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Innovative strategy based on combined microencapsulation technologies for food application and the influence of wall material composition

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ABSTRACT

It is widely reported in the literature that polyunsaturated fatty acids (PUFAs) have positive effects on human health, therefore it is important to have alternatives to their direct addition into food products. This study investigated the use of combined microencapsulation technologies for fish oil and sacha inchi oil protection and food application. The microparticles were first obtained by spray drying. The evaluated wall materials were skimmed milk powder (SM), acacia gum (AG) and a mixture of acacia gum and grape juice (AGG). The SM:AG:AGG (ratio of 1:1:1) showed a positive effect on encapsulation efficiency (85.26–88.09%). The obtained microparticles had no unpleasant odour but did have a pronounced fish oil taste, likely due to the high solubility of the wall materials. A second shell formed by spray chilling overcame this limitation. In this stage, the spray drying microparticles were used as the core and a mixture of vegetable fat and hydrogenated palm oil was the wall material, providing microparticles with no oil taste and a sufficient amount of PUFAs to be incorporated into chocolate panned products with a positive sensory evaluation. The use of combined microencapsulation technologies was considered promising for encapsulating functional oils and increasing their use in processed foods.

1. Introduction

The beneficial health effects of long-chain eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids from fish oil are very well established, but the application of fish oil (FIO) into foods is associated with decreased sensory acceptance of those foods (Bannikova, Evteev, Pankin, Evdokimov, & Kasapis, 2018; Lu & Norziah, 2010; Torres-Giner, Martinez-Abad, Ocio, & Lagaron, 2010) due to oxidation reactions that reduce shelf life and readily produce hydroperoxides, unpleasant taste and odour (Aghbashlo, Mobli, Madadlou, & Rafiee, 2013; Kaushik, Dowling, Barrow, & Adhikari, 2015).

Another source of polyunsaturated fatty acids is vegetable oils, including sacha inchi (*Plukenetia volubilis* L.) oil (SIO), the seed of which originates from the Amazon Rainforest of Peru. The amounts of alpha-linolenic (ALA) and linoleic (LA) acids in SIO vary between 47 and 51%, and 34–37%, respectively (Gutiérrez, Rosada, & Jiménez, 2011). The intake balance of omega-3, omega-6 and their long chain derivatives is important for preventing diseases with an inflammatory-immune component (Wahle, Heys, & Rotondo, 2015).

Table 1 presents some regulations of claims related to EPA, DHA and ALA.

According to the Food and Drug Administration (USA), manufacturers can use the claim “x grams of omega-3 fatty acids” outside of the required Nutrition Facts.

Microencapsulation has been studied by many authors as an alternative to improve oil oxidative stability. The amorphous structure of a wall material containing carbohydrates can delay lipid oxidation (Morales-Medina, Tamm, Guadix, Guadix, & Drusch, 2016). Thus, to achieve higher core compound retention, it is important to choose the correct encapsulating matrix (Oriani et al., 2016) and a combination of

Abbreviations: AG, Acacia gum; AGG, mixture of acacia gum and grape juice; ALA, alpha-linolenic acid; Aw, water activity; DHA, docosahexaenoic acid; EE, encapsulation efficiency; EPA, eicosapentaenoic acid; FIO, fish oil; LA, linoleic acid; SIO, sacha inchi oil; SCM, spray chilling microparticles; SDM, spray drying microparticles; SM, skimmed milk powder; SO, surface oil; PO, 100% hydrogenated palm oil; TO, total oil; PUFAs, polyunsaturated fatty acids; VF, vegetable fat obtained from fully hydrogenated and interesterified vegetables oils

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Table 1
Claims related to EPA, DHA and ALA.

Claim	European Commission	Brazil
Source of omega-3	At least 40 mg of the sum of EPA and DHA per 100 g and per 100 kcal At least 0.3 g of ALA per 100 g and per 100 kcal	Minimum of 40 mg of the sum of EPA and DHA per serving Minimum of 300 mg of ALA per serving
High in omega-3	At least 80 of mg the sum of EPA and DHA per 100 g and per 100 kcal At least 0.6 g of ALA per 100 g and per 100 kcal	Minimum of 80 mg of the sum of EPA and DHA per serving Minimum of 600 mg of ALA per serving

EPA: eicosapentaenoic acid. DHA: docosahexaenoic acid. ALA: alpha-linolenic acid.
(Brasil, 2012; Drusch, 2012).

Table 2
Real levels of independent variables in mixture experimental design.

Independent variables (g/100 g, db)	Coded variables[a]	0	0.5	1.0
Skimmed milk powder	x_1	0	50	100
Acacia gum	x_2	0	50	100
Acacia gum and grape juice[b]	x_3	0	50	100

$x_1 + x_2 + x_3 = 100\%$ of mixture design ($\Sigma x = 1.0$).
[a] Sum of component fractions is equal to 1.0.
[b] Ratio of 2:3, on dry weight basis.

polymers could give the desired protection.

Acacia gum is a suitable wall material for oils microencapsulation, has emulsifying and film-forming properties, and can fulfil the roles of both a surface-active substance and drying matrix that prevents contact with oxygen in the atmosphere (Madene, Jacquot, Scher, & Desobry, 2006; Tonon, Grosso, & Hubinger, 2011). Dairy proteins have been used as encapsulating materials in spray-dried emulsions, and the concept of using milk origin products for this purpose has been established (Augustin et al., 2015; Goula & Adamopoulos, 2012).

