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Microencapsulation of linseed oil by spray drying for functional food application

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ABSTRACT

Health benefits associated to ω-3 fatty acids consumption together with the high susceptibility to oxidation of ω-3 containing oils have led to the development of microencapsulated oils for nutraceutical and food enrichment applications. The aim of this work is to obtain different formulations for linseed oil microencapsulation by spray-drying with high encapsulation efficiency and evaluate their resistance to oxidation through the accelerated Rancimat test. Four formulations were tested; using different combinations of gum arabic (GA), maltodextrin (MD), methyl cellulose (MC) and whey protein isolate (WPI). Microcapsules made of 100% GA and ternary mixtures of GA, MD and WPI presented the highest protection from oxidation and microencapsulation efficiencies higher than 90%. They also presented spherical structures with smooth surfaces which kept unaltered after 10-month storage. GA containing formulation was included in bread manufacturing. Fortified bread resulted similar in appearance to control bread without microcapsules, but α-linolenic acid content was reduced significantly after preparation.

1. Introduction

Health benefits associated with ω-3 fatty acids consumption have been extensively demonstrated, especially those related to cardiovascular diseases prevention (Astrup et al., 2011; Beltrán, 2010; Riediger, Othman, Suh, & Moghadasian, 2009). The low ω-3 intake in occidental diets (Simopoulos, 2011) has led to the development of nutraceuticals and functional foods in recent years, particularly those containing polyunsaturated fatty acids (PUFA). The most relevant ω-3 fatty acids are alpha-linolenic acid (ALA, C18:3), eicosapentaenoic acid (EPA, C20:5) and docosahexaenoic acid (DHA, C22:6), which are present in animal sources such as fish oil (DHA and EPA) or vegetable sources such as algae (DHA and EPA) or vegetable oils (ALA). Fish oil has been chosen as a preferential source of long chain ω-3 fatty acids due to their proven positive role in infant development and mental illnesses (Balanza-Martinez et al., 2011; Hadders-Algra, 2011), and their effects against inflammation, platelet aggregation, hypertension, and hyperlipidemia (Kris-Etherton, Harris, & Appel, 2002).

A good vegetable alternative ω-3 source is linseed oil, also known as flaxseed oil, which contains more than 50% of ALA (Bozan & Temelli, 2008). Even though it is assumed that ALA cannot replace the consumption of long chain fatty acids (Wang et al., 2006) its role in cardiovascular health (Mozaffarian, 2005) and some mental disorders such as depression (Lucas et al., 2011) has been reported. These beneficial effects may be associated to its partial in vivo conversion to EPA as previously described (Anderson & Ma, 2009).

However, ω-3 PUFA are easily oxidized due to their high degree of unsaturation, a process which involves the formation of off-flavor compounds (Kolanowski, Jaworska, & Weißbrodt, 2007) as well as toxic products (Guillén & Ruiz, 2005). Microencapsulation has appeared as a key technology in delaying or inhibiting oxidation and masking undesirable odors and flavors in the final product. The process converts the oil into a free flowing powder which can be easily handled and used for nutraceuticals and/or food fortification. Microencapsulation can be defined as a process in which tiny droplets, namely core, are surrounded by a coating of a microencapsulating agent. This coating wall can be made of a great variety of food grade materials and protects the entrapped core by providing a physical barrier against environmental conditions. Spray-drying is the most common microencapsulation technology used in food industry due to low cost and available equipment (Gharsallaoui, Roudaut, Chambin, Voilley, & Saurel, 2007). The process involves the atomization of emulsions into a drying medium at a high temperature, resulting in very fast water evaporation.

Microencapsulated fish oil has been largely obtained by spray-drying, a product referred to as Dried Microencapsulated Fish Oil (DMFO). Many different wall materials have been used, such as skim milk powder or mixtures of Na/Ca caseinate with lactose (Keogh et al., 2001), Maillard reaction products obtained by heat treatments of mixtures of proteins and carbohydrates (Augustin, Sanguansri, & Bode, 2006), sugar beet pectin (Drusch, 2007; Polavarapu, Oliver, Ajlouni, & Augustin, 2011), barley protein (Wang, Tian, & Chen, 2011), cellulose

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derivatives such as methylcellulose (MC) and hydroxypropyl methylcellulose (HPMC) (Kolanowski, Laufenberg, & Kunz, 2004), maltodextrin in combination with branched cyclodextrin and casein (Kagami et al., 2003), among others.

