Article

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Optimization of the production of double-shell microparticles containing fish oil

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Abstract

Fish oil incorporation into food products is a challenge because long-chain fatty acids are susceptible to oxidation. Microencapsulation is an alternative for protecting and delivering fish oil besides masking undesirable flavours. This work aimed to produce spray-chilled microparticles using spray-dried microparticles loaded with fish oil as the core material and evaluate the effects of core concentration and lipid wall material composition on the apparent viscosity of the feeding material (suspension), microparticle mean diameter (D_{50}), moisture content and eicosapentaenoic acid and docosahexaenoic acid losses. Double-shell microparticles containing fish oil were successfully obtained. Higher core concentrations resulted in higher feeding material viscosities and microparticles with higher D_{50} values and higher moisture content, but suitable for food applications. Less eicosapentaenoic acid and docosahexaenoic acid loss was achieved with lipid matrixes containing palm fat/vegetable fat ratios of up to 40/60 or a ratio of 50/50 when associated with a low concentration of core material. The remaining eicosapentaenoic acid and docosahexaenoic acid content observed in the final double-shell microparticles and its good oxidative stability can be considered sufficient for the successful application of these microparticles in foods. These findings may contribute to expanding the use of microencapsulated fish oil.

Keywords

Central composite rotatable design, fish oil, spray drying, spray chilling, double shell

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INTRODUCTION

The health benefits of polyunsaturated fatty acids (PUFAs), such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), especially from fish oil sources, have been widely recognized (Arab-Tehrany et al., 2012; Di Giorgio et al., 2019; Kaushik et al., 2015; Sanguansri et al., 2016; Timilsena et al., 2017). However, PUFAs are susceptible to oxidation, which results in off-flavour, toxic compounds and a decrease in their nutritional value (Alamed et al., 2009; Kaushik et al., 2015; Tamjidi et al., 2014) making their incorporation into foods a challenge. Microencapsulation is an alternative to protect PUFAs against oxidation while masking their typical flavours (Arab-Tehrany et al., 2012; Encina et al., 2016).

Spray drying and spray chilling are considered to be microencapsulation technologies that provide good protection to sensitive substances, and both can be

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affordably used in industrial-scale production (Alvim et al., 2016). The wall material and the microencapsulation method must be carefully chosen for the protection and delivery of bioactive compounds (Sahari et al., 2016).

Spray-drying microencapsulation is one of the most suitable techniques for oil encapsulation, and many studies have reported its use for fish oil protection and delivery (Di Giorgio et al., 2019; Encina et al., 2016; Kurek et al., 2018). For food applications, generally hydrophilic wall materials (carbohydrate or protein) are used in spray-drying microencapsulation, and the obtained product is water soluble. For fish oil, in most cases, this solubility could lead to rapid flavour perception when consumed. Furthermore, depending on the emulsion characteristics and wall material composition, some surface oil will remain on the microparticles (Encina et al., 2016; Wang et al., 2011), which could negatively impact their stability during storage and flavour perception. The surface oil should be below 0.1% (w/w) in order to provide better protection during storage (Kaushik et al., 2015), although many studies on fish oil microencapsulation by spray drying have reported higher values (Fadini et al., 2018; Sanguansri et al., 2016; Vishnu et al., 2017).

On the other hand, the spray-chilling technique uses wall materials such as waxes and fats and results in hydrophobic microparticles but some core material may remain at or near the surface (Oxley, 2012). During storage, the crystallinity of the lipid matrix may change, and the core material may be released, changing the barrier properties (Tulini et al., 2017). However, due to its highly protective effect, the spray-chilling technique has recently been used more often in food research (Arslan-Tontul and Erbas, 2017).

Therefore, the purpose of this study was to optimize the lipid suspension composition used for producing double-shell microparticles containing fish oil using a central composite rotatable design based on the response surface methodology and characterize the obtained microparticles. Spray-dried microparticles loaded with fish oil were used as the core material in a spray-chilling process and double-shell microparticles were obtained. The independent variables were the core material concentration used in the spray-chilling microencapsulation process and the ratio between 100% hydrogenated palm oil (palm fat) (PF) and vegetable fat (VF) used as wall materials to produce the second shell. The responses under observation were the apparent viscosity of the spray-chilling feeding material (suspension), solid lipid microparticle mean diameter (D_{50}), moisture content and the EPA and DHA losses due to the microencapsulation process.

MATERIAL AND METHODS

Material

For the production of spray-dried microparticles (first shell), fish oil (W3, MEG-3® 4020 EE Oil, DSM, Mulgrave, Canada), acacia gum (Encapsia®, Nexira, Rouen, France), skimmed milk powder (Nestlé, Araçatuba, Brazil), grape juice (Aurora, Bento Gonçalves, Brazil) and polysorbate 80 (Tween® 80, Synth, São Paulo, Brazil) were used. For the production of spray-chilled microparticles (second shell), 100% hydrogenated palm oil (palm fat) with the following fatty acid composition: 0.42% 12:0; 0.88% 14:0; 38.19% 16:0; 0.12% 17:0; 55.19% 18:0; 0.14% 18:1; 0.51% 20:0; 0.07% 22:0 and 0.08% 24:0 (A. Azevedo Óleos Vegetais, Itupeva, Brazil), vegetable fat obtained from fully hydrogenated and interesterified vegetable oils with the following fatty acid composition: 0.14% 6:0; 2.65% 8:0; 2.71% 10:0; 40.29% 12:0; 13.67% 14:0; 10.26% 16:0; 24.44% C:18; 0.49% 18:1; 0.72% 18:2 and 0.24% 20:0 (AL Lette K39LT®, Cargill, Itumbiara, Brazil) and polyglycerol polyricinoleate (PGPR 4150®, Palsgaard, Juelsminde, Denmark) were used.

