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Microencapsulation by spray drying of omega-3 lipids extracted from oilseeds and microalgae: Effect on polyunsaturated fatty acid composition

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ABSTRACT

In this work, microencapsulation of omega-3 lipids from sustainable sources, including oilseeds such as *Salvia hispanica* L., *Echium plantagineum* L. and *Camelina sativa* L., microalgal lipids (from *Nannochloropsis gaditana*) and enzymatically produced fatty acid ethyl esters (FAEEs), was successfully done by spray drying and their outcomes studied. Previous to microencapsulation process, lipid extracts from new omega-3 sources (chia, camelina and echium oilseeds and wet microalgal biomass of *Nannochloropsis*) were produced using Pressurized Liquid Extraction. The best microencapsulation efficiency was achieved for chia FAEEs (76.9%), while the lowest was obtained for camelina oil (58.8%) and *Nannochloropsis gaditana* lipids (57.0%) microcapsules, which also showed low emulsion stability. Analysis by GC-MS showed that microencapsulation process did not negatively affect omega-3 fatty acid profile of microencapsulated extracts. The percentage of omega-3 in each used lipid was similar before and after spray drying (no significant differences at 5% level). Under the optimal conditions, produced microcapsules ranged from 1.5 to 30 μm and showed spherical shape and smooth surface without cracks. Thus, the proposed method provides an original approach to produce omega-3 microencapsulated lipid extracts from different nature, with high microencapsulation efficiency and payload, for potential applications in the development of foods enriched in omega-3 fatty acids.

1. Introduction

In recent years, there has been a growing interest by the scientific community in the nutritional and pharmaceutical properties of omega-3 polyunsaturated fatty acids (omega-3 PUFAs), due to their several functions in promoting human health. The essential role played by omega-3 PUFAs in the organism is extensively recognized for the beneficial effects of omega-3 PUFAs related to maintenance of normal blood pressure and triglyceride levels, prevention of cardiovascular diseases, and also, for the development of infant brain and visual systems (Shahidi & Ambigaipalan, 2018; Zhang, Fulgoni, Kris-Etherton, & Mitmesser, 2018). The omega-3 α-linolenic acid (ALA, 18:3 ω-3) is considered an essential fatty acid from which all other omega-3 PUFA are metabolically derived. ALA can be converted in the human body by elongation and desaturation pathways into more bioactive acids found in marine sources: stearidonic (SDA, 18:4 ω-3), eicosapentaenoic (EPA, 20:5 ω-3), docosapentaenoic (DPA, 22:5 ω-3) and docosahexaenoic (DHA, 22:6 ω-3). A lack of omega-3 PUFAs in a normal diet is associated with the development of pathologies including vascular diseases and inflammatory disorders, because of their role in the biosynthesis of eicosanoids (Calder, 2015). Moreover, based on recent epidemiological findings, the deficiency of omega-3 PUFAs, especially EPA and DHA, is also associated with depressive disorders and Alzheimer's disease (Song et al., 2016).

The most widely available dietary source of omega-3 PUFAs is fish oil. However, there is a concern regarding the sustainability of fish stocks because of over-fishing and high global demand. Moreover, some fish species contain high levels of toxic heavy metals, such as copper or mercury, and organic contaminants, such as PCBs or dioxins (Thompson & Darwish, 2019). Over the last years, scientific studies have been diverted towards the search of alternative and sustainable sources of omega-3 fatty acids, such as little-known oilseeds and microalgae. Despite the fact that plant-based sources of omega-3 are mainly composed of ALA, they could provide a sustainable, renewable, and cost-effective source of omega-3 PUFAs, compared to fish oils (Maurer, Hatta-Sakoda, Pascual-Chagman, & Rodriguez-Saona, 2012). On the other hand, marine microalgae are the primary producer of EPA and DHA and are considered one of the most promising alternative sources to

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meet the world demand for omega-3 PUFAs (Khozin-Goldberg, Leu, & Boussiba, 2016). In the present work, novel oilseeds containing significant amounts of high-quality oil such as chia (*Salvia hispanica* L.), echium (*Echium plantagineum* L.) and camelina (*Camelina sativa* L.), and wet microalgal biomass of *Nannochloropsis gaditana* were investigated as sustainable sources of omega-3 PUFAs.

The World Health Organization (WHO) and health authorities in many countries have promoted the intake of foods that contain high amounts of omega-3 fatty acids and an optimal omega-6/omega-3 ratio. In general, modern western diets are not only deficient in omega-3 fatty acids but also rich in pro-inflammatory omega-6 fatty acids (Spector & Kim, 2019). Therefore, there is a need to promote the consumption of omega-3 fatty acids and, in consequence, the development of functional foods enriched in these bioactive compounds is of great importance for the food industry. However, due to its hydrophobicity and low oxidative stability, which leads to the formation of oxidation products (such as volatile ketones and alcohols) implying unpleasant off odors and flavors, the addition of omega-3 oils to hydrophilic foods represents an important challenge (Comunian & Favaro-Trindade, 2016).

In recent years, microencapsulation technologies have increased their importance in the food industry, specifically in the development of functional foods with high-added value ingredients (Encina, Vergara, Giménez, Oyarzún-Ampuero, & Robert, 2016). Microencapsulation is the envelopment of small solid particles, liquid droplets or gases in a coating, and can be used to protect, transport or control the release of active compounds (Nazzaro, Orlando, Fratianni, & Coppola, 2012). In the field of food processing, microencapsulation technology is extensively involved in preventing food spoilage, volatile loss and loss of properties of essential oils (Shao, Pan, Liu, Teng, & Yuan, 2018). In this sense, microencapsulation is an effective method to protect omega-3 fatty acids from oxidation and extend their shelf life. At the same time, this technology offers a practical solution for the stabilization and improvement in the administration of these bioactive ingredients for the enrichment of new food products such as dairy products, beverages, and bakery products (Kaushik, Dowling, Barrow, & Adhikari, 2015; Ye, Georges, & Selomulya, 2018).

