). Each sample was covered with a fine layer of gold through Sputer Coating Attachment of Quorum Q150R ES in vacuumed evaporators. The equipment used for observations was a scanning electron microscope (Hitachi, S-3400N, Tokyo, Japan) working with a voltage of 20 kV. The microphotographs were carried out with a camera coupled to the microscopic. The samples were systematically observed with 500 × and 2000 × magnification. The particle size was determined by examination of SEM micrographs.

Chemical composition of phenolic compounds of powder particle extracts

Total phenolic content (TPC) by Folin-Ciocalteau. Total phenolic content was measured using a modified Folin-Ciocalteu assay Slinkard and Singleton (1977). Briefly, water (5 mL), the sample (1–3 mL) and a Folin-Ciocalteu Reagent (0.5 mL) were mixed, allowed to stand for 5–8 min at room temperature, followed by the addition of 1.5 mL sodium carbonate (20%, w/v) together with water to obtain a final volume of 10 mL. The solution was mixed, allowed to stand for 2 h and filtered (0.45 µm poly-tetrafluoroethylene filter, Whatman #4) prior to an absorbance reading at 750 nm in a spectrophotometer (Hitachi U2010, UV-vis spectrophotometer). All determinations were performed in triplicate. Negative controls were performed with water. Total phenol content was quantified by comparison of the sample absorbance values with those of the gallic acid reaction. The calibration curve of gallic acid was prepared in the 5–25 mg·L^{-1} range, and results were expressed as mg of gallic acid per g of sample.

Phenolic composition by Liquid Chromatography and Tandem Mass Spectrometry (LC–HRMS/MS). Samples were analyzed by Liquid Chromatography (UHPLC Elute) interfaced with a QqTOF Impact II mass spectrometer equipped with an ESI source (Bruker Daltonics). Chromatographic separation was carried out on a C18 reversed-phase Kinetics column 100 Å (150 mm × 2.1 mm, 2.7 µm particle size; Phenomenex). The mobile phase consisted of water containing 0.1% formic acid (A) and acetonitrile containing 0.1% formic acid (B). The elution gradient used (A:B, v/v) was as follows: 93:7 from 0 to 1 min; 0:100 at 10 min to 14 min; 93:7 at 16 min to 22 min. The injected volume was 8 µL, the flow rate was 300 µL·min^{-1}, and the temperature of the column and autosampler were maintained at 40°C and 8°C, respectively.

The high-resolution mass spectra were acquired in both ESI positive and negative modes. The optimized parameters were set as follows: ion spray voltage,

+4.5 / –2.5 kV; and plate offset, 500V, nebulizer gas (N_2), 2.8 bars; dry gas (N_2), 8 L·min^{-1}; dry heater, 200°C. Internal calibration was performed on the high-precision calibration mode (HPC) with a solution of sodium formate 10 mM introduced to the ion source via a 20 µL loop, at the beginning of each analysis using a six-port valve. Acquisition was performed in full scan mode in the m/z 100–1000 range, and in a data--depending MS/MS mode, with an acquisition of 5 Hz using a fixed cycle time of 2 s, a dynamic exclusion duration of 0.5 min and a *m/z*-dependent isolation window of 0.03 Da.

To evaluate the variation of the signal intensity across the entire measurement intensity raw files, the samples were spiked with a solution of gallic acid 10-4M, and replicate injections were analyzed. Data acquisition and processing were performed using DataAnalysis 4.2 software (Bruker Daltoniks).

Statistical analysis

Results were expressed as mean and relative standard deviation (RSD) of two measurements for each parameter. The variation within each process was analyzed using one-way analysis of variance (ANOVA) for the parameter in question and the mean separations were performed using the Tukey test ($P < 0.05$) performed by Statistica TM v8.0 (StatSoft, 2007). Correlations among the data were calculated using the MS Excel software correlation coefficient statistical option.

RESULTS AND DISCUSSION

Microcapsule powder analysis

The results of the physicochemical analysis (yields, water activity, solubility, higroscopicity, color and encapsulation efficiency) which were obtained for the powder particles of the oleoresin extracts for fish and meat encapsulated with inulin and maltodextrin for the SD and FD processes are presented in Table 1.