The stabilization of ω-3 PUFA by spray-drying microencapsulation has been assessed by complementary methods, such as peroxide value (PV) and p-anisidine value determination (Omar, Shan, Zou, Song, & Wang, 2009), headspace propanal determination (Augustin et al., 2006) and non-isothermal differential scanning calorimetry (Pedroza Islas, Macías Bravo, & Vernon Carter, 2002). Velasco et al. has also applied and validated the accelerated test Rancimat to evaluate the oxidative stability of dried microencapsulated oils (Velasco, Dobarganes, Holgado, & Márquez-Ruiz, 2009; Velasco, Dobarganes, & Márquez-Ruiz, 2000). The application of this test enables to predict shelf-life and efficacy of antioxidants without the need to go through tedious time-consuming solvent extractions.

Microencapsulation of linseed oil has been hardly described. The production of this vegetal oil is being promoted in Argentina for nutritional purposes due to its high level of alpha-linolenic acid. Omar et al. obtained microencapsulated flaxseed oil using gum arabic, maltodextrin and xantham gum with high encapsulation efficiencies (Omar et al., 2009). More recently, it has been encapsulated by spray-drying using gum arabic and some critical parameters, such as inlet air temperature, total solid content and oil concentration, were evaluated and optimized (Tonon, Grosso, & Hubinger, 2011). Flax oil was also encapsulated using zein as coating material and spray-drying was compared to freeze-drying (Quispe-Condori, Saldaña, & Temelli, 2011). Nanocomplexes of flaxseed oil with high amylase corn starch have been developed for bread fortification, but the oil content of the powder was lower than 12% w/w (Gokmen et al., 2011). Linseed oil has also been encapsulated using mixtures of gum arabic and maltodextrin and a ω-3 fortified soup powder was obtained by mixing the solid ingredients with the obtained microcapsule powder (Rubilar et al., 2012).

The aim of this study is the evaluation of different formulations to encapsulate linseed oil by spray-drying with a high efficiency and an increased protection from oxidation. In particular a commercial whey protein isolate (WPI) has been included as an ingredient of the formulation due to its reported antioxidant properties (Hu, McClements, & Decker, 2003). The Rancimat accelerated test was used to predict the stability of microencapsulated powders to temperature, which has not been used for microencapsulated linseed oil to the best of our knowledge. We finally prepared ω-3 fortified bread by including microencapsulated linseed oil (MLO) in its manufacture and determined ALA content after the process.

2. Material and methods

2.1. Materials

Whey Protein Isolate (WPI) (BiPro, 90% protein) was obtained from Davisco Foods International Inc. (Le Sueur Food Ingredient Company, Minnesota, USA). Methyl cellulose (MC) Methocel™ A15 Premium LV (viscosity 12–18 mPa.s; MeO% 30) was kindly donated from Colorcon S.R.L. (México D.F., México). Maltodextrin (MD) 10 DE (MW approx. 80,000) was from Inamalt (Industrializadora de maíz S.A., México D.F., México). Gum Arabic Instant Gum BA (GA) (MW approx. 600,000) was obtained from Coloides Naturales S.A. (México D.F., México). Soya lecithin Ultralec F was supplied by Helm S.A. (Naucalpam, México). Flaxseed oil was purchased from Droguería Cosmopolita (México D.F., México), showing the following fatty acid composition: 5.1% C16:0, 3.5% C18:0, 19.5% C18:1, 17.2% C18:2 and 53.9% C18:3. The oil had no added antioxidants as declared by the supplier. Fresh yeast (Calsa S.A., Argentina), refined salt (Dos Anclas S.A., Argentina) and refined sugar (Ledesma, Argentina) were used for bread manufacturing. Ethyl ether, petroleum ether and hydrochloric acid were from Sintorgan S.A. (Buenos Aires, Argentina). All other reagents were of analytical grade.

