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Title**CHARACTERISATION OF FREEZE-DRIED FLAXSEED OIL
MICROCAPSULES OBTAINED BY MULTILAYER EMULSIONS****Authors**

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1. INTRODUCTION

Long chain omega-3 polyunsaturated fatty acids (PUFAs) have been related to several cardioprotective mechanisms, development of visual and cognitive functions in foetus and young children as well as prevention of inflammatory and autoimmune disorders [1,2,3]. Despite the proven health benefits, the intake of these nutraceuticals does not meet the recommended intake in most countries worldwide [4]. Therefore, there is a growing interest to enrich food products with ω -3 PUFAs. Flaxseed oil is a good vegetable omega-3 source in nature, since it contains high levels of α -linolenic acid (50-60% of total fatty acids) [5]. Nevertheless, there are some limitations to incorporate these oils into aqueous food matrices due to their lipophilic nature and high oxidation rates. Thereby, microencapsulation comprises an excellent tool for obtaining a powdered functional ingredient which could be then incorporated into a food matrix. There is a novel technology called layer-by-layer deposition technique which consists in designing multilayers around oil droplets through electrostatic self-assembly between oppositely charged biopolymers, thereby obtaining emulsions with interfacial layers of greater thickness [6]. In previous work we focused on obtaining stable emulsions to address successful microencapsulation of flaxseed oil by applying a two-step method which comprised obtaining a coarse emulsion through mechanical agitation in a blender and then applying an ultrasound treatment to reduce droplet sizes, using whey proteins (WP) and sodium alginate (SA) as encapsulation materials. The best conditions to produce emulsions with high stability against creaming, flocculation and coalescence mechanisms were determined [5]. The next step would require dehydration of emulsions to obtain powdered microcapsules. Most of the research work found in the literature about microencapsulation of flaxseed oil used spray-drying to obtain microcapsules [7,8,9,10]. Since flaxseed oil contains heat-sensitive compounds,

lyophilisation would be an interesting technique to apply as drying is carried out at temperatures lower than ambient temperatures [11]. In this case, it is necessary to add a cryoprotectant (e.g. maltodextrin) when formulating emulsions to increase the physicochemical stability of the systems during freezing [12]. Nevertheless, there is little information about microencapsulation of flaxseed oil by freeze-drying. Quispe-Condori, Saldaña and Temeri [13] compared the quality of flaxseed oil microcapsules using spray and freeze drying. They reported reduced microencapsulation efficiencies using freeze-drying, but they worked with monolayer emulsions obtained by high speed homogenization. We hypothesize that formulation of multilayer emulsions with interfacial layer of greater thickness could provide better protection for the bioactive agent entrapped within the core.

It is also interesting to highlight that although some authors have applied ultrasonication to obtain highly stable submicron emulsions for oil encapsulation [14,15], they focused on obtaining small droplet sizes so as to reduce creaming and coalescence destabilization mechanisms, without assessing oxidative stability of the oil. To the best knowledge of the authors, there are no studies concerning about the oxidative stability of flaxseed oil entrapped in freeze-dried microcapsules obtained by ultrasonically generated multilayer emulsions.

In the present contribution, we aim to obtain and characterise lyophilized flaxseed oil microcapsules containing variable maltodextrin (MD) concentrations, and to evaluate the influence of microencapsulation processing steps on the oxidative stability of the oil, from emulsions formation to powdered microcapsules obtention.

2. MATERIALS AND METHODS

2.1. Materials

Whey protein isolate (WPI) was donated by Davisco Food International, Inc. (USA). Maltodextrin (DE 15) was kindly donated by Productos de Maíz SA (Argentina). Low density sodium alginate (SA) was provided by Cargill (Argentina) (MW 135 kDa). Flaxseed oil was purchased from Sigma Aldrich (USA) and it was used without further purification. According to literature, α -linolenic acid content of flaxseed oil is within the range of 48-57% of total fatty acids [14,15,16].

2.2. Microcapsule formation from oil-in-water multilayer emulsions

Preparation of multilayer emulsion was done by the procedure described in previous work [12]. Briefly, a primary emulsion was obtained by blending 20% (w/w) oil phase with 80% (w/w) aqueous phase having WPI-MD dispersions using a high-speed blender (Waring Blender, USA) for 2 min at the highest speed (24000 rpm), followed by a sonication step (75% Amplitude, 150 s) performed by a 20 KHz ultrasonic probe with 13 mm diameter tip (Sonics & Materials, USA). Samples were placed in an ice bath to avoid over-heating during sonication, and the final temperature of primary emulsions was 25°C. These fixed conditions for ultrasonic processing were previously chosen on the basis of preliminary experiments where different combinations of wave amplitudes (AMP) and times of sonication were assessed, with the aim to produce droplet sizes in the range of 1 μ m to enhance emulsion stability against creaming and coalescence mechanisms. Primary emulsion was then diluted by adding sodium alginate (SA) dispersion and adjusting pH to 5 with HCl 2N to form secondary emulsion, with final composition presented in Table 1. These

systems were then frozen at -20°C and lyophilized for 3 days at a pressure of 40 Pa, using a HETO FD 25 freeze dryer (China). Two individually prepared replicates were assayed for each condition.