Drying yield – DY values obtained by SD with inulin and maltodextrin carrier agents ranged between 61.59% to 62.19% for fish, and 71.30% to 55.89% for meat respectively; the highest DY values were obtained with FD for the same carrier agents, with an average of 74.54% to 72.36% for fish, and 80.80% to 79.09% for meat, respectively. The lower DY of the microcapsules in the SD process might be associated

with the deposition of the powder on the wall of the drying chamber or cyclone. Similar observations were also reported by other authors (Jimenez-Gonzalez et al., 2018; Santana et al., 2013).

Water activity is a critical parameter for powder stability during storage, as its biochemical and microbiological stability could be compromised if values are high enough, thus reducing its shelf life. All the powder samples showed water activity values (0.3) comparable to those found by Fennema (1996), which is very positive for powder stability, and exhibiting less water available for microbiological and biochemical development during storage, thus increasing the shelf life.

With respect to solubility, the powder particles of the oleoresin extracts for fish and meat encapsulated with inulin and maltodextrin for the two drying processes were lightly soluble and did not show significant differences, which was expected, since these materials are largely used in SD and FD processes. Those results showed the same trend as was found by Lacerda et al. (2016), who observed a similar solubility with tapioca starch in açai powder particles.

However, we can observe a solubility increase during food preparation that can be attributed to disruption of the powder particles, facilitating the migration of water inside, and the additional leaching out of soluble components. Similar results were observed by Tonon et al. (2009).

According to the GEA Niro Research Laboratory (2010), the powder particles obtained by SD were not hygroscopic (<10%), while the powder particles produced by the FD process were revealed to be lightly hygroscopic (10.1–15%). So, every particle powder showed a low higroscopicity, which is a desirable characteristic for dehydrated products regardless of the agents used. The lower hygroscopicity value obtained for SD (8.25 ±0.17) and FD (13.42 ±0.04) can be attributed to the different drying processes. A similar observation was found by several authors: Ahmed et al. (2010) and Cai and Corke (2000).

The *L** color parameters of the powder particles of the oleoresin extracts for fish and meat encapsulated with inulin and maltodextrin for the SD process (94.67 ±0.3 to 95.54 ±0.02) were higher than those obtained by the FD process (80.86 ±0.16 to 81.46 ±0.40), regardless of the encapsulating agent. The higher *L**

Table 1. Yields and physical-chemical analysis (mean ±SE) of powder particles of oleoresin extracts for fish and meat encapsulated with inulin and maltodextrin for SD and FD processes

	SD				FD			
	inulin		maltodextrin		inulin		maltodextrin	
	meat	fish	meat	fish	meat	fish	meat	fish
DY, %	71.38[a] ±0.03	61.59[a] ±0.02	55.89[a] ±0.05	62.19[a] ±0.05	80.80[a] ±0.02	74.54[a] ±0.02	79.09[a] ±0.02	72.36[a] ±0.02
a_w	0.290[d] ±0.008	0.272[c] ±0.004	0.317[f] ±0.003	0.355[e] ±0.004	0.276[d] ±0.003	0.246[bc] ±0.004	0.224[a] ±0.001	0.228[a] ±0.002
Solubility, %	48.22[abc] ±1.34	46.90[bc] ±0.71	46.00[c] ±0.40	50.15[ab] ±0.42	49.72[ab] ±0.40	48.58[abc] ±0.78	50.36[a] ±1.12	50.68[a] ±1.02
Hygroscopicity, %	8.25[a] ±0.17	10.54[a] ±0.13	9.03[a] ±0.07	8.87[a] ±1.05	12.28[a] ±0.10	10.83[a] ±3.57	11.79[a] ±1.68	13.42[a] ±0.04
Color								
L^*	95.54[b] ±0.02	94.67[b] ±0.30	95.33[b] ±0.39	94.92[b] ±0.05	81.08[a] ±0.01	81.46[a] ±0.40	80.86[a] ±0.16	81.13[a] ±0.20
a^*	−1.16[c] ±0.00	−3.08[a] ±0.04	−1.09[c] ±0.06	−3.14[a] ±0.04	−2.24[b] ±0.16	−3.36[a] ±0.19	−2.20[b] ±0.17	−4.47[d] ±0.02
b^*	6.46[b] ±0.06	11.89[a] ±0.08	6.51[b] ±0.43	11.54[a] ±0.15	11.34[a] ±0.34	16.72[c] ±0.18	10.78[a] ±0.83	19.77[d] ±0.37
C^*	6.56[a] ±0.06	12.28[c] ±0.09	7.61[a] ±1.88	11.95[d] ±0.16	11.53[b] ±0.40	17.05[a] ±0.22	11.04[b] ±0.79	20.26[a] ±0.37
H°	100.10[ab] ±0.14	104.40[de] ±0.00	99.25[a] ±0.35	105.10[a] ±	100.50[ab] ±1.27	101.25[abc] ±0.49	101.50[cd] ±0.00	102.70[e] ±0.28
EE, %	86.20[ab] ±5.23	88.37[ab] ±2.07	90.77[b] ±2.17	85.52[ab] ±2.79	80.94[ab] ±0.12	78.69[a] ±1.65	82.63[ab] ±2.42	78.88[a] ±2.66