Even though a range of microencapsulation techniques have been applied to omega-3 oils, spray drying and complex coacervation are the primary methods used for commercial purposes (Xia et al., 2019). Spray drying has several advantages over complex coacervation including the ability to handle heat-sensitive ingredients, fast drying speed, high throughput and yield, good keeping qualities of microcapsules and possibility to use a wide range of formulations and wall materials (Ray, Raychaudhuri, & Chakraborty, 2016). In spray drying, operating conditions used depend on the characteristics of the material to be dried and the desired powder specifications (Medina-Torres et al., 2013). In that respect, it is important to highlight that studying the effect of operating parameters on the physical properties of microcapsules helps to find the optimum operating conditions, and their effect on the characteristics of the final product.

Therefore, the main objective of this study was to develop an integrated and feasible extraction and microencapsulation process to produce microencapsulated lipid extracts from new omega-3 sources, including vegetable oils, microalgal lipids and fatty acid ethyl esters (FAEEs), for potential applications in the production of novel functional foods enriched in omega-3 fatty acids. The effect of operating parameters of spray drying on omega-3 microcapsules was studied during process development to identify the optimal conditions. Microcapsules were characterized using different physicochemical parameters such as microencapsulation efficiency, microencapsulation payload, and evaluation of microcapsules shape and size by SEM images. Furthermore, fatty acid compositions of microencapsulated extracts were analyzed to assess the effect of microencapsulation process on the fatty acid profile and especially on the omega-3 composition.

2. Materials and methods

2.1. Materials

Commercial chia seeds (*Salvia hispanica* L.) from Bolivia were purchased from Dietica (Cuenca, Spain). Echium seeds (*Echium plantagineum* L.) were provided by Technology Crops International (Essex, United Kingdom). Camelina seeds (*Camelina sativa* L.) were provided by Camelina Company España (Madrid, Spain). Seeds were ground with a particle size less than 500 μm using a grinder (Moulinex-A320R1 700 W) and stored at 4 °C until the oil extraction process. Commercial wet paste biomass from oleaginous microalga *Nannochloropsis gaditana* was provided by AlgaEnergy S.A. (Alcobendas, Spain). The wet biomass contained a concentration of 27% w/w of dry microalgae biomass. The percentage of water in the wet biomass paste was determined by drying it (50 °C, overnight) to a constant weight.

Commercial lipase Lipozyme TL IM (lipase from *Thermomyces lanuginosus*) were kindly donated by Novozymes (Bagsvaerd, Denmark). Absolute ethanol (PRS grade), sodium hydrogen carbonate and potassium hydroxide were purchased from Panreac Quimica S.A (Barcelona, Spain). Molecular sieves pore size 4 Å, *n*-hexane, isopropanol, acetic acid and ethyl acetate were purchased from Scharlau (Barcelona, Spain). Wall materials for microencapsulation (sodium caseinate and lactose) were purchased from Acros Organics (New Jersey, USA). The water used was Milli-Q grade (Millipore, USA). Fatty acid methyl esters standard (Supelco 37 FAME Mix) was from Supelco (Bellefonte, PA, USA). All other reagents and solvents used were of analytical or HPLC grade.

2.2. Lipid extraction from different omega-3 sources using pressurized liquids

Lipid extracts from new omega-3 sources (chia, camelina and echium oilseeds and wet microalgal biomass of *Nannochloropsis gaditana*) were produced in our laboratory using pressurized liquids and results were compared with traditional methods.

2.2.1. Soxhlet method

5 g of ground oilseeds (chia, camelina and echium) were extracted with hexane in a Soxhlet apparatus by continuous series of cycles of boiling and condensation of solvent for 8 h. Samples were evaporated in a rotary evaporator (Heidolph Hei-Vap Value HB/G3, Germany) under reduced pressure at 40 °C and dried under a nitrogen stream to constant weight. The lipid content was determined gravimetrically and was calculated as weight percentage of dry biomass. Lipid extracts obtained were stored in dark vessels with nitrogen atmosphere at 4 °C until their analysis.

2.2.2. Folch method

The Folch extraction method was done following the original procedure described by Folch et al., (Folch, Lees, & Sloane Stanley, 1957). Wet microalgal biomass (equivalent to 1 g dry biomass) was extracted with 20 mL of chloroform:methanol (2:1 v/v) vortexing for 2 min. The mixture was centrifuged at 1207 g for 10 min and the organic layer was collected. The extraction process was carried out 3 times on the same microalga biomass. The collected organic layers were purified washing with water and centrifuged at 1207 *g* for 10 min. Finally, the chloroform layer contained the extracted lipids. Samples were evaporated and treated as described above for other extraction methods.

2.2.3. Pressurized liquid extraction

Pressurized liquid extraction (PLE) was carried out with an ASE 350 DIONEX (Sunnyvale, California) extractor. Lipid extraction was performed using 3 g of ground oilseeds (chia, camelina and echium) and equivalent to 3 g of dry biomass for the wet microalgal biomass of *Nannochloropsis gaditana*. Stainless steel extraction cells were used with a capacity of 10 mL. Extracts were collected under a nitrogen stream in

different vials of 50 mL. Extraction conditions used were performed according to previous studies of the group using ethyl acetate as solvent at 120 °C for oilseeds (Castejón et al., 2017, 2018) and a mixture of hexane:isopropanol (2:1 v/v) at 120 °C for the microalgal biomass (Castejón & Señoráns, 2019).

Samples were evaporated in a rotary evaporator (Heidolph Hei-Vap Value HB/G3, Germany) under reduced pressure at 40 °C and dried under a nitrogen stream until constant weight. The lipid content was determined gravimetrically and expressed as dry weight percentage. Lipid extracts obtained were stored in dark vessels with nitrogen atmosphere at 4 °C until their use.

2.3. Enzymatic synthesis of fatty acid ethyl esters from chia oil

Enzymatic synthesis of fatty acid ethyl esters from extracted chia oil was performed as previously described (Castejón et al., 2019). Produced omega-3 ethyl esters were stored in dark vessels with nitrogen atmosphere at 4 °C until their use.