Spray drying is one of the most commonly used technique for microencapsulation of oils (Bakry et al., 2016). It offers many advantages over other drying methods, such as low operational costs, the ability to handle heat-sensitive materials, available machinery, scale-up and reliable operation (Kaushik et al., 2015). One disadvantage is that part of the core may remain on the capsule surface, cause changes in the flavour balance of the finished products and increase the potential for oxidation (Kolanowski, Ziolkowski, Weiβbrodt, Kunz, & Laufenberg, 2006).

As SIO and FIO have unpleasant flavours, even a minimal amount of these oils on the microparticle surface will negatively affect their use in processed foods. Therefore, a second shell could provide extra protection to the core.

Spray chilling technology is based on the atomisation of a suspension containing the active substance and a melted lipid wall material in a cold chamber, where solid lipid microparticles are formed (Alvim, Stein, Koury, Dantas, & Cruz, 2016) and the degradation of bioactive compounds is avoided because milder temperatures are used (Tulini et al., 2017).

The aim of this work was to combine the use of spray drying and spray chilling technologies to obtain microparticles loaded with FIO and SIO with a double shell to improve the protection of these oils, mask undesirable flavours, and decrease the water solubility of the microparticles for food applications.

2. Materials and methods

2.1. Materials

The following materials were used to produce the microparticles:

First shell formed by spray drying: FIO (W3, MEG-3™ 4020 EE Oil, DSM); SIO (Embrapa Amazônia Ocidental, AM, Brazil); acacia gum (AG) (EncapsiaTM, Nexira); skimmed milk powder (SM); mixture of acacia gum and grape juice (AGG) (ratio of 3:2); and soy lecithin (Solec SG, DuPont).

Second shell formed by spray chilling: 100% hydrogenated palm oil (PO) (melting point 58.32 ± 0.16 °C) (A. Azevedo Óleos Vegetais) and vegetable fat obtained from fully hydrogenated and interesterified vegetable oils (VF) (melting point 35.83 ± 0.15 °C) (AL Lette K39LT™, Cargill). Panned product: milk chocolate and extruded cereals.

The following analytical-grade chemicals were used: absolute ethanol, methanol, chloroform and hexane. A FAME MIX37 standard was obtained from Sigma-Aldrich.

2.2. Statistical design and data analyses

A simplex-centroid design for three components was used to achieve the optimal composition of the encapsulating material. The core material was the FIO and the three independent variables were SM (x_1), AG (x_2) and AGG (x_3), with three replicates at the central point and 2^{q-1} mixture combinations, where q is the number of independent variables whose sum is equal to 1 (i.e., 100%) (Table 2). Each component was studied at three levels, and nine assays were performed. The following polynomial Equation (1) was fitted to the data. The responses under observation were total oil, surface oil, and encapsulation efficiency.

$$y = \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \beta_{12} \times 1 \times {}_2 + \beta_{13} \times 1 \times {}_3 + \beta_{23} \times 2 \times {}_3 + \beta_{123} x_1 x_2 x_3 \quad (1)$$

Statistica® 12 (StatSoft Inc., Tulsa, USA) software was used for data analyses through ANOVA variance, regression coefficient calculations, response surfaces, and Tukey's test, with confidence intervals of 90 and 95%.

2.3. First shell formed by spray drying

The emulsion solid concentration was fixed at 30 g/100 g. The wall materials were mixed with distilled water at 25 °C and homogenized at 15,000 rpm for 10 min using an Ultra Turrax® (T18, IKA, Germany). The core to coating ratio was 1:5. Soy lecithin was used as the emulsifier.

The spray drying microparticles (SDM) were produced with a mini spray dryer (B-290, Büchi, Flawil, Switzerland). The operating conditions are described in Table 3.

Table 3
Operating conditions for microencapsulation processes.

Operating conditions	Spray drying	Spray chilling
Sample temperature (°C)	21 ± 0.01	80 ± 0.01
Inlet temperature (T_{in}; °C)	150 ± 2	6 ± 1
Outlet temperature (T_{out}; °C)	80 ± 2	15 ± 2
Feed sample rate (mL/min)[a]	10	6
Aspiration (m^3/h)	35[b]	24.5[c]
Nozzle diameter (mm)	0.7	2.0
Atomisation gas flow (L/min)	600	742

[a] Spray Dryer: peristaltic pump; Spray Chiller: gravity feed.
[b] Equivalent to 100% of aspirator rate.
[c] Equivalent to 70% of aspirator rate.

2.4. Second shell formed by spray chilling

The spray chilling microparticles (SCM) were produced in the mini spray dryer linked to a Dehumidifier B296 (Büchi, Flawil, Switzerland) to produce cool air. The process was adapted from Alvim et al. (2016) (Table 3).

The wall material was composed of a mixture of VF and PO in a ratio of 3:2, with a softening point of 52.05 ± 0.11 °C. The SDM (core) were mixed with the melted wall material (80.00 ± 1.00 °C). The ratio of wall material to core was 3:2.