2.2. Preparation of microencapsulated linseed oil

Four formulations were prepared to encapsulate linseed oil with different wall materials. They were designed to contain a high solid content in the O/W emulsions (>30% w/v) and a content of linseed oil higher than 20% w/w in the final dry powder. Soya lecithin was used as emulsifier in all cases. Microcapsules were formulated according to Table 1: 1) M–GA contained a 100% gum arabic as wall material; 2) M–GA/MD was a binary mixture of 56% GA and 44% MD; 3) M–GA/MD/WPI was a ternary mixture of 17% GA, 66% MD and 17% WPI; 4) M–MD/MC contained 33% MD and 66% MC. This was the only case in which the solid content of O/W emulsion had to be kept below 10% due to MC high viscosity.

The dissolution of wall materials was achieved by different procedures according to formulation. M–GA aqueous phase was obtained by dissolving gum arabic in deionized water at 30 °C. The solution was kept at 8 °C overnight. Maltodextrin was added afterwards to obtain M–GA/MD aqueous solution. Whey Protein Isolate (WPI) was dispersed in deionized water with magnetic stirring for 30 min at 80 °C and then kept overnight at 8 °C. GA and MD were further added to WPI solution. Methyl cellulose was dispersed in deionized water at 40 °C with magnetic stirring. Cold water (10 °C) was subsequently added to achieve full dissolution and the solution was kept overnight at 8 °C. Maltodextrin was separately dissolved in deionized water at 40 °C with magnetic stirring, and then cooled to 10 °C. Both solutions were mixed with magnetic stirring in cold bath water.

Oil-in-water (O/W) emulsions were obtained by adding flax seed oil previously mixed with lecithin to each aqueous phase. Samples were processed by high shear homogenization for 5 min at 9000 rpm in a Silverson L5M (Silverson Machines Ltd., UK) at 25 °C. The emulsions were spray dried in a Niro Mobile Minor™ Atomizer (GEA Niro, Søborg, Denmark) at a pressure of 2.8 bar. The inlet air temperature was adjusted to 175 ± 5 °C and the outlet was kept at 75 ± 5 °C by controlling the flow rate (15.0 mL/min).

2.3. Microcapsule characterization

2.3.1. Size and morphology

Particle size and morphology were analyzed by scanning electron microscopy (SEM) Phillips 505 (Amsterdam, Holland). Microphotographs with high magnification were acquired with a SEM LEI Quanta 250 (Hillsboro, Oregon, USA). Samples were previously gold sputtered with an Edwards Sputter Coater S150B (Crawley, England).

2.3.2. Moisture content

The moisture content in microencapsuled oil was determined by isothermal drying using a HR83 Halogen Moisture Analyzer Mettler-Toledo (Ohio, USA) with a readability of 0.001% moisture content.

Table 1
Description of formulations.

	M–GA	M–GA/MD	M–GA/MD/WPI	M–MD/MC
Gum Arabic (g)	112	72	22	–
Maltodextrin (g)	–	56	85	10
Whey Protein Isolate (g)	–	–	22	–
Methyl cellulose (g)	–	–	–	20
Linseed oil (g)	28	33	32	15
Soya lecithin (g)	4	5	3	3
Deionized water (g)	260	240	240	502
Solid % w/v[a]	35.6	40.9	40.6	8.7
O/W Ratio (g/g)[b]	0.55	0.70	0.68	0.10
Wall material/oil ratio (g/g)	4	3.9	4	2

[a] Solid content in emulsion including linseed oil.
[b] Ratio between dispersed (O) and continuous phases (W).