The chemicals used were methanol, chloroform and n-hexane of analytical grade obtained from Merck, (Darmstadt, Germany), and absolute ethanol obtained from Synth (São Paulo, Brazil). The Supelco 37 FAME Mix standard was obtained from Sigma-Aldrich (Pennsylvania, USA).

Statistical design and data analysis

A central composite rotatable design (2^2) was adopted to determine the influence of two independent variables on the apparent viscosity of the spray chilling-feeding material at 100 rpm (Y_1), solid lipid microparticle mean diameter (D_{50}) (Y_2), moisture content (Y_3) and EPA and DHA losses (Y_4). The complete design consisted of 11 experiments with 3 central and 4 axial points (Table 1). The independent variables were the core material concentration (spray-dried microparticles) (x_1) used in the spray-chilling microencapsulation process and the ratio between the palm fat (PF) and vegetable fat (VF) (x_2) used as the lipid wall material. The Statistica® 12 (StatSoft Inc., Tulsa, USA) program was used for data analyses with a 95% confidence interval. The following polynomial equation (1) was fitted to the data.

$$Y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_{11} x_1^2 + \beta_{22} x_2^2 + \beta_{12} x_1 x_2 \quad (1)$$

where Y is the predict response (the apparent viscosity of the spray chilling-feeding material at 100 rpm, solid lipid microparticle mean diameter (D_{50}), moisture

Table 1. Real levels of independent variables according to their values codified from a full factorial design (2^2)

	Values codified				
Parameters	−1.41	−1	0	1	+1.41
Core material[a] concentration (g/100 g, d.b.)	26	30	40	50	54
Lipid wall material composition (PF/VF ratios)	36/64	40/60	50/50	60/40	64/36

PF: 100% hydrogenated palm oil; VF: vegetable fat obtained from fully hydrogenated and interesterified vegetable oils.
[a]Microparticles loaded with fish oil and obtained by spray drying.

content and EPA and DHA losses). β_0 is the model constant, β_1 and β_2 the linear effects terms, β_{11} and β_{22} are the squared effects, β_{12} is the interaction effects and x_1 and x_2 correspond to independent variables, namely core material concentration used in the spray-chilling microencapsulation process and the ratio between PF and VF, respectively.

Microencapsulation procedure

The wall material composition used for the spray-dried microparticles (SD-M) production (first shell) was adapted from a previous study (Fadini et al., 2018) and composed, on dry weight basis, by acacia gum, skimmed milk powder and grape juice at a ratio of 48.4/33.3/18.3. The first two components were selected to provide the desired protection to the fish oil, and grape juice was used to mask unpleasant flavours. Additionally, these three components are typical items found in food products composition. The surfactant was Tween® 80 (0.48 g/100 g total emulsion). The wall material was dissolved in distilled water and the fish oil was added (20 g/100 g total emulsion, d.b.). The emulsion with a solid concentration of 30 g/100 g was obtained using an Ultra Turrax® (T18, IKA, Germany) (15,000 rpm/3 min). SD-M were produced in a mini spray dryer (B-290, Büchi, Flawil, Switzerland) with inlet and outlet temperatures of 150 ± 2 °C and 76 ± 3 °C respectively and feed sample rate of 10 mL/min. The spray nozzle diameter was 0.7 mm and it was maintained at 21 °C in order to protect the fish oil. The obtained microparticles were used as the core material in a second microencapsulation process performed by spray chilling.

For the production of spray-chilled microparticles (second shell) the mini spray dryer (B-290, Büchi, Flawil, Switzerland) was linked to a dehumidifier (B-296, Büchi, Flawil, Switzerland) for producing cool air. Different concentrations of core material (SD-M) were added to the melted lipid blends containing different ratios of PF and VF (Table 1). PGPR (4.8 g/100 g total feeding material) was used to maintain the feeding material viscosity low enough to be gravity-fed in the spray chiller using a spray nozzle with 2.0 mm diameter. The suspension was atomized through the cold chamber, and solid lipid microparticles (SLMs) were obtained. The process conditions were adapted from Alvim et al. (2016) with feeding material temperature of 80 °C and inlet temperature of 7 ± 1 °C.

Slip melting point

The slip melting points of the lipid blends used as wall materials were determined according to AOCS (2017a), method Cc.3.25 ($n = 10$).

Apparent viscosity of the spray-chilling feeding material

Apparent viscosity was measured at a shear rate of 100rpm in triplicate using a Brookfield rotational rheometer (Brookfield RVDV-III+, Stoughton, USA) with a small sample adapter and a cylinder spindle (S15) with an external to internal radius ratio of 0.75. The sample temperature was maintained at 80 °C using a water bath. This temperature was the same as that used in the spray-chilling process to keep the lipid matrix molten.

Moisture content and water activity (a_w)

Microparticle moisture content was measured gravimetrically by drying the samples in an oven at 105 °C/20 h (AOAC, 2006), and a_w was analysed using dew point water activity meter (AquaLab 4TEV, Decagon Devices Inc., Pullman, USA) at 25.0 ± 0.5 °C in triplicate.