Mean values ($n = 2$) ±standard deviation. Different letters indicate significant differences ($p < 0.05$).

values for the particles obtained by the SD process may be due to the volatility of some compounds, with the increase of the temperature leading to lighter powders. The a^* parameter for the powder particles obtained for both processes showed a predominant green hue with a^* values ranging from −4.47 ±0.02 to −1.09 ±0.06, and b^* values corresponding to a yellow hue ranging from 6.46 ±0.06 to 19.77 ±0.37. The a^* parameter decreased in the fish mixtures in both drying processes regardless of the carrier used, indicating an increase in greenish hue, while the b^* parameter increased positively in fish indicating a yellowish hue. The cylindrical coordinate C^* followed the same trend as the a^* and b^* parameters, while H° showed no clear trend in

either encapsulating agent obtained by the two drying processes. Similar results were observed by Kha et al. (2010) with SD of Gac fruit powder.

The encapsulation efficiency of the polyphenols was significantly better in the inulin and maltodextrin obtained in the SD process (88.37% ±2.07 and 86.20 ±5.23% (85.52 ±2.79 and 90.77 ±2.17) in fish and meat oleoresins, respectively, than in the FD process (78.69 ±1.65 and 80.94 ±0.12, 78.88 ±2.66 and 82.63 ±2.42) in fish and meat oleoresins, respectively. In the SD process, inulin showed better EE for fish oleoresin polyphenols (88.37 ±2.07), while maltodextrin showed better EE for meat oleoresins (90.77 ±2.17). While the EE of the polyphenols in the FD process
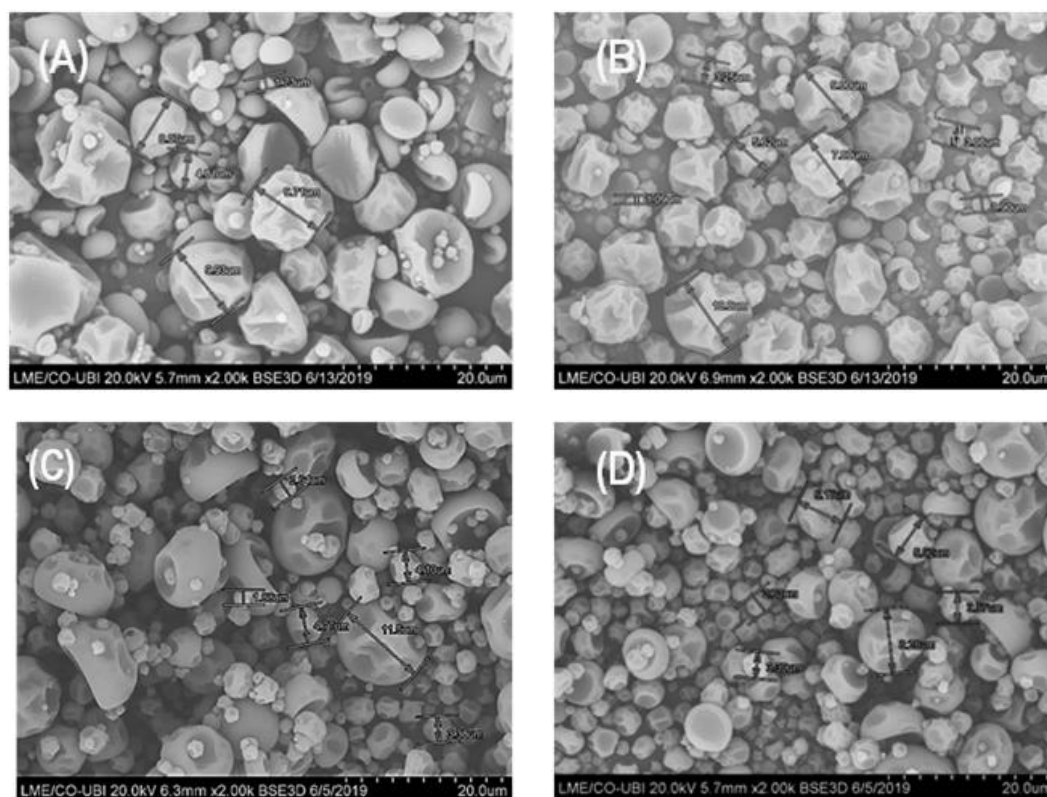
Fig. 1. Microphotographs of powder particles produced with: A – inulin, B – maltodextrin for meat oleoresins, C – inulin, D – maltodextrin for fish oleoresins; obtained by SD (magnification 2000×)