2.3. Freeze-dried powders characterization

2.3.1. Encapsulation Efficiency

Encapsulation efficiency of powder microcapsules was determined by the method described in McClements, Decker and Weiss [6], with modifications. Extraction of free oil (FO) was performed by adding 12 mL of hexane to 2 g powder, the mixture was left for 5 min at room temperature and then centrifuged (3000 g, 5 min) (Heal Force, China). The supernatant was filtered through filter paper (Munktell 00R, Sweden) and the powder residue was rinsed twice with hexane. The filtrate was then placed in a rotary evaporator at 60°C for 15 min and then the solvent-free extract was dried at 105°C . FO was determined gravimetrically. Total oil (TO) was quantified using the AOAC Official Method 925.32, with some modifications. Emulsions were first reconstituted by adding the required amount of water to 1 g of freeze-dried powder and vortexing the mixture for 3 min, then 10-mL of 10N HCl were added and the tubes were placed in a 70°C water bath which was immediately heated to 100°C for 30 min. After cooling, the resulting dispersion was extracted with 25 mL hexane/isopropanol (3:1 v/v), vigorously shaken for 5 min in a vortex mixer and centrifuged (5000 g, 10 min). The clear organic phase was collected and the aqueous phase reextracted. Solvent was then evaporated using the same conditions described above. The solvent-free extract was dried at 105°C and TO was determined gravimetrically. Both TO and FO determinations were done in triplicate. Finally, encapsulation efficiency was obtained from the following formula:

$$\%EE = [TO \text{ (g/100g powder)} - FO \text{ (g/100g powder)}] / [TO \text{ (g/100g powder)}] \times 100 \quad Eq. 1$$

2.3.2. Water activity

Water activity of powdered microcapsules was determined at ambient temperature (25°C) by an Aqualab system (Washington, USA). Samples were measured in triplicate.

2.3.3. Morphological analysis

The microstructure of powdered samples was investigated by scanning electron microscopy (JSM-35C, JEOL, Japan). Samples were placed on the SEM stubs using a two-sided adhesive tape, and were then coated with gold using a magnetron sputter coater. Coated samples were analyzed at an accelerating voltage of 20 kV.

2.4. Oxidative stability of flaxseed oil

2.4.1. Primary oxidation compounds

Lipid hydroperoxides were determined weekly using a method adapted from Shanta and Decker [19] (IDF method) which comprised an additional extraction step in which 0.3 mL of reconstituted emulsion was added to a mixture of 1.5 mL isooctane/isopropanol (3:2 v/v) followed by vortexing three times for 10 s each and centrifugation (3400 g, 5 min). Next, 0.2 mL of the upper

organic phase (0.9 mL total volume) was added to 2.8 mL of chloroform/methanol (7:3 v/v), followed by 15 μ L of ammonium thiocyanate solution (3.94 M) and 15 μ L of ferrous iron solution (prepared by mixing 0.132 M BaCl₂ and 0.144 M FeSO₄ in acidic solution). The solution was vortexed for 4 s and the absorbance at 500 nm was measured after 10 min incubation at room temperature. The entire procedure was conducted in subdued light. Lipid hydroperoxide concentrations were determined using a Fe³⁺ standard curve. The peroxide value (PV) was expressed as milliequivalents of peroxide per kilogram of sample as follows:

$$PV = (A_S - A_{SB} - A_{RB}) / (m \times 55.84 \times m_0 \times 2) \times 4.5 \quad \text{Eq. 2}$$

where A_S is the absorbance of the sample, A_{SB} is the absorbance of the sample blank, A_{RB} is the absorbance of the reagent blank, m is the slope obtained from the calibration curve, m_0 is the mass of the oil in grams, 55.8 is the atomic weight of iron, 2 is a division factor necessary to express the peroxide value as milliequivalents of peroxide instead of milliequivalents of oxygen, 4.5 corresponds to the inverse of the dilution factor related to the isooctane organic phase (0.9/0.2).

Each sample was measured in duplicate.

2.4.2. Secondary oxidation compounds

Thiobarbituric acid-reactive substances (TBARS) were also determined weekly, using the method described in Tong, Sasaki, McClements and Decker [20], where 1 mL of reconstituted emulsion was combined with 2 mL of TBA reagent (15% w/v trichloroacetic acid and 0.375% w/v thiobarbituric acid in 0.25 M HCl) in test tubes and placed in a boiling water bath for 15 min. The

tubes were cooled to room temperature for 10 min and then centrifuged (12000 g, 15 min). The absorbance was measured at 532 nm. Concentrations of TBARS were determined from a standard curve prepared using 1,1,3,3-tetramethoxypropane.

2.8. Statistical analysis

Each experiment was carried out with its corresponding replication (two replicates). All assays were performed at least in duplicate. Averages and standard deviations were calculated from these measurements. Differences between means were determined by applying analysis of variance using LSD test at $p < 0.05$ significance level. When homogeneity of variance assumption was not satisfied, Kruskal Wallis test at $p < 0.05$ and boxplots were used to identify significant differences (Statgraphics Centurion XV).

3. RESULTS AND DISCUSSION

3.1. Freeze-dried powders characterization

Encapsulation efficiency was determined to evaluate the degree of protection of the oil within the powdered microcapsules. This parameter depends on numerous factors such as the nature of the core material, type and composition of wall material, ratio of core material to wall material, droplet size distribution in the emulsions [21,22]. Table 2 shows the values of free oil (FO), total oil (TO) and encapsulation efficiency (EE) for each powder containing different MD concentrations. FO represents the portion of flaxseed oil present on the surface of the microcapsules. Overall, FO

was found to decrease when increasing MD concentration in the aqueous phase of emulsions. It is crucial to minimize the amount of FO in lipid encapsulation, as this material can undergo oxidative deterioration at more rapid rates than the encapsulated oil, thereby reducing shelf life of the functional ingredient [23].

In this regard, it can be seen that EE values were also affected by MD concentration. Powders containing no MD presented the lowest EE, which was below 30%, this being related to higher levels of unencapsulated oil (FO). However, when adding MD, EE increased significantly, obtaining values above 90% in M20. This behavior might be related to total solids content of emulsions (Table 1). According to Gharsallaoui, Roudaut, Chambin, Voilley, and Rémi [24], microencapsulation efficiency can be increased by increasing wall solution solids concentration as they become part of the wall structure of the powdered microcapsules once emulsions are dehydrated, acting as a coated supporting matrix. Moreover, it has been suggested that increasing total solid concentration leads to a higher viscosity of the aqueous phase of former emulsions and smaller droplet sizes [25], which could minimize the internal circulation of oil inside de droplets, preventing their migration to the surface and, consequently improving oil encapsulation [21]. This is consistent to our previous work, where we reported lower droplet sizes (D_{32}) for emulsions containing 20% MD [12]. In that contribution it was observed that whereas emulsions containing 0 and 10% MD exhibited droplet sizes around $1\mu\text{m}$, 20% MD systems showed droplet sizes of $0.4\mu\text{m}$. This latter was attributed to MD concentration, that could have modified the viscosity ratio of dispersed to continuous phase.