2.3.3. Microencapsulation efficiency

Total oil content in microcapsules was quantified using the AOAC Official Method 925.32 which consists in an acid hydrolysis, solvent extraction and gravimetric analysis. Briefly, 1 g of powder was transferred to a fat-extraction tube and 10 mL HCl (4 + 1) was slowly added. The tubes were set in a water bath at 70 °C, heated to 100 °C and then boiled for 30 min. After cooling to room temperature (25 °C), 25 mL ethyl ether and 25 mL petroleum ether were added and the tubes were vigorously shaken for a minute. The ether solution (supernatant) was separated and filtered through packed cotton. The remaining aqueous phase was further extracted twice 15 mL of ethyl ether and 15 mL of petroleum ether. The solvent was rota-evaporated (Hei-VAP-Value, Heidolph Instruments GmbH, Schwabach, Germany) and the oil was dried in a vacuum oven at 100 °C to constant weight. Extractable oil, usually referred to as surface oil, was determined according to Davidov-Pardo, Roccia, Salgado, Leon, and Pedroza-Islas (2008). This non-encapsulated oil can be defined as the fraction that is easily extracted with organic solvents without disruption of the solid matrix. Briefly, 4 g microcapsule powder was drip washed with 75 mL of ethyl ether for 15 min at 25 °C. The suspension was filtered through a Whatman No. 1 filter paper and the powder on the filter was rinsed three times with ethyl ether. The solvent was dried and rota-evaporated to obtain the surface oil mass. Encapsulation efficiency (EE%) was calculated from the following equation:

$$EE\% = \left(\frac{TO - EO}{TO}\right) \times 100$$

where TO is the total oil content in microcapsules and EO is the extractable oil content determined as previously described.

2.3.4. Fatty acids profile of linseed oil by gas chromatography

ALA content was determined by gas chromatography in bulk and microencapsulated oil. In this case, extracted oil resulting from total oil determination was used for GC analysis after fatty acid derivatization to obtain the corresponding methyl esters. Chromatographic profiles were registered and ALA percentage was determined by area normalization with a GC coupled to a FID detector in a Perkin Elmer Clarus 500 (Shelton, USA) using a column Supelco SP-2560 100 m × 0.25 mm × 0.2 μm. The injector and detector temperature was set at 240 °C and 280 °C respectively. The column gradient was 140 °C for 5 min (4 °C/min), 230 °C for 20 min and the carrier gas was H_2.

2.3.5. Linseed oil stability with accelerated Rancimat test

The accelerated Rancimat test was used to estimate linseed oil stability to oxidation before and after microencapsulation. The Rancimat apparatus was used with one evaluation mode: the Induction Period, (IP), defined as the time corresponding to the inflection point of the curve Conductivity vs. Time. It was determined at 100 °C and 20 mL air/h using a 743 Rancimat apparatus (Metrohm, Herisau, Switzerland) according to Velasco et al. (2000). 3 g of microencapsulated linseed oil were weighted in each reaction vessel taking care not to push the solid into the oxygen glass tube. Bulk oil was used as control (3 g). IP values were automatically detected and registered. Analyses were performed in duplicates.

2.3.6. Characterization of microencapsulated linseed oil after storage

Microencapsulated linseed oil was stored at 5 °C in hermetic bags (no vacuum) for 10 months. Rancimat test was performed on these samples and SEM microphotographs were obtained to detect changes in morphology compared to freshly prepared samples.

2.4. Bread formulation and manufacturing

Flour was obtained in our laboratory by milling wheat grains (Bühler MLU-202, IRAM 15864 I y II) in order to avoid any possible interference from additives. Bread was prepared with the following formulation: white flour 300 g, microcapsules M–GA 19.8 g, salt 5.25 g, sugar 15 g, yeast 9 g, and water 170 mL. Flour was mixed with microcapsules and placed in a farinograph (Brabender® GmbH & Co. KG, Germany) for 5 min at a low speed. Salt, sugar and yeast were dissolved in water at 33 °C and added to the farinograph. Immediately after stopping the mixer, two pieces of dough (150 g) were scaled off and rounded for twenty revolutions in the extensigraph. They were then placed on the baking pans. The dough formed was rested for 105 min at 32 °C with humidity of 80% in a fermentation chamber (W. Ehret, Germany). The bread was baked in an oven (Argental, Argentina) for 20 min at 220 °C.