showed no significant difference for either encapsulating agent. This behavior could be related with the nature of the bioactive compounds (i.e. negative charge for polyphenols) and with the polyelectrolyte structure (type and density charge), with inulin having a fructose polysaccharide, and maltodextrin a glucose polysaccharide, the conditioning of the bioactive–polymer interaction will be similar (Paz et al., 2010).

Morphology and particle size

Scanning electronic microscopy – SEM microphotographs of the powder particles of the oleoresin extracts for fish and meat encapsulated with inulin and maltodextrin are presented in Figure 1 for the SD process, and in Figure 2 for the FD process.
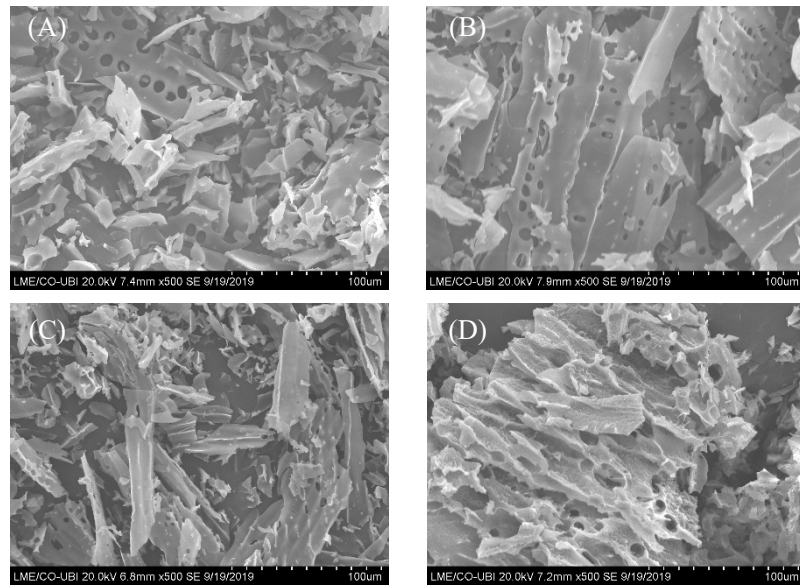
The powder particles produced with maltodextrin and inulin obtained using the SD process were very similar, exhibiting a rounded external surface with continuous walls and an absence of cracks or fissures in the microstructure. Similar results were observed by Carneiro et al. (2013). However, some wrinkled powder particles could also be observed. Beirão-da--Costa et al. (2013), observed similar morphologies in oregano oil inulin powder particles obtained using the SD process. This kind of structure is advantageous in terms of offering protection to the core by providing low permeability to gas.

The morphology of inulin and maltodextrin powder particles obtained using FD exhibit a skeletal and porous structure (amorphous), which may be due to the formation of ice on the surface of the material during freeze drying, which helps to prevent shrinkage and the collapse of the structure, resulting in an insignificant change in volume (Caparino et al., 2012; Ratti, 2001).