It should also be noted that TO decreased significantly when increasing MD concentration. Although a high-oil-load would be desirable in the powder, it is important to ensure the complete protection of the oil, so as to minimize the amount of unencapsulated oil. As discussed previously,

higher amounts of wall solids would prevent collapse of the powder and the leaking of the oil. Several authors have reported oil loads between 20-40% [7,8,26,27] in spray-dried powders.

When examining the appearance of freeze-dried microcapsules it was clearly seen that powders without MD presented a bright yellow oily consistency, with the formation of agglomerates of sponge-like texture (Fig. 1). This could be related to emulsion destabilization during the freezing step prior to freeze-drying, as reported in Fioramonti, Arzeni, Pilosof, Rubiolo and Santiago [12], and the excessive amount of FO showed in Table 1 (60%) might have led to aggregation of powder particles [8]. However, addition of MD greatly improved the macroscopic appearance of the powders, as the yellow color associated with unencapsulated oil gradually disappeared when increasing its concentration.

SEM is a commonly used technique to observe the external microstructure of microcapsules [8]. Images of freeze-dried powders containing different MD concentrations are shown in Fig. 2. It bears noting that figures on the left side were taken at low magnification in order to analyze the general topography of the surface of the powders, thus examining a larger area (Fig. 2A, C, E). On the other hand, figures on the right correspond to a greater magnification of the systems so as to take a closer look to identify individual microcapsules structures (Fig. 2B, D, F).

The external structure of powders containing no MD revealed an irregular continuous surface with multiple folds (Fig. 2A), this probably being related to oily consistency of the powders and the high amounts of unencapsulated oil reported. Fig. 2B presents an individually existing microcapsule exhibiting a smooth surface with some holes on it, probably caused by destabilization of the emulsion during the freezing step. As we discussed in previous work [12], ice crystals formed in the aqueous phase of emulsions without MD stored at -20°C might have penetrated into oil droplets, thus disrupting their interfacial membranes. This latter would promote coalescence and

leaking of the oil from the core to the surface, leading to excessive amounts of free oil in the powders. This is consistent with results presented in Table 2, where more than 70% of FO was observed.

On the other hand, when adding MD, the powders presented a structure that resemble flakes, similar to that describe in the literature for freeze-dried powders [26,28,29], where microcapsules appeared to be buried within the MD matrix surrounding them as shown in Fig. 2D and 2F. In M20 systems, round shaped microcapsules can be clearly seen embedded within the MD matrix (Fig. 2F). In these latter systems, the oil would be entrapped inside the WPI-SA double protective layer and this structure might be underpinned by the coating wall matrix [5]. So, addition of MD could contribute to reduce levels of free oil content as there would be less opportunity for the core material to come onto the surface of the particles. It bears noting that in Fig. 2C there are some visible pores within the structure of M10 powders, and this could be related to sublimation process during freeze-drying where ice would be replaced by air [26,46].

Fig. 3 shows a_w values of powders, where an increase of water activity was observed with increasing MD concentration. These results were opposite to what it was expected, as several authors have reported a decrease of a_w when increasing MD concentration in powders [30,31,32,33]. Water activity measures the availability of free water in a food system [32] and polysaccharides are known to modify and control the mobility of water in food systems. As MD are polyols in which each hydroxyl group have the possibility of hydrogen bonding to bind to one or more water molecules [34], it was expected that an increase in MD concentration could reduce the water activity of the powders. However, the observed behavior in Fig. 3 might be associated with the freeze-drying process itself. This is, the higher the solids content in frozen emulsions, the higher the resistant of water molecules to diffuse, thereby diminishing mass-transfer during freeze-drying

process [35,36]. Hence, less water removal would occur when dehydrating emulsions at higher MD concentrations. This hypothesis could be also supported by the results reported by several authors where particles obtained by freeze-drying treatments had higher moisture and water activity than the same samples subjected to spray drying [37, 31,30].

In addition, it is interesting to note that powders containing no MD showed a_w values lower than 0.2 (Fig. 3). This has to be taken into account as such low values of a_w might promote an increase in lipid oxidation rates during storage [38,39]. Nevertheless, when adding MD, a_w of powders was between the range 0.20-0.40 which is considered as stable for browning, lipid oxidation, microbial growth and enzymatic reactions [31].

3.2. Flaxseed oil oxidative stability through microencapsulation processing steps

Lipid oxidation of oils rich in ω -3 polyunsaturated fatty acids limits storage and affects product quality as it leads to rancid taste and off flavors. The complicated thing about oxidation is that once initiated, it produces free radicals and propagates in a chain reaction [40]. To monitor oxidative stability of flaxseed oil during microencapsulation process, we measured both primary and secondary oxidation products. The peroxide value (PV) determines the amount of hydroperoxides which are among the primary products, that first increase and reach a maximum, but as they are not stable, they further decompose into secondary oxidation products (aldehydes, ketones). These latter are determined by measuring thiobarbituric reactive substances (TBARS).

Fig. 4 and 5 show PV and TBARS, respectively, of flaxseed oil after different microencapsulation processing steps: (i) emulsion preparation and (ii) freeze-dried powders obtention. Initially, unencapsulated oil presented low values of PV (0.55 meq/kg oil) and TBARS