Encapsulation efficiency (EE)

The SD-M EE was calculated by quantifying the microparticles' surface oil according to Carneiro et al. (2013) and was expressed as the percentage of the surface oil (SO) in relation to the total oil (TO). The SO was extracted by adding 15 mL of hexane to 1.5 g of SD-M in a glass jar with a lid, and shaking by hand for

2 min at room temperature. The mixture was filtered through Whatman N° 1 filter paper and the powder retained on the filter was rinsed three times with 20 mL of hexane. The filtrate solution containing the extracted oil was transferred to a clean flask, which was allowed to evaporate and then dried at 60 °C. The SO was calculated based on the difference between the weight of the initial clean flask and that containing the extracted oil (Jafari et al., 2008). The TO was measured by adding chloroform/methanol/water to 2.5 g of SD-M to obtain a separation of the two phases. The chloroform phase containing the extracted oil was transferred to a clean flask, which was allowed to evaporate at 100 °C and the TO calculated (Bligh and Dyer, 1959). The EE was calculated according to equation (2)

$$\%EE = ((TO - SO)/TO) \times 100 \quad (2)$$

EPA and DHA analysis

The content and composition of the fatty acid methyl esters were analysed by gas chromatography (Agilent 7890A – FID), using a fused silica capillary column of 100 m length, 0.25 mm inner diameter and 0.20 μm film thickness (CP-Sil 88, Agilent). Hydrogen was used as carrier gas (30 mL/min) and nitrogen as the make-up gas (30 mL/min). A split injector was used with a split ratio of 75:1 and a temperature of 260 °C. The flame ionization detector (FID) was operated at 260 °C. The column temperature was held at 140 °C for 6 min, and then programmed to 230 °C at 4.0 °C/min, and held for 24.5 min. at 230 °C. The fatty acids were identified by comparing the retention times of the sample to those of the standards (Supelco 37 Component FAME Mix) (AOCS, 2017b) and were quantified by internal normalisation. The results were expressed in g/100 g of sample, calculated from the percentage of area and using equation (3), where Ci = concentration of component i in the sample, expressed in g/100 g; % Ai = Area (in percentage) of component i expressed as the methyl ester; %L = percentage of total fat in the product; F = conversion factor (0.956 for fats and oils) (Food Standards Agency, 2002).

$$C_i = \frac{\%A_i \times \%L \times F}{100} \quad (3)$$

EPA and DHA losses after the double-shell microparticles production were calculated according to equation (4)

$$\%Losses = 100 - ((C_r/C_0) \times 100) \quad (4)$$

where C_r is the remaining concentration of EPA and DHA in the double-shell microparticles and C_0 is the initial concentration of EPA and DHA in the bulk fish oil.

Microparticle morphology

The surface morphology of the microparticles was observed by using a scanning electron microscope (SEM) (DSM 940 A FOCUS, Zeiss, Jena, Germany) with a digital camera (SMZ 745 T, Nikon, Tokyo, Japan). A layer of gold was sputtered on the microparticle surfaces (Goldstein et al., 1992), and accelerating voltages of 10 kV and 5 kV were used to observe the microparticles produced by spray drying and spray chilling, at magnifications of 500× and 200×, respectively.

Optical microscopy was performed using an Olympus BX41 microscope with a digital camera Olympus Q-Color3 at a magnification of 1000×.

Mean diameter and size distribution

The mean diameter and size distribution were determined by a light scattering instrument (LV 950-V2, Horiba, Kyoto, Japan). The samples were prepared according to Alvim et al. (2016). The size reported is the D_{50} (mean diameter relative to 50% of the accumulated size distribution), and the polydispersity was given by a span index calculated according to equation (5), where $D_{0.1}$, $D_{0.5}$ and $D_{0.9}$ are the percentiles of the undersized particle distribution curve ($n = 6$).

$$Span = D_{0.9} - D_{0.1}/D_{0.5} \quad (5)$$

Oxidative stability under accelerated conditions

The accelerated Rancimat test is an indirect measure of oxidative stability that has been increasingly accepted and used to evaluate microencapsulated oils (Di Giorgio et al., 2019; Gallardo et al., 2013; Noello et al., 2016; Velasco et al., 2000). The bulk fish oil, the SD-M (core material) and the double-shell microparticles were analysed after the particles production and at seven months of storage at 6 °C. The analysis was performed in a 679 Rancimat apparatus (Metrohm, Switzerland) with a 3 g sample at 100 °C and 20 mL air/h. The short-chain volatile acids formed under these controlled conditions are collected in distilled water increasing its conductivity (Di Giorgio et al., 2019). The oxidative stability was expressed as induction period (IP) that corresponds to the inflection point in the oxidation curve (Velasco et al., 2000). As higher is the IP, more stable is the sample (Gallardo et al., 2013). Analyses were performed in duplicates.

RESULTS AND DISCUSSION

Spray-dried microparticle characterization

The encapsulation efficiency (EE) of the SD-M was $96.60 \pm 0.07\%$. Higher EE values correspond to lower surface oil contents and, thus, better core material stability against oxidation reactions. Oil microencapsulation EE values between 60.00% and 90.00% are considered satisfactory and are influenced by the feeding emulsion, wall material characteristics and process drying rate (Botrel et al., 2017; Vishnu et al., 2017). Other studies that have microencapsulated fish oil by spray drying, obtained EE values of 74.90% using barley β-D-glucan modified starch complex as wall materials (Kurek et al., 2018) and from 57 to 90% using soybean protein as wall material (Di Giorgio et al., 2019).