The particle size distribution of the inulin (Fig. 3A) and maltodextrin (Fig. 3C) for the meat oleoresin powder particles obtained using the SD process showed microcapsules with a diameter ranging from 2.6 to

Fig. 2. Microphotographs of powder particles produced with: A – inulin, B – maltodextrin for meat oleoresins, C – inulin, D – maltodextrin for fish oleoresins; obtained by FD (magnification 500×)
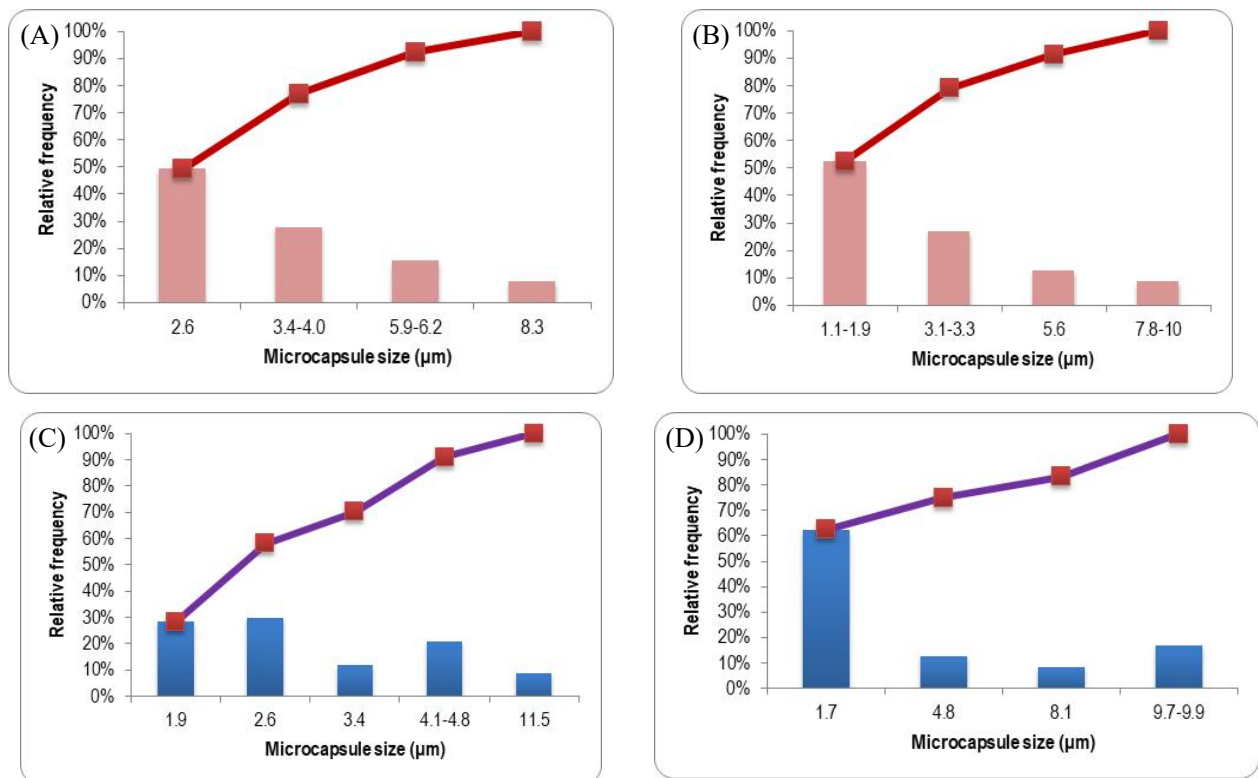
Fig. 3. Relative frequency (bars) and cumulative frequency (lines) equivalent to the diameter of powder particles: A – inulin and C – maltodextrin meat oleoresins, and B – inulin and D – maltodextrin fish oleoresins obtained using SD

8.3 μm and 1.1 to 10.0 μm, respectively. 50% of inulin microcapsules showed a diameter less than about 2 μm, 30% about 3.5 μm, and 20% with 6.0 μm, while 50% of maltodextrin microcapsules were less than 1.9 μm, 20% about 3.3 μm, and 10% about 5.6 μm. Regarding the particle size distribution of the inulin (Fig. 3B) and maltodextrin (Fig. 3D) for the fish oleoresin powder particles, microcapsules were presented with a diameter ranging from 1.9 to 11.5 μm and 1.7 to 9.9 μm, respectively. 30% of the inulin microcapsules had a diameter of about 2 μm, and 60% of about 2.0 μm for maltodextrin, while 60% of the maltodextrin microcapsules presented a diameter of about 1.7 μm. The diameters of inulin and maltodextrin microcapsules for the meat oleoresins were more regular than the inulin and maltodextrin microcapsules for the fish powder particles and for both oleoresins, maltodextrin presented more regular particles than inulin. Concerning the results obtained, the existence of a large population of small particles was found, showing that the drying process was adequate in all stages, which is favorable for application in food products. Tonon et al. (2009), observed similar results for açai powder particles obtained by spray drying.