2.4. Microencapsulation of lipids rich in omega-3 by spray drying

Microcapsules of different omega-3 lipids (chia, camelina, echium, and microalgal lipid extract) were produced by spray drying.

2.4.1. Emulsions preparation

Oil-in-water (O/W) emulsions were prepared using sodium caseinate and lactose as wall materials (oil to wall ratio 1:4 (w/w)). O/W emulsions were prepared as follows using different omega-3 lipids. Briefly, 5 g of sodium caseinate were dissolved in 80 mL of Milli-Q water. Then, the mixture was homogenized using a high-speed homogenizer equipment (Ultra turrax®, IKA, Staufen, Germany) for 5 min. At that time, 2.5 g of omega-3 lipid and 5 g of lactose were added to the mixture. Then, the water/oil mixture was homogenized until a stable emulsion was formed. Finally, the homogenized emulsions were immediately fed into a spray-dryer.

2.4.2. Spray drying process

The spray drying process was performed in a laboratory-scale Spray Dryer Büchi B-191 (Buchi Labortechnik AG, Flawil, Switzerland). Optimal process conditions were the following: air inlet temperature 170 °C, compressed air pressure 5 bar, air flow 700 L/min and aspiration 70%. The equipment was feed by a peristaltic pump and feed rate was controlled by the rotational speed of the pump (10% of its capacity). The emulsions were under continuous stirring throughout process (using a magnetic stirrer). The obtained microcapsules were stored in glass vials and kept in darkness until further analysis.

2.5. Microencapsulation efficiency (MEE)

The microencapsulation efficiency (MEE) is defined as the ratio of core material in the final dried microcapsules to that in the original emulsion (Zilberboim, Kopelman, & Talmon, 1986) and was calculated according to Eq. (1) and Eq. (2), as provided elsewhere (Ahn et al., 2008; Quispe-Condori, Saldaña, & Temelli, 2011).

$$MEE\ (\%) = \frac{microencapsulated\ oil\ (mg/100\ mg\ microcapsules)}{total\ oil\ (mg/100\ mg\ microcapsules)}\ x\ 100 \quad [1]$$

$$Microencapsulated\ oil = total\ oil - surface\ oil\ (mg\ /\ 100\ mg\ microcapsules) \quad [2]$$

Microencapsulation efficiency was calculated at least in duplicate.

2.5.1. Surface oil

Surface oil, also known as non-encapsulated oil fraction, was determined by extracting the microcapsules with hexane according to previously described (Klinkesorn, Sophanodora, Chinachoti, Decker, & McClements, 2006). Briefly, 100 mg of microcapsules were extracted with 3 mL of hexane by vortexing for 2 min. Samples were centrifuged for 10 min at 2147 *g* at room temperature. The supernatant was collected, filtered and evaporated. The amount of non-encapsulated oil was determined gravimetrically. Duplicate determinations were performed for each sample.

2.5.2. Total oil

Total oil content of microcapsules was quantitatively determined. 2 mL of acetic acid (1% v/v) was added to 100 mg of microcapsules and vortexed for 2 min. Then, the sample was extracted with 3 mL of hexane: isopropanol (1:1 v/v) by vortexing for 2 min. Samples were centrifuged for 10 min at 2147 *g* at room temperature. The supernatant was collected, filtered and evaporated. The extraction step with hexane: isopropanol (1:1 v/v) was repeated one more time to assure complete extraction. The amount of total oil was determined gravimetrically. Duplicate determinations were performed for each sample.

2.6. Payload

Payload is the amount in mg of omega-3 oil or active ingredient per gram of microcapsules. It was calculated by taking the ratio of mass of microencapsulated oil to total mass of powder (Kaushik et al., 2015).

2.7. HPLC-ELSD analysis

HPLC-ELSD analyses were performed using an Agilent 1260 Infinity HPLC equipped with an Agilent 385 (Palo Alto, CA, USA) ELSD instrument. The chromatographic separation of the different species of lipids (neutral and polar lipids) was carried out with a silica normal phase ACE (250 mm × 4.6 mm i. d., 5 μm) column maintained at 30 °C using a ternary gradient as follows (Castejón & Señoráns, 2019): 0–2 min, 99.5% A and 0.5% B; at t = 6.5 min, 70% A and 30% B; at t = 11 min, 63% A, 27% B and 10% C; at t = 18 min, 99.5% A and 0.5% B; and at t = 20 min, 99.5% A and 0.5% B. Eluent A consisted of 2,2,4-trimethylpentane, eluent B consisted of methyl tertbutyl ether, and eluent C consisted of 2-propanol. The flow rate was 2.0 mL/min except for minutes 13 to 16 which was 1.0 mL/min. Optimal signal and resolution were attained with the following ELSD conditions: evaporator temperature of30 °C; nebulizer temperature of30 °C; and evaporator gas N_2 of 1.6 SLM.

2.8. Determination of fatty acids in microencapsulated lipid extracts by GC–MS

Fatty acids composition of microencapsulated lipid extracts was analyzed on an Agilent GC-MS series 5975 MSD (Palo Alto, Cal., USA) using a HP 88 capillary column (100 m × 0.25 mm, i. d. 0.2 μm) (Agilent, CA, USA). Previous to analysis fatty acid methyl esters (FAMEs) were prepared by base-catalyzed methanolysis of the glycerides (KOH in methanol). 1 μL sample was injected using a split ratio of 1:100. The column was held at 175 °C for 10 min after injection, the temperature programmed at 3 °C/min to 220 °C and held for 20 min more. Helium was used as gas carrier, at a constant column flow rate of 1.5 ml/min. The injector temperature was 250 °C and the detector temperature was 230 °C. The mass spectrometer was operated at 70 eV with a mass range from 30 to 400 amu. Fatty acids were identified comparing their retention times and their mass spectra with those obtained from the standards (Supelco 37 FAME Mix) and the NIST MassSpectral Library (Version 2.0).

