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Impact of wall materials on physicochemical properties of microencapsulated fish oil by spray-drying

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Abstract

The aim of the present study was to investigate the effect of wall materials composition on physicochemical characteristics of fish oil microcapsule produced by spray-drying (180^{oC}). Four different combination of coating materials (fish gelatin, chitosan, combination of gelatin and chitosan and a mixture of microbial transglutaminase (MTGase) with maltodextrin) were applied to two different fish oils to produce 40% solid emulsions. Scanning electron microscopy and extraction of surface and encapsulated oils revealed that fish gelatin had the highest ability of covering fish oil ; Meantime, addition of MTGase to gelatin could also increase this ability and reveled less surface oil (2.63% than 5.23% on chitosan treatment (P<0.05). Mixture of gelatin and maltodextrin with MTGase as the wall material led to a high encapsulation efficiency 88%, so was selected as the best microcapsule. However, application of chitosan with maltodextrin had lower encapsulation efficiency ~67% (P<0.05). All indices of powders (encapsulation efficiency, surface morphology and particle size) showed that powders prepared from gelatin and gelatin with MTGase increased efficiency of encapsulation and will increase stability of the microcapsules powders.

Key words: Fish oil, microencapsulation, wall material, SEM, encapsulation efficiency

1. INTRODUCTION

In recent decades, attention to the n-3 long-chain fatty acids in human health has been continuously increased. Many clinical and epidemiologic studies have displayed positive roles of n-3 fatty acids (Riediger et al., 2009); According to Bao et al., (2011) regular consumption of these fatty acids has ameliorative effects. However, n-3 fatty acids are highly susceptible to oxidative deterioration, and it is a limiting agent for using them in foods because of flavor and color degradation. Furthermore, hydroperoxides, the primary product of lipid oxidation, also have been considered to be toxic. Preventing oxidation of the n-3 fatty acids is essential in allowing them to accomplish their original physiological functions (Kagami et al., 2003).

To control or reduce the negative attributes of fish oil, it can formulated to solid microcapsules. Microencapsulation is a technique to protect unsaturated fatty acids against oxidation and can help in masking the odor and/or taste of encapsulated materias. It simplifies the handling, storage, and delivery of the powder like materials (Kagami et al. 2003; Ramakrishnan et al. 2012, 2013).This method has been reported to enable the supplementation of encapsulated fish oil to foods like instant products and salad dressing, milk and yoghurt (Let et al., 2007).

The main technologies used for encapsulation of lipophilic food ingredients are spray-drying, coacervation and extrusion (Drusch & Berg, 2008). Nowadays, microencapsulation by spray-drying has been executed in the food industry and is still the overshadowing technology as it is rather inexpensive and straightforward and is the most popular technique to prepare microcapsules of good quality (Jafari et al., 2007; Gharsallaoui et al., 2007; Bao et al., 2011).

Oil encapsulation includes a two-step process of emulsion preparation and drying. Emulsion plays a key role in optimizing the oil encapsulation efficiency because the emulsion droplet size distribution correlates with this parameter. Some studies have represented that emulsion droplet size has a considerable effect on the oil encapsulation efficiency of microcapsules obtained by spray drying. Jafari et al., (2008) investigated the effect of the emulsion size of fish oil droplets produced by different emulsification systems. Their results showed that smaller droplet size yields higher encapsulation efficiency. Based on Soottitantawat et al.(2003) the emulsions with the smallest droplet size distribution, had the highest retention of orange oil. These reports clearly indicated that emulsions with small droplet size distribution had higher oil encapsulation efficiencies than those with larger droplets (Ramakrishnan et al., 2012). The

other effective process parameter on oil encapsulation efficiency was the core-wall ratio. Recent studies have shown that when the oil-wall ratio increases, so does the oil encapsulation efficiency (Ramakrishnan et al., 2012).

The other process parameter that controls oil encapsulation efficiency is the composition of wall material. According to the Jafari et al., (2008) properties of fish oil microcapsules including encapsulation efficiency, peroxide value, size, shape, density, and moisture content are greatly affected by the composition of wall material, core , infeed emulsion (total solids, viscosity, droplets size) and the conditions of the spray drying process. So, the basic step for encapsulating a food ingredient is the selection of an appropriate wall material, principally a film-forming biopolymer, from a wide variety of natural or synthetic polymers, depending on the core material and the characteristics desired in the final microcapsules. Many investigations have been conducted to evaluate different wall materials on fish oil encapsulation, such as sugar beet pectin, chitosan coating (Shen et al., 2010), cross-linked sodium caseinate (Bao et al, 2011).

Some of the most common wall materials used for fish oil microencapsulation include gelatin, maltodextrin, sugars, starch, skimmed milk, milk and whey protein and plant gums, chitosan. Combination of wall materials is often used to increase the efficiency of microencapsulation (Kolanowski, Laufenberg, Kunz & 2004). Carbohydrates such as lactose and maltodextrin are effective in final product by altering the time and procedure of crust formation, but cannot be used as wall materials due to poor emulsification properties (Kagami et al., 2003). In spite of many published works in regarding effect of different wall composition, little information is available in using of fish gelatin, combination with chitosan and adding enzyme with carbohydrate on microcapsule properties.

The present study has therefore focused to evaluate coating fish oil by different wall material, including fish gelatin, chitosan with maltodextrin alone or combination of them and also effects of adding microbial transglutaminase (MTGase) to gelatin in the form of a fish oil powder. We postulate that utilization of the multilayer interfacial membrane emulsion system in combination with maltodextrin may prove to be an effective means of improving the stability of microencapsulated oils. Consequently, a mixture of fish gelatin and chitosan, both derived from marine sources, would seem to be especially suitable as wall materials.

2. MATERIALS AND METHODS

2.1. Chemicals

Cod (*Gadus morhua*) liver oil (CL-oil), fish gelatin (gelatin from cold water fish skin), powdered chitosan (medium molecular weight, deactylation 75-85%) were purchased from the Sigma Chemical Co. (St. Louis, MO). Microbial transglutaminase (ACTIVA TG-K) was a kind gift from Ajinomoto Co., Inc. (Tokyo, Japan). Maltodextrin (DE 16-20) was purchased from Iran Dextrose Industry (Tehran, Iran). Other analytical grades of chemical and reagent were

purchased from Merck (Darmstadt, Germany). Omega-3 rich oil (ω_3-oil), containing EPA (180 mg/1000 mg) and DHA (120 mg/1000 mg) was purchased from a local pharmacy (Golden Sea, California, USA).