Despite the high EE obtained in our study, the hydrophilic wall materials used led to a fast perception of fish oil taste, according to our previous sensorial screening (Fadini et al., 2018).

The SD-M moisture content and a_w were 3.10 ± 0.01 g/100 g and 0.18 ± 0.00, respectively. Bakry et al. (2017) microencapsulated tuna oil by spray drying (inlet temperature of 170 °C) and obtained moisture content values of 1.67 and 1.90 (g/100 g) and an a_w of 0.14 and 0.18. The moisture content must be sufficient to prevent the caking of the powder particles and particle collapse, which would release the core material and expose it to degradation reactions (Wang et al., 2011). For good stability during storage, food-grade powders should have a moisture content below 3 g/100 g (Thirundas et al., 2012) and an a_w between 0.1 and 0.4, depending on the dry product type (Tontul and Topuz, 2014).

The mean diameter (D_{50}) of the SD-M was 7.46 ± 0.61 μm, and the span index was 3.0. Di Giorgio et al. (2019) microencapsulated fish oil by spray drying and obtained mean apparent diameters between 15 and 20 μm. The small particle size observed in our study is particularly interesting since the SD-M were used as core material in the second microencapsulation step performed by spray chilling.

Slip melting point of the lipids applied as wall materials in the production of spray-chilled microparticles

The slip melting point of all lipid blends containing different ratios of PF/VF (36/64, 40/60, 50/50, 60/40 and 64/36) increased proportionally with increasing PF concentration (Figure 1) and ranged from 51.15 ± 0.13 to 55.47 ± 0.10 °C. This result can be explained by the higher concentration of palmitic and stearic acids in the PF (93.38%) when compared to VF (34.70%). Procopio et al. (2018) observed that a decrease in the lipid wall material melting point led to agglomeration of the microparticles. Generally, the SLMs obtained in our study were not soft or sticky, showed no aggregates and the powder obtained could be easily handled, what may be explained by the high slip melting point of all lipid blends studied.

Apparent viscosity of spray-chilling feeding material, microparticle properties and EPA and DHA losses

The experimental conditions and results are shown in Table 2 and the mathematical models for the responses

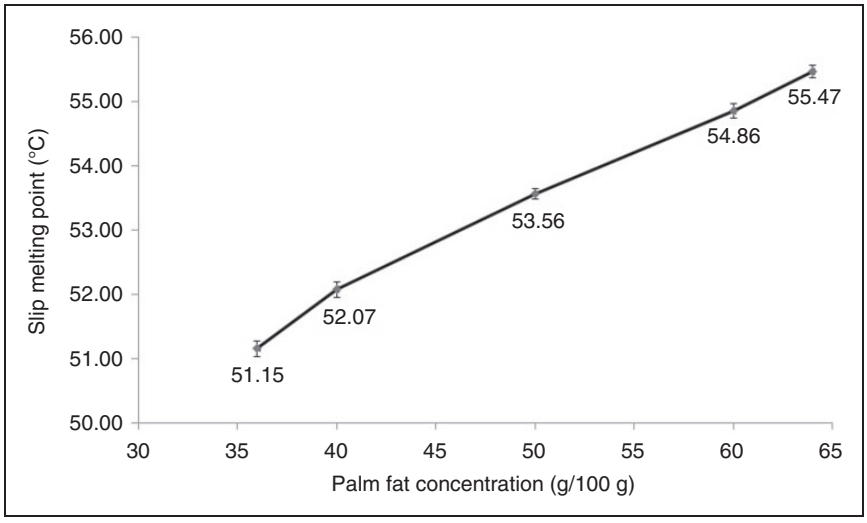

Figure 1. The slip melting point profile of the lipid blends according to different concentrations of palm fat in the wall material.

Table 2. Matrix of experimental central composite rotatable design for the optimisation of the production of double-shell microparticles containing fish oil

Trial	Core material concentration (g/100 g dispersion) x^1	PF/VF (ratio) x^2	η (mPa.s)	Microparticle mean diameter (D^{50})	Moisture content (g/100 g)	EPA and DHA losses (%)
			Y^1	Y^2	Y^3	Y^4
1	−1 (30)	−1 (40/60)	30.00 ± 0.00	16.57 ± 0.30	1.10 ± 0.01	5.45
2	1 (50)	−1 (40/60)	108.33 ± 2.89	51.56 ± 0.98	2.00 ± 0.03	1.68
3	−1 (30)	1 (60/40)	30.00 ± 0.00	15.38 ± 0.38	1.30 ± 0.02	15.63
4	1 (50)	1 (60/40)	110.00 ± 0.00	57.21 ± 0.55	2.18 ± 0.02	17.24
5	−1.41 (26)	0 (50/50)	30.00 ± 0.00	14.67 ± 0.53	1.22 ± 0.02	8.62
6	1.41 (54)	0 (50/50)	146.67 ± 5.77	81.31 ± 1.15	1.96 ± 0.02	14.50
7	0 (40)	−1.41 (36/64)	50.00 ± 0.00	32.59 ± 0.29	1.59 ± 0.02	4.70
8	0 (40)	+1.41 (64/36)	50.00 ± 0.00	37.81 ± 2.15	1.58 ± 0.04	28.68
9	0 (40)	0 (50/50)	50.00 ± 0.00	37.64 ± 0.82	1.51 ± 0.03	20.48
10	0 (40)	0 (50/50)	50.00 ± 0.00	35.95 ± 0.58	1.79 ± 0.05	19.01
11	0 (40)	0 (50/50)	50.00 ± 0.00	36.62 ± 0.53	1.77 ± 0.05	19.01

Values codified and in () are real values of the independent variables.
PF: 100% hydrogenated palm oil; VF: vegetable fat; η: apparent viscosity; EPA: eicosapentaenoic acid; DHA: docosahexaenoic acid.