2.9. Scanning electron microscopy (SEM)

The morphological characteristics of microcapsules were analyzed by scanning electron microscopy (SEM) using a Hitachi TM-1000

Tabletop Microscope (Hitachi High-Technologies Corporation, Tokyo, Japan). The size distribution of microcapsules was estimated from SEM images using the ImageJ software, version 2.0 (license-free tool for image treatment). The average size was determined by measuring the diameter of microcapsules (assuming that they were spherical) using a known length segment.

2.10. Statistical analysis

All the experiments were carried out at least in duplicate. Results are expressed as mean ± standard deviation Statistical analysis was performed using SISA online software (Hilversum, Netherlands). The effect of microencapsulation process on the fatty acid composition were analyzed by Student's T-Test. In all cases, differences were considered statistically significant at $p < 0.05$.

3. Results and discussion

3.1. Production of lipid extracts rich in omega-3 fatty acids from different sources

Previous to microencapsulation process, lipid extracts from new omega-3 sources (chia, camelina and echium oilseeds and wet microalgal biomass of *Nannochloropsis gaditana*) were produced in our laboratory. Lipid yields using PLE were 12.3 ± 0.9% for *Nannochloropsis gaditana*, 28.7 ± 3.7% for chia seeds, 28.9 ± 1.1% for echium seeds and 39.3 ± 0.1% for camelina seeds. PLE results were compared with traditional extraction methods, such as Soxhlet extraction for oilseeds and Folch method for the microalgal biomass due to its heterogeneous composition. Soxhlet extraction achieved similar oil yields than PLE for camelina (39.5 ± 0.6%) and echium (31.3 ± 0.2%) oilseeds (no significant differences at 1% level), and slightly higher for chia oilseeds (33.8 ± 0.2%) (significant differences at 5% level). Regarding to the microalgal biomass, Folch method rendered a lipid yield of 15.0 ± 1.4%, similar than PLE results (no significant differences at 1% level). The advantages of using PLE are shorter extraction times, full automation and less solvent use, while offering similar extraction yields. Thus, in this work, lipid extracted using PLE were used for the microencapsulation study. Additionally, enzymatic synthesis of FAEEs from extracted chia oil was done in our laboratory by ethanolysis reaction with a yield up to 97.7% using a commercial biocatalyst. The production of omega-3 FAEEs is interesting from a commercial point of view due to most commercial omega-3 concentrates on the marked are omega-3 ethyl esters derivatives, instead of triacylglycerols. Even most evidence indicates that omega-3 PUFAs are more efficiently digested as TAGs, omega-3 FAEEs could be a great strategy to enrich food products (Castejón & Señoráns, 2020).

Due to the different nature of the microalgal lipid extract (composed by neutral and polar lipids) and to evaluate the effect of the lipid composition on the microencapsulation process, lipid classes from the *Nannochloropsis gaditana* extract were analyzed by HPLC-ELSD. Results showed a heterogeneous composition in terms of lipid classes in comparison with chia, camelina and echium oils (composed only by triacylglycerols): 22.3% triacylglycerols, 12.6% free fatty acids, 9.3% diacylglycerols and 39.9% glycolipids.

3.2. Production of microencapsulated omega-3 lipids by spray drying

In a first step, spray-drying conditions were selected to microencapsulate omega-3 lipid extracts. Optimization of experimental conditions is a key factor for developing an efficient microencapsulation process (Paulo & Santos, 2017). The main factors to optimize during process development in spray drying are: air inlet temperature, feed rate, air flow, aspiration rate and coating material ratio (Keshani, Daud, Nourouzi, Namvar, & Ghasemi, 2015). Previous unpublished studies of the research group found that 1:4 (w/w) oil to wall ratio using sodium caseinate and lactose (both of food grade) and spray drying conditions applied (see Materials and Methods section) were the optimal conditions to microencapsulate fish oil with a high content of omega-3 PUFAs. The utilization of emulsions containing different proteins and lactose has been previously reported for other authors, showing that incorporation of lactose in the protein-based wall system allowed enhanced core retention, stability of the matrix structure and microencapsulation efficiency (Rosenberg, Rosenberg, & Zhang, 2018).

To the best of our knowledge, this is the first time that microencapsulation of camelina oil, echium oil and *Nannochloropsis gaditana* lipid extracts and omega-3 FAEEs from chia oil has been described by spray drying. Microencapsulation of chia oil by spray drying and freeze drying has been described in recent years by some authors (Alcântara et al., 2019; González, Martínez, Paredes, León, & Ribotta, 2016; Ixtaina, Julio, Wagner, Nolasco, & Tomás, 2015; Martínez et al., 2015; Rodea-González et al., 2012; Us-Medina, Julio, Segura-Campos, Ixtaina, & Tomás, 2018). Microencapsulation of echium oil has been described, though using other encapsulation techniques, such as chemical coacervation, freeze drying or using nanostructured lipid carriers (Azizi, Kierulf, Connie Lee, & Abbaspourrad, 2018; Comunian, Boillon, et al., 2016; Comunian, Gomez-Estaca et al., 2016).

Table 1 shows different operating parameters during microencapsulation process for all emulsions prepared with the different lipid extracts rich in omega-3 investigated in the present study. The outlet temperature can be considered a key parameter for the microencapsulation of food ingredients, which are highly susceptible to oxidative degradation, and it mainly depends on the inlet temperature and drying efficiency. The outlet temperature has been reported to vary from 50 to 80 °C for the microencapsulation of different bioactive compounds (Edrisi Sormoli & Langrish, 2016; Gharsallaoui, Roudaut, Chambin, Voilley, & Saurel, 2007; Murugesan & Orsat, 2012). As can be seen in Table 1, the outlet temperature never exceeded 80 °C and, in general, varied from 67 to 71 °C, except for the microalgal extract.

