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Encapsulation of carrot waste extract by freeze and spray drying techniques: An optimization study

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ABSTRACT

Carotenoids were recovered from carrot processing waste using sunflower oil. Simplex centroid mixture design was applied for the optimization of wall material formulations (whey protein/maltodextrin/inulin) for the encapsulation of carrot waste extract by freeze drying (FD) and spray drying (SD). The optimal wall materials were pure whey protein, and 71 g/100 g whey protein - 29 g/100 g inulin mixture, respectively, showing total carotenoids of 1.31 and 0.87 mg β-carotene/100 g, encapsulation efficiencies of 63.69 g/100 g and 53.78 g/100 g, and β-carotene bleaching antioxidant capacities of 70.06 and 41.23 μmol TE/100 g. The freeze dried encapsulate showed the best hygroscopicity, oxidative stability and colour properties, while the spray dried encapsulate had the lowest water activity, moisture content and particle size. The morphological properties of the optimal encapsulates were assessed. The techniques and the formulations tested showed a big potential for the preparation of functional food with improved nutritional, colour and bioactive properties.

1. Introduction

Large amounts of waste and by-products are generated annually by the food industry and are extensively used as cheap animal feed and fertilizers. Recent studies indicate that waste from fruits and vegetable processing still contains valuable molecules (antioxidants, dietary fibres, natural colorants, aroma compounds, etc.) which can be reused as functional ingredients in food, pharmaceutical, cosmetic, and healthcare products (Ravindran & Jaiswal, 2016).

The worldwide trend toward the use of natural colorants as alternatives to synthetic colours in food applications is boosted by legislative actions and consumer concerns (Mahdavi, Jafari, Assadpoor, & Dehnad, 2016). The interest and the scientific research in carotenoid pigments have increased in recent years, mainly due to the role that natural antioxidants can have on health quality. To recover valuable compounds from plant matrices, conventional methods are usually employed; however, as the food industry emphasizes the need for environment-friendly and sustainable processes, new methods are being developed. One promising alternative to the traditional methods for carotenoids extraction is the use of edible oils as solvents (Li, Fabiano-Tixier, Tomao, Cravotto, & Chemat, 2013; Sachindra & Mahendrakar, 2005).

The oil extracts are however susceptible to oxidation and isomerization, owing to the high number of conjugated double bonds in the chemical structure of fatty acids and carotenoids, leading to significant deterioration of sensory characteristics and drastic reduction in nutritional and functional values. Encapsulation is an effective method to improve the phytochemical stability by entrapping the core material with a coating agent (wall material). Encapsulation also modifies the solubility of targeted compounds (most carotenoids are lipophilic) and hence its application may be limited; to facilitate it, standardization, handling and storage settings could be improved (Indrawati, Sukowijoyo, Indriatmoko, Wijayanti & Limantara, 2015).

The selection of an encapsulation method depends upon specific applications and parameters, such as particle size, physicochemical properties of the core and coating materials, release mechanisms, costs, etc. Some of the most suitable preservation methods for carotenoids are the freeze and spray drying processes; their main advantages over other

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encapsulation techniques are simplicity, continuity, effectiveness, availability, and applicability (Nedović, Kalušević, Manojlović, Petrović, & Bugarski, 2013). Freeze drying is the most useful method for the encapsulation of thermosensitive substances, because minimizes thermal degradation reactions. Although freeze drying is a time-intensive and costly method, it is widely used for carotenoids encapsulation (Desorby, Netto & Labuza, 1997; Ogrodowska, Tanska, & Brandt, 2017; Tang & Chen, 2000). On the other hand, spray drying is a very attractive technique for the food industry because it is cheap and flexible. Over the last years, the applicability of spray drying for carotenoids encapsulation, e.g. for chia oil (Us-Medina, Julio, Segura-Compos, Ixtaina, & Tomas, 2018), soybean oil (Jones et al., 2013), rosa mosqueta oleoresin (Robert, Carlsson, Romero, & Masson, 2003) etc., has been frequently tested.

The wall material (WM) plays a crucial role in the encapsulation process. The different types of wall materials include polysaccharides (starches, maltodextrins, and gum Arabic), lipids (stearic acid, mono- and diglycerides) and proteins (gelatin, casein, milk serum, soy and wheat); the structure and characteristics of each coating agent impart different physicochemical properties to the encapsulate (Mahdavi et al., 2016). Maltodextrin is widely used because of its great water solubility, low viscosity and low sugar content; its greatest drawback is the low emulsifying capacity, therefore is often used in blends with other wall materials (Šturm et al., 2019). Another coating agent, whey protein (WP), has high nutritional qualities (e.g. great protein content, with abundant essential amino acids) and excellent bioavailability. Additionally, β-lactoglobulin and α-lactalbumin (the main components of whey protein) provide good emulsification and protective film-forming abilities that meet the demands of encapsulation (Khan, Wang, Sun, Killpartrick, & Guo, 2019). Inulin, a polysaccharide obtained from chicory root (*Cichorium intybus*), is widely known for its prebiotic effects, dietary fibre action, and improvements of calcium bioavailability (Šturm et al., 2019). Supplementing inulin in foods does not increase their glycemic index, which makes inulin a potential ingredient in diabetic foods (Fernandes, Boares, Botrel & de Oliveira, 2014). However, like maltodextrin, it has low emulsifying capacity and poor water solubility, and consequently is rarely used alone.

The aim of the present study was to identify optimal wall material formulations to encapsulate carrot waste extracts by the freeze and spray drying techniques by comparing total carotenoid content, encapsulation efficiency and antioxidant capacity of the formulations. Furthermore, the physicochemical properties of optimal encapsulates were determined, to assess their stability and their possible utilization as food additives.

2. Materials and methods

2.1. Materials

The carrot waste, acquired from the “Nectar” beverage industry (Bačka Palanka, Serbia), was immediately packed, freeze-dried and stored at −20 °C. The sunflower oil used for carotenoid extraction, purchased from a local supermarket, was from the edible oil manufacturing company “Dijamant” (Zrenjanin, Serbia). The whey protein concentrate was purchased from Olimp Laboratories (Debica, Poland), having 77 g/100 g minimum protein content. The maltodextrin was from Battery Nutrition Limited (London, U.K.) and the inulin was purchased from Elephant Pharma (Belgrade, Serbia).

2.2. Preparation of carrot waste extract

Briefly, freeze dried carrot waste and sunflower oil (1:10 w/v) were mixed with by stirring for 30 min at 25 °C with a B800E high-speed blender (Gorenje, Velenje, Slovenia), using time shifts of 10 min blend and 5 min pause to avoid heating. The mix was then centrifuged at 4000 rpm for 10 min (model Lace 24, Colo Lab Experts, Novo Mesto, Slovenia), the supernatant was recovered and was stored in a dark glass bottle, wrapped in foil, at 4 °C for further use.