2.5. Bread characterization

Since there are no official methods for the determination of fat in bread, AOAC Official Method 922.06, used to determine fat in flour, was adapted for bread application by previously lowering its water content. Portions of bread were accurately weighed and lyophilized (L-I-E300-CRT, Rificor, Buenos Aires, Argentina) at constant temperature (−45 °C) and vacuum (0.008 mm Hg) during 16 h. 2 g of each lyophilized sample were transferred to a Mojonnier tube and 2 mL of alcohol was added for sample dispersion. After stirring, 10 mL HCL (25 + 11) was further added and mixed with the suspension. The tubes were set in a water bath at 70 °C, heated to 100 °C and then boiled for 30 min. 10 mL alcohol was added and the samples were cooled to 25 °C. Oil was extracted with a mixture of ethyl and petroleum ether as previously described for total oil determination in microcapsules. ALA content was determined in fortified bread by gas chromatography as previously described and fatty acid profiles were recorded. The appearance of treated bread was compared to control bread with no microencapsulated oil addition by a non-trained panel.

2.6. Statistical analysis

IP values and encapsulation efficiencies were evaluated by one-way ANOVA and significant differences between treatments determined by Tukey's test a $P = 0.05$ using Minitab 15 (Minitab, Softonic, USA).

3. Results and discussion

3.1. Formulation and preparation of microencapsulated linseed oil

Table 1 presents a description of the feeding formulations. The biopolymers have been selected according to different, complementary criteria. A suitable wall material should be highly water-soluble and exhibit a good surface activity to favor emulsion stabilization. Second, it must have suitable drying profiles to achieve a rapid formation of a dense skin and a good protection of core against oxygen transfer (Gharsallaoui et al., 2007). This is usually achieved by biopolymers which form dense networks during drying, such as some globular proteins (Nicolai, Britten, & Schmitt, 2011). The selected wall material should also protect the core from possible degradation during storage and/or incorporation to food. In our case, the challenge for the selected system is to protect linseed oil from oxidation upon storage and during bread manufacture. Finally, the costs and availability need to be considered.

Gum Arabic was the first biopolymer chosen for encapsulation due to its recognized film forming and emulsifying properties (Madene, Jacquot, Scher, & Desobry, 2006). In fact it produces stable emulsions with most oils over a wide pH range (Gharsallaoui et al., 2007). It has been previously selected as a low-cost alternative for cod liver oil encapsulation with a high efficiency (Pedroza Islas et al., 2002) and preferred to maltodextrins and modified starch for the encapsulation of cardamom resin (Krishnan, Bhosale, & Singhal, 2005). Another desirable characteristic of this gum is its slow dispersion in water at room temperature, which is promising for food fortification with

microcapsules. This material was used alone or combined with other biopolymers at different proportions (Table 1). In fact, as the expected functional properties may not be provided by a single wall material, many authors have proposed and demonstrated the synergistic effect of biopolymer blends (Kagami et al., 2003; Pedroza-Islas, Vernon-Carter, Durán, & Trejo-Martínez, 1999).

Maltodextrin was mainly chosen as it provides good oxidative stability to encapsulated oil (Gharsallaoui et al., 2007); it is also very water soluble and exhibits a low viscosity even at concentrated solutions, which allows increasing the solid content of emulsions. It is also considered as an alternative to gum arabic due to its low cost. This carbohydrate, however, exhibits poor emulsifying capacity and low oil retention (Gharsallaoui et al., 2007). Therefore, the use of GA and MD mixtures has been proposed for the microencapsulation of oils as a good compromise between cost and effectiveness as wall materials (Rubilar et al., 2012). Pedroza-Islas et al. demonstrated that a 50:50 mixture of GA and MD presented the highest thermo-oxidative stability as evaluated by dynamic differential scanning calorimetry (Pedroza Islas et al., 2002).

Whey proteins have been found to inhibit lipid oxidation due to different antioxidant mechanisms such as the formation of thick viscoelastic films at emulsion droplets interfaces and chelation of prooxidative metals (Hu et al., 2003). Globular proteins present in whey modify their secondary structure upon heating above 60 °C. This state is often referred to as "molten globule" because the protein chains are more labile but do not unfold completely. Internal functional groups can then become exposed and may interact with other neighboring molecules. Consequently, bonds are formed between proteins leading to aggregation. Nicolai et al. have described the optimal conditions for this aggregation to occur and its beneficial effects for bioactive microencapsulation (Nicolai et al., 2011). In the present work, we have favored this aggregation process by heating WPI at 80 °C at pH > 5.8. WPI was included in one of the formulations (see Table 1) based on previous works from other authors. In fact, a blend composed of WPC (whey protein concentrate)–MG (mesquite gum)–MD 17:17:66 showed the highest activation energy values for microencapsulated red chili oleoresin (Pérez-Alonso et al., 2008). In this case, WPC and MG were replaced by WPI and GA respectively, which are available in our country.