The highest flow rate (mL/min) was found for chia FAEEs due to the excellent homogenization of the sample, since it is known that the solubility of ethyl esters in water is higher than other lipid extracts investigated in the present study. The lower viscosity of ethyl esters in comparison with the other used oils could also explain these favorable results. However, the lipid extract of *Nannochloropsis gaditana* showed the lowest flow rate during microencapsulation process. Although the feed rate was fixed by controlling the rotation of the peristaltic pump, other factors such as the rheological properties of the emulsion and, in particular, the viscosity of the sample, strongly affect the flow rate. The heterogeneous composition of the microalgal extract (see analysis of microalgal lipids classes by HPLC-ELSD in section 3.1) could be the reason why the emulsion formed was unstable, showing greater resistance and longer processing time.

The amount of microcapsules collected after microencapsulation process (product recovery) varied from 4.8 to 6.0 g, implying a production yield from 38.4 to 48.0%. Product recovery is a control parameter of the quality of the process. Nevertheless, in this work, it was not an absolute value, due to the difficulties involved in collecting all microcapsules from the equipment for a great number of short time tests of different experimental conditions. Probably, this value would increase in larger scale or continuous productions.

As a result, omega-3 microcapsules were successfully produced by spray drying (Fig. 1). In general, produced microcapsules had the appearance of a fine, dry and white powder, except for *Nannochloropsis gaditana* microcapsules, which presented a characteristic green color consistent with the algal extract (Fig. 1(b)). After microencapsulation process, microcapsules were characterized using different physicochemical parameters such as microencapsulation efficiency, microencapsulation payload, determination of the fatty acid composition of microencapsulated oil by GC-MS, and evaluation of microcapsules shape and size by SEM images.

Table 1
Operating parameters during microencapsulation by spray drying process of lipid extracts rich in omega-3 fatty acids.

Lipid extract	Operating parameters				
	Outlet temperature[a] (°C)	Time[b] (min)	Product recovery[c] (g)	*Flow*[d] *(mL/min)*	Production yield[e] (%)
Chia oil	68–70	21.1	5.7	3.8	45.6
Camelina oil	68–71	21.2	6.0	3.8	48.0
Echium oil	67–69	23.6	5.6	3.4	44.8
Chia FAEEs	68–71	20.5	5.9	3.9	47.2
N. gaditana extract	78–80	30.1	4.8	2.6	38.4

[a] Air outlet temperature during microencapsulation process.
[b] Time to complete microencapsulation process.
[c] Amount of microcapsules (g) collected after the microencapsulation process. The maximum amount of microcapsules corresponds to the total grams of solids in the emulsion: grams of sodium casein, lactose and oil, 12.5 g.
[d] Flow rate = emulsion volume per minute (mL/min) that passes through the spray drying equipment during test time. The used emulsion volume was 80 mL for all experiments.
[e] Production yield (%) = percentage of initial mass recovered as microcapsules.

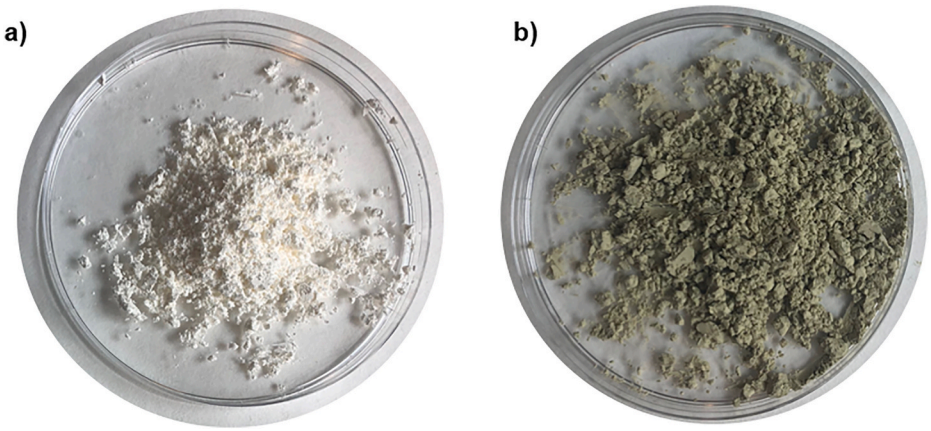

Fig. 1. Examples of omega-3 microcapsules produced by spray drying: chia oil microcapsules (a) and *Nannochloropsis gaditana* extract microcapsules (b).

3.3. Characterization of microcapsules: microencapsulation efficiency and payload

Table 2 shows characterization results of produced omega-3 microcapsules including total and free oil determination, microencapsulated oil values, microencapsulation efficiency and payload.

Table 2
Characterization of produced omega-3 microcapsules: microencapsulation efficiency (MEE) and payload.

Microcapsule type	Oil determination			***MEE***[d] ***(%)***	***Payload***[e] ***(mg/g)***
	Total oil$_t$[a] *(mg/100 mg)*	*Surface oil*[b] *(mg/100 mg)*	Microencapsulated oil[c] (mg/100 mg)		
Chia oil	15.2 ± 1.9	4.8 ± 0.4	10.5 ± 1.5	68.7 ± 1.6	104.8 ± 15.4
Camelina oil	14.1 ± 0.6	5.8 ± 0.3	8.3 ± 0.3	58.8 ± 0.4	82.9 ± 2.8
Echium oil	19.9 ± 3.5	5.7 ± 0.8	14.2 ± 2.7	71.3 ± 1.2	142.4 ± 27.5
Chia FAEEs	14.9 ± 1.7	3.4 ± 0.0	11.5 ± 1.7	76.9 ± 2.7	115.2 ± 17.1
N. gaditana extract	3.1 ± 0.2	1.3 ± 0.1	1.8 ± 0.3	57.0 ± 6.7	17.6 ± 3.1

Mean ± standard deviation based on duplicate determinations.
[a] Total oil (mg oil/100 mg of microcapsules).
[b] Surface oil (mg oil/100 mg of microcapsules).
[c] MEE, Microencapsulation efficiency (%).
[d] Payload (mg of active compound/1000 mg of microcapsules).