2.3. Experimental design for the optimization of wall material formulations

Response surface methodology was used to evaluate the effect of different wall materials and their combinations on the response variables total carotenoid content (TCar), encapsulation efficiency (EE) and β-carotene bleaching (BCB) assay for the two encapsulation techniques (freeze drying and spray drying). The experiments were carried out following a simplex centroid mixture design (SCMD), considering three wall materials (maltodextrin, whey protein, and inulin). The SCMD for each technique consisted of 8 trials with 7 different formulations and one replicated point. To avoid systematic errors, the experiments were performed randomly.

2.3.1. Preparation of the emulsions for encapsulation

The wall materials formulations, defined by experimental design, were prepared as follows: for freeze drying, 5 g/10 mL distilled water at 60 °C kept under stirring until the temperature reached 30 °C; for spray drying, 12.5 g/100 mL distilled water, prepared in the same way. Eight different formulations were prepared, for freeze drying by the addition of 3 mL carrot oil extract, and for spray drying by the addition of 7.5 mL carrot oil extract. The wall material-carrot oil extract solutions were homogenized at 11,000 rpm for 3 min at room temperature and dried.

2.3.2. Freeze drying encapsulation

The formulations were kept overnight at −80 °C in a deep freezer (Snijders Labs, Tilburg, the Netherlands) and then freeze dried (Christ Alpha 2–4 LSC, Martin Christ, Germany) at −40 °C for 48 h, to ensure complete drying. The freeze dried encapsulates were stored at −20 °C until further use.

2.3.3. Spray drying encapsulation

The formulations were spray dried using a lab-scale spray dryer (Büchi mini B-290, Büchi Labortechnik, Switzerland) at an inlet temperature of 130 °C and an outlet temperature of 65 ± 2 °C. The spraying air flow rate and rate of liquid feed were 600 L/h and 8 mL/min, respectively. The spray dried encapsulates were stored at −20 °C until further use.

2.4. Chemical and physical analyses

The **s**unflower oil and the carrot waste extract were analysed for carotenoids, tocopherols and fatty acids content. For carotenoids determination the oil samples were diluted in methanol:dichloromethane (50:50 v/v), filtered through a 0.2 μm PTFE membrane, and immediately analysed by reverse phase HPLC as detailed in Alfieri, Hidalgo, Berardo, and Redaelli (2014). All carotenes were quantified using the β-carotene standard curve. The tocopherols were analysed by normal phase HPLC (Varas Condori et al., 2020). Carotenoid and tocopherol contents (mg/kg) are the average of two analyses. Fatty acid methyl esters were prepared from the extracted lipids by transesterification using 14 g/100 L boron (III)-fluoride in methanol (Karlović et al., 1996). The samples were analysed by a GC Agilent 7890A system with FID according the method of Csengeri et al. (2013).

The response variables, total carotenoid content of encapsulates (TCar) and encapsulation efficiency (EE; the ratio between total carotenoids and carotenoids on the encapsulate surface) were determined following Barbosa, Borsarelli, and Mercadante (2005). The determination of the carotenoid levels for these purposes were carried out by a spectrophotometric method (Nagata & Yamashita, 1992). The antioxidant capacity was evaluated by the β-carotene bleaching assay (Al-Saikhan, Howard, & Miller, 1995), and expressed as μmol Trolox equivalent (TE) per 100 g sample.

The water activity (a_w) was determined by placing approximately 3 g encapsulate in the sample holder of a LabSwiftawmeter “Novasina” (Switzerland) at 25 °C. The moisture content of the encapsulates was measured by the air oven drying at 105 °C until a constant weight was obtained. To gauge the hygroscopicity, the samples of each encapsulate (about 2 g) were placed at 25 °C in an airtight plastic container filled with NaCl saturated solution (75.29% RH); after 1 week, the hygroscopic moisture (hygroscopicity) was measured and expressed as g moisture per 100 g dry solids (g/100 g). The solubility of the encapsulates was evaluated based on the method described by Yamashita et al. (2017). The particle size distribution of the powders was determined using the Mastersizer 2000 laser diffraction size analyzer (Malvern Instruments, Worcestershire, UK) equipped with a Scirocco2000 dispersion unit. The size distribution, quantified as relative volume of particles in size bands, was presented as size distribution curves using the Mastersizer 2000 Software (Mastersizer, 2000). The bulk density (Db), tapped density (Dt), compressibility ratio (CR), and Hausner Ratio (HR) were determined as outlined by Šeregelj et al. (2019). Compressibility ratio multiplied with 100 represent Carr’s index (data not shown). The classification of the encapsulate flowability and cohesiveness was made according to Jinapong, Suphantharika, & Jamnong, 2008. The colour measurements were made with a Minolta reflectance colorimeter (Minolta ChromaMeter CR-300, Minolta, Osaka, Japan) considering the CIELab colour system. Chroma or saturation (C*), and hue angle (h°) were calculated according to the formulas: $C^* = \sqrt{a^{*2} + b^{*2}}$ and $h^\circ = \arctan(b^*/a^*)$. The oxidation stability of the carrot waste extract and the encapsulated carrot waste extract were investigated by induction time (h) on a Rancimat 670 equipment, at 100 and 110 °C and air flow of 18–20 L/h (ISO 6886:2016). FTIR spectra were recorded by an IRAffinity-1 spectrometer (SHIMADZU, Japan). The solid samples were compressed into pellets with KBr, while the carrot oil extract was analysed on the surface of the blank KBr pellet. Measuring parameters were: spectral range 4000–500 cm^{-1}; resolution of 4 cm^{-1}. Raman spectra were acquired using XploRA Raman spectrometer from Horiba Jobin Yvon under the following conditions: laser at 785 nm (power reduced at 25%); grating of 1800 gr/mm; 20 s acquisition time; long working distance microscope objective (magnification × 50). The spectra were smoothed (Savitzky–Golay filters with 10 points and a second-order polynomial function) and baseline corrected using the Spectragryph software. The morphological properties of the encapsulates were assessed by JEOL JSM-6390LV scanning electron microscope (SEM). Before the analysis, the samples were covered with Au using a Baltec scd 005 sputter coater (30 mA for 100s).

2.5. *Statistical analysis*

The data were processed by one-way analysis of variance (ANOVA); when significant differences at $P \leq 0.05$ were found, Fisher’s Least Significant Difference (LSD) at $P \leq 0.05$ was determined. The statistical analyses were performed with the Statgraphics® Centurion XVI program (Statpoint Technologies, USA); mean and standard error values were computed using the software Excel (Microsoft® Office Excel 2016). When applicable, the results were analysed by *t*-test ($P \leq 0.05$) with the OriginPro 8 SR2 software (OriginLab Corporation, MA, USA). The optimizations were carried out using Design-Expert version 10 (Stat- Ease, Inc., MN, USA). The data are presented as mean ± standard deviation (SD) of three independent experiments.