2.5. Characterization of microparticles

2.5.1. Moisture content and water activity (a_w)

The moisture content was determined gravimetrically by drying the microparticles in an oven at 105 °C/20 h (AOAC, 2006), and the a_w was measured using a water activity meter (AquaLab 4TEV, Decagon Devices Inc., Pullman, USA) at 25.00 ± 0.50 °C.

2.5.2. Surface oil (SO), total oil (TO), and encapsulation efficiency (EE)

The SO was measured according to Carneiro, Tonon, Grosso, and Hubinger (2013) and the TO according to Bligh and Dyer (1959). The EE was calculated as showed in Equation (2) (Bae & Lee, 2008):

$$\%EE = ((TO - SO)/TO) \text{ X } 100 \quad (2)$$

2.5.3. Mean diameter and size distribution

The measurements were made using a light scattering instrument (LV 950-V2, Horiba, Kyoto, Japan). SDM were suspended in absolute ethanol and SCM in polyoxyethylene (20) sorbitan monolaurate solution (0.5 g/100 g) (Alvim et al., 2016). The mean particle size was expressed as $D_{4,3}$ and D_{50}, and the polydispersity was given by the span index according to Equation (3), where $D_{0.1}$, $D_{0.5}$ and $D_{0.9}$ correspond to the diameters relative to 10, 50 and 90%, respectively, of the accumulated size distribution.

$$Span = D_{0.9} - D_{0.1} / D_{0.5} \quad (3)$$

2.5.4. Fatty acid composition

TO was determined by the cold extraction method (Bligh & Dyer, 1959). The dried lipid extract was esterified with a solution of ammonium chloride and sulphuric acid in methanol (Hartman & Lago, 1973). The fatty acid methyl esters were separated in a gas chromatograph (Varian 3900) with a split injector (75:1), a flame ionization detector (GC-FID), and a fused silica capillary column (100 m × 0.25 mm i.d., 0.20 μm film thickness, with 88% of cyanopropyl aryl polysiloxane-CP-SIL 88, Chrompack, Netherlands). The column temperature programme was set as follows: 140 °C held for 2 min, then ramped to 235 °C at 2.5 °C/min, and held for 10 min. The injector temperature was set at 270 °C and the detector temperature at 310 °C. The carrier gas was hydrogen at a flow rate of 0.5 mL/min, and nitrogen was used as the make-up gas at 30 mL/min. The fatty acids were identified by comparing the retention times of the sample to those of the standards (FAME MIX37 Sigma-Aldrich) (AOCS, 2014a,b). Quantification was achieved by normalisation, and transformation of the area percentage into g/100 g was calculated using Equation (4), 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).

$$Ci = \frac{\%Ai \times \%L \times F}{100} \quad (4)$$

2.5.5. Morphology

This characterization was performed using a scanning electron microscope (DSM 940A FOCUS, Zeiss, Jena, Germany). The samples were fixed on metal surfaces and coated with gold (Goldstein et al., 1992). The observations were made using electronic image capture with 500× and 1000× magnification.

2.6. Microparticle incorporation into a food matrix and sensory analysis

The microparticles loaded with SIO were not used for food applications because oil extraction was conducted using a Soxhlet apparatus with n-hexane as the solvent.

The products were prepared to ensure 40 mg of the sum of EPA and DHA per 25 g serving, according to the microparticle fatty acid compositions. The microparticles were incorporated into melted chocolate, which was used to cover extruded cereals in a rotating pan (JAA110E, Incal, Brazil). Three samples were produced: without microparticles (A), with SDM (B) and with SCM (C).

For sensory analysis, the FIO off-flavour was evaluated through the discrimination method using an intensity scale and 12 trained assessors. The assessors were chosen according to their discriminating capability between samples ($p < 0.30$) and their repeatability ($p > 0.05$), as verified by an analysis of variance (ANOVA) of two factors (sample and repetition) for each assessor and attribute (Stone & Sidel, 2004) at $p < 0.05$, using the software Statistical Analysis System (SAS Institute Inc., 2013). The consensus between the assessors was considered. The sensory intensities were scored on a 9-cm non-structured linear scale (Stone & Sidel, 2004). The extreme points of the scale were represented by the minimum intensity of FIO flavour (none) and the maximum (very intense). The means obtained were compared by Turkey's test at $p < 0.05$ using SAS 2013 software (SAS Institute Inc. Cary, US).

This research was approved by the Research Ethics Committee of the Max Planck Faculty in Brazil (CAAE 59078216.5.0000.8053).

3. Results and discussion

3.1. First encapsulant, spray drying coating

The moisture content and a_w of the SDM ranged from 1.10 to 7.92 g/100 g and from 0.12 to 0.19, respectively (Table 3). Run 3, with the highest level of AGG, resulted in the highest moisture, followed by run 5; this result can be attributed to the higher hygroscopicity of AGG compared to SM and AG. Ixtaina, Julio, Wagner, Nolasco, and Tomás (2015) reported a_w values ranging from 0.20 to 0.32 and moisture content from 1.48 to 3.52 g/100 g for particles containing chia oil microencapsulated by spray drying. The authors noted that a powdered food product should have a moisture between 3 and 4 g/100 g. The inlet temperature has a moderate influence in the final product moisture and a high solid content or high viscosity feeding material decrease this parameter (Patel, Patel, Chakraborty, & Shukla, 2015).