(0.19 meq MDA/kg oil), which was within the range allowed for fresh oils (max. 10 meq/kg oil) (Codex Alimentarius, FAO). However, encapsulation process significantly increased oil PV after preparation of multilayer emulsions for M0 and M20 systems (Fig. 4) indicating lipid oxidation in both systems. Presumably, mechanical agitation in the blender and the subsequent sonication step could have promoted the incorporation of oxygen and the generation of temperature gradients during emulsions preparation, leading to oil oxidative deterioration. As reported in literature, cavitation is the main phenomena related to ultrasonic emulsification [15, 41]. It comprises the rapid formation and collapse of micro-bubbles that occur at the oil-water interface under the influence of high-intensity acoustic field produced by sound waves. The collapse of the cavitation bubble could have created a transitory hot spot with elevated localized temperature, which might have accelerated the chemical reactivity of heat-sensitive compounds [41, 42]. Particularly, addition of 10% MD seemed to have protected oil from oxidative deterioration during emulsions formation, as PV values were low for these systems (1.49 meq/Kg oil). Chua [43] postulates that sonication in aqueous medium is believed to promote the formation of highly reactive hydroxyl radicals from water molecules, and that the combination of these radicals would produce hydrogen peroxide, thereby enhancing PUFAs oxidative degradation. When adding 10% MD to the systems, this latter phenomenon could have been slowed down, as polysaccharides such as MD have the ability to bind water molecules, thus making them unavailable for hydroxyl radical generation during cavitation. It is noteworthy that higher MD concentrations produced exactly the opposite effect. As shown in Fig 3A, M20 emulsions exhibited PV close to that observed for systems without MD. This might be related to results reported in previous work, in which emulsions containing 20% MD showed lower droplet sizes (D_{32}) and higher surface specific area [12]. This might have played an important role in oxidation of the oil as larger interfacial surface area would facilitate interactions between the oil

and water-soluble prooxidants – such as transition metals – that could have been present in the continuous phase of emulsions [44,45]. It bears noting that, we also reported higher energy inputs during sonication step of primary emulsions at higher MD concentrations in previous work (Fioramonti), which was attributed to the ultrasonic processor that was designed to deliver constant wave amplitude. So, the greater the resistance to the movement of the probe due to higher viscosity of 20% MD emulsions, the greater the amount of power delivered to the probe to maintain the amplitude. As a result of these higher energy inputs, formation of greater temperature gradients and transitory hot spots could have increased lipid oxidation rates in M20 systems (Fig. 4).

As regards to freeze drying influence on lipid oxidation, it can be seen that there was no significant ($p < 0.05$) difference between PV of M0 powders and former emulsions. In fact, M0 freeze-dried powders showed the highest PV value (Fig 4) which was consistent with the high levels of unencapsulated oil observed in these systems (Table 2). So, it might be possible that flaxseed oil had been completely oxidized during emulsion preparation in systems containing no MD.

When freeze-drying M10 emulsions, PV significantly ($p < 0.05$) increased from 1.5 to 46.5 meq/Kg oil. In this case, it seems like the drying process promoted further oxidation of flaxseed oil, thus spoiling the protective effect against oxidative deterioration performed by MD when preparing former emulsions. This latter might be related to the structure of freeze-dried powders which could be highly porous, thereby increasing surface area. Some authors have suggested that after sublimation, ice would be replaced by air, thereby facilitating the diffusion of oxygen within the inner part of the freeze-dried particles and promoting lipid oxidation [26, 46]. So, the easy access of oxygen onto the particle surface and also the access into the inner part of the porous structure might have promoted further lipid oxidation in M10 powders.

Powders containing 20% MD slightly decrease the PV as regards to M20 emulsions (Fig. 4). But as this does not seem to be a huge difference, it might be related to slight variations inherent to the method applied to determine lipid hydroperoxides in fresh emulsions or powder microcapsules.

When determining TBARS to monitor secondary oxidation compounds formation, a similar trend was observed. The results are shown in Fig. 5. A significant increase in TBARS values of flaxseed oil was observed after preparing emulsions M0 and M20. The amounts of TBARS developed within M0 were significantly larger than those formed within M20, whereas M10 emulsions showed similar TBARS values to those corresponding to initial oxidation state of unencapsulated oil. This latter is in agreement with low PV observed in M10 emulsions, thereby indicating that at this stage, M10 systems could be at initial states of oxidation as there was not an increase in secondary oxidation compounds concentration [40]. Freeze-drying of M0 and M20 emulsions did not produce an increment in TBARS, whereas it did for M10 systems, which is also consistent with the results shown for PV.

Finally, it should be realized that all powders presented PV beyond the range allowed for food matrices, as a consequence of high oxidation rates promoted during ultrasonic emulsification (M0 and M20 systems) and freeze-drying processing (M10 systems) . In this regard, freeze-dried microcapsules had a high content of peroxide radicals once obtained.

Several authors obtained flaxseed oil stable emulsions by applying ultrasonic emulsification, but they have focused on reducing droplet size so as to increase colloidal stability of these systems [47, 48, 49] without assessing oxidative stability of the oil during processing. So this is an important issue as high input energies during ultrasound emulsification can promote an increase in lipid oxidation rates. Furthermore, cavitation abrasion of the metallic sonotrode could induce the emission of ions or particles into the bulk [47], which could then act as prooxidants thereby

enhancing oxidative deterioration of the oil [50,44]. Therefore, extreme care should be taken during formulation of emulsions to maintain low levels of oxidation, since once initial phase of lipid autooxidation had begun, free radicals generation rates increases exponentially [50]. This might be achieved by (i) obtaining emulsions in a high pressure homogenizer, as continuous application of ultrasound in a water medium could promote generation of highly reactive hydroxyl radicals and (ii) adding antioxidants and chelating compounds to emulsion formulation to better protect the bioactive lipid core from processing conditions. As a result, microcapsules oxidative stability would be improved during storage.

4. CONCLUSIONS

Flaxseed oil powdered microcapsules were obtained through freeze-drying of multilayer emulsion. Powders containing no maltodextrin presented high contents of unencapsulated oil whereas encapsulation efficiencies above 90% were achieved when formulating emulsions with 20% maltodextrin. However, the methods applied to obtain microcapsules by ultrasonic emulsification and freeze-drying processes contributed significantly to oxidation of the oil, with the consequent formation of highly reactive peroxides, whose concentration was beyond the limit allowed for food matrices. Systems formulated with 10% maltodextrin seemed to have a protective effect against oxidation during emulsion formation, however when freeze-drying this emulsion, peroxide values increased significantly. So, extreme care should be taken when formulating oil-in-water emulsions using ultrasonic emulsification as there is concern that the high local intensities in cavitation process can promote lipid oxidation. Although the presence of thicker interfacial layers in multilayer emulsions could inhibit interactions between continuous phase prooxidant components

and the lipids in emulsion droplets, if the oil is already oxidized during ultrasonic processing to form primary emulsions, then further encapsulation could not prevent deterioration of flaxseed oil by oxidation. Additional studies should be carried out to limit the oxidation rate of flaxseed oil at this stage, so as to enhance stability of microcapsules during storage.