3. Results and discussion

3.1. *Chemical characterization of sunflower oil and carrot waste extract*

Due to their non-toxicity, the vegetable oils are considered as green solvents in the extraction processes of carotenoids; depending on their composition and fatty acid profile, their consumption might even improve the human health. However, the relatively high viscosity of vegetable oil presents a limiting factor for their effective application in the extraction process, because a high solvent viscosity is generally associated with heightened solvent migration through the matrix, which affects the extraction efficiency. Sachindra and Mahendrakar (2005) studied the extractability of carotenoids from shrimp wastes and observed that the highest yield was obtained using sunflower oil; therefore, in this study sunflower oil was selected as the “green” solvent for the recovery of carotenoids from carrot processing waste. The carotenoids, tocopherols, fatty acids composition, and antioxidant capacity of sunflower oil and carrot waste extract are presented in Table 1.

The carotenoids were completely absent in sunflower oil. Similarly, Rafalowski, Zegarska, Kuncewicz, and Borejszo (2008) reported the nonexistence of carotenoids in sunflower, grapeseed, and sesame oils, but spotted a high β-carotene content in pumpkin seed oil and linseed oils. The presence of carotenoids in edible oils is very important because of the natural antioxidant properties; that is why sunflower oil is a perfect medium for carotenoids enrichment from carrot waste. The total carotenoid content in the carrot waste extract was 65.64 mg/kg oil; the β-carotene was predominant (45.10 mg/kg), followed by α-carotene (13.97 mg/kg), and cis β-carotene (6.56 mg/kg). Li et al. (2013) used ultrasound-assisted extraction and sunflower oil to isolate carotenoids from fresh carrots, and obtained the highest β-carotene yield (334.75 mg/L) after 20 min; additionally, β-carotene represented 97% of the total carotenoid content, while the other carotenoids (α-carotene, lutein, and 9-cis-carotene) were scarce.

In sunflower oil, α-tocopherol was the most abundant tocol (559.41 mg/kg), followed by β-tocopherol (22.8 mg/kg) and γ-tocopherol (4.86 mg/kg). Zaunschirm et al. (2018) found higher levels of α- and γ-tocopherol (788.0 and 35.7 mg/kg, respectively), and β-tocopherol was not analysed, while da Silva et al. (2020) identified only α-tocopherol (149.76 mg/100 g). A total tocopherol content between 303.8 and 1187.9 mg/kg for different sunflower varieties grown in various French locations is reported; the tocopherols content is modified by genotype and meteorological conditions, and the tocopherol content is highly correlated to temperature (Ayerdi Gotor et al., 2015). No significant differences for β- and γ-tocopherols were found between sunflower oil and carrot waste extract, while α-tocopherol decreased from 559.41 to 488.82 mg/kg. After the enrichment of sunflower oil with β-carotene from carrots, a slight decrease in α-tocopherol content was detected (da Silva et al., 2020).

No significant differences for fatty acids content were observed between sunflower oil and carrot waste extract. Linoleic acid with ~54.5

Table 1
Carotenoids, tocopherols, fatty acids composition, and antioxidant activity of sunflower oil and carrot waste extract. The results are presented as means ± standard deviation.

Characteristics	Sunflower oil	Carrot waste extract
Carotenoids (mg/kg)		
α-carotene	nd	13.97 ± 1.49
β-carotene	nd	45.10 ± 2.80
cis β-carotene	nd	6.56 ± 0.70
Tocopherols (mg/kg)		
α-tocopherol	559.4^{a} ± 6.69	488.82^{b} ± 4.19
β-tocopherol	22.8^{a} ± 1.54	21.13^{a} ± 0.63
γ-tocopherol	4.86^{a} ± 0.12	4.76^{a} ± 0.01
Fatty acids (g/100 g)		
C 16:0 (Palmitic)	5.84^{a} ± 0.13	5.95^{a} ± 0.12
C 16:1 (Palmitoleic)	0.07^{a} ± 0.01	0.08^{a} ± 0.01
C 18:0 (Stearic)	3.47^{a} ± 0.06	3.21^{a} ± 0.04
C 18:1n9c (Oleic)	35.84^{a} ± 0.24	36.42^{a} ± 0.11
C 18:2n6c (Linoleic)	54.78^{a} ± 0.33	54.33^{a} ± 0.26
Antioxidant activity (μmol TE/100 g oil)		
BCB	31.69^{a} ± 1.71	159.54^{b} ± 6.95

Different letters in the same row indicate a significant difference among means ($P \leq 0.05$) following *t*-test; nd - not detectable; BCB - β-carotene bleaching assay; TE - Trolox equivalents.

g/100 g was the predominant fatty acid, followed by oleic acid ~35.5 g/100 g, while only traces of palmitic, stearic, and palmitoleic acids were found. A similar fatty acid composition was reported for sunflower oil (Panda, Sridhar, Prakash, Rama Rao, & Raju, 2016), that is constituted by about 90% unsaturated fatty acids (linoleic and oleic acid) and 10% saturated acids (palmitic and stearic acid). The fatty acid composition, which affects the physical and chemical characteristics of the oils, is influenced by species, environment and growing conditions. Studying sunflowers grown in the East Mediterranean region, Akkaya, Cil, Cil, Yuce, and Kola (2019) reported that high temperatures and low amounts of rains during the seed filling period increase oleic acid content. A high oleic acid content improves oil resistance to high temperatures and oxidation, an advantageous feature for the food industry.

The β-carotene bleaching assay (Table 1) has a high specificity for lipophilic compounds. The highly unsaturated β-carotene is oxidized by the free radicals, but this process can be minimized by the presence of antioxidants. In fact, the enrichment of sunflower oil with carotenoids from carrot waste led to a significant increase, from 31.69 to 159.54 μmol TE/100 g, in antioxidant capacity. The α-tocopherol is considered as an efficient antioxidant in a hydrophobic milieu because inhibits lipid peroxidation and scavenges lipid peroxyl radicals, thus preventing the propagation of free radical-mediated chain reactions. The β-carotene and α-tocopherol can act synergistically as an effective "radical-trapping antioxidant", and the lipid peroxidation inhibition by a combination of the two fat-soluble antioxidants is superior to the sum of their individual inhibitions (Fiedor & Burda, 2014).