2.2. Emulsion preparation

Aqueous solutions of the wall materials (Gelatine, Maltodextrin) were prepared by dispersing them in distilled water. In the case of chitosan, a stock buffer solution (pH 3.0) was prepared (100 mM by 2 mM sodium acetate and 98mM acetic acid). One day before emulsification, powder solutions were kept in a shaking water bath (Memmert GmbH, Schwabach, Germany) overnight to warrant hydration of the polymer molecules. Total concentration of dissolved solid was 40% (w/v); formulations of the different treatments are mentioned in Table 1. Gelatin treatment was composed of 30 wt% maltodextrin and 10 wt% fish gelatin. In treatment of Gelatin and MTGase[1] (Ge-MTG-MD), wall solutions containing 10% fish gelatin and MTGase (0.025%) (Cho et al., 2003) was added into the protein wall solution. The core material (CL-oil and ω_3-oil), prepared in the ratio of 1:4 (core: wall), was progressively added to the continuous phase during pre-emulsion preparation and stirred for 10 min by a laboratory mixer. During the homogenizing step, emulsions were kept in a beaker and submerged in water/ice to keep the emulsion cold.

These coarse emulsions were then further emulsified using an ULTRA-TURRAX homogenizer (T25 Digital IKA-Werke Stuttgart Staufen/Germany) with 22mm diameter that operated at 24000 rpm for 5 min and resulted in the formation of a fine emulsion.

Spray-drying was carried out on laboratory scale equipment. The emulsions were fed to a Buchi mini spray dryer (Model B-191; Buchi Laboratorioums-Tecknik, Flawil, Switzerland) containing a 0.5 mm atomizer, inside a chamber at 44 cm height and 10.5 cm in diameter. The inlet and outlet air temperatures were maintained at 180±0.5^{o}C and 90±5^{o}C, respectively. The feed pump was set at 25% and compressed air for the spraying flow was established at 0.6MPa. The microcapsules stored in a sealed plastic bag for further analysis.

2.3. Analyses

2.3.1. Fatty acid composition in fish oils and microcapsules

Fatty acid methyl esters of fish oils and surface and encapsulated oils were prepared according to the method by Metcalfe & Schmitz (1961) using boron-trifluride in methanol. The fatty acid composition was then analyzed by gas chromatography (GC) (Hewlett Packard 5890 series II, Ramsey, MN, USA) equipped with a flame ionization detector and a fused silica capillary (25m x 0.2mm, film thickness, BPX70 SGE Australia Pty. Ltd., analytical products) (Unicam 4600 gas chromatograph, England, UK). C19:0 fatty acid was employed as internal quantitative standard.

[1] Microbial transglutaminase

Operating conditions were as follows: temperatures-injection port 250°C; detector temperature 300°C; oven programmed from 160 to 200°C at 20°C/min. Helium was employed as carrier gas. Pressure of gas at first was 20 psi and gas pressure of makeup was 15 psi. The polyene index (PI) was calculated as described by Memon et al. (2010), according to the following fatty acid ratio: C20:5 + C22:6/ 16:0.

2.3.2. Emulsion stability

Each emulsion (10 ml) was placed in a test tube and stored at ambient temperature (20ºC) for one month in replicate. The evaluation was daily observation in order to assess the depths (centimeter units) of a distinctive clear serum lower phase. The results were expressed as creaming index (%) of total emulsion height in the tubes, according to the following formula: Creaming index = 100 × (the height of formed serum layer/total height of the emulsion) (Klaypradit & Huang, 2008).

2.3.3. Size distribution of emulsion

The droplet size distributions of emulsions were measured by using a laser based static light scattering particle size analyzer (Mastersizer 2000 Ver. 5.1, Malvern Instruments Ltd., Worcestershire, U.K.). Distilled water was used as the dispersant. Particle size was expressed as d 3,2 ; each sample was analyzed in triplicate and the average data were reported. Results are reported as 50^{th} and 90th percentile of the distribution.

2.3.4. Extraction of encapsulated oil in fish oil microcapsules

Acetate buffer (2 ml) (pH 3.0) was added to 0.5 g of fish oil powders and samples were vortexed for 1 min. The resulting solution was then extracted with 25 mL hexane/ isopropanol (3:1 v/v). The tubes were then shaken for 15 min at 160 rpm using an automatic shaker (Orbital shaker OS 10 control, IKA, Germany) and centrifuged (Eppendorf centrifuge 5810 R, Germany) for another 15 min. The clear organic phase was collected and the aqueous phase re-extracted with the solvent mixture. After filtration through anhydrous Na_2SO_4, the solvent was evaporated in a rotary evaporator (IKA, Germany) at 70°C, and the solvent-free extract was dried at 105°C. The amount of encapsulated oil was determined gravimetrically (Klinkesorn et al., 2006).

2.3.5. Extraction of surface oil from fish oil microcapsules

For extraction of surface oil, hexane (15 ml) was added to 2.5 g of fish oil powder. The mixture was mixed with a vortex mixer (Fisher Vertex Genie 2, Scientific Industries, Inc., Bohemia, Czech Republic) for 2 min and then centrifuged (Eppendorf centrifuge 5810 R, Germany) at 8000 rpm for 20 min. The supernatant was filtered, the filter paper (Whatman, Maidstone, Kent, UK) washed twice with hexane and hexane eliminated in a rotary

evaporator (IKA, Germany) at 70°C; the solvent-free extract was finally dried at 105°C. The amount of encapsulated oil was determined gravimetrically (Klinkesorn et al., 2006).

2.3.6. Extraction of total oil from fish oil microcapsules

For extracting total oil, 2 mL of acetate buffer (pH 3.0) was added to 0.5 g of fish oil powders and vortexed for 1 min. Total oil was extracted using the same method as described above for extraction of encapsulated oil (Klinkesorn et al., 2006).