3.1.1. Surface oil (SO), total oil (TO) and encapsulation efficiency (EE)

Table 4 shows the values obtained for SO, TO and EE. The regression coefficients for the special cubic model, *F* values and determination coefficients (R^2) are presented in Table 5. The non-significant terms were eliminated, and the resulting equations were tested for adequacy and fitness by analysis of variance (ANOVA). The fitted models were suitable, showing significant regression, low residual values, no lack of fit and satisfactory determination coefficients.

The SO responses for single variables showed different behaviours. As expected, AG (run 2) presented the lowest levels of SO, since this material shows good emulsifying capacity. SM and AGG (runs 1 and 3) showed SO above 40%, which could increase microparticles FIO flavour and oxidation rate (Fig. 1). The combinations SM.AG.AGG (runs 7, 8, 9 - central points) and SM.AGG (run 5) resulted in SO levels below 15%, indicating that these microparticles could be more stable during storage

Table 4
Responses of dependent variables according to composition of encapsulating material, moisture content, and a_w.

Run	Coded Values			Real Values (x) (g/100 g, db)			Responses (Y)			Moisture content (g/100 g)	a_w
	x_1	x_2	x_3	SM	AG	AGG	SO (%)	TO (%)	EE (%)		
1	1	0	0	100.0	0	0	41.11 ± 2.75	16.18 ± 0.68	58.89 ± 2.75	1.62 ± 0.12^{f}	0.19 ± 0.01^{a}
2	0	1	0	0	100.0	0	18.11 ± 0.59	16.70 ± 0.33	81.89 ± 0.59	2.26 ± 0.16^{e}	0.16 ± 0.01^{a,b}
3	0	0	1.0	0	0	100.0	42.99 ± 2.35	13.96 ± 2.22	57.01 ± 2.35	7.92 ± 0.06^{a}	0.19 ± 0.01^{a}
4	0.5	0.5	0	50.0	50.0	0	60.40 ± 2.12	15.40 ± 0.11	39.60 ± 2.12	1.10 ± 0.06^{g}	0.12 ± 0.00^{b}
5	0.5	0	0.5	50.0	0	50.0	12.75 ± 1.78	10.46 ± 2.49	87.25 ± 1.78	3.73 ± 0.12^{b}	0.18 ± 0.00^{a,b}
6	0	0.5	0.5	0	50.0	50.0	25.36 ± 0.99	18.31 ± 0.59	74.64 ± 0.99	2.31 ± 0.07^{e}	0.16 ± 0.01^{a,b}
7	0.3	0.3	03	33.3	33.3	33.3	14.74 ± 0.96	15.37 ± 0.85	85.26 ± 0.96	3.11 ± 0.02^{c}	0.19 ± 0.02^{a}
8	0.3	0.3	0.3	33.3	33.3	33.3	11.91 ± 0.33	16.33 ± 0.85	88.09 ± 0.33	2.79 ± 0.03^{d}	0.18 ± 0.03^{a}
9	0.3	0.3	0.3	33.3	33.3	33.3	12.20 ± 0.31	15.00 ± 0.96	87.80 ± 0.31	2.59 ± 0.02^{d}	0.19 ± 0.04^{a}

Response values represent mean of three replications ± standard deviation and are averages of three measurements.
SM: skimmed milk powder. AG: acacia gum. AGG: mixture of acacia gum and grape juice.
Note: Values followed by different letters in same column are significantly different ($p < 0.05$) according to Tukey's test.

Table 5
Coded regression coefficients for SO, TO and EE.

Coefficient	SO	TO	EE
β_1	41.11	15.93	58.89
β_2	16.40	16.43	83.62
β_3	41.26	13.88	58.74
β_{12}	126.62	N.S.**	- 126.62
β_{13}	- 113.74	- 15.68	113.74
β_{23}	N.S.*	14.52	N.S.*
β_{123}	- 577.74	N.S.**	582.69
R^2	0.9910	0.9340	0.9920
F	63.87	8.49	70.13

N.S.*: non-significant ($p < 0.05$).
N.S.**: non-significant ($p < 0.10$).
SO: surface oil. TO: total oil. EE: encapsulation efficiency.

than other samples. The amount of SO may reflect the stability of the emulsion. If the emulsion is not stable, partial phase separation can occur, contributing to an increased amount of oil on the particle surface after drying (Munoz-Ibanez, Azagoh, Dubey, Dumoulin, & Turchiuli, 2015). According to the results, SO was lower when the three independent variables were used together.

During the spray drying of oil/water emulsions, some FIO likely remains on the inner wall of the equipment chamber (Kolanowski et al., 2006). Therefore, as TO may also be affected by the process, parameters with $p < 0.10$ were considered significant. The results showed that TO ranged from 13.96 to 16.70 g/100 g (db).

According to Fig. 1 the increasing in SM and AG concentrations increased TO. This result was expected, since both have good emulsification capability and film-forming properties. The use of SM.AG.AGG (central point) resulted in TO amounts close to those obtained when SM and AG were used individually. The combination of SM.AGG negatively affected TO content; i.e., TO was reduced.