3.2. *Evaluation of the experimental design for optimization of wall material formulations*

The properties of the wall material and the drying parameters are the main factors that can affect the efficiency of encapsulation (Jafari, Assadpoor, He, & Bhandari, 2008). Hence, response surface methodology was applied to determine the optimal wall material formulations for the encapsulation of the carotenoids from carrot waste extract by freeze and spray drying. The experimental design performed by SCMD, along with the responses (Tcar, EE, and BCB), is given in Table 2. Maltodextrin and whey protein were chosen as coating agents based on a previous study (Šeregelj et al., 2017), while inulin was used due to its promising encapsulation potential and prebiotic nature. The freeze drying technique was selected because of its suitability for heat-sensitive molecules, and the spray-drying because of its simplicity, cheapness, usefulness and wide practical use (Özkan & Bilek, 2014).

All the models were significant, with the exception of BCB for spray drying ($P = 0.0516$). The regression coefficients and significance test results are reported in Supplementary Table 1 and the response surface plots are shown in Fig. 1. All the characteristics for freeze and spray dried encapsulates were significantly influenced by the wall formulation. The combination maltodextrin + inulin increased Tcar and BCB in both processes, particularly in freeze drying. The combination whey protein + inulin had a positive effect on spray dried encapsulates and a negative influence on freeze dried encapsulates. The special cubic term (maltodextrin + whey protein + inulin) was significant only for spray drying. With reference to the encapsulation efficiency, all the models were linear, depicting the positive and relevant influence of whey protein on both encapsulates.

As seen in Table 2 and Fig. 1, in freeze dried encapsulates Tcar ranged from 0.68 to 1.35 mg β-carotene/100 g; the WM2 formulation (pure whey protein) exhibited the highest content. On the other side, in spray dried encapsulates the highest Tcar (0.93 mg β-carotene/100g) was from a binary whey protein and inulin blend (WM4). Generally, the Tcar values for spray dried samples are significantly lower than those for freeze dried encapsulates. During spray drying, the atomization of the feed material results in a very fine mist which increases the surface area, more exposed to heat; additionally, parts of the wall material may be removed from the core material even after homogenization. Such partially covered encapsulates are easily affected by heat. On the other hand, the freeze-dried samples after homogenization were dried without atomization and heat exposure (Saikia, Mahnot, & Mahanta, 2015). The pure maltodextrin formulation (WM5) exhibited the lowest Tcar for both techniques; the encapsulation efficiency results for this formulation indicated that the carotenoids remained on the particle surface and were susceptible to faster degradation. Pure maltodextrins present a very low surface activity, leading to poor encapsulation; therefore, maltodextrins are usually blended to materials with good emulsifying properties (Cano-Higuita, Velez & Teliz, 2015). The best encapsulation efficiency was achieved by the whey protein-inulin blend (WM4) and the pure whey protein (WM2 and WM6) formulations. The encapsulates changes in antioxidant capacity under the influence of the wall material mixtures were comparable to those for Tcar for both encapsulation techniques.

Table 2

Experimental design and results (mean ± standard deviation) for total carotenoid contents encapsulation efficiency, and antioxidant capacity of wall material mixtures for freeze and spray drying encapsulation of carrot waste extract.

WM formulation	Independent variables Share in WM			Response variables		
	Maltodextrin	Whey protein	Inulin	TCar	EE	BCB
Freeze drying						
WM1	0.5	0	0.5	$1.13^{cd} \pm 0.14$	$15.00^{b} \pm 0.82$	$65.89^{d} \pm 1.50$
WM2	0	1	0	$1.35^{e} \pm 0.10$	$54.03^{e} \pm 3.32$	$69.43^{e} \pm 2.76$
WM3	0.33	0.33	0.33	$1.01^{bc} \pm 0.17$	$47.89^{d} \pm 1.17$	$52.41^{c} \pm 1.73$
WM4	0	0.5	0.5	$0.94^{b} \pm 0.03$	$59.77^{f} \pm 3.52$	$50.88^{bc} \pm 0.88$
WM5	1	0	0	$0.68^{a} \pm 0.07$	$0.00^{a} \pm 0.00$	$37.94^{a} \pm 1.12$
WM6	0	1	0	$1.30^{de} \pm 0.12$	$56.14^{ef} \pm 1.58$	$69.85^{e} \pm 3.10$
WM7	0.5	0.5	0	$1.00^{bc} \pm 0.06$	$21.59^{c} \pm 3.29$	$49.63^{bc} \pm 1.21$
WM8	0	0	1	$0.99^{bc} \pm 0.10$	$21.80^{c} \pm 4.81$	$48.52^{b} \pm 1.52$
Spray drying						
WM1	0.5	0	0.5	$0.62^{bc} \pm 0.05$	$3.56^{b} \pm 0.01$	$32.85^{d} \pm 1.54$
WM2	0	1	0	$0.69^{c} \pm 0.08$	$65.81^{g} \pm 0.32$	$32.42^{d} \pm 1.31$
WM3	0.33	0.33	0.33	$0.58^{b} \pm 0.01$	$36.00^{d} \pm 0.41$	$27.42^{c} \pm 0.89$
WM4	0	0.5	0.5	$0.93^{e} \pm 0.02$	$49.87^{e} \pm 0.21$	$41.90^{f} \pm 1.13$
WM5	1	0	0	$0.35^{a} \pm 0.10$	$0.00^{a} \pm 0.00$	$12.44^{a} \pm 1.90$
WM6	0	1	0	$0.68^{bc} \pm 0.03$	$62.97^{f} \pm 0.54$	$31.45^{d} \pm 1.30$
WM7	0.5	0.5	0	$0.71^{c} \pm 0.04$	$29.64^{c} \pm 0.31$	$37.07^{e} \pm 1.25$
WM8	0	0	1	$0.42^{a} \pm 0.09$	$0.00^{a} \pm 0.00$	$20.04^{b} \pm 0.51$

WM: Wall material, TCar: Total carotenoid content (mg β-carotene/100 g encapsulates), EE: Encapsulation efficiency (g/100 g), BCB: β-carotene bleaching assay (μmol TE/100 g encapsulates). For each encapsulation technique, different letters in the same column indicate significant differences ($P \leq 0.05$) among samples following LSD test.