2.3.7. Calculation of encapsulation efficiency

The encapsulation efficiency (EE) was calculated from the quantitative determinations detailed below

2.3.8. Particle size of spray-dried powders

Assessment of particle size of fish oil powders was performed using a laser diffraction spectometer (Mastersizer 2000 Ver. 5.1, Malvern Instruments Ltd., Worcestershire, UK). The powders were suspended in ethanol and sonicated for 2 min before each determination. Particle size was expressed as d 3,2 ; each sample was analyzed in triplicate and the average data were reported. Results are reported as 50^{th} and 90th percentile of the distribution.

2.3.9. Scanning electron microscopy (SEM)

Scanning electron microscopy (LEO 1455VP, Cambridge, UK) was used for determination of surface morphology of the fish oil microparticles. The samples were placed directly onto the SEM sample holder using double-sided sticking tape and was gold spray-coated.

2.3.10. Color measurements of fish oil microcapsules

The color of spray dried fish oil powders with different treatments and wall materials was determined. The color parameters of lightness (L*), redness (a*), and yellowness (b*) were measured using a Lovibond CAM-System 500 Imaging Colorimeter (Tintometer Ltd., Amesbury, UK).

2.4. Statistical analysis

Data reported in this study are averages of triplicate experiments (n=3). Analysis of the data was carried out by analysis of variance (ANOVA) using SPSS statistical software (version 16.0 for windows; SPSS, Chicago, IL, USA). Comparison among means were made using the Duncan's multiple range analysis at $P<0.05$.

3. RESULTS AND DISCUSSION

The oil droplet size (10^{th}, 50^{th} and 90^{th} percentile μm) of emulsions and the moisture, total oil, surface oil, encapsulation efficiency and particle size (10^{th}, 50^{th} and 90^{th} percentile μm) of microcapsules with different wall materials are reported in Tables 2.

3.1 Emulsion droplet size analysis

The 50^{th} percentile of the oil droplet size of some parent emulsions was nearly below 1.5 μm, providing a sufficient stability of the parent emulsions for spray drying. Droplet size of emulsions varied from 2.41 to 3.12μm (Table 2). The emulsion prepared by chitosan as one of ingredient from continues aqueous phase had smaller droplet size than those prepared with contribution of gelatin. Incorporating the chitosan to emulsion formula decreased the droplet size to some extent for Ge-Cs treatment. However, a higher oil droplet size was measured for emulsions prepared with Ge-MD (3.09 μm) and Ge-MTG-MD (3.12 μm) when compared with other emulsions (Table 2). In Ge-MTG-MD treatment, addition of MTGase to the wall material affected the droplet size of emulsion and reached to 1.78 μm that was significantly higher than other treatments ($P<0.05$). It may be cause of huge crossed-linked protein molecules (Bao et al., 2011). Ramakrishnan et al. (2013) showed an increase in the droplet size results to bigger microcapsules. According to the finding of Ramakrishnan et al. (2013), there is a relationship between the droplet size of the emulsion and the particle size, the smallest microcapsules always obtained from the emulsions with the smallest droplet size.

3.2. Physical analyses of fish oil microcapsules

3.2.1. Encapsulation efficiency (EE) of fish oil microcapsules

The amount of entrapped fish oil in microcapsules is so important for the shelf life and storage. The encapsulation efficiency value for the multilayer emulsion system varied from a minimum value of 67.35% a maximum value of 88%. The Ge-MTG-MD and Ge-MD coated microcapsules had highest encapsulation efficiency ($p<0.05$), due probably to the presence of gelatin, while microcapsules Cs-MD had the lowest encapsulation efficiency ($p<0.05$). Affixing gelatin to chitosan showed satisfying effect on encapsulation efficiency ($p<0.05$). O'Regan & Muvihill (2010) stated that protein and carbohydrate composition influence on physical properties of coating material and improve the encapsulation. It can be result of glass transition temperature (T_g), crust formation around droplets containing wall materials with lower T_g begins earlier that for droplets composing of material with higher T_g (Mezhericher et al., 2010).

It was found that encapsulation efficiency of two type fish oil in gelatin spray-dried powders increased slightly by addition of MTGase from 85.36 to 88.01% and from 83.55 to 87.03%, respectively. The lower EE values obtained indicates that higher surface oil would be detrimental to the oxidative stability of microencapsulated fish oil. Gelatin worked both as an emulsifier and as a membranous material, crust formation in droplets contacting gelatin was quick and fish oil could not spread out to the surface and enhanced encapsulation efficiency to a value of 85 % (Table 2). According to Liu et al. (2001), augmentation of gelatin (1% w/w) enhanced the retention of ethyl butyrate by arabic gum, because enhanced crust formation on the surface of droplets. It is argued that the presence of gelatin and maltodextrin in the chemical composition of Ge-MTG-MD and Ge-MD can alter the properties of the wall, therefore facilitating crust formation and reducing the diffusion of the entrapped oil to the surface of the particles. Development of nitrogenous polymers and melanoidins as a result of the reaction between the amino groups of proteins and the carbonyl group of maltodextrin (Millard reaction) might also have a significant contribution to the formation of the tough skin. Protein–carbohydrate conjugates formed by the Maillard reaction have a good potential for the stabilization of fish oil microcapsules by changing the physical properties of the wall (Aghbashlo et al. 2012). The treatments containing gelatin formed a dried crust layer on the surface of the droplets which hindered the shrinkage of microparticles, and therefore larger capsules were produced (Reineccius 2004; Aghbashlo et al. 2012).

In the cases of addition of MTGase to gelatin treatment compact film may be formed due to the effective covalent cross-link by MTGase, and the free oil was less than others and improved encapsulation efficiency up to 88%. Bao and others (2011) confirmed that by adding MTGase to sodium caseinate encapsulation efficiency increased to more than 90% and expressed it is by cross-linked of MTGase. The employment of the highest possible core concentration that provides a high core retention in the microcapsules is advantageous because less wall materials would be needed; additionally, an increase of the yield and output would lead to advantages from the economical point of view. In general, there is an optimum core concentration that can be encapsulated efficiently (Jafari et al., 2007). Previous workers have reported EE values from 0% to 95% depending on the type and composition of the wall material, the ratio of core material to wall material, the drying process used, and the stability and physicochemical properties of the emulsions (Shaw et al., 2007). As well, Aghbashlo et al. (2012) has suggested that changing the encapsulating wall composition is a good way for improving the encapsulation efficiency.