Table 3. Analysis of variance (percentage of explained variance (R^2), $F_{calculated}$ value and $F_{tabulated}$) for the responses of apparent viscosity of the spray-chilling feeding material at 100 rpm, microparticle mean diameter (D_{50}), moisture content and EPA and DHA losses

Response	R^2 (%)	$F_{calculated}$	$F_{tabulated}$	Mathematical equation
Apparent viscosity (η) (mPa.s)	99.9	8292.00	4.46	$Y_1 = 50.10 + 40.42x_1 + 19.25x_1^2$
Mean diameter (D_{50})	91.9	102.52	5.12	$Y_2 = 37.95 + 21.37x_1$
Moisture content (g/100 g)	85.4	52.62	5.12	$Y_3 = 1.64 + 0.35x_1$
EPA and DHA losses (%)	84.4	21.67	4.46	$Y_4 = 17.20 \times 4.28\ x_1^2 + 7.46\ x_2$

x_1, x_2: codified independent variables for core concentration (microparticles obtained by spray drying) (g/100 g) and lipid wall material composition (PF/VF ratio).

are presented in Table 3. The R^2 and the $F_{calculated}$ indicated that adequate second-order models (equations Y_1, Y_2, Y_3 and Y_4) could be obtained for the responses evaluated.

The feeding material apparent viscosity (Y_1) ranged from 30.00 to 146.67 mPa.s (Table 2). Higher core (SD-M) concentrations corresponded to higher apparent viscosities and the PF/VF ratio showed no influence on this result (Figure 2(a)) what could be explained by the temperature used during the viscosity analyses (80 °C), ensuring fusion of the lipids. According to Oxley (2012) the active ingredient composition and concentration may increase or decrease the feeding material viscosity and low viscosities will result in smaller particle sizes. Some atomization mechanisms allow for working at high viscosities, but viscosities below 200 mPa.s are desirable for most processes. Matos-Jr et al. (2017) observed that the addition of ascorbic acid powder in the molten lipid matrix significantly increased the particle size, what could be attributed to an increase in the viscosity of the feeding material.

Factors as temperature and viscosity of the feeding material, atomization pressure, nozzle diameter and lipid blends and the relationships between them may affect the SLM particle size (Okuro et al., 2013; Oriane et al., 2016; Ribeiro et al., 2012). The microparticles exhibited D_{50} (Y_2) values ranging from 14.67 ± 0.53 to 81.31 ± 1.15 μm (Table 2), and presented a span index ranging from 1.4 to 5.0 showing variation in the particle size. Higher core concentrations (Figure 2(b)), independently of the lipid wall material composition, resulted in higher mean diameters (D_{50}). Procopio et al. (2018) produced SLM loaded with cinnamon oleoresin with D_{50} ranging