EE ranged from 39.60 to 88.09% (Table 3). The individual use of AG (run 2) resulted in a good EE, likely due to its good emulsifying and film-forming properties. The best results were observed for SM.AGG (run 5) and SM.AG.AGG (central point). The EE can be influenced by the polymer matrices formed, their retention properties and their film-forming capacity (Carneiro et al., 2013). As expected, the highest EE values were obtained for samples with the lowest SO content. Similar EE results for FIO and flaxseed oil microencapsulated by spray drying have been reported (Aghbashlo et al., 2013; Carneiro et al., 2013; Kolanowski et al., 2006).

The combination SM.AG (run 4) negatively affected EE by decreasing it. Emulsion stability tests (data not shown) revealed that samples containing milk (runs 1, 4, 5) were highly aerated, even after 120 min of observation. The occluded air and use of AG hindered the release of air and impaired good homogenisation of the FIO, with a consequent reduction in EE.

Therefore, the encapsulating material composed of SM.AG.AGG (central point) had a positive effect on the SO, TO and EE of microparticles loaded with FIO obtained by spray drying.

3.1.2. Mean diameter and size distribution

The samples showed high polydispersity and the run 1 microparticles were the least polydisperse.

The $D_{4.3}$ of particles ranged from 6.54 ± 0.11 to 14.50 ± 0.26 μm and the mean diameter D_{50} from 5.03 ± 0.02 to 12.38 ± 0.10 μm (Table 6). The D_{50} may be influenced by factors such as the physical properties of the emulsions and process variables. As the process variables were similar for all trials, the difference could be due to the wall material composition. The highest D_{50} were obtained for samples in which the components were used individually. In studies where FIO and flaxseed oil were microencapsulated by spray drying (Aghbashlo et al., 2013; Carneiro et al., 2013; Kolanowski et al., 2006; Tonon et al., 2011) comparable results were obtained.

Some process parameters affect the final product characteristics. Higher gas flow is correlated with smaller particle size, higher feed material viscosity is correlated with larger particle size while the inlet temperature has no influence (Patel et al., 2015).

Although the microparticles obtained from runs 5, 7, 8 (central point) and 9 showed the highest EE and lowest SO, only the central point samples had the highest TO levels. Therefore, their wall material composition was also used for the microencapsulation of SIO by spray drying, resulting in microparticles with a moisture content of 1.76 ± 0.07 g/100 g; a_w of 0.20 ± 0.00 and an EE of 84.60%. The microparticles loaded with SIO were slightly larger than those containing FIO, with a D_{50} of 9.50 μm and a $D_{4.3}$ of 24.17 μm, which are typical sizes for spray-dried microparticles. Sanchez-Reinoso and Gutiérrez (2017) observed a moisture content of 1.00% for SIO microencapsulated by spray drying, with an a_w of 0.247.

3.1.3. Particle morphology

The SDM obtained from the combination of SM.AG.AGG as wall materials and loaded with FIO and with SIO showed spherical structures, typical of materials subjected to atomisation (Fig. 2). Structures with these characteristics were also observed by other authors (Alvim et al., 2016; Rocha, Fávaro-Trindade, & Grosso, 2012; Tonon et al., 2011). The microparticles will be more spherical and without inside void spaces at a low outlet temperature (Patel et al., 2015).

3.2. Second encapsulant, spray chilling coating

3.2.1. Moisture content and water activity (a_w)

The SCM moisture content was affected by the core material moisture content. The moisture content and the a_w of SCM containing