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REFERENCES

- [1] Q. Chen, D. McGillivray, J. Wen, F. Zhong, S.Y. Quek, Co-encapsulation of fish oil with phytosterol esters and limonene by milk proteins. *J. Food Eng.* 117 (2013) 505-512.
- [2] A. Goyal, V. Sharma, N. Upadhyay, A.K. Singh, S. Arora, D. Lal, L. Sabikhi, Development of stable flaxseed oil emulsions as a potential delivery system of ω -3 fatty acids. *J. Food Sci. Technol.* 52 (2015) 4256-4265.
- [3] A. Goyal, V. Sharma, M.K. Sihag, S. Arora, A.K. Singh, L. Sabikhi, Effect of microencapsulation and spray drying on oxidative stability of flaxseed oil and its release behaviour under simulated gastrointestinal conditions. *Drying Technol.* 34 (2016) 810-821.
- [4] C. Lemahieu, C. Bruneel, E. Ryckebosch, K. Muylaert, J. Buyse, I. Foubert, Impact of different omega-3 polyunsaturated fatty acid (n-3 PUFA) sources (flaxseed, *Isochrysis galbana*, fish oil and DHA Gold) on n-3 LC-PUFA enrichment (efficiency) in the egg yolk. *J. Funct. Foods* 19 (2015) 821-827.

- [5] S.A. Fioramonti, M.J. Martínez, A.M.R. Pilosof, A.C. Rubiolo, L.G. Santiago, Multilayer emulsions as a strategy for flaxseed oil microencapsulation: Effect of pH and alginate concentration. *Food Hydrocolloid* 43 (2015) 8-17.
- [6] D.J. McClements, E.A. Decker, J. Weiss, Emulsion-based delivery systems for lipophilic bioactive components. *J. Food Sci.* 72 (2007) 109-124.
- [7] G. Gallardo, L. Guida, V. Martinez, M.C. López, D. Bernhardt, R. Blasco, R. Pedroza-Islas, L.G. Hermida, Microencapsulation of linseed oil by spray drying for functional food application. *Food Res. Int.* 52 (2013) 473-482.
- [8] A. Goyal, V. Sharma, M.K. Sihag, S.K. Tomar, S. Arora, L. Sabikhi, A.K. Singh, Development and physico-chemical characterization of microencapsulated flaxseed oil powder: A functional ingredient for omega-3 fortification. *Powder Technol.* 286 (2015) 527-537.
- [9] R.V. Tonon, C.R.F. Grosso, M.D. Hubinger, Influence of emulsion composition and inlet air temperature on the microencapsulation of flaxseed oil by spray drying. *Food Res. Int.* 44 (2011) 282-289.
- [10] I. Tontul, A. Topuz, Influence of emulsion composition and ultrasonication time on flaxseed oil powder properties. *Powder Technol.* 264 (2014) 56-60.
- [11] V.M. Esquerdo, G.L. Dotto, L.A.A. Pinto, Preparation of nanoemulsions containing fatty acid concentrate-chitosan capsules. *J. Colloid Interf. Sci.* 445 (2015) 137-142.
- [12] S.A. Fioramonti, C. Arzeni, A.M.R. Pilosof, A.C. Rubiolo, L.G. Santiago, Influence of freezing temperature and maltodextrin concentration on stability of flaxseed oil-in-water multilayer emulsions. *J. Food Eng.* 156 (2015) 31-38.
- [13] S. Quispe-Condori, M.D.A. Saldaña, F. Temelli, Microencapsulation of flax oil with zein using spray and freeze drying. *LWT–Food Sci. Technol.* 44 (2011) 1880-1887.

- [14] E.K. Silva, V.M. Azevedo, R.L. Cunha, M.D. Hubinger, M.A.A. Meireles, Ultrasound-assisted encapsulation of annatto seed oil: Whey protein isolate versus modified starch. *Food Hydrocolloid* 56 (2016) 71-83.
- [15] M. Kaci, S. Meziani, E. Arab-Tehrany, G. Guillet, I. Desjardins-Lavisse, S. Desobry, Emulsification by high frequency ultrasound using piezoelectric transducer: Formation and stability of emulsifier free emulsion. *Ultrason. Sonochem.* 21 (2014) 1010-1017.
- [16] L.G. Gillingham, S.V. Harding, T.C. Rideout, N. Yurkova, S.C. Cunnane, P.K. Eck, P.J.H. Jones, Dietary oils and FADS1-FADS2 genetic variants modulate [¹³C]α-linolenic acid metabolism and plasma fatty acid composition. *Am. J. Clin. Nutr.* 97 (2013) 195-207.
- [17] A. Lewinska, J. Zebrowski, M. Duda, A. Gorka, M. Wnuk, Fatty acid profile and biological activities of linseed and rapeseed oils. *Molecules* 20 (2015) 22872-22880.
- [18] A. Bayrak, M. Kiralan, A. Ipek, N. Arslan, B. Cosge, K.M. Khawar, Fatty acid compositions of linseed (*Linum Usitatissimum* L.) genotypes of different origin cultivated in Turkey. *Biotechnol. & Biotechnol. Eq.* 24 (2010) 1836-1842.
- [19] N.C. Shanta, E.A. Decker, Rapid, sensitive, iron-based spectrophotometric methods for determination of peroxide values of food lipids. *J. AOAC Int.* 77 (1994) 421-424.
- [20] L.M. Tong, S. Sasaki, D.J. McClements, E.A. Decker, Mechanisms of the antioxidant activity of a high molecular weight fraction of whey. *J. Agr. Food Chem.* 48 (2000) 1473-1478.
- [21] A. González, M.L. Martínez, A.L. Paredes, A.E. León, P.D. Ribotta, Study of the preparation process and variation of wall components in chia (*Salvia hispanica* L.) oil microencapsulation. *Powder Technol.* 301 (2016) 868-875.