Total phenolic compounds (TPC) of powder particle extracts

The TPC of oleoresin extracts for meat encapsulated with inulin, varied from 110.0 to 228.0 mg·100 g^{-1}

GAE (gallic acid equivalent), for FD and SD, respectively, while for maltodextrin they ranged from 127.0 to 232.0 mg·100 g^{-1} GAE (Fig. 4). These results showed losses of TPC after encapsulation ranging from 16.33 to 33.85% for inulin and 18.86 to 34.45% for maltodextrin. In the extracts of fish oleoresins encapsulated with inulin it varied from 114.0 to 205.0 mg·100 g^{-1} GAE for the FD and SD process, respectively, while for maltodextrin it varied from 102.0 to 208.0 mg·100 g^{-1} GAE. The SD process showed a higher TPC for both extracts of oleoresins for meat and fish encapsulated with the two carrier agents, while in the FD process the maltodextrin presented a higher TPC for extracts of meat oleoresins, but the differences were not significant. These results can be attributed to the shape of the particles obtained in the SD process that originated as uniform spherical particles, leading to a greater encapsulation of the TPC than in the FD process, where the particles do not present a definitive shape.

Polyphenol composition of powder particle extracts by LC-MS

The polyphenol composition of the powder particle extracts was assessed by UPLC-ESI(-)/MSMS. The distribution of polyphenol compounds in the extracts of inulin, and maltodextrin powder particles of fish and meat oleoresins obtained using the two drying processes are presented in Tables 2 and 3. In the fish oleoresin extracts, 11 flavonoid derivatives, and
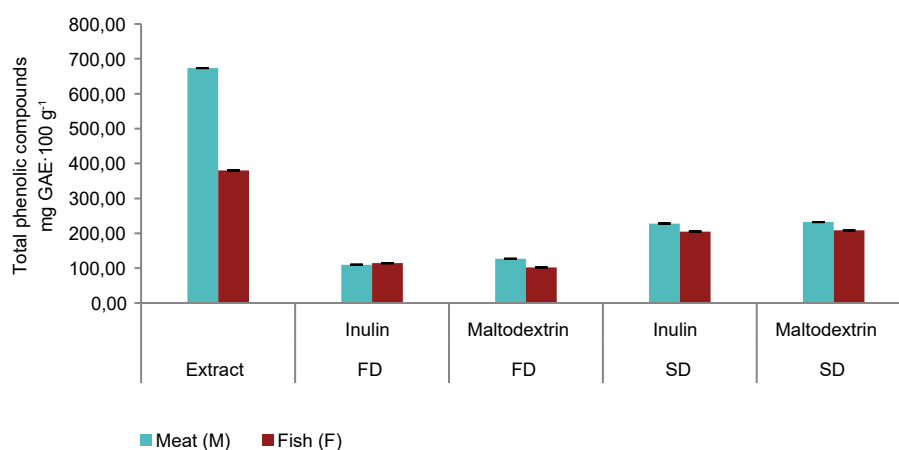
Fig. 4. TPC of inulin and maltodextrin fish (A) and meat (B) oleoresin powder particle extracts obtained from FD and SD processes

Table 2. Distribution of polyphenol compounds of inulin and maltodextrin in fish oleoresin powder particle extracts, µg·100 g^{-1}