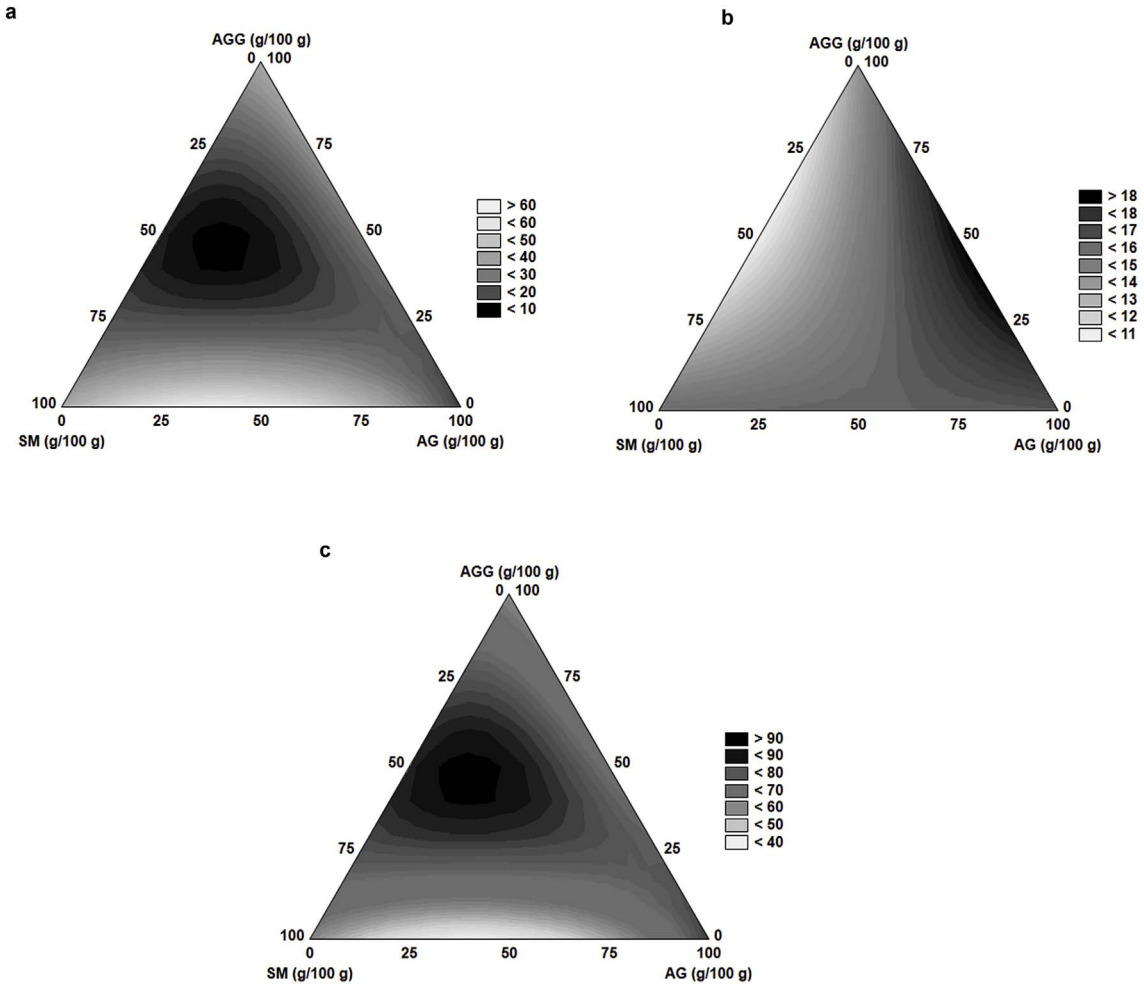


Fig. 1. Contour plot of experimental values obtained for Surface Oil (a), Total Oil (b) and Encapsulation Efficiency (c) of SDM as a function of SM, AG and AGG. SDM: spray drying microparticles. SM: skimmed milk powder. AG: acacia gum. AGG: mixture of acacia gum and grape juice.

Table 6
Mean diameter D_{50}, $D_{4.3}$ and polydispersity index (Span) for microparticles obtained with microencapsulation by SD.

Run	$D_{4.3}$ (μm)	D_{50} (μm)	Span
1	8.23 ± 0.08^{c}	7.72 ± 0.01^{c}	0.99 ± 0.05^{e}
2	10.50 ± 1.15^{b}	8.17 ± 0.11^{b}	$1.84 \pm 0.04^{b,c}$
3	14.50 ± 0.26^{a}	12.38 ± 0.10^{a}	$1.88 \pm 0.04^{b,c}$
4	$7.10 \pm 0.11^{c,d}$	6.02 ± 0.02^{e}	1.58 ± 0.04^{d}
5	8.24 ± 0.12^{c}	7.02 ± 0.13^{d}	$1.91 \pm 0.01^{b,c}$
6	$7.28 \pm 0.30^{c,d}$	5.73 ± 0.01^{f}	$1.99 \pm 0.10^{a,b}$
7	6.71 ± 0.27^{d}	5.03 ± 0.02^{h}	2.16 ± 0.14^{a}
8	$7.10 \pm 0.04^{c,d}$	$5.88 \pm 0.04^{e,f}$	$1.76 \pm 0.07^{c,d}$
9	6.54 ± 0.11^{d}	5.24 ± 0.08^{g}	$1.89 \pm 0.01^{b,c}$

Each value represents mean ± standard deviation and is the average of three measurements.
Note: Values followed by different letters in the same column are significantly different ($p < 0.05$) according to Tukey's test.

FIO was 3.25 ± 0.06 g/100 g and 0.33 ± 0.01 and for SCM loaded with SIO was 1.71 ± 0.02 g/100 g and 0.23 ± 0.00, respectively. According to the stability map of foods as a function of a_w, the lipid oxidation rate increased below 0.2 and showed the lowest rate at a_w from 0.2 to 0.3 (Labuza, McNally, Gallagher, Hawkes, & Hurtado, 1972). The results obtained in our study are favourable for achieving microparticles with great stability.

3.2.2. Mean diameter and size distribution

In the spray chilling, the droplet size usually is the final particle size since there is no solvent to be evaporated. A two-fluid nozzle can result in a particle size range of 10–450 μm (Oxley, 2012). For food applications the particle sizes should be smaller than 100 μm (Kaushik et al., 2015). The $D_{4.3}$ of SCM loaded with FIO was 24.01 ± 0.37 μm and the D_{50} was 24.08 ± 0.04 μm. For SCM loaded with SIO the $D_{4.3}$ was 25.80 ± 1.10 μm and D_{50} was 22.20 ± 0.40 μm. The samples showed polydispersity (span 1.71 ± 0.05 and 2.10 ± 0.14, respectively). Our results are in accordance with those reported in the literature (Salvim et al., 2015; Sartori, Consoli, Hubinger, & Menegalli, 2015).

3.2.3. Fatty acid composition

The losses of EPA and DHA (FIO) and ALA and LA (SIO) before and after microencapsulation (Fig. 3) can be attributed to the heated feed system (80.00 ± 1.00 °C) in the spray chilling microencapsulation step.