Microencapsulation efficiency was significantly influenced by the type of lipid extract used: values varied from 57.0 to 76.9%. Moreover, all produced microcapsules were characterized by a minimal surface oil content, which is related to a low oxidation rate. Measurement of surface oil is important as this oil can oxidize extremely rapidly, so that high surface oil tends to correlate with off-flavor of microcapsules and poor food application stability (Kaushik et al., 2015). The best microencapsulation efficiency was achieved for chia FAEEs microcapsules (76.9%) (significant differences at 5% level), which also showed the best microencapsulation conditions according to the operating parameters (see section 3.2). On the other hand, the lowest efficiency was found for microcapsules prepared with microalgal lipids (57.0%) and camelina oil (58.8%) (no significant differences at 5% level). *Nannochloropsis gaditana* microcapsules showed the lowest amount of total oil (3.1 mg/100 mg of microcapsules) (significant differences at 1% level). These results suggest that the microencapsulation of microalgal extracts involves an added difficulty to the process due to the heterogeneous composition of the sample. Thus, it was concluded that the microencapsulation of various lipid extracts rich in omega-3 PUFAs using the above process was feasible. The results achieved regarding to microencapsulation efficiency in the current research were in agreement with previous findings reported for other natural sources of omega-3 fatty acids (efficiency values reported from 49 to 95%) (Carneiro, Tonon, Grosso, & Hubinger, 2013; Kolanowski, Ziolkowski, Weißbrodt, Kunz, & Laufenberg, 2006; Timilsena, Wang, Adhikari, & Adhikari, 2017).

Using the same wall materials (sodium caseinate and lactose), Ixtaina et al. reported a microencapsulation efficiency of 90%, but the emulsion was formed using a high pressure homogenizer (Ixtaina et al., 2015). These results emphasize the importance of emulsion formation and homogenization during microencapsulation by spray drying. Other

authors have investigated the use of whey protein and gum arabic as wall materials for the microencapsulation of chia oil, obtaining efficiencies from 70 to 80% (Rodea-González et al., 2012). Less common wall materials have been studied for the encapsulation of chia oil (soy protein and maltodextrin), obtaining efficiencies from 52.2 to 60.2% (González et al., 2016). Therefore, wall material, emulsion preparation and process conditions used seem to significantly affect the microencapsulation efficiency.

In addition, payload or load capacity of produced microcapsules was also calculated. Payload value is important to determine the economic viability and efficacy of microencapsulation process. A high payload means that the process becomes more effective and economically feasible (Islam, Mahmud, Nawas, Fang, & Xia, 2018). Due to the oil to wall ratio 1:4 (w/w) used in this work, the maximum loading capacity is 200 mg of active ingredient per gram of microcapsules. As can be seen in Table 2, payload ranged from 17.6 to 142.4 mg; *Nannochloropsis gaditana* microcapsules showed the lowest payload value (significant differences at 1% level). Although the microcapsules composed by chia FAEEs showed the highest microencapsulation efficiency, echium oil microcapsules reached the highest payload (significant differences at 5% level). From a technical point of view, a high payload implies a smaller amount of microcapsules in the formulation of functional foods (Oxley, 2014). According to the results of the current research, the addition of echium oil microcapsules would have better economic viability to produce a novel functional food enriched in omega-3 fatty acids. Nevertheless, the composition of omega-3 PUFAs and their percentage in the microencapsulated oil should be evaluated and considered, as discussed in the following section.

3.4. Effect of microencapsulation process on the fatty acid composition

Microencapsulated extracts were analyzed by GC-MS in order to evaluate the effect of microencapsulation process on fatty acid composition. Table 3 shows the fatty acid composition of lipid extracts before and after microencapsulation process. In general, oilseeds were characterized by a low percentage of saturated fatty acids (SFAs) and monounsaturated fatty acids (MUFAs), except for camelina oil, which showed a higher percentage of MUFAs due to the identification of an uncommon fatty acid in vegetable oils, gondoic acid (20: 1 *cis*-11). Among the main types of fatty acids, it should be emphasized the high percentage of PUFAs identified in all investigated vegetable oils (chia, camelina and echium oils) and chia FAEEs, and specifically, the high content of omega-3 fatty acids (30.7, 48.0, 63.4 and 64.2% for camelina oil, echium oil, chia FAEEs and chia oil, respectively). Two omega-3 PUFAs were identified in seed oils: ALA and SDA, the latter exclusively in echium oil, which is considered the highest natural source of this special fatty acid by several authors (Castejón, Luna, & Señoráns, 2018; Zanetti, Monti, & Berti, 2013). Regarding the fatty acid profile of the lipid extract of *Nannochloropsis gaditana* (data not shown in Table 3, as further explained), microalgae extracts were mainly characterized by palmitic acid (16:0) (25.8%), palmitoleic acid (16:1 *cis*-9) (26.6%) and omega-3 EPA (29.2%), being the major component of the total fatty acids identified.

Furthermore, GC-MS analysis showed that microencapsulation by spray drying did not negatively affect the fatty acid profile of vegetable oils (chia, camelina and echium oils) and chia FAEEs. The fatty acid profile of microencapsulated lipids was similar to the natural profile (non-microencapsulated extracts) found for these extracts (see Table 3). However, a different behavior was found for the lipid extract of *Nannochloropsis gaditana*. After microencapsulation, the fatty acid profile of *Nannochloropsis gaditana* was different from the original extract with a large dispersion between analyses. This result could be caused by the different composition in neutral and polar lipids of this microalga extracts as mentioned before (Castejón & Señoráns, 2019), which could affect emulsion formation and subsequent microencapsulation. The lack of homogeneity and the small amount of sample analyzed (low lipid recovery from microcapsules) could lead to analysis errors. Therefore, results of the fatty acid composition of *Nannochloropsis gaditana* after microencapsulation were not considered conclusive for this study.