3.2.2. Emulsion stability

Ecording to Ramakrishnan et al. (2013), emulsion stability and composition determine some of the key quality parameters of oil microcapsules such as surface free oil contents, morphology, encapsulation efficiency, and oxidation

stability.The optimal concentrations of the different emulsion treatments were found to be 30g/100g MD and 1.5 g/100g CS (CS-MD), 10g/100g Ge (Ge-MD), 1g/100g CS and 6g/100g Ge (Cs-MD). The stability of fish oils in emulsion containing gelatin showed the lowest creaming index (0%) values. A small higher creaming rate was observed in emulsions made from Cs-MD ($P<0.05$). Our experiments for selecting the best concentrations were in agreement to the results obtained by Klaypradit and Huang (2008). According to Laplante and others (2005) and Klaypradit & Huang (2008), CS cannot individually produce the stable emulsions and should be mixed with other components for getting stable emulsion. It has been also showed that the emulsification capacity of CS combines both electrosteric and viscosifying stabilization mechanisms. In addition, CS is a polysaccharide with a cationic nature with hydrophilic zones rich in glucosamine and hydrophobic zones rich in N-acethyl-glucosamine which enables it to be adsorbed in the oil/water interfaces. In general, all the selected concentrations tested were stable. Stability of emulsion droplets during the encapsulation process is an important factor. In fact, it is crucial that emulsion droplets not only have the minimum size but are also stable enough without any coalescence or flocculation so that they can be embedded in the shell of powder particles inside the capsules with maximum protection (Jafari et al., 2007). Interaction between CS and Ge can be co-adsorbed on the oil/water interface, resulting in a higher emulsion stability caused by an increased interfacial electrostatic stability (Laplante et al., 2005).

Gelatin is surface-active and is capable of acting as an emulsifier in oil-in-water emulsions and these characteristics can be successfully worked during the emulsification process. Gelatin stability and other characteristics are useful during the subsequent drying and encapsulation stages, so it was chosen for rheological and gelling properties and prepared emulsions were stable (Karim & Bhat, 2009). MTGase cross-linked polymers of gelatin and maltodextrin and can improve stability of fish oil emulsions. Similar finding have been reported by Bao et al. (2011) for spray drying of oil that adding MTGase increase emulsion activity ability.

3.2.3. Surface oil of microcapsules

The amount of surface oil in powdered emulsions is usually defined as that part of oil that can be extracted with organic solvents. However, it should be noted that the amount of surface oil measured relies on the precise extraction conditions used. In this sense, most previous studies have considered the ‘‘surface oil’’ of powdered emulsions to be equivalent to the hexane extractable oil (Danviriyakul et al., 2002). Extractable oil is partially located at the particle surface and is directly associated to the flowability and wetting properties of the powder as well as to the stability toward oxidation (Drusch, 2007). Extractable oil ranged from 2.26% in the Ge-MD treatment to 5.23% in the Cs-MD based microcapsules; surface oil in Cs and Cs-Ge treatment were thus higher than Ge and Ge-MTG based microcapsules (Table 2). Our findings showed an increase in the droplet size results in bigger microcapsules. According to Soottitantawat et al. (2003), large emulsion droplets can be sheared into smaller droplets because extractable oil is

located on the surface of the particle and may oxidize rapidly during storage. A higher oil droplet size was measured for emulsions prepared from Ge-MTG compared to emulsions prepared from other treatments. In contrast of our findings, a good correlation was reported by Klinkesorn et al. (2006) between the droplet size in the parent emulsion and the extractable oil content in the dried powder. The stabilization of the newly formed interface during homogenization is comparably slow, and oil droplet coalescence may occur prior to complete coverage of the newly formed interface with emulsifier reflected by an increase of the oil droplet size. Thus, as a result of its good emulsifying ability, gelatin has shown to be more successful in the coverage of oil during homogenization.

3.2.4. Particle size of fish oil microcapsules

Drusch & Schwarz (2006) indicated that the particle size would significantly be the result of chemical composition molecules included and of the emulsion and drying conditions. The 50^{th} percentile particle size of powders in all treatments varied from 14.76 μm (Ge-Cs) to 20.61 μm (Ge-MTG). Composite wall material of Ge-Cs produced the smallest and Ge-MTG largest microcapsules, respectively (Table 2). According to Aghbashlo et al. (2012), it could be attributed to glass transition temperature (Tg) of coating materials, that effectively on the drying behavior of droplets. Crust formation for droplet containing materials with lower Tg commences earlier than that for droplets composing of material with higher Tg (Mezhericher et al., 2010). Therefore, the second drying stage for droplet containing chitosan begun later than that for droplets with gelatin, which in turn postponed the crust formation and thus particles with smaller sizes were produced. Presence of fish gelatin, accelerated the skin formation, and on the other hand, produced an emulsion with a lower viscosity. The lower viscosity of emulsion prepared with composite Ge-MD accelerated the migration of crust forming materials toward the surface of droplet and thus the crust formation prompted and particles with larger size were finished. According to Aghbashlo et al. (2012) encapsulated oil which had the smallest particle size, had the lowest encapsulation efficiency due to the postponed crust formation.