	Fish extract	Inulin	Maltodextrin	Inulin	Maltodextrin
			FD		SD
CQA isomers	26.1	6.93	3.43	8.54	7.21
Hydroxyjasmonic acid glucoside	12.3	4.76	2.24	3.75	3.26
Coumaryl-Quinic isomers	12.8	2.05	1.89	3.91	3.61
Hydroxybenzoic acid derivative	39.2	14.5	12.2	19.7	17.3
Rosmarinic acid	98.6	27.3	22.4	44.4	37.0
Total hydroxycinnamic acids	189.0	56.0	42.0	80.0	68.0
Quercetin-diglucoside	32.0	6.83	10.1	20.8	18.6
Quercetin-3-glucoside	29.6	15.9	135	22.5	19.3
Quercetin-3-glucuronic	2.56	–	–	0.69	0.48
Luteolin-7-apiosyl-glucoside	16.5	12.5	11.7	18.5	15.1
Luteolin-7-glucoside	13.3	8.99	6.35	10.7	9.30
Luteolin-7-glucuronic	5.43	1.54	0.98	2.48	3.66
Luteolin-7-apiosyl-malonyl-glucoside	11.0	22.5	3.18	7.57	6.03
Apiin	10.5	11.0	9.45	14.1	6.04
AcetylApiin	7.73	4.14	3.00	6.72	5.33
MalonylApiin	3.65	1.49	1.06	2.58	2.04
Nepetin-7-glucoside	2.61	2.07	1.44	2.66	2.08
Total flavonoids	135.0	87.0	61.0	109.0	88.0

5 hydroxycinnamic acid (HCAs) derivatives were identified (Table 2). The total relative concentration of flavonoid derivatives for inulin powder particles ranged from 109.0 to 87.0 µg·100 g^{-1} dry weights, while for maltodextrin powder particles ranged from 88 to 61 µg·100 g^{-1}, for the SD and FD processes, respectively. Concerning HCA derivatives, a total relative concentration for inulin powder particles was measured ranging from 80.0 to 56.0 µg·100 g^{-1} dry weights, and for maltodextrin from 68.0 to 42.0 µg·100 g^{-1} for the SD and FD processes, respectively. However, in meat oleoresins 9 HCA derivatives and 7 flavonoid derivatives were detected (Table 3). The total relative concentration of HCAs for inulin powder particles ranged from 83 to 36 µg·100 g^{-1} dry weights, while for maltodextrin powder particles, it ranged from 128 to 62 µg·100 g^{-1} dry weights, for the SD and FD processes, respectively. Regarding flavonoid derivatives, the total relative concentration for inulin powder particles ranged from 56 to 23 µg·100 g^{-1} dry weights, while for maltodextrin powder particles, it ranged from 56 to 30 µg·100 g^{-1}, for the SD and FD processes, respectively.

The identified compounds in the encapsulated oleoresin for use in fish products by inulin and maltodextrin obtained using the SD and FD process, contain mainly non-volatile flavoring components like quercetin derivatives, responsible for the bitterness of many foods (Drewnowski and Goméz-Carneros, 2000; Panche et al., 2016), while nepetin and apiin compounds provide flavors, and colorants, like luteolin derivatives, the yellow color of the powder particles.

Table 3. Distribution of polyphenol compounds of inulin and maltodextrin in meat oleoresin powder particle extracts, $\mu g \cdot 100\ g^{-1}$

|	Meat extract	Inulin	Maltodextrin	Inulin	Maltodextrin
		FD		SD	
PCA-4-gluc	6.09	0.71	0.83	2.07	1.58
CQA isomers	34.3	3.78	4.32	11.2	10.9
Hydroxyjasmonic acid gluc	16.5	2.97	4.32	6.16	5.55
Gallocatechin	52.1	5.13	5.77	14.0	11.5
Rosmaniric acid gluc	6.43	0.82	0.83	2.19	1.90
di-CQA	19.9	3.87	5.19	12.7	11.3
Rosmarinic acid	141.0	16.1	35.9	16.6	70.1
Lithospermic acid	12.8	0.43	2.62	4.03	2.96
Salvianolic acid A	41.6	2.04	2.02	13.8	11.9
Total hydroxycinnamic acids	331.0	36.0	62.0	83.0	128.0
Carnasol	44.4	24.9	29.8	31.9	30.2
Rutin	6.31	1.21	1.56	3.26	3.08
Querc-gluc	10.6	2.24	2.87	5.69	5.72
Querc-ram	9.05	2.02	3.42	6.52	6.46
Lut-O-glucur	10.2	1.79	2.62	5.85	6.45
NG-O-hex-dhex	4.43	0.89	1.15	2.39	2.30
Apiin	78.6	8.35	9.40	16.6	17.0
6''-acetyl-Apiin	18.2	6.56	8.55	15.3	15.0
Total flavonoids	137.0	23.0	30.0	56.0	56.0

While oleoresins for application in meat products that are encapsulated in inulin and maltodextrin microcapsules obtained using the SD and FD process, contain mainly non-volatile flavoring components like HCAs, generating aromas and active flavors. All these compounds have demonstrated antioxidant properties.