Additionally, in order to deeply understand the effect of microencapsulation on the omega-3 content, the percentage of total omega-3 fatty acids before and after microencapsulation process has been studied. As can be seen in Table 3, the percentage of omega-3 was similar after and before spray drying, and only a slight variation in the omega-3 fatty acids was found after the microencapsulation process (no significant differences at 5% level), with the mentioned exception of the lipid extract of *Nannochloropsis gaditana*. Once the omega-3 content of microencapsulated extracts was determined, it was related to the

Table 3
Fatty acid composition (as percentage of total fatty acids) of lipid extracts determined by GC–MS before and after microencapsulation process.

Fatty acid	% Fatty acids[a]							
	Chia oil		Camelina oil		Echium oil		Chia FAEEs	
	Before[b]	After[c]	Before[b]	After[c]	Before[b]	After[c]	Before[b]	After[c]
16:0	7.6 ± 0.2^{A}	6.2 ± 0.0^{B}	5.2 ± 0.2^{B}	6.0 ± 0.0^{A}	7.7 ± 0.2^{B}	8.9 ± 0.3^{A}	8.1 ± 0.1^{A}	8.1 ± 0.0^{A}
18:0	3.6 ± 0.1^{B}	5.6 ± 0.2^{A}	2.6 ± 1.3^{A}	3.4 ± 0.5^{A}	4.2 ± 0.5^{A}	5.9 ± 0.5^{A}	3.8 ± 0.1^{A}	3.1 ± 0.0^{B}
18:1 ω-9	7.2 ± 0.1^{B}	7.7 ± 0.3^{A}	17.8 ± 0.3^{A}	17.6 ± 0.3^{A}	16.6 ± 0.0^{A}	16.3 ± 0.2^{A}	6.7 ± 0.3^{A}	7.3 ± 0.3^{A}
18:2ω-6c	18.2 ± 0.0^{A}	17.9 ± 0.2^{A}	17.5 ± 0.2^{A}	17.5 ± 0.5^{A}	13.5 ± 0.3^{A}	13.0 ± 0.2^{A}	17.2 ± 0.0^{A}	17.7 ± 0.0^{A}
20:0	–	–	1.6 ± 0.1^{A}	1.8 ± 0.1^{A}	–	–	–	–
18:3 ω-6	–	–	–	–	10.0 ± 0.1^{A}	10.1 ± 0.1^{A}	–	–
18:3 ω-3	**63.4 ± 0.3**^{A}	**62.6 ± 0.3**^{A}	**30.3 ± 0.6**^{A}	**28.4 ± 0.3**^{B}	**33.3 ± 0.3**^{A}	**31.7 ± 0.1**^{B}	**64.2 ± 0.2**^{A}	**63.8 ± 1.0**^{A}
20:1 ω-9	–	–	19.1 ± 0.1^{A}	19.1 ± 0.2^{A}	–	–	–	–
18:4 ω-3	-	-	-	-	**14.7 ± 0.6**^{A}	**14.1 ± 0.2**^{A}	-	-
20:2 ω-6	–	–	1.7 ± 0.1^{A}	1.8 ± 0.1^{A}	–	–	–	–
20:3 ω-3	-	-	**0.4 ± 0.0**^{A}	**0.4 ± 0.2**^{A}	-	-	-	-
22:1 ω-9	–	–	3.8 ± 0.0^{A}	4.0 ± 0.0^{A}	–	–	–	–
SFAs	11.2	11.8	9.4	11.2	11.9	14.8	11.9	11.2
MUFAs	7.2	7.7	40.7	40.7	16.6	16.3	6.7	7.3
PUFAs	**81.6**	**80.5**	**49.9**	**48.1**	**71.5**	**68.9**	**81.4**	**81.5**
ω-6	18.2	17.9	19.2	19.3	23.5	23.1	17.2	17.7
ω-3	**63.4**	**62.6**	**30.7**	**28.8**	**48.0**	**45.8**	**64.2**	**63.8**
ω-6/ω-3 ratio	0.3	0.3	0.6	0.7	0.5	0.5	0.3	0.3

SFAs, saturated fatty acids; MUFAs, monounsaturated fatty acids; PUFAs, polyunsaturated fatty acids.
Different capital letters for the same sample indicate statistically significant differences before and after microencapsulation process (Student's *t*-Test, $p < 0.05$).
[a] Results expressed as percent over the total content (relative content). Values are the mean ± SD of two determinations.
[b] Original oil analyzed before microencapsulation process.
[c] Oil analyzed after microencapsulation process (total oil extracted from microcapsules).

payload in order to calculate the percentage of microencapsulated omega-3 PUFAs per gram of microcapsules. The results were 23.9% for camelina oil, 65.3% for echium oil, 65.5% for chia oil and 72.9% for chia FAEEs. Consequently, the highest omega-3 fatty acids payload was obtained for the microcapsules formed by chia FAEEs, followed by chia and echium oils.

Therefore, it can be concluded that the microencapsulation process did not affect the fatty acid profile of microencapsulated extracts, and specifically the omega-3 percentage. The present results were in agreement with other authors (Comunian, Gomez-Estaca et al., 2016; Sugasini & Lokesh, 2013), who also concluded that there was no significant change in the fatty acid composition after microencapsulation process.

3.5. Evaluation of particle size and morphology of microcapsules by scanning electron microscopy (SEM)

SEM images of microencapsulated omega-3 lipid extracts were used to analyze the size and morphology of produced microcapsules (see Fig. 2). Particle size (diameter of microcapsule) inferred from microphotographs ranged from 1.5 to 30 μm with a relatively high polydispersity, depending on the type of omega-3 lipid microencapsulated. The inferred size was in accordance with results reported by other authors for microcapsules produced by spray drying ranging up to 50 μm (Desai & Jin Park, 2005; Gharsallaoui et al., 2007; Nedovic, Kalusevic, Manojlovic, Levic, & Bugarski, 2011).