- [22] U. Klinkesorn, P. Sophanodora, P. Chinachoti, E.A. Decker, D.J. McClements, Characterization of spray-dried tuna oil emulsified in two-layered interfacial membranes prepared using electrostatic layer-by-layer deposition. *Food Res. Int.* 39 (2006) 449-457.
- [23] A.C. Karaca, M. Nickerson, N.H. Low, Microcapsule production employing chickpea or lentil protein isolates and maltodextrin: Physicochemical properties and oxidative protection of encapsulated flaxseed oil. *Food Chem.* 139 (2013) 448-457.
- [24] A. Gharsallaoui, G. Roudaut, O. Chambin, A. Voilley, S. Rémi, Applications of spray-drying in microencapsulation of food ingredients: An overview. *Food Res. Int.* 40 (2007) 1107-1121.
- [25] S.M. Jafari, E. Assadpoor, Y. He, B. Encapsulation efficiency of food flavours and oils during spray drying. *Drying Technol.* 26 (2008) 816-835.
- [26] S.H. Anwar, B. Kunz, The influence of drying methods on the stabilization of fish oil microcapsules: Comparison of spray granulation, spray drying, and freeze drying. *J. Food Eng.* 105 (2011) 367-378.
- [27] R.V. Tonon, P.B. Ranie, C.R.F. Grosso, M.D. Hubinger, Microencapsulation of flaxseed oil by spary drying: Effect of oil load and type of wall material. *Dry.Technol.* 30 (2012) 1491-1501.
- [28] M. Sousdaleff, M.L. Baesso, A.M. Neto, A.C. Nogueira, V.A. Marcolino, G. Matioli, Microencapsulation by freeze-drying of potassium norbixanate and curcumin with maltodextrin: stability, solubility and food application. *J. Agric. Food Chem.* 61 (2013) 955-965.
- [29] V. Kaushik, Y.H. Roos, Limonene encapsulation in freeze-drying of gum arabic-sucrose-gelatin systems. *LWT-Food Sci. Technol.* 40 (2007) 1381-1391.
- [30] D.P.S. Oberoi, D.S. Sogi, Effect of drying methods and maltodextrin concentration on pigment content of watermelon juice powder. *J Food Eng.* 165 (2015) 172-178.

- [31] G. Caliskan, S.N. Dirim, The effect of different drying processes and the amounts of maltodextrin addition on the powder properties of sumac extract powders. *Powder Technol.* 287 (2016) 308-314.
- [32] S.Y. Quek, N.K. Chok, P. Swedlund, The physicochemical properties of spray-dried watermelon powders. *Chem. Eng. Process.* 46 (2007) 386-392.
- [33] A. Ekpong, W. Phomkong, E. Onsaard, The effects of maltodextrin as a drying aid and drying temperature on production of tamarind powder and consumer acceptance of the powder. *Int. Food Res. J.* 23 (2016) 300-308.
- [34] O.R. Fenemma, *Food Chemistry*, third ed., Marcel Dekker, New York, 1997.
- [35] A. Jedlinska, K. Samborska, D. Witrowa-Rajchert, D. Properties of powders received by spray drying and freeze drying of solutions of honey. *Acta Agrophys.* 19 (2012) 563-574.
- [36] H.M.P. Canuto, M.R.A. Afonso, J.M. Correia da Costa, Hygroscopic behavior of freeze-dried papaya pulp powder with maltodextrin. *Acta Sci. Technol.* 36 (2014) 179-185.
- [37] C.M. Galanakis, *Nutraceutical and functional food components: Effect of innovative processive techniques*, first ed., Elsevier, Amsterdam, 2017.
- [38] H.K. Leung, H. K, Influence of water activity on chemical reactivity, in: L.B. Rockland, L.R. Beuchar (Eds.), *Water Activity: Theory and Applications to Food*, Chicago, 1987, pp. 27-54.
- [39] E.N. Frankel, *Lipid oxidation*, second ed., Woodhead Publishing Limited, Cambridge, 2012.
- [40] S. Sampels, Oxidation and antioxidants in fish and meat from farm to fork, in: I. Muzzalupo (Ed.), *InTech*, 2013, pp. 115-144.
- [41] D. Pingret, A. Fabiano-Tixier, F. Chemat, Degradation during application of ultrasound in food processing. A review. *Food Control* 31 (2013) 593-606.

- [42] S. Abbas, K. Hayat, E. Karangwa, M. Bashari, X. Zhang, An overview of ultrasound-assisted food-grade nanoemulsions. *Food Eng Rev*, 5 (2013) 139-157.
- [43] L.S. Chua, L. S. A review on plant-based rutin extraction methods and its pharmacological activities. *J. Ethnopharmacol.* 150 (2013) 805-817.
- [44] T. Waraho, D.J. McClements, E.A. Decker, Mechanisms of lipid oxidation in food dispersions. *Trends Food Sci. Tech.* 22 (2011) 3-13.
- [45] L. Atarés, L.J. Marshall, M. Akhtar, B.S. Murray, Structure and oxidative stability of oil in water emulsions as affected by rutin and homogenization procedure. *Food Chem.* 134 (2012) 1418-1424.
- [46] M.S. Rahman, O.S. Al-Amri, I.M. Al-Bulushi, Pores and physico-chemical characteristics of dried tuna produced by different methods of drying. *J. Food Eng.* 53 (2002) 301-313.
- [47] S. Freitas, G. Hielscher, H.P. Merkle, B. Gander, Continuous contact- and contamination-free ultrasonic emulsification – a useful tool for pharmaceutical development and production. *Ultrason. Sonochem.* 13 (2006) 76-85.
- [48] S. Ganta, M. Amiji, Coadministration of paclitaxel and curcumin in nanoemulsion formulations to overcome multidrug resistance in tumor cells. *Mol. Pharm.* 6 (2009) 928-939.
- [49] S. Kentish, T.J. Wooster, M. Ashokkumar, S. Balachandran, R. Mawson, L.Simons, The use of ultrasonics for nanoemulsion preparation. *Innov. Food Sci. Emerg.* 9 (2008) 170-175.
- [50] R. Walker, E.A. Decker, D.J. McClements, Development of food-grade nanoemulsions and emulsions for delivery of omega-3 fatty acids: opportunities and obstacles in the food industry. *Food. Funct.* 6 (2015) 42-55.