Methyl cellulose (MC) has been largely used as coating material for pharmaceutical applications. In contrast to other biopolymers, it is soluble in cold water and becomes almost insoluble over 35–40 °C. This feature might lead to a higher resistance during bread manufacturing, as previously reported for fish oil microencapsulated with a blend of MC–MD 2:1 (Davidov-Pardo et al., 2008). Kolanowski et al. had previously used the same mixture to demonstrate that the oxidative stability of fish oil was significantly improved by microencapsulation with MC–MD (Kolanowski et al., 2004). Moreover, they achieved to obtain a 40% w/w of oil which is higher than the contents usually obtained in fish oil powders (20–30% w/w).

The wall material to core ratio was kept at 4:1 as previously described for better protection against oxidation (Pérez-Alonso, 2008), except for MC-containing formulation, which was obtained at 2:1, according to previous results from bibliography (Davidov-Pardo et al., 2008; Kolanowski et al., 2004). Though most of the selected wall materials exhibit emulsifying properties, lecithin was included in all formulations to assure the stability of feeding emulsions before spray drying.

Spray drying conditions were selected based on preliminary experiments performed at different inlet temperatures, feed rates and air flows so as to maximize powder yield and minimize surface oil. Some generally accepted criteria have also been considered. It has been stated, for instance, that the best spray-drying conditions are a compromise between high air temperature and high solid concentration (Gharsallaoui et al., 2007). Higher solid contents lead to an increased viscosity reducing the migration of oil to the surface and resulting in a rapid skin formation (Jafari, Assadpoor, He, & Bhandari, 2008). In this work the solid content of O/W emulsions was higher than 30% w/v except for M–MD/MC, which was formulated with a solid percentage lower than 10% w/v due to the high viscosity of methylcellulose even at low temperature. The emulsions were spray-dried at 175 ± 5 °C, a compromise between high temperatures and oil protection. In fact, most of the authors have used temperatures in the range of 160–180 °C for spray drying of highly unsaturated oils (Davidov-Pardo et al., 2008; Drusch, Serfert, Scampicchio, Schmidt-Hansberg, & Schwartz, 2007; Pedroza Islas et al., 2002; Tonon et al., 2011).

Linseed oil was microencapsulated with a high powder yield (>80%), except for the formulation containing methylcellulose. In this case, even though the feed temperature was maintained below 10 °C to keep a low viscosity of the emulsion, the yield was very low as the sample soon blocked the atomizer through the formation of a thick solid wall. This result is in contrast to previous works performed with fish oil encapsulated with the mixture MD/MC in similar conditions, which may be a consequence of the different nature of the oil or some small variations in the preparation procedure (Davidov-Pardo et al., 2008).

3.2. Microcapsule characterization

Microencapsulated linseed oil was obtained with encapsulation efficiencies around 90%, independent of the formulation (Table 2), which can be considered an adequate level for oil powders. Encapsulation efficiency determines the grade of oil protection and is dependent on many factors. The importance of high pressure homogenization in the preparation of the feeding o/w emulsion has been largely described. The reduction of the vacuole volume of fish oil in the feeding emulsion, achieved through a high energy process, resulted in reduced surface oil in the dried powder and consequently an increased shelf life was observed (Keogh et al., 2001). The wall material to core ratio is another variable to consider. It is generally accepted that a wall material to core ratio between 2 and 4 (w/w) should be suitable for most applications. A ratio lower than 2 would probably lead to an unacceptable increase of surface oil, while a ratio higher than 4 would result in a powder with a very low oil content, which is not desirable for food applications. Polavarapu et al. have recently studied the influence of wall material to core ratio in surface oil by encapsulating fish and olive oil with sugar beet pectin using high pressure homogenization to prepare the emulsions (Polavarapu et al., 2011). The solvent-extractable oil increased from 2% (EE% = 98) of the total oil in the microcapsules having 25% w/w oil load to 10% (EE% = 90) of the total oil in the microcapsules having 50% w/w oil load. The decreased microencapsulation efficiency for microcapsules formulated at 50% w/w may be explained by the lower amount of continuous matrix in these formulations, which was insufficient to form a dense, tightly packed matrix around the dispersed oil droplets.