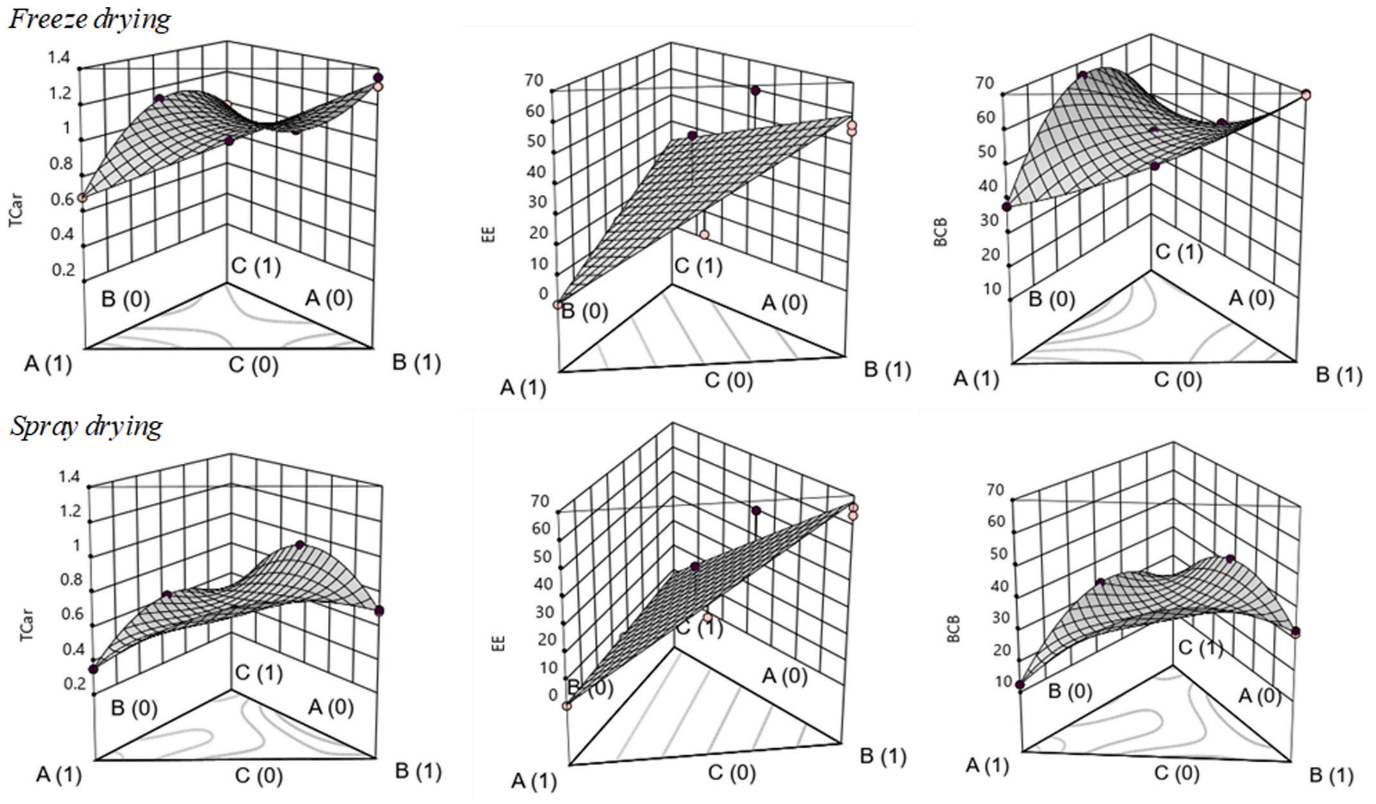

Fig. 1. Response surface plots for total carotenoid content (Tcar, mg β-carotene/100 g encapsulate), encapsulation efficiency (EE, g/100 g), and BCB (β-carotene bleaching assay (μmol TE/100 g encapsulate) as a function of wall material formulation (A, maltodextrin; B, whey protein; C, inulin) for carrot waste oil encapsulation by freeze drying and spray drying techniques.

The ability of the carotenoids to inhibit the bleaching of β-carotene by linoleic acid was reported also by Hanachi and Naghavi (2016), and by Hidalgo et al. (2019).

To identify the optimal wall materials for carrot waste encapsulation, the maximum values of the response variables within the ranges of the independent variables were considered. The multicriteria methodology (multi-response), was used to find an optimal point considering different responses at the same time. The multi-response optimization of wall materials with predicted and observed response variables is presented in Table 3. The most suitable wall material for freeze drying was pure whey protein; the predicted values of Tcar, EE, and BCB were 1.33 mg β-carotene/100 g encapsulates, 59.21g/100 g, and 69.64 μmol TE/100 g encapsulates, respectively. On the other hand, the most suitable wall material for the spray drying technique was the blend of 71 g/100 g whey protein and 29 g/100g inulin; the predicted values of Tcar, EE, and BCB were 0.92 mg β-carotene/100 g encapsulate, 50.74 g/100 g, and 41.58 μmol TE/100 g encapsulates, respectively. To validate the accuracy of the optimization model, the Tcar, EE, and BCB observed values of the optimal freeze dried (FDE Opt) and optimal spray dried (SDE Opt) encapsulates were compared with the predicted values: all the observed response values were not significantly different from the predicted values at $P < 0.05$.

Wall material nature is one of the most influential factors in the encapsulation efficiency of bioactive compounds. In this case, the high lactose amounts in whey protein enhanced capsule solidification ratio during the drying process, and the carrot waste oil extract droplets were locked in the dry matrix. Moreover, by adsorbing to the oil-water interface, or by binding, entrapping, coating a bioactive compound, the proteins form a crust, essential for protecting the encapsulated bioactives (de Barros Fernandes, Vilela Borges, Alvarenga Botrel, & de Oliveira, 2013).

The functionality of wall materials that are optimal for spray drying is conditioned by their effective emulsification, which was not the case when the proportion of inulin or maltodextrin in the blends was high. Due to the absence of surface-active wall constituent and the lack of emulsification properties, carbohydrates cannot be used as wall materials; however, incorporating carbohydrates into the wall improves the drying properties of the wall matrix, most likely by enhancing the formation of a dry crust around the drying droplets. In general, carriers that are effective emulsifiers or film formers exhibit better bioactives retention than carriers that lack those properties. However, carriers do not often possess both properties, hence, to improve the efficiency of encapsulation wall materials blends may be used (de Barros Fernandes et al., 2013).

Table 3
Multi-response optimization of wall material (WM) for freeze and spray drying encapsulation of carotenoids present in carrot waste extract.

	Predicted share in WM			Predicted response variables			Observed response variables		
	MDx	Whey protein	Inulin	TCar	EE	BCB	TCar	EE	BCB
Freeze drying	0	1	0	1.33	59.21	69.64	1.31 ± 0.02	63.69 ± 3.69	70.06 ± 5.13
Spray drying	0	0.71	0.29	0.92	50.74	41.58	0.87 ± 0.01	53.78 ± 1.26	41.23 ± 2.69

MDx: maltodextrin, TCar: Total carotenoid content (mg β-carotene/100 g encapsulates), EE: Encapsulation efficiency (g/100 g), BCB: β-carotene bleaching assay (μmol TE/100 g encapsulates).