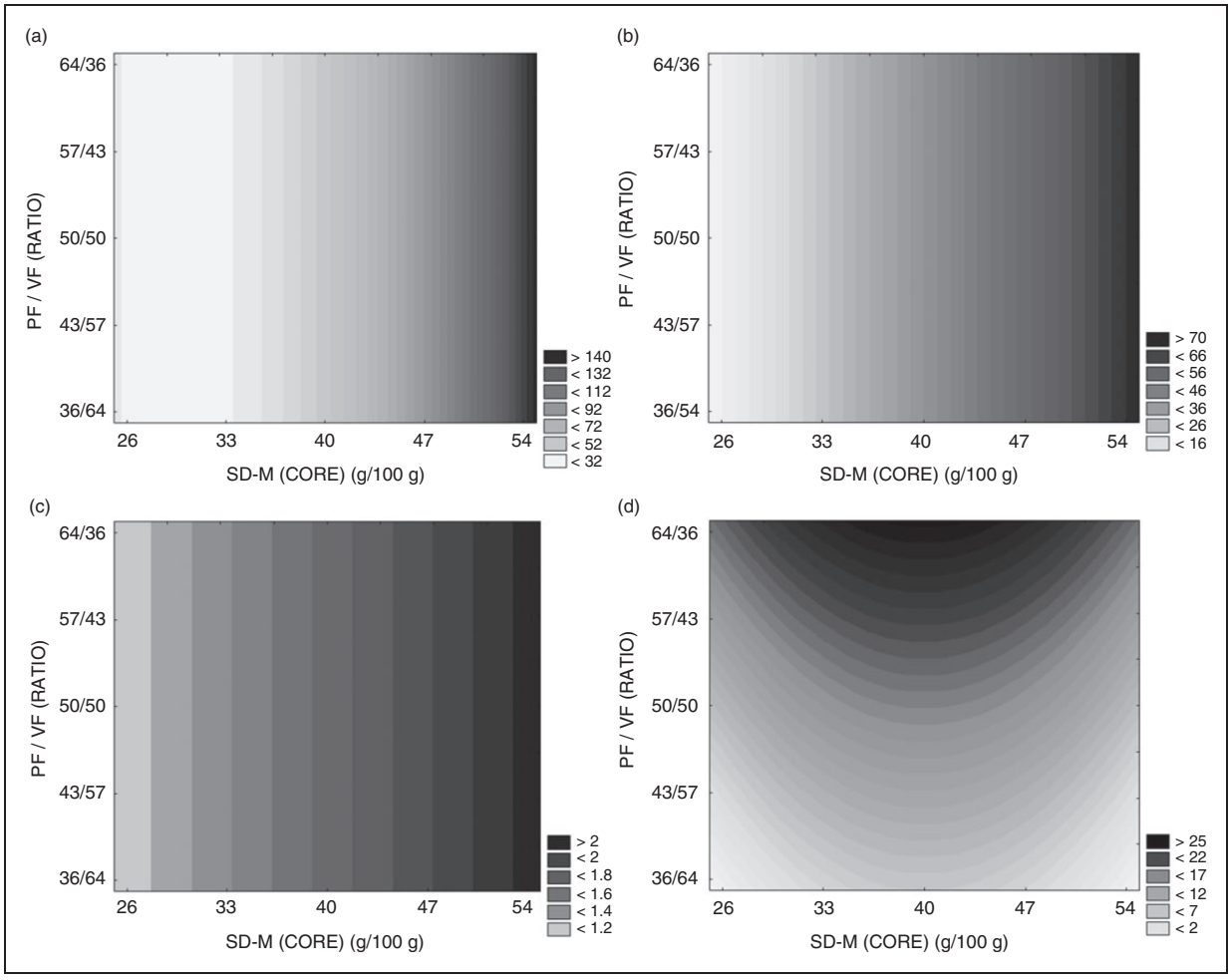

Figure 2. Contour curves for the apparent viscosity of the spray-chilling feeding material at 100 rpm (mPa.s) (a), microparticle mean diameter (D_{50}) (b), moisture content (g/100 g) (c), and EPA and DHA losses (%) (d) as a function of SD-M (CORE) (microparticles obtained by spray drying) concentration and lipid wall material composition (palm fat (PF) and vegetable fat (VF) at different ratios).

between 8.42 ± 1.4 and 72.21 ± 11.27 μm (span index ranging from 1.92 to 3.79) and pointed out that the size variation is typical of microparticles produced by spray chilling. Sartori et al. (2015) produced SLM loaded with ascorbic acid with D_{50} between 17 ± 1 and 35 ± 11 μm. Therefore, the double shell did not affect the microparticles D_{50} which is in agreement to those reported in the literature for spray-chilled microparticles.

Although for some food applications a small particle size is desirable, Appelqvist et al. (2015) reported that the combination of particle size and shape influences sensory perception and that particles of approximately 80 μm may not be considered gritty when they are soft and rounded, whereas angular and hard particles may be considered gritty even at small sizes (11–22 μm). Therefore, the particle sizes obtained in our study ensure the potential of these double-shell microparticles for food application.

The morphology evaluation (Figure 3) showed that all trials resulted in microparticles with spherical shape and surface without pores. The pores are undesirable because the oxygen can enter and accelerate degradation reactions (Matos-Jr et al., 2017). Optical microscopy (Figure 4) revealed the SD-M (core) distribution inside the lipid matrixes. The small size of the core material (SD-M) might have facilitated its accommodation inside the SLM.

The core concentration (SD-M) was the responsible for the samples moisture content. As shown in Figure 2(c), the SLM moisture content (Y_3) increased with the increase in core material concentration and ranged from 1.10 to 2.18 g/100 g (Table 2), values that are considered adequate for good stability of food-grade powders (Thirundas et al., 2012). The a_w ranged from 0.21 ± 0.01 to 0.27 ± 0.01, values that should ensure the oxidative stability of the microparticles (Labuza et al., 1972). Salvim et al. (2015) produced