3.2.5. Scanning electron microscopy (SEM) of fish oil microcapsules

To investigate the microstructure of powders, analysis by scanning electron microscopy (SEM) of the microcapsules was achieved. Outer topography of microcapsules is presented in Figure 1. Occasionally, we observed some cracks in all samples (Figures 1) that may be due to weak viscoelastic properties of the wall materials during expansion at the final stages of spray drying (Jafari et al., 2007). In almost all the cases in powder particles, a mixture of spherical particles and a few traces of shrinkage of the outer surface were observed. These results are consistent with Jafari et al (2007), Sheu & Rosenberg (1998) and Klinkesorn et al. (2006); such authors employed a high amount of maltodextrin

or sodium caseinate and maltodextrin and stated that solidification of the wall material is prior to expansion of the microcapsules. Klinkesorn et al. (2006) indicated that wrinkle or scars on surface of particles would be the result of mechanical stresses proved by uneven drying at different parts of liquid droplets during the first steps of drying. In some particles (Figure 1c), pore formation can be observed as a result of the final drying phase (Klinkesorn et al., 2006).

Formation of cracks on the surface of microcapsules had been attributed to high cross-linking density that rendered microcapsules fragile. Ballooning is a phenomenon which is caused by fast fixing of the particle structure in the early stages of drying with subsequent steam formation inside particles, this rendering particles inflated. Based on Jafari et al.(2007), wall material properties and drying conditions are more important in surface morphology than other factors such as emulsification method (Kagami et al., 2003; Klikesorn et al., 2006); such authors indicated that this may be the mechanism of moisture movement during the drying process of nonsaturated surfaces, leading to shirinkage of particles. Kagami et al. (2003) found that microcapsules by 83% oil load had less shrinkage and shallow dents on surface particles in all types of wall materials; further, an increase of the oil load would improve the microcapsule resistance. Such conclusions agree to our present results where, Ge-MD (Figures 1A-1B) and Ge-MTG-MD (Figures 1G-1H) treatments showing a higher oil load and encapsulation efficiency (more than 83%) led to particles showing less shrinkage and cracks. Meantime, microcapsules corresponding to Cs-MD treatment provided more shrinkages and dents, especially at 67% oil load.

3.2.6. Color measurement of fish oil microcapsules

Color data of the microcapsules are shown in Table 3. Spray drying of CS-MD treatment produced a yellowish powder (b* scores of 3.53 and 3.79). CS-MD treatment for both kinds of fish oils provided the lowest values for lightness (L*) and the highest values in redness (a*) and yellowness (b*) ($p<0.05$) when compared to the remaining treatments. For Ge-Cs-MD treatments, b-value was higher as in the case of the Cs-MD treatment. Klaypradit & Hugan (2008) detected a greater whitish formation in Cs-MD powders than our experiment; they observed a higher b-value in treatments (specify the kind of treatments) (5.2±3.1 and 5.3±3.0) than in the present results; such a higher b* value obtained was explained by the authors as a result of non-enzymatic browning product formation during drying. Additionally, chitosan has been reported to include small protein fractions so that its reaction with sugar molecules would be likely to occur, this leading to a higher yellowness formation (Klaypradit & Hugan, 2008).

3.2.7. Fatty acids composition

The individual fatty acid composition of starting fish oils (CL-oil and ω3-oil), and encapsulated and surface fish oils of sprayed samples is expressed in Tables 4 and 5. Some composition differences were observed between both starting oils. Thus, the most abundant fatty acids in CL-oil were C18:1n9 and C16:0, followed by C18:3n3, C16:1 and C22:6n3;

meantime, ω3-oil showed C16:0 as the most abundant, followed by C22:6n3, C18:1n and C16:1, while C18:3 showed a relatively low proportion.

Related to the presence of individual fatty acids in both locations (surface and encapsulated) of spray dried CL-oil-samples (Table 4), C16:0 showed in all cases higher proportions in surface than in encapsulated oils, while C22:6n3 provided the opposite behavior. For C18:1n9, C18:3n3 and C16:1, slight differences were obtained or a general tendency could not be concluded for all kinds of spray dried treatments. Concerning the ω3-oil-samples (Table 5), C22:6n3 fatty acid showed in all cases markedly higher values in encapsulated oils than in their counterparts corresponding to surface oils. C18:1n and C16:1 also showed higher values in encapsulated oils, although differences with their counterpart surface oils were small. A general trend was not obtained for C16:0, since higher proportions were obtained in surface oils, except in the case of Ge-MTG-MD-treated samples.

Fatty acid analysis was also considered according to the composition on saturated (SFA), monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids, as well as to the ω3/ω6 and polyene (PI) ratios (Tables 4 and 5). ω3/ω6 ratio has recently attracted a great attention because of its important influence on the development of several health human problems. Complementary, Pl is considered as an index for studying damage of polyunsaturated fatty acids during process; it provides a meaningful tool to measure oxidative stability of fishery products as it includes two major long chain essential polyunsaturated fatty acids (Memon et al., 2010).

Starting CL-oil showed higher levels in MUFA and PUFA groups than for the SFA one. Differentially, ω3-oil provided higher values in SFA and PUFA groups; additionally, higher ω3/ω6 and PI ratios were detected in starting ω3-oil than in CL-oil. Concerning the effect of spray drying of CL-oil (Table 4), SFA showed higher values in surface oils than in their counterpart encapsulated oils; contrary PUFA proportion was greatly higher in encapsulated oils, this leading to higher PI scores. In the case of employing the ω3-oil, no significant differences ($p>0.05$) were detected for the MUFA presence when comparing contents in surface and encapsulated oils, except for Ge-MTG-MD treatment ($p<0.05$), where encapsulated oil provided a 28.93 and 39.87 scores, respectively. Related to the other type of fish oil (CL-oil), significant difference was observed among surface and encapsulated oils in all treatments, except for treatment with MTGase. Finally, ω3/ω6 ratio was found higher in surface oils than in encapsulated ones, except for Ge-MD-treated samples.

Related to ω3-oil analysis in treated samples (Table 5), SFA content was found higher in encapsulated oils than in their counterparts placed in the surface. The contrary result was found for PUFA proportion, except for the case of Ge-Cs-MD-treated samples, where its content was found higher in the encapsulated oils. MUFA group distribution between surface and encapsulated oils provided the same results as in the case of the PUFA group. Finally, no general tendency could be concluded for the ω3/ω6 ratio as a result of the spray-drying treatment and the PI showed higher values in surface oils, except for samples corresponding to the Cs-MD-treated ones.