3.3. Physicochemical characterization of FDE Opt and SDE Opt

Table 4 shows the water activity (0.18 and 0.16) and moisture content (4.05 and 3.35 g/100 g) of FDE Opt and SDE Opt respectively: their low values contribute to a long shelf life of the encapsulates, due to decreased chemical reactions, enzymatic activity and inhibition of microorganisms growth. Ramakrishnan, Adzahan, Yusof, and Muhammad (2018) reported that low moisture content and water activity are desirable to prevent agglomeration and caking, which may result in wet powders, bioactive compounds degradation, and flowability and dispersion hindrance, in order to extend the shelf life of the powders. The moisture content and a_w values of the spray dried samples were lower than those obtained by freeze drying. Similar results were reported by Quispe-Condori, Aranda Saldana, and Temelli (2011), which concluded that at higher inlet temperature the heat transfer rate is greater and provides the driving force for moisture evaporation.

Since water mobility causes a high rate of enzymatic or chemical reactions and results in some quality deteriorations and changes in the physical properties in the encapsulates, hygroscopicity is one of the main factors to predict stability. FDE Opt showed a low hygroscopicity: after seven days, water absorption was 2.10 g/100 g encapsulate; SDE Opt instead exhibited an hygroscopicity four-time higher than FDE Opt, indicating its stronger capacity to attract water molecules when in contact with the surrounding air. Tonon, Brabet, and Hubinger (2008) reported that the low-moisture spray dried encapsulates has the greatest capacity to absorb water from the environment and hence were more hygroscopic. However, the moisture–hygroscopicity relationship cannot be generalized for all commodities. In fact, Ahmed, Akter, Lee, and Eun (2010) found that the hygroscopicity of spray-dried sweet potato was greatly affected by wall material, with no direct relationship to varying moisture content, while Caparino et al. (2012) reported that maltodextrin greatly influences the hygroscopicity of spray-dried mango powder. Our findings are in agreement with their conclusions, because the wall material with inulin and whey protein influenced the hygroscopicity of the spray-dried sample, probably for the high water affinity of inulin, which has hydrophilic groups that strongly bind to water (Fernandes, Boares, Botrel & de Oliveira, 2014). With respect to solubility, i.e. the ability of powders to form a solution in water, which is considered as the key determinant that influences functional characteristics of encapsulates in food systems, the results of both samples were similar (>50%).

Table 4
Physicochemical characteristics (mean ± standard deviation) of optimal freeze dried (FDE Opt) and spray dried (SDE Opt) encapsulates.

Characteristics		FDE Opt	SDE Opt
Water activity (aw)		$0.18^b \pm 0.00$	$0.16^a \pm 0.00$
Moisture content (g/100 g)		$4.05^b \pm 0.03$	$3.35^a \pm 0.01$
Higroscopicity (g/100 g)		$2.10^a \pm 0.01$	$8.45^b \pm 0.11$
Solubility (g/100 g)		$58.72^b \pm 0.27$	$56.79^a \pm 0.98$
Mean diameter; Dsr (μm)		$234.95^b \pm 76.00$	$30.99^a \pm 12.47$
d (0.1)		$45.80^b \pm 0.92$	$7.28^a \pm 1.00$
d (0.5)		$132.47^b \pm 24.90$	$16.67^a \pm 5.32$
d (0.9)		$591.97^b \pm 224.97$	$80.91^a \pm 29.64$
Polydispersity index (Span)		$4.01^a \pm 1.02$	$4.37^b \pm 0.70$
Bulk density; Db (g/mL)		$0.26^a \pm 0.02$	$0.34^b \pm 0.06$
Tapped density; Dt (g/mL)		$0.38^a \pm 0.04$	$0.49^b \pm 0.05$
Compressibility ratio; CR		$0.32^b \pm 0.05$	$0,31^a \pm 0.07$
Hausner ratio; HR		$1.46^a \pm 0.05$	$1.44^a \pm 0.07$
Flowability		Fair	Fair
Cohesiveness		High	High
CIE Lab			
*L**		$82.44^a \pm 0.03$	$89.04^b \pm 0.00$
*a**		$-0.10^a \pm 0.03$	$-2.12^b \pm 0.01$
*b**		$37.72^b \pm 0.07$	$24.90^a \pm 0.05$
C*		$37.72^b \pm 0.04$	$24.99^a \pm 0.05$
h°		$90.15^a \pm 0.04$	$94.86^b \pm 0.01$
Oxidative stability (h)			
Temperature	**Carrot waste oil extract**	**FDE Opt**	**SDE Opt**
100 °C	6.10 ± 0.03^a	$8.55^c \pm 0.4$	$7.10^b \pm 0.3$
110 °C	3.00 ± 0.2^a	$4.25^c \pm 0.2$	$3.50^b \pm 0.1$

Different letters in the same row indicate a significant difference among means ($P \leq 0.05$) following *t*-test.

Particle size and its distribution are the most important characteristics of encapsulates, influencing the properties of intermediate and final products. In some cases, large particles can be undesirable because of unpleasant mouthfeel, while in others they may be desired, like when the goal is to obtain products with visible elements. Particle size, particle size distribution, as well as span values showed significant variation according to the encapsulation technique (Table 4) ($P < 0.05$); the FDE Opt showed greater particle size (from 45.80 to 591.97 μm) than the SDE Opt (from 7.28 to 80.91 μm). Similar results were published for wild blueberry pomace extracts encapsulated with soy protein isolate (Correia, Grace, Esposito, & Lila, 2017) and beetroot juice encapsulated with pumpkin oil cake protein (Čakarević et al., 2020). The greater particle size of freeze-dried samples can be explained by the low processing temperature and the lack of strength to break the frozen drops or to alter the surface during drying (Chen, Chi, & Xu, 2012). Conversely, the smaller and more uniform particles of the spray dried samples are due to the transit through a nozzle before entering the drier chamber that initiates the formation of relatively uniform particles (Correia et al., 2017). The polydispersity of encapsulates also has a critical impact on the release kinetics of encapsulated ingredients, as similar particles tend to have the same release rate (Ramos, 2011). The Span values, that summarise polydispersity, were >1, indicating a very broad distribution of particle sizes for both encapsulates. The probable reason for this high polydispersity is the presence of surface oil on both encapsulates and their agglomeration.