FIGURE CAPTIONS

Figure 1. Visual appearance of microencapsulated powders obtained from emulsions containing different MD concentrations (% w/w): 0% (A), 10% (B) and 20% (C).

Figure 2. SEM of freeze-dried flaxseed oil microcapsules produced from multilayer emulsions containing 0% MD (A, B), 10%MD (C, D) and 20% MD (E, F).

Figure 3. Effect of MD concentration water activity of freeze-dried powders. Different letters indicate significant statistical differences ($p < 0.05$).

Figure 4. Peroxide Values (PV) of flaxseed oil encapsulated in multilayer emulsions (■), and in freeze-dried powders (■) containing different MD concentrations. Initial PV of flaxseed oil was included as a control and PV of powders was determined the same day they were removed from the lyophilizer. Different letters indicate significant statistical differences ($p < 0.05$).

Figure 5. TBARS values of flaxseed oil encapsulated in multilayer emulsions (■), and in freeze-dried powders (■) containing different MD concentrations. Initial TBARS of flaxseed oil was included as a control and TBARS of powders was determined the same day they were removed from the lyophilizer. Different letters indicate significant statistical differences ($p < 0.05$).

Table 1. Combination of coating materials to prepare flaxseed oil multilayer emulsions.

Condition Code	Flaxseed oil (%)	WPI (%)	SA (%)	MD (%)	Total Solids* (g/100g emulsion)
M0	10	1	0.25	0	11.05
M10	10	1	0.25	10	20.24
M20	10	1	0.25	20	29.24

*Solid content in emulsions including flaxseed oil

Table 2. Influence of maltodextrin concentration on Free Oil (FO), Total Oil (TO) and Encapsulation Efficiency (EE) of powdered microcapsules.

Condition Code	FO (g/100g powder)	TO (g/100g powder)	EE (%)
M0	60.29 ± 4.16 ^a	82.64 ± 5.45 ^a	27.01 ± 0.88 ^a
M10	18.14 ± 1.15 ^b	50.08 ± 3.20 ^b	63.70 ± 2.24 ^b
M20	1.56 ± 0.84 ^c	34.20 ± 2.67 ^c	95.44 ± 2.69 ^c

Data represents means ± standard deviations. Different letters indicate significant statistical differences (p<0.05)

Fig. 1



ACCEPTED MANU

Fig. 2

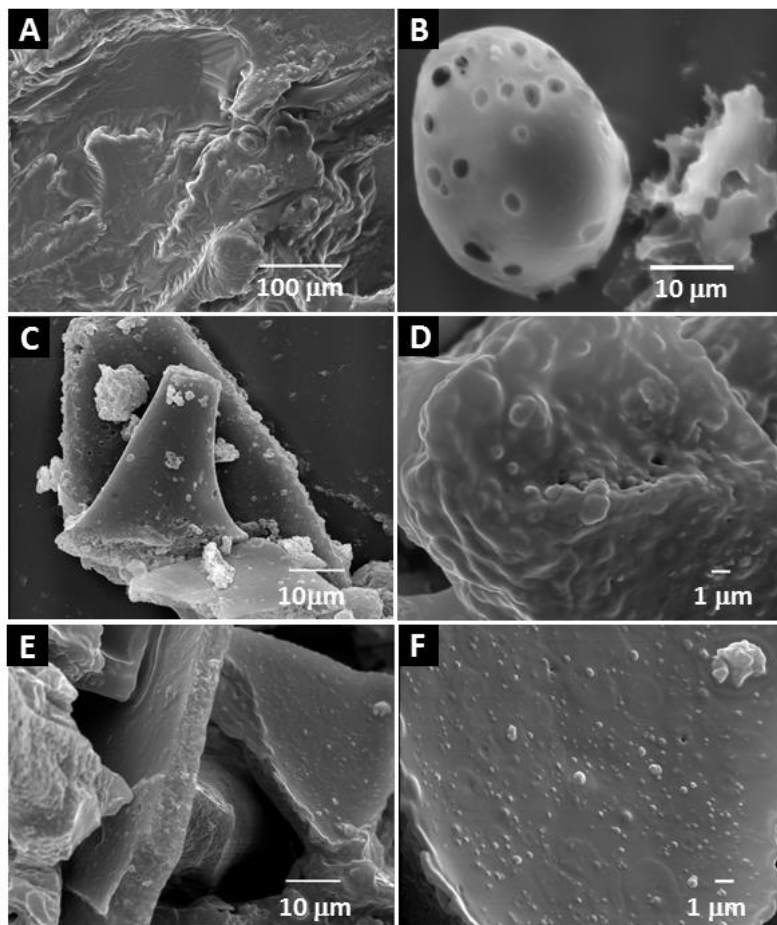
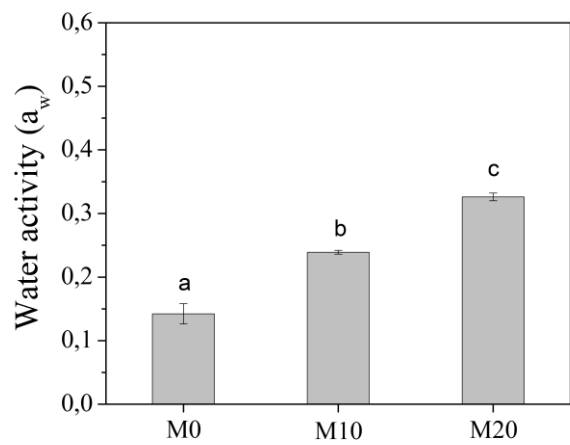