Related to PUFA retention during encapsulation, the employment of CL-oil showed that all kinds of spray-drying treatments tested led to higher PUFA content mean values in the encapsulated oils, this leading also to higher PI scores. Concerning ω3-oil employment, no significant decrease ($p>0.05$) was observed in PUFA content of encapsulated oils in Ge-MD and Cs-MD treatments. Previous research (Memon et al., 2010) has reported that storage of fish oil could decrease the Pl as result of lipid oxidation development during storage. The present study demonstrates that encapsulated oil had higher amount of PI than the surface oil in all treatments. Klaypradit & Huang (2008) studied the encapsulation of fish oil by chitosan and maltodextrin as wall material by ultrasonic technology; such authors measured fatty acids of encapsulated fish oil and found that EPA and DHA content (240 mg/g) in encapsulated powder were slightly higher when compared to commercial information provided (100 mg/g). Sun-Waterhouse et al. (2011) detected differences among surface and encapsulated oils in 30-day storage time at 20 and 37^{o}C; as a result, no significant differences ($p>0.05$) in SFA scores could be depicted, while MUFA and PUFA contents provided significant differences ($p<0.05$); additionally, it was observed that MUFA contents in surface oil was lower than in the encapsulated counterpart and SFA to PUFA ratio decreased after 30 days.

Most previous literature agrees that PUFA are more susceptible to oxidation than SFA (Zhang et al., 2007). However, encapsulation process and antioxidant addition would help to protect such labile fatty acids (Sun-Waterhouse et al., 2011; Klaypradit & Huang, 2008).

4. CONCLUSION

Therefore, knowledge about wall composition can help the research to design appropriate encapsulating coating materials. Coating of fish oil with fish gelatin mixture of gelatin and chitosan and using MTGase produced stable emulsion. The mixtures of maltodextrin and gelatin with MTGase and also compound of gelatin with maltodextrin were found superior to other two formulas with respect to encapsulation efficiency and surface oil AND seems to have a protective effect against oxidation. Spray drying has proved to be suitable and economical process for drying. Results confirmed addition of combination of fish gelatin and maltodextrin with MTGase and fish gelatin with chitosan improved retention of fish oil. Surface oil in all wall compositions had higher saturate fatty acids than in encapsulated oil. Concerning the ω3-oil, C22:6n3 fatty acid showedramatkably higher values in encapsulated oil than in its corresponding counterpart. So, encapsulation was effective on fish oil quality and surface oil had less PUFA than encapsulated oil and had protective effects.

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Figure captions page

Figure 1. Scanning electron micrograph of microencapsulated fish oil spray-dried at 180/90°C: **A** and **B (**gelatin-maltodextrin; **core**; CL-oil and ω3-oil, respectively), **C** and **D (**chitosan-maltodextrin; **core;** CL-oil and ω3-oil, respectively), **E** and **F (**gelatin-chitosan-maltodextrin; **core;** CL-oil and ω3-oil, respectively), **G** and **H (**gelatin-MTGase-maltodextrin; **core;** CL-oil and ω3-oil, respectively).

Table 1

Experimental design of different fish oil microcapsules: Percentage content of components

Code [A]	Type of wall material				Fish oil [B]	
	Fish gelatin	Chitosan	Maltodextrin	MTGase	CL-oil	ω3-oil
Ge-MD	10	-	30	-	10	-
					-	10
Cs-MD	-	1.5	38.5	-	10	-
					-	10
Ge-Cs-MD	6	1	33	-	-	10
					10	-
Ge-MTG-MD	10	-	30	0.025	-	10
					10	-

[A] Treatment abbreviations employed: Ge-MD (fish gelatin-maltodextrin), Cs-MD (chitosan-maltodextrin), Ge-Cs-MD (fish gelatin-chitosan-maltodextrin), Ge-MTG-MD (fish gelatin-MTGase-maltodextrin).

[B] Oil abbreviations employed: CL-oil (cod liver oil) and ω3-oil (omega-3-enriched oil**).**

Table 2

Moisture, encapsulation efficiency, surface and total oil contents and particle size assessment of initial emulsions and spray-dried microcapsules[A]

Parameter	unit	Ge-MD[B]		Cs-MD[B]		Ge-Cs-MD[B]		Ge-MTG-MD[B]	
		Fish oil	ω3	Fish oil	ω3	Fish oil	ω3	Fish oil	ω3
Total oil	(g /100g powder)	16.05±0.05^{a}	19.06±2.56^{b}	16.56±0.68^{a}	16.04±0.84^{a}	20.03±0.05^{bc}	16.83±0.76^{a}	21.98±0.80^{d}	21.48±0.91^{d}
Surface oil	%	2.26±0.25^{a}	3.10±0.10^{b}	4.66±0.40^{cd}	5.23±0.40^{d}	4.23±0.05^{c}	3.96±0.23^{c}	2.63±0.35^{ab}	2.80±0.40^{ab}
Encapsulation efficiency	%	85.36±1.91^{de}	83.55±2.03^{d}	71.84±1.57^{b}	67.35±2.56^{a}	79.16±0.28^{c}	79.7±1.26^{c}	88.01±1.61^{e}	87.03±2.21^{de}
Oil droplet size	50th percentile, μm	1.73±0.01^{d}	1.67±0.22^{c}	1.46±0.00^{a}	1.48±0.11^{a}	1.45±0.15^{a}	1.57±0.08^{b}	1.72±0.01^{de}	1.78±0.06^{e}
Oil droplet size	90th percentile, μm	2.60±0.42^{c}	3.09±0.06^{e}	2.45±0.01^{a}	2.41±0.14^{a}	2.51±0.19^{b}	2.51±0.00^{b}	2.86±0.08^{d}	3.12±0.07^{f}
Particle size	50th percentile, μm	17.84±0.21^{c}	18.88±0.03^{b}	16.01±0.42^{e}	15.38±0.14^{f}	14.29±0.06^{h}	14.76±0.34^{g}	20.61±0.0^{a}	17.99±0.21^{d}
Particle size	90th percentile, μm	36.87±0.41^{g}	38.73±0.58^{f}	32.40±1.02^{e}	31.98±0.32^{a}	33.74±0.27^{b}	31.76±0.14^{a}	40.07±0.18^{c}	37.60±0.24^{d}
Oil droplet size	D3,2	1.72	1.64	1.46	1.39	1.45	1.57	1.57	1.64
Particle size	D3,2	6.54	6.61	5.92	5.42	6.15	6.11	8.76	8.55

[A] Values expressed as average of triplicate (n=3) analyses ± standard deviation. For each row, mean values followed by different letters denote significant (p<0.05) differences.