The most homogenous particle size was found for chia oil microcapsules with a size average of approximately 3.5 μm (see Fig. 2(a)), followed by chia FAEEs microcapsules (size average of approximately 4.2 μm, see Fig. 2(d)). Particle size highly depends on the efficacy of microencapsulation process. Several authors have described a relation between particle size and microencapsulation efficiency (Drusch, Serfert, Scampicchio, Schmidt-Hansberg, & Schwarz, 2007; Gallardo et al., 2013). In this work, the best microencapsulation efficiency also corresponded with the smallest particle size found. Nevertheless, the highest dispersion size was found for camelina oil microcapsules (see Fig. 2(b)), which agrees with the lowest experimental result of microencapsulation efficiency.

Regarding to microcapsules morphology and surface, most of produced microcapsules showed spherical shape and smooth surface with no cracks or pores, which is important to provide a better protection of the active ingredient. Several authors also observed spherical microcapsules without cracks or pores on the surface of fish or chia oil microparticles prepared by using sodium caseinate, lactose and whey proteins (Aghbashlo, Mobli, Rafiee, & Madadlou, 2012; Anandharamakrishnan, Rielly, & Stapley, 2007; Ixtaina et al., 2015). Moreover, surfaces were concave and wrinkled, which is typical of microcapsules produced by spray drying. This type of morphology has also been observed by other authors (Karaca, Nickerson, & Low, 2013; Tonon, Grosso, & Hubinger, 2011). According to Goyal et al. it can be explained by irregular drying or droplet collapse during the initial stages of drying, when there is a slow process of film formation and a high content of solids (Goyal et al., 2015). In Fig. 2(c) and (d) corresponding to echium and *Nannochloropsis gaditana* microcapsules, it could be easily observed this typical type of irregularity on particle surface. Another characteristic observed was the aggregation of the microcapsules, as it is evident from Fig. 2(b). The aggregation of microcapsules could be due to presence of oil surface and/or moisture (Shivakumar, Chetana, & Reddy, 2012). The preceding results are directly in line with previous findings by other authors describing similar microcapsule aggregation (Partanen et al., 2008).

4. Conclusions

In conclusion, the present work is an original research on microencapsulation of different lipid extracts rich in omega-3 PUFAs from several sources, such as novel vegetable oils, fatty acid ethyl esters and microalgal extracts, showing the versatility of microencapsulation process by spray drying and its effect on PUFA composition. Microencapsulation efficiency depended on the type of lipid extract to encapsulate and varied from 57.0 to 76.9%. The highest microencapsulation efficiency was found for chia FAEEs microcapsules (76.9%), while echium microcapsules showed the highest payload (142 mg/g). The analysis by GC-MS showed that the microencapsulation process did not negatively affect the fatty acid profile of microencapsulated extracts, and specifically the omega-3 fatty acid composition (no significant differences at 5% level). Under the optimal conditions, produced microcapsules ranged from 1.5 to 30 μm and SEM images showed spherical shape and smooth surface with no cracks or pores, which indicated the microencapsulation of omega-3 lipid extracts with the above process was suitable. Hence, the present study shows an integrated process starting from raw materials of different nature to the production of microencapsulated lipids, without affecting the omega-3 fatty acid composition and with

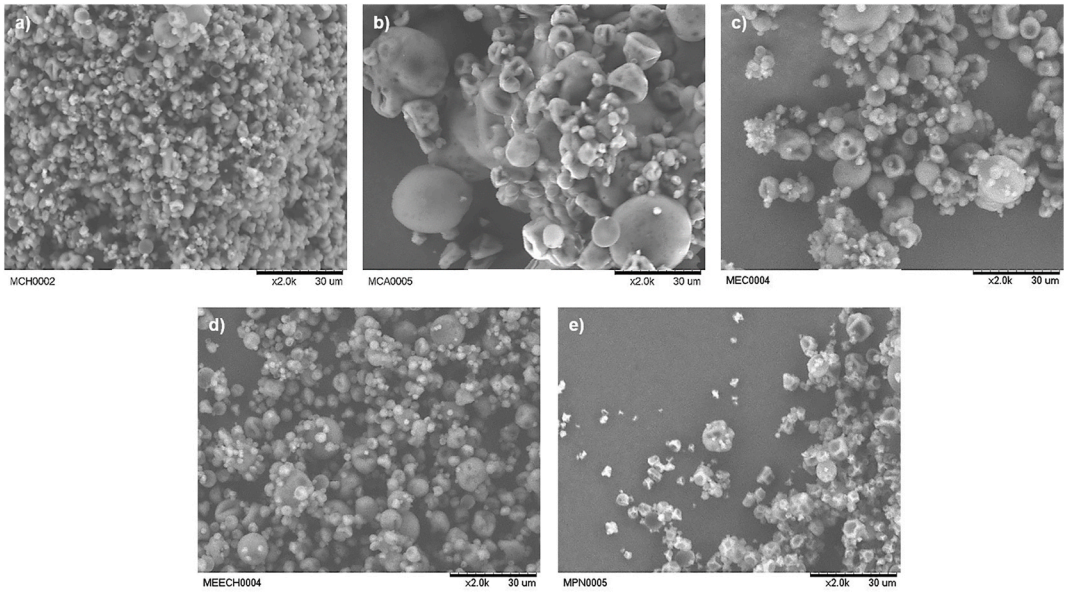

Fig. 2. SEM images of omega-3 microcapsules (x3000): chia oil (a), camelina oil (b), echium oil (c), FAEEs from chia oil (d) and *Nannochloropsis gaditana* lipid extract (e).

high microencapsulation efficiency and payload, which are valuable ingredients for potential applications in the development of functional foods enriched in omega-3 fatty acids.

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.

CRediT authorship contribution statement

Natalia Castejón: Methodology, Investigation, Validation, Writing – original draft, Visualization, Writing – review & editing. **Pilar Luna:** Conceptualization. **F. Javier Señoráns:** Supervision, Conceptualization, Resources, Writing – review & editing, Project administration, Funding acquisition.

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