3.2.4. Morphology

The SCM (Fig. 4) showed spherical and polydispersed structures without agglomeration that could be attributed to the high melting point of the lipid wall material. Similar characteristics were observed in other studies (Alvim et al., 2016; Oriani et al., 2016; Sartori et al., 2015).

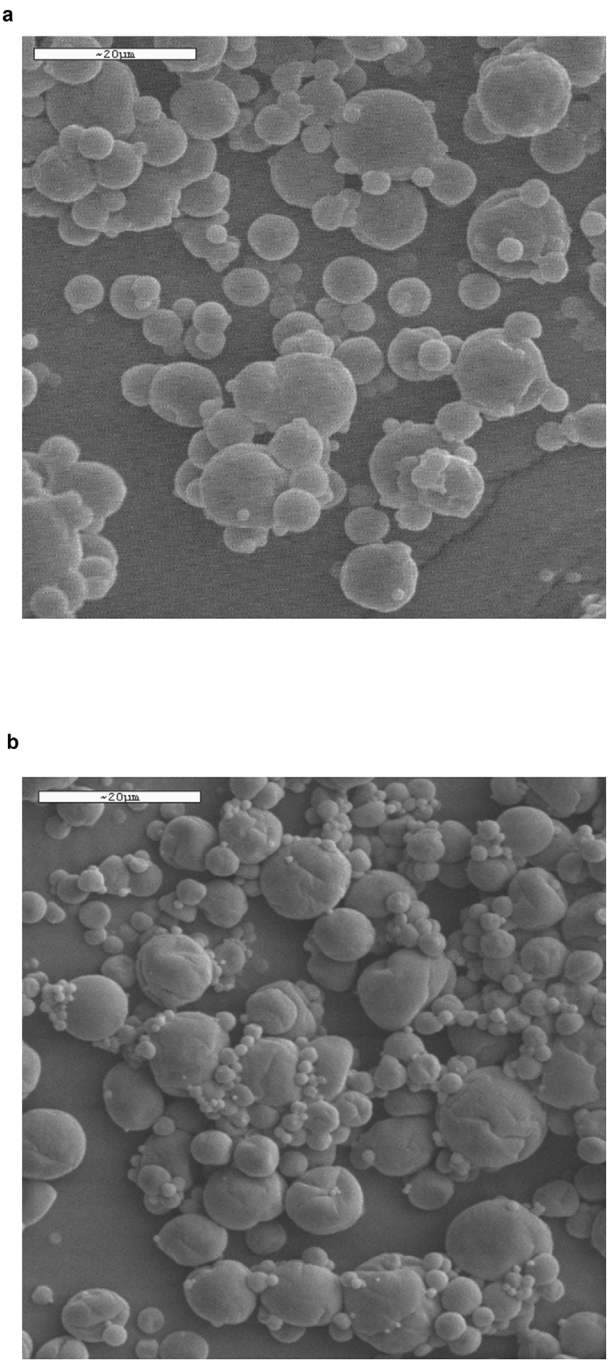


Fig. 2. Surface morphology of SDM with wall material containing SM.AG.AGG, loaded with FIO (a) and SIO (b) (SEM/Magnification: ×1000/Bar = 20 µm).
SDM: spray drying microparticles. SM: skimmed milk powder. AG: acacia gum. AGG: mixture of acacia gum and grape juice. SEM: scanning electron microscopy.

3.3. Incorporation of SDM and SCM loaded with FIO into a food matrix and sensory analysis

There was no need for adjustments in the panning process, and no fish odour was noticed during production. However, the sample B had a very unpleasant taste attributed to SDM water-solubility and was not used for the sensory evaluation.

The mean for FIO flavour for sample A (without microparticles) was 0.06 ± 0.10 (on an intensity scale of 0–9), whereas for sample C (with SCM containing FIO) it was 0.99 ± 1.30. The results indicated that these samples were different ($p < 0.05$), with a minimum significant difference of 0.0765. According to the comments by the assessors, some noticed a slightly different flavour between the samples but did not characterize it as a FIO flavour. The grape juice may have had an influence on this perception.