[B] Treatment name abbreviations as described in Table 1.

No moisture data are included !!

Table 3

Color parameter measurement[A] obtained in samples corresponding to the different treatments[B]

Treatments		Color Parameter		
		L*	a*	b*
Ge-MD	CL-oil	93.60±0.23^{bc}	3.10±0.00^{bc}	0.80±1.38^{a}
	ω3-oil	93.20±0.86^{bc}	3.10±0.00^{bc}	0.00±0.00^{a}
Cs-MD	CL-oill	92.30±1.75^{a}	2.86±0.40^{b}	3.53±0.46^{c}
	ω3-oil	89.66±0.23^{a}	3.63±0.46^{c}	3.76±1.00^{b}
Ge-Cs-MD	CL-oill	93.56±0.23^{bc}	2.63±0.40^{ab}	1.30±1.60^{a}
	ω3-oil	93.96±0.46^{c}	2.63±0.40^{ab}	0.83±0.40^{a}
Ge-MTG-MD	CL-oill	93.40±0.43^{bc}	3.10±0.00^{bc}	0.00±0.00^{a}
	ω3-oil	93.40±0.43^{bc}	2.40±0.00^{a}	0.80±0.00^{a}

[A] Values are average of triplicate (n=3) analyses ± standard deviation.

In each row, means followed by different letters (a-e) denote significant (p<0.05) differences.

[B] Treatment name abbreviations as described in Table 1.

Table 4

Fatty acid composition[A] of surface and encapsulated cod liver oil resulting from spray-dried microencapsulation

Fatty acids	Cod liver oil (CL- oil)	Ge-MD		Cs-MD		Ge-Cs-MD		Ge-MTG-MD	
		Surface	Encapsulated	Surface	Encapsulated	Surface	Encapsulated	Surface	Encapsulated
C 14:0	5.95±0.06	4.11±1.56	4.05±0.21	5.00±0.00	4.73±0.05	5.01±0.01	4.95±0.18	5.62±0.05	5.21±0.70
C16:0	17.14±0.00	19.63±0.04	13.64±1.40	14.28±0.09	13.31±0.29	19.32±0.15	13.94±0.01	15.29±0.33	13.77±0.40
C17:0	1.67±0.00	4.04±0.21	2.88±0.54	0.90±0.00	2.27±0.53	4.91±0.45	0.29±0.00	2.02±0.08	2.24±0.60
C18:0	2.51±0.00	3.22±0.06	3.21±0.56	3.02±0.02	2.89±0.02	2.77±0.05	2.98±0.12	2.91±0.07	2.98±0.12
C16:1	10.09±0.02	15.29±0.12	9.22±0.90	8.26±0.06	10.48±0.20	11.61±0.02	8.58±0.60	9.69±0.26	9.44±1.71
C17:1	1.77±0.00	0.05±0.07	1.05±0.10	0.55±0.00	1.16±0.03	1.79±0.02	0.55±0.00	1.46±0.00	1.58±0.42
C18:1n9	22.08±0.00	22.17±0.05	23.58±0.80	24.82±0.01	24.06±0.08	21.30±0.02	22.94±0.32	23.91±0.28	24.35±0.50
C 20:1n	1.82±0.01	1.23±0.08	1.74±0.12	1.61±0.02	1.67±0.13	1.72±0.03	1.83±0.00	1.71±0.14	1.79±0.04
C18:2n6	1.36±0.02	1.94±0.07	2.27±0.80	1.10±0.00	2.11±0.30	1.40±0.02	4.39±0.00	1.43±0.21	2.01±0.12
C18:3n6	0.63±0.02	0.01±0.00	0.59±0.01	1.00±0.00	0.59±0.02	0.58±0.09	0.06±1.11	0.53±0.08	0.69±0.12
C18:3n3	10.41±0.02	7.54±0.18	10.73±0.85	11.40±0.03	10.73±0.09	8.31±0.50	10.20±0.24	10.07±0.70	10.69±0.14
C20:3n3	6.71±0.00	5.10±0.13	6.91±0.60	5.55±0.62	7.53±0.15	5.98±0.13	6.90±0.14	6.55±0.73	7.51±0.20
20:4n6	8.44±0.01	5.92±0.08	7.61±0.45	7.89±0.02	8.23±0.00	6.89±0.19	8.46±0.02	7.22±0.97	8.37±0.20
C20:5n3 (EPA)	0.32±0.00	ND	1.24±0.20	0.25±0.01	0.27±0.00	0.19±0.00	0.33±0.02	0.20±0.10	0.27±0.00
C22:6n3 (DHA)	9.01±0.02	6.63±0.03	9.78±1.22	8.63±0.20	9.19±0.00	7.69±0.24	9.62±0.30	8.41±0.80	9.20±0.01
Σ SFA[B]	27.25±0.07^{c}	33.00±1.89^{d}	23.78±2.76^{ab}	23.21±0.13^{ab}	23.18±0.90^{ab}	32.02±0.66^{d}	22.15±0.32^{a}	25.82±0.54^{bc}	24.18±1.76^{ab}
Σ MUFA	35.76±0.05^{bcd}	38.73±0.33^{f}	35.59±2.03^{bc}	35.23±0.10^{b}	37.37±0.50^{e}	36.43±0.09^{cde}	33.09±0.90^{a}	36.78±0.69^{e}	37.06±2.65^{e}
Σ PUFA	36.88±0.09^{de}	27.15±0.52^{a}	39.08±4.15^{e}	31.84±0.90^{bc}	38.66±0.50^{e}	31.06±1.23^{b}	39.97±1.31^{e}	34.42±3.60^{cd}	38.75±0.80^{e}
ω3/ ω6	2.05±0.01^{cd}	2.45±0.00^{cd}	2.81±0.58^{d}	2.18±0.07^{bc}	1.01±0.17^{a}	2.50±0.02^{cd}	1.87±0.04^{b}	2.76±0.12^{d}	2.50±0.13^{cd}
Polyene index)	0.54±0.00^{bc}	0.34±0.00^{a}	0.83±0.16^{e}	0.62±0.01^{cd}	0.71±0.02^{de}	0.41±0.02^{ab}	0.64±0.06^{de}	0.56±0.05^{cd}	0.69±0.02^{cde}

[A] Values are average of triplicate (n=3) analyses ± standard deviation. In each row and for fatty acid groups and ratios, means followed by different letters (a-e) denote significant (p<0.05) differences.