The comparative diagrams of the relative concentration values of polyphenols (flavonoids derivatives and HCAs) in the SD and FD powders obtained from the encapsulated fish and meat oleoresin extracts are shown in Figure 5. The recovery percentages of flavonoid derivatives were higher for the SD microcapsules of fish oleoresins encapsulated with inulin (81.03%), followed by maltodextrin (65.18%), while HCAs presented no difference for microcapsules of inulin (42.48%) and maltodextrin (42.10%). Regarding the meat oleoresins, the percentages of recovery of HCAs were higher for the SD with maltodextrin (42.10%), followed by inulin (30.56%), while flavonoid derivatives showed no difference for inulin (40.41%), and maltodextrin (40.72%) microcapsules.

Therefore, we can conclude that inulin showed a more protective effect of polyphenol compounds for fish flavors, while maltodextrin is the best meat flavor transporter in the SD process.

The presence of non-volatile flavoring components (that is, taste substances) encapsulated in inulin and maltodextrin from oleoresins will allow a reduction in
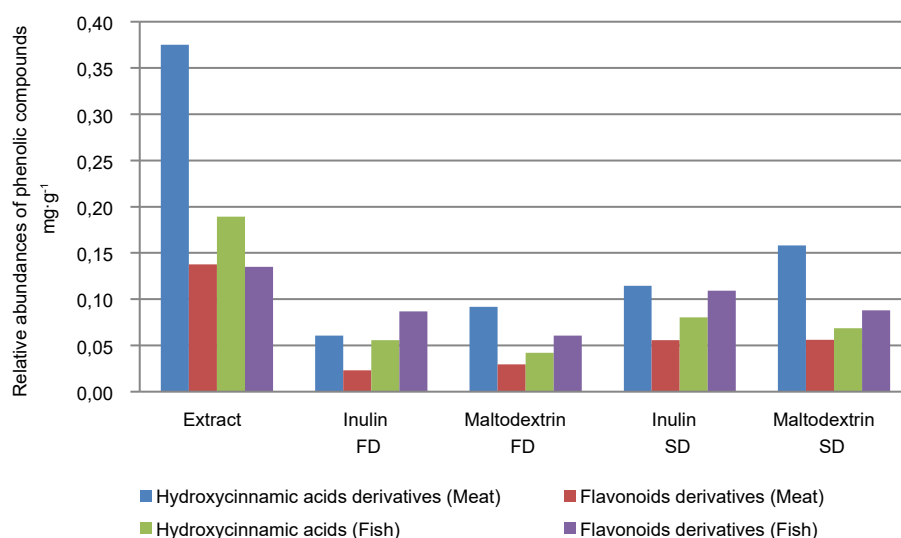
Fig. 5. Comparative diagram of the relative concentration of polyphenol compounds from inulin and maltodextrin fish (A) and meat (B) oleoresin powder particle extracts, obtained from FD and SD processes

the salt content of fish and meat products. However, sensory analysis must be performed to test oleoresins in meat and fish products in a real context.

CONCLUSIONS

The non-volatile compounds from oleoresins of aromatic plants and spices provide initial evidence that they can be encapsulated in polymeric materials of inulin and maltodextrin obtained through spray drying and freeze-drying processes.

The microcapsules showed high encapsulation efficiency, low water activity, and similar solubility to sodium chloride, and the morphology of those obtained using the SD process presented a rounded outer surface, without cracks, while those obtained using FD showed an amorphous structure. The absence of pores / cracks on the particles' surface prevented the inward diffusion of oxygen, and provided better protection for the encapsulated compounds.

The analysis by LC-MS / MS led to the conclusion that inulin guaranteed protection from environmental conditions to the flavonoid compounds from the fish oleoresins, and maltodextrin offered protection to the HCAs from the meat oleoresins.

The results demonstrate a new strategy for the additional industrial application of encapsulated complex oleoresin mixtures to use as an additive to reduce salt consumption, which has a growing market value. However, functional properties of powders under specific conditions (for example, heat and acidity) and sensory analysis must be performed in a real context in order to evaluate their use in specific product formulations.

The data obtained refer to powders produced on a laboratory scale. A good scale up can be achieved by making improved test work in pilot plants in order to produce powders with similar characteristics and other flavoring properties on an industrial scale.

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