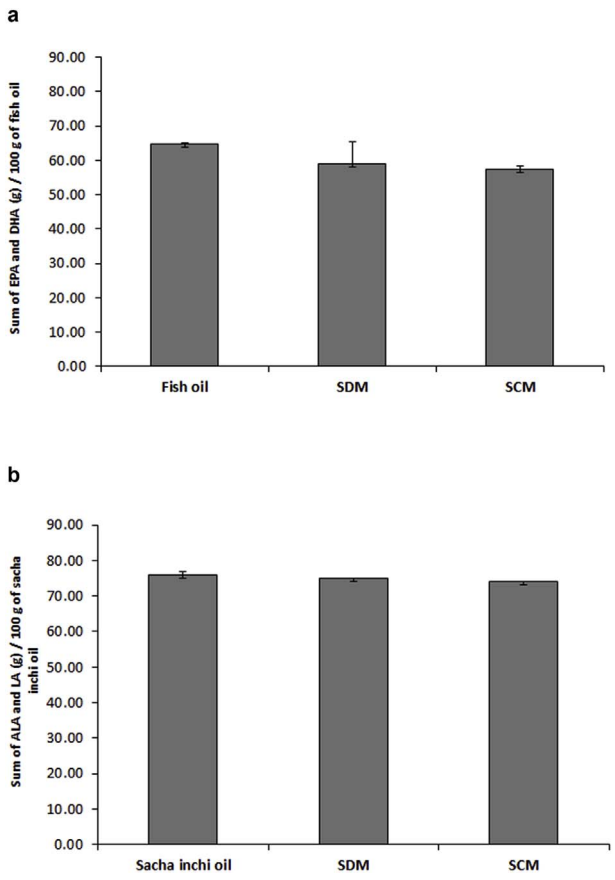


Fig. 3. EPA and DHA (a) and ALA and LA (b) contents before and after the use of combined technologies for FIO and SIO microencapsulation.
Bars represent the standard deviation of two measurements.
FIO: fish oil. SIO: sacha inchi oil. SDM: spray drying microparticles. SCM: spray chilling microparticles.

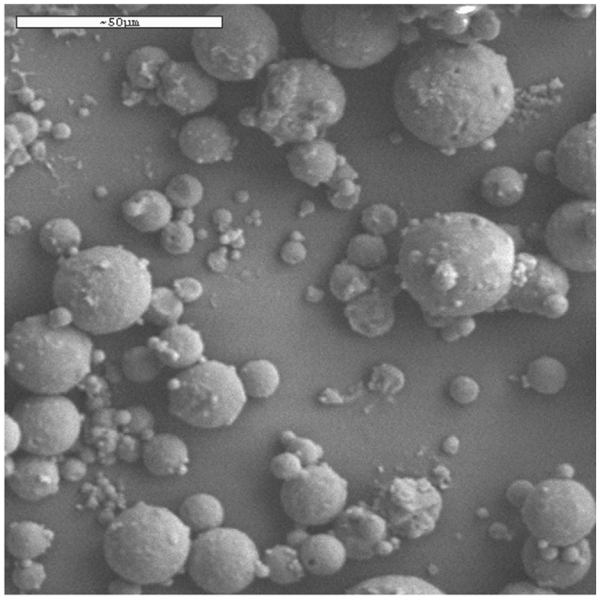
4. Process yield

Considering the organic materials and the laboratory level of the equipment used in our study, the process yield for spray drying was about 45% in relation to the initial raw material in dry basis and for the spray chilling about 60%. These percentages may not represent the industrial yields levels since the equipment used in larger production have more efficient product recovery mechanism.

5. Conclusions

The mixture of SM.AG.AGG (ratio of 1:1:1) used as the wall material in microencapsulation by spray drying resulted in microparticles with low levels of superficial oil and had a positive effect on encapsulation efficiency (85.26–88.09%). The mean diameter, size distribution and morphology of the microparticles were typical of the technique employed, but the microparticles presented an undesirable fish oil flavour. A second microencapsulation step performed by spray chilling, using the spray drying microparticles as the core material was a good alternative to overcome this limitation. This strategy was also efficient for SIO microencapsulation. The final microparticles had a higher D_{50} when compared with the spray drying microparticles, but suitable for food applications. The moisture content (1.71–3.25 g/100 g) and water activity (0.33–0.23) were considered favourable for a great stability of

a



b

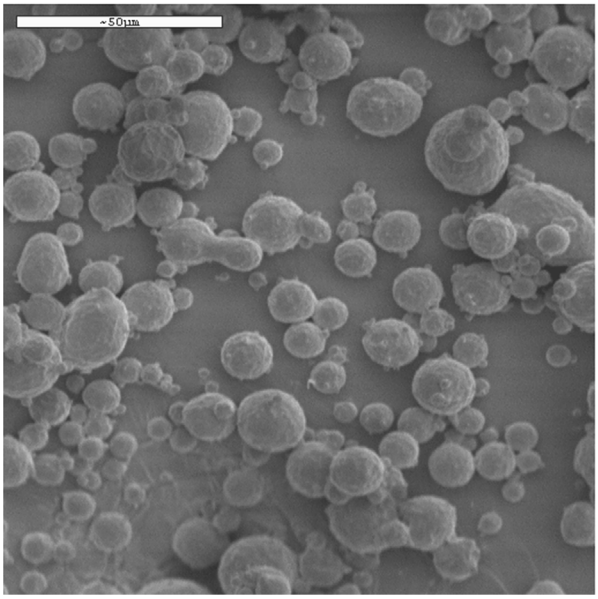


Fig. 4. Surface morphology of SCM containing FIO (a) and SIO (b) produced by combined microencapsulation technologies (SEM/Magnification: ×500/Bar = 50 µm).
SCM: spray chilling microparticles. FIO: fish oil. SIO: sacha inchi oil. SEM: scanning electron microscopy.

these microparticles. Although losses in the PUFAs content were observed after the two microencapsulations steps, the microparticles had a sufficient amount of omega-3 and omega-6 for incorporation into food products. The chocolate panned product produced with the addition of the double shell microparticles loaded with FIO oil had no undesirable flavour. The use of combined microencapsulation technologies proved to be an innovative and promising tool for delivering functional oils, providing microparticles with a good prospect for food applications.

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