[B]Abbreviations employed: SFA (saturated fatty acids), MUFA (monounsaturated fatty acids), PUFA (polyunsaturated fatty acids). Treatments name abbreviations as described in Table 1.

Table 5

Fatty acids composition[A] of surface and encapsulated **ω**3-oil resulting from spray-dried microencapsulation

Fatty acids	ω3-oil	Ge-MD		Cs-MD		Ge-Cs-MD		Ge-MTG-MD	
		Surface	Encapsulated	Surface	Encapsulated	Surface	Encapsulated	Surface	Encapsulated
C 14:0	9.19±0.00	7.93±0.87	8.57±0.40	5.76±0.05	8.97±0.01	C 14:0	9.19±0.00	7.93±0.87	8.57±0.40
C16:0	21.13±0.02	19.39±0.50	21.06±0.50	15.67±0.06	19.10±0.02	C16:0	21.13±0.02	19.39±0.50	21.06±0.50
C17:0	1.84±0.00	1.19±0.08	1.09±0.50	1.91±0.00	1.33±0.10	C17:0	1.84±0.00	1.19±0.08	1.09±0.50
C18:0	3.76±0.00	4.82±0.70	4.26±0.07	3.01±0.03	4.39±0.05	C18:0	3.76±0.00	4.82±0.70	4.26±0.07
C16:1	12.17±0.10	10.35±0.10	11.13±1.08	10.47±0.28	10.74±0.07	C16:1	12.17±0.10	10.35±0.10	11.13±1.08
C17:1	0.71±0.02	1.34±0.05	1.74±0.60	0.63±0.00	1.95±0.50	C17:1	0.71±0.02	1.34±0.05	1.74±0.60
C18:1n9	15.33±0.03	15.84±0.37	16.32±0.04	14.03±0.04	14.66±1.20	C18:1n9	15.33±0.03	15.84±0.37	16.32±0.04
C 20:1n9	0.60±0.01	2.81±0.17	ND	3.04±0.00	ND	C 20:1n9	0.60±0.01	2.81±0.17	ND
C18:2n6	1.48±0.01	1.70±0.45	1.00±0.00	2.91±0.08	1.43±0.01	C18:2n6	1.48±0.01	1.70±0.45	1.00±0.00
C18:3n6	2.81±0.01	0.74±0.20	1.68±1.10	0.94±0.10	2.67±0.03	C18:3n6	2.81±0.01	0.74±0.20	1.68±1.10
C18:3n3	2.10±0.04	2.12±0.13	1.92±0.05	1.57±0.06	1.09±0.06	C18:3n3	2.10±0.04	2.12±0.13	1.92±0.05
C20:3n3	1.08±0.00	1.14±0.17	1.48±0.20	0.98±0.03	1.37±0.00	C20:3n3	1.08±0.00	1.14±0.17	1.48±0.20
C20:4n6	2.22±0.01	2.82±0.25	2.07±0.07	0.92±0.10	3.37±0.10	C20:4n6	2.22±0.01	2.82±0.25	2.07±0.07
C20:5n3 (EPA)	9.24±0.01	8.72±0.70	9.25±0.01	6.03±0.07	9.25±0.00	C20:5n3 (EPA)	9.24±0.01	8.72±0.70	9.25±0.01
C22:6n3 (DHA)	16.07±0.20	15.87±0.12	17.11±0.60	14.35±0.60	16.55±0.20	C22:6n3 (DHA)	16.07±0.20	15.87±0.12	17.11±0.60
Σ SFA[C]	35.92±0.03^{e}	33.33±2.12^{c}	34.97±1.50^{d}	26.35±0.14^{a}	33.80±0.18^{c}	29.37±0.64^{b}	30.24±0.43^{b}	33.77±1.33^{c}	33.88±0.18^{c}
Σ MUFA	28.78±0.16^{ab}	30.32±0.7^{b}	29.18±1.70^{ab}	28.42±0.33^{ab}	27.32±1.70^{a}	28.86±0.90^{ab}	27.10±3.20^{a}	28.93±1.25^{ab}	39.87±0.23^{c}
Σ PUFA	35.00±0.24^{f}	33.12±2.05^{def}	34.51±2.9^{ef}	26.93±1.10^{a}	34.13±0.4^{def}	31.51±1.23^{cde}	29.34±1.53^{abc}	28.16±2.67^{ab}	31.11±0.30^{bcd}
ω3/ ω6	4.38±0.04^{ab}	5.36±0.70^{bc}	6.52±1.75^{c}	4.81±0.11^{abc}	3.30±0.01^{a}	13.75±0.06^{e}	11.68±1.06^{d}	4.29±0.27^{d}	13.26±0.53^{de}
Polyene index)	1.20±0.01^{bc}	1.25±0.01^{cd}	1.27±0.08^{bcd}	1.30±0.04^{d}	1.17±0.00^{b}	1.18±0.03^{bc}	1.29±0.06^{d}	1.00±0.00^{a}	1.30±0.00^{d}

[A] Values are average of triplicate (n=3) analyses ± standard deviation. In each row and for fatty acid groups and ratios, means followed by different letters (a-e) denote significant (p<0.05) differences.

[C] Abbreviations employed: SFA (saturated fatty acids), MUFA (monounsaturated fatty acids), PUFA (polyunsaturated fatty acids). Treatments name abbreviations as described in Table 1.