Flowability measures the free-flow characteristics of the encapsulates, and its knowledge is important for handling and processing operations, for the design of reliable storage systems, for the packaging and for the conditions of distribution. Encapsulates flowability is tested by bulk and tapped density, compressibility ratio and Hausner ratio (Table 4). The bulk and tapped density of FDE Opt (0.26 and 0.38 g/mL, respectively) were significantly lower ($P < 0.05$) than those of SDE Opt (0.34 and 0.49 g/mL, respectively). Generally, the results were expected to follow a common trend, influenced by different drying parameters. Calín-Sanchez et al., 2015 reported that freeze dried pomegranate rind samples exhibited the lowest bulk density, followed by connective, vacuum microwave and combined dried rinds, due to their low shrinkage. The trend in the increase of bulk and tapped density is also followed by smaller particle sizes. Similar results were reported by Šeregelj et al. (2019) and by Caliskan and Dirim (2016). In addition, Fernandes, Boares, Botrel & de Oliveira (2014) reported that encapsulates produced at greater inulin concentration showed the highest bulk and tapped density. A higher proportion of small inulin particles increases the density, as their accommodation in the inter-particles spaces is easier. The encapsulates flowability and cohesiveness, based on the Compressibility ratio and Hausner ratio, showed that both samples had fair flowing properties and high cohesiveness. Generally, the flowability depends on the properties of wall materials. Thus, in the study of Kulthe, Thorat, and Mhalaskar (2016) the β-carotene encapsulated with maltodextrin showed good flow characteristics, while the β-carotene encapsulated with potato starch and gelatin showed fair flow characteristics.

The colour is a parameter influencing sensory attractiveness and is an important quality indicator, especially for supplemented products where encapsulates are used as colorant. FDE Opt was characterized by lower lightness ($L^* = 82.44$) and greenness ($a^* = -0.10$), as well as greater yellowness ($b^* = 37.72$) compared to SDE Opt ($L^* = 89.04$, $a^* = -2.12$, $b^* = 24.90$). The darker colour and superior yellowness of FDE Opt may be explained by the higher surface oil content; additionally, the colour differences may be related to particle size diversity, because smaller particles increase lightness and decrease yellowness. A similar trend was observed by Ogrodowska, Tanska, Brandt, and Czaplicki (2019), who investigated the influence of the same drying techniques on bio-oil encapsulates quality. The chroma (C*), or degree of colour saturation, was higher for FDE Opt, confirming its higher colour intensity. The hue angle (h°) range was within the 90° region, hinting to an apparent yellow colour. Our results agreed with those of mango pulp encapsulates (Sharma, Kadam, Chadha, Wilson, & Gupta, 2013), and of pumpkin seed oil encapsulates (Ogrodowska et al., 2017).

The oxidative stability is represented by the induction time values: a greater induction time indicate a greater oxidative stability. The pure carrot waste extract showed induction times of 6.1 and 3.0 h at 100 °C and 110 °C, respectively. According to EN141112 (2003), an oil can be considered stable if has at least 6 h induction time, measured with a Rancimat equipment, thus our carrot waste extract complied to the rule. The induction time of the optimal encapsulates was higher than that of the oil, proving that the carrot waste extract was indeed encapsulated. FDE Opt had a larger impact on oxidation delay, increasing the induction time ~30%, while SDE Opt raised it only ~15%, probably because of lower carotenoid content. A greater induction time increase for the freeze dried sample when encapsulating non-dewaxed propolis with different wall materials by different techniques is reported (Šturm et al., 2019). Oxidative stability and induction time may be influenced by the wall materials: the oxidative stability of the pitaya seed oil encapsulated by spray drying showed variations of the induction time from 5.20 to 38 h, depending on the wall material (Lim, TanBakar & Ng, 2012).

Fig. 2A shows the FTIR spectra of inulin, WP and carrot sunflower oil extract, and of the corresponding encapsulates i.e. FDE Opt and SDE Opt. Our results indicate that bands assigned to carotenoids are overlapped primarily by bands from the extraction medium i.e. sunflower oil, and this trend can be noticed also in the spectra of the encapsulates. Additionally, the bands from WP, as dominant carrier, further overlapped the spectra of the encapsulated carrot extract. Considering the chemical properties of carriers and carrot extract, and the results of FTIR analysis, the compounds most probably formed a physical mixture inside the encapsulates, without significant chemical interactions. Also, in our previous research on spray drying and freeze drying as encapsulation methods we found that certain amounts of carriers are necessary for a successful finalization of the encapsulation process and an adequate protection of the carotenoids (Šeregelj et al., 2019).

Raman microscopy was employed in order to evaluate the efficiency of the encapsulation process through the analysis of surface-bounded extract. Since the carriers (i.e. maltodextrin, WP and inulin) dominate in formulations, we used their “marker” bands to distinguish them from the carrot extracts bands. The bands from inulin and WP clearly separate these two carriers from carrot oil extract, dominated by bands originated from sunflower oil (Fig. 2B). However, the 1157 cm^{-1} and 1525 cm^{-1} bands in the spectrum of carrot oil extract are due to the carotenoids, while the characteristic carotenoid band at ~1008 cm^{-1} is probably overlapped by the spectra of sunflower oil and WP (~1006 cm^{-1}) (Camorani et al., 2015), close to the position of the phenylalanine band (Zhao, Ma, Yuen, & Phillips, 2004). Raman spectra of FDE Opt and SDE Opt mainly exhibited the bands from sunflower oil and extracted carrot carotenoids, indicating the presence of active compounds on the surface of encapsulates. This is expected since both encapsulation techniques provide encapsulates with a certain amount of surface-bound active compounds. Nevertheless, the addition of sufficient quantity of carriers provides an efficient protection and minimizes the loss due to free active compounds on the surface of encapsulates.

Fig. 3 presents the SEM micrographs of the FDE and SDE optimal encapsulates. The FDE sample did not have a specific shape. Generally, the irregularities (dents and wrinkles) on the surface of freeze-dried samples may be associated with a low drying temperature Moayyedi et al., 2018). On the other side, the formation of concavities on the surfaces of the spray dried encapsulates is attributed to the shrinkage of the particles due to a dramatic moisture loss after cooling (Silva, Stringheta, Teófilo, & de Oliveira, 2013). Both samples appear agglomerated, possibly for the presence of surface oil.