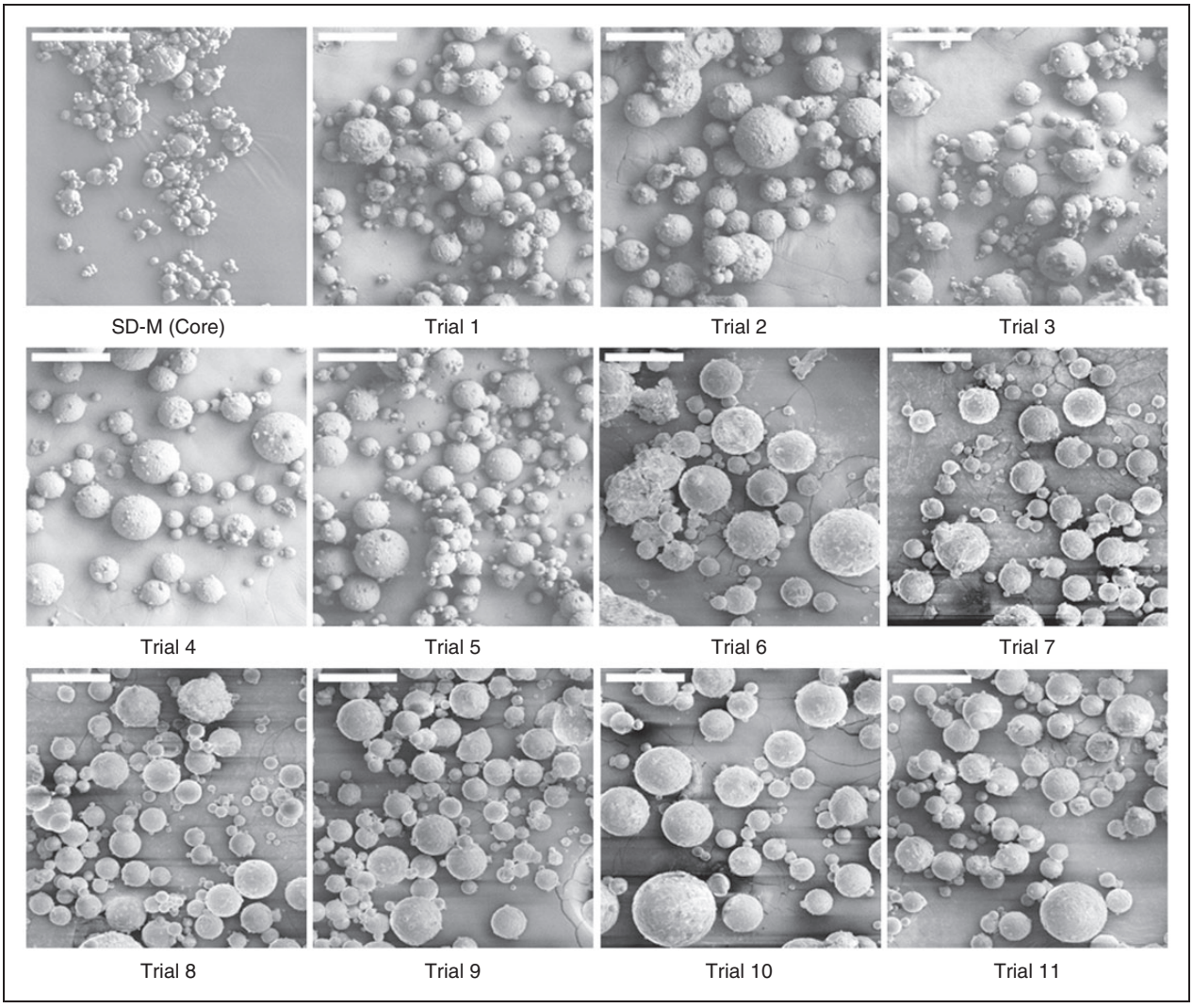

Figure 3. Images of the microparticles by scanning electron microscopy (200× magnification and bars of 100 μm, except SD-M (core) with 500× magnification and bar of 50 μm).

SLM using a suspension containing soybean protein hydrolysate and obtained similar moisture content (1.17 ± 0.23 g/100 g) but higher a_w values (0.43 ± 0.03).

Monitoring the changes in the core material is important because knowing the microparticle fatty acid concentration after the two microencapsulation steps will allow for the successful food application of these microparticles. The difference between the initial EPA and DHA content in the bulk fish oil and the remaining EPA and DHA content in the double-shell microparticles indicated the losses occurred during the microencapsulation process (Y_4). According to the results, EPA and DHA losses increased with increasing PF/VF ratios (Figure 2(d)). For all the core material concentrations used, the losses were below 10% for PF/VF ratios in the lipid matrixes equal to or less than 40/60. A PF/VF ratio of 50/50 together with 26 g/100 g core material also showed low loss of EPA and DHA during microparticle production. Bakry et al. (2017) microencapsulated fish oil by spray drying and reported that the losses in EPA and DHA content observed in their study were possibly due to oxidation during the emulsification and/or spray-drying microencapsulation process. In addition to that, the losses observed in our study could also be attributed to the time necessary for the core material homogenization in the molten lipid matrix, to the spray-chilling feeding material temperature, and to the difficulties faced during the atomization processes because higher concentrations of PF in the wall material were more likely to harden inside the feeding system.

Oxidative stability

The sample from trial 5 was selected to be evaluated since its characteristics such as good maintenance of