Fig. 3



ACCEPTED MANUSCRIPT

Fig. 4

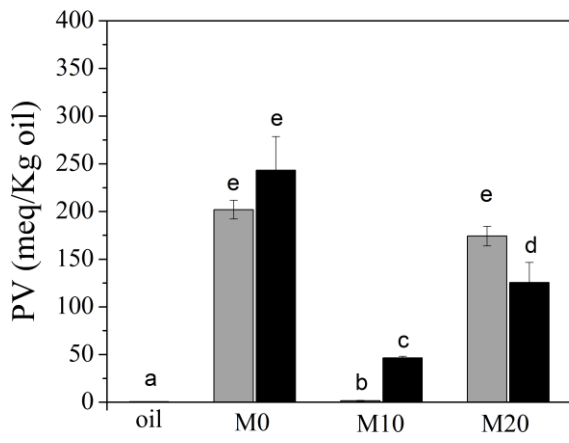
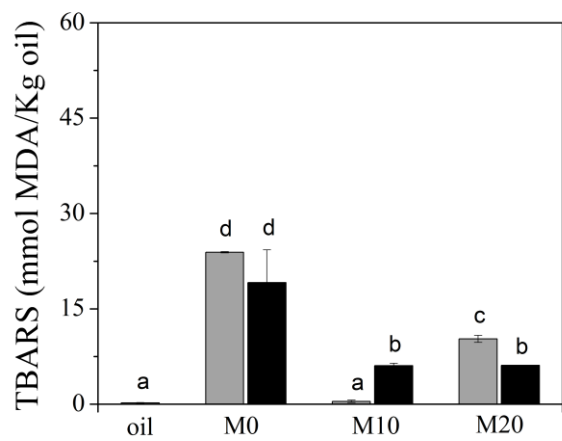
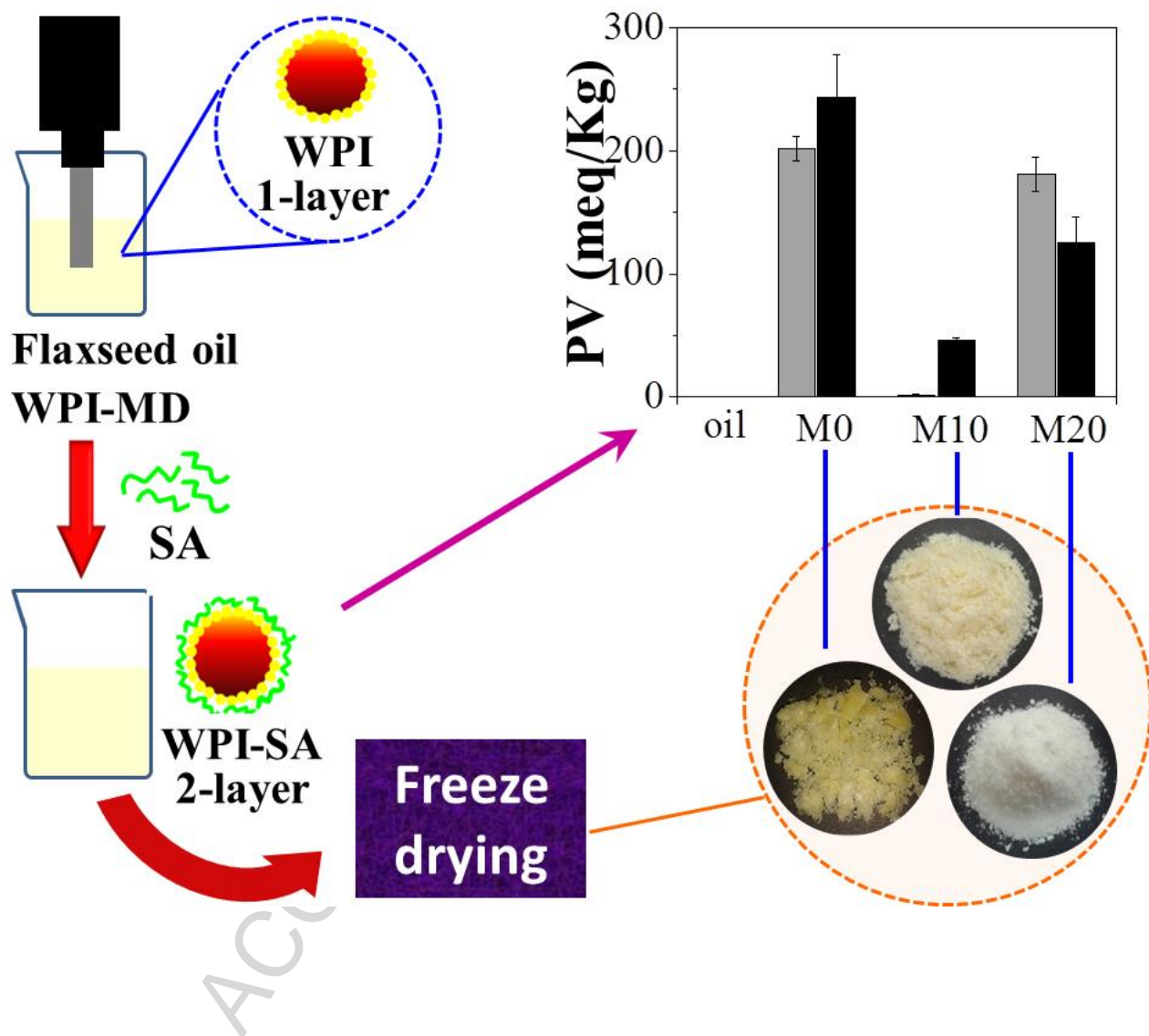


Fig. 5



Graphical abstract



HIGHLIGHTS

- Sonication was used to encapsulate flaxseed oil through double-layer emulsions.
- Powdered microcapsules were obtained by freeze-drying of emulsions.
- Microencapsulation efficiency above 90% with 33% oil load was achieved.
- Maltodextrin concentration influenced powders structure and properties.
- Sonication and freeze-drying process contributed significantly to oil oxidation.