4. Conclusion

The carrot waste extract was successfully encapsulated by freeze and spray drying techniques with different wall materials. The optimization study to find the most suitable wall material for these techniques was performed by simplex centroid mixture design. The optimal wall materials which predict the highest values for the analysed responses (Tcar, EE, and BCB) were pure whey protein for freeze drying, and 71 g/100 g whey protein – 29 g/100 g inulin mixture for spray drying. The physicochemical characteristics of the optimal carrot waste encapsulates were significantly affected by the different drying techniques: thus, freeze drying gave a better encapsulate in terms of encapsulation efficiency, antioxidant capacity, hygroscopicity, and oxidative stability, while spray drying provided an encapsulate with lower water activity, moisture content and particle size. The high temperature of spray drying

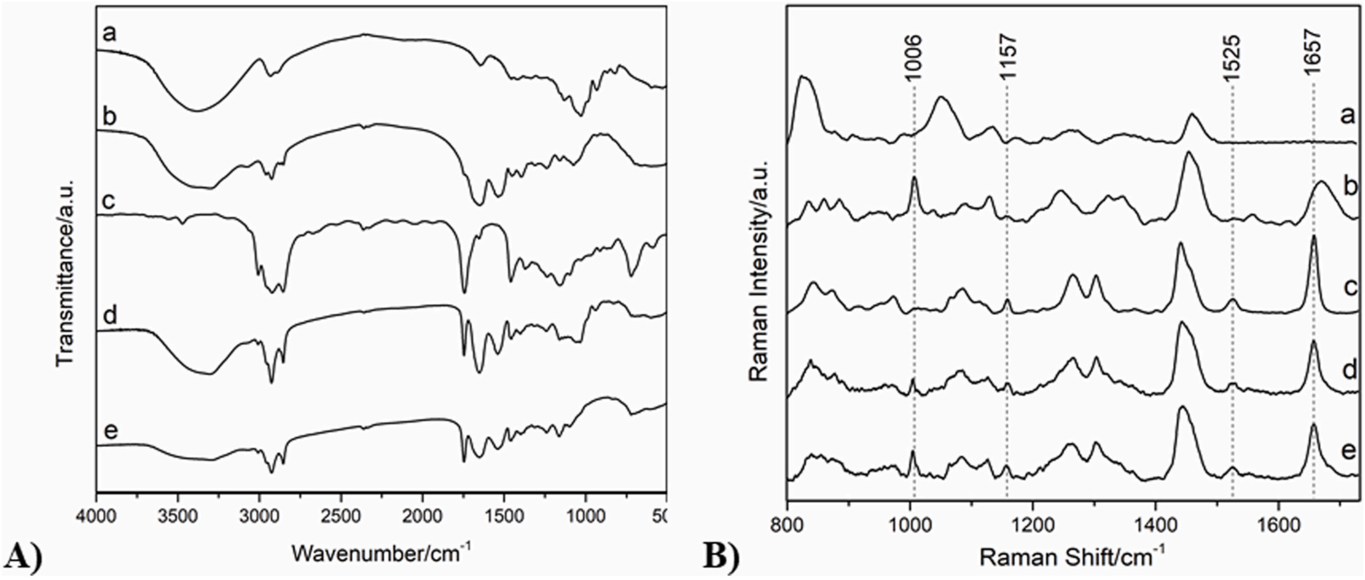

Fig. 2. - FTIR spectra (A) and Raman spectra (B) of inulin (a), WP (b), carrot oil extract (c), optimal spray dried encapsulates (SDE Opt) (d), and optimal freeze dried encapsulates (FDE Opt) (e).

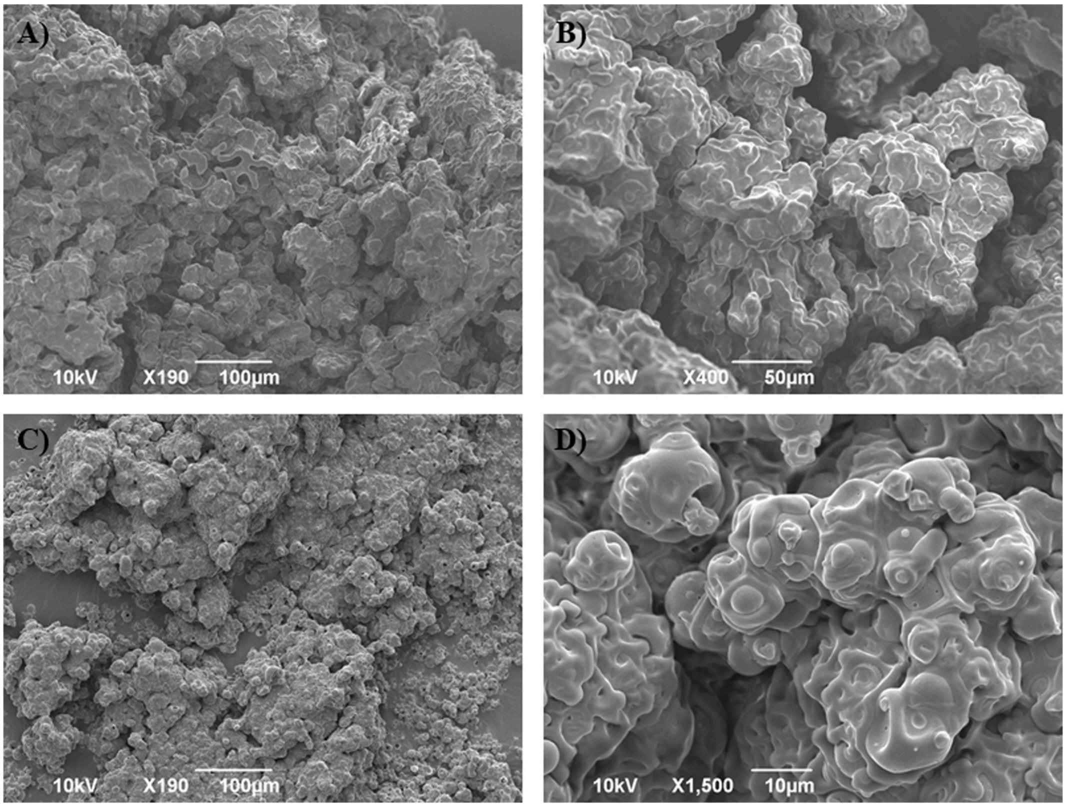

Fig. 3. - Scanning electron morphology of optimal freeze dried encapsulates (FDE Opt) magnified 190X (A) and 400X (B), and of optimal spray dried encapsulates (SDE Opt) magnified 190X (C) and 1500X (D).

influenced the colour properties of encapsulates, who had higher *L** (lightness) than the freeze-dried samples. The techniques and the formulations used in the present study are appropriate to obtain carotenoid-rich additives in encapsulated form, particularly appropriate for the preparation of functional food with improved nutritional, colour and bioactive properties.

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CRediT authorship contribution statement

Vanja Šeregelj: Writing - original draft. **Gordana Ćetković:** Conceptualization. **Jasna Čanadanović-Brunet:** Conceptualization. **Vesna Tumbas Šaponjac:** Data curation, Methodology. **Jelena Vulić:** Visualization, Investigation. **Steva Lević:** Software, Validation. **Viktor Nedović:** Supervision. **Andrea Brandolini:** Writing - review & editing. **Alyssa Hidalgo:** Supervision, Writing - review & editing, Methodology, Software.

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.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lwt.2020.110696.

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