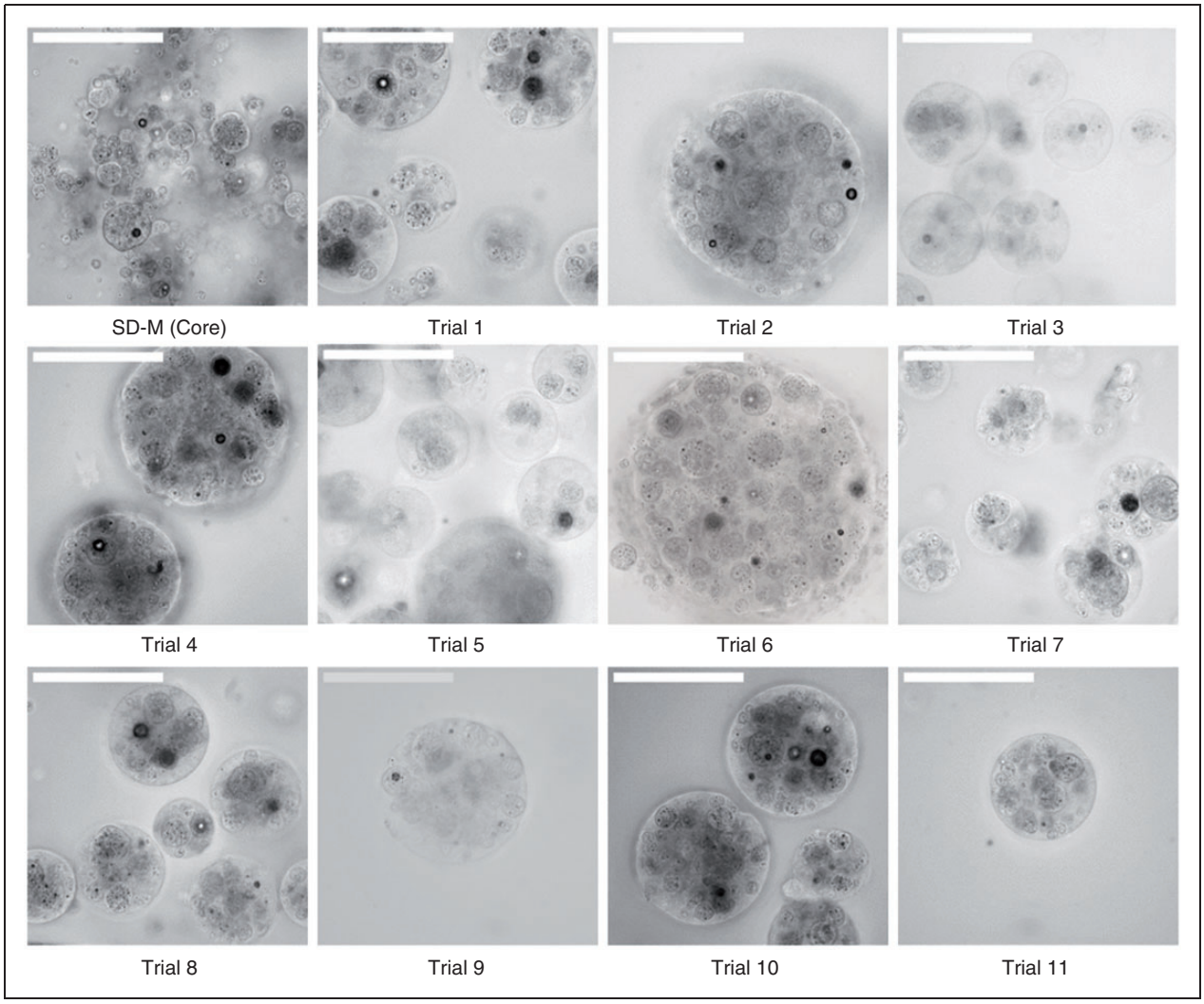

Figure 4. Images of the microparticles by optical microscopy (1000× magnification and bars of 50 μm).

EPA and DHA content, low moisture content and small particle size suggest potential use for food application. Besides that, the lower core material concentration (SD-M) of this sample facilitated the spray-chilling process and can guarantee a higher amount of continuous matrix to form a dense, tightly packed matrix around the core material. The bulk fish oil induction period (IP) was 1.96 ± 0.01 h. The literature reports IP for fish oil of 3.9 h (Di Giorgio et al., 2019) and 1.05 ± 0.05 h (Gallardo et al., 2013). Noello et al. (2016) pointed out on their study that oil can be considered stable if the IP obtained in Rancimat equipment is of at least 6 h.

The SD-M (core) and the SLM (trial 5) showed no inflection point in the oxidation curve until 44 hours of analysis. After seven months of storage the bulk fish oil IP was 0.80 ± 0.03 h and again the SD-M and the SLM (trial 5) did not show any inflection point in the oxidation curve until 44 hours of analysis. Noello et al. (2016) observed that spray-dried microparticles loaded with chia oil had the IP increased by four times when compared to the bulk oil IP and Gallardo et al. (2013) also proved the protective effect of the polymer matrix against linseed oil oxidation as the microparticles obtained by spray drying showed an IP 3.9 to 4.7 times higher than that observed for the bulk oil, depending on the matrix composition. Our results showed a protective effect of the microencapsulation against fish oil oxidation. According to Gallardo et al. (2013) the oxygen accessibility can be affect by the presence of pores and by the moisture content. The first shell composition might have resulted in a wall matrix less permeable to the oxygen and could be responsible for the result observed in the SLM.

CONCLUSION

All trials studied resulted in regular-shaped SLM that could be easily handled, underlining the potential use of

the combined microencapsulation technologies for fish oil microencapsulation. Higher core material concentrations resulted in higher apparent viscosity of the feeding material and, consequently, larger SLM size. However, the obtained particle sizes were suitable for food applications. The microparticles moisture content were adequate for good powder stability, and the a_w observed assures stability against lipid oxidation. The EPA and DHA losses were reduced when the lipid matrix had PF/VF ratios up to 40/60, primarily corresponding to core concentrations of 30, 40 and 50 g/100 g. For a PF/VF ratio of 50/50, the use of 26 g/100 g core material resulted in low EPA and DHA losses, and the final double-shell microparticles showed a protective effect against the fish oil oxidation. Therefore, these findings may contribute to expanding the use of microencapsulated fish oil in food products.

DECLARATION OF CONFLICTING INTERESTS

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