In the present work, we prepared the emulsions using a high shear homogenizer. This process produces emulsions with higher vacuole volumes than high pressure homogenizers, which may be one of the main causes for the decrease in encapsulation efficiency. On the other hand, the EE% values may have remained around 90% due to the adequate wall material to core ratio selected for microencapsulation (Table 1). Contrary to GA containing microcapsules, the formulation based on methylcellulose encapsulated a low percentage of the oil,

Table 2
Characterization of microencapsulated linseed oil: encapsulation efficiency.

	M–GA	M–GA/MD	M–GA/MD/WPI	M–MD/MC
Extractable oil (% w/w)	2.2 ± 0.4	1.9 ± 0.2	2.7 ± 0.3	24.5 ± 0.1
Total oil (% w/w)	23.2 ± 0.2	22.1 ± 0.6	22.2 ± 0.3	32.9 ± 0.1
Encapsulation efficiency % (EE%)	90.5 ± 0.1	91.4 ± 0.1	87.8 ± 0.1	25.5 ± 0.1

probably due to the experimental drawbacks already mentioned in Section 3.1.

Powders presented moisture contents from 1.8 to 3.1% w/w which is under the minimum specification for most dried powders used in the food industry (3–4%). It has been observed that these low water contents are usually associated with low water activities, which might prevent lipid oxidation (Klaypradit & Huang, 2008).

Fig. 1 shows size and morphology of the four formulations. Fig. 1d is a microphotograph of M–MD/MC microcapsules. Most agglomerates appear instead of well-formed microcapsules which is coincident with experimental results of low yield and EE% (Table 2). On the other hand, well-defined spherical microcapsules were observed in the other cases. The size range inferred from microphotographs is 10–50 μm with a relatively high polydispersity. WPI containing microcapsules present more homogeneous size distribution and a higher mean size (Fig. 1c). Some differences in morphology can be appreciated. M–GA presented the highest percentage of wrinkled microcapsules with concave surfaces which is typical of microcapsules produced by spray-drying. Tonon et al. observed the same morphology for linseed oil encapsulated in GA, but in that case the effect was more pronounced (Tonon et al., 2011). This might be explained by the higher solid content used in the present work. In fact, the formation of wrinkled structures has been explained by the formation of vacuoles inside the particles after the crust development (Nijdam & Langrish, 2006). The magnitude of this process which involves the inflation of the crust at high temperature and subsequent shrinkage of the particle is inversely related to solid concentration in the emulsion. On the other hand, M–GA/MD/WPI presented a spherical morphology and smooth surface with very few wrinkled capsules (Fig. 1c). It has been described that thermal pre-treatment of WPI at 80 °C generated more cohesive films which seem to be the consequence of interaction between WPI and carbohydrates as carboxymethyl cellulose (Villa García, 2009). The improved films resulted in smoother and more uniform surfaces, as observed in the present work. M–GA/MD presented an intermediate morphology between the other two formulations (Fig. 1b).

It is also interesting to observe a broken microcapsule to appreciate its internal porous structure (Fig. 2). Small spherical holes of the size of oil droplets can be observed in a cross cut of a microcapsule corresponding to M–GA/MD formulation, which indicates that oil was homogeneously distributed in the wall matrix. This was previously reported for flaxseed oil encapsulated in WPI (Partanen et al., 2008) and fish oil microencapsulated with barley protein (Wang et al., 2011).

Total oil content was well in the range of microencapsulated oils obtained by spray drying (Table 2) and very similar to previous works using flaxseed oil (Omar et al., 2009; Tonon et al., 2011). Chromatographic ALA profiles were similar to bulk oil (Fig. 3) though ALA content was 90% of the initial content for all formulations (Table 3, ALA% in fresh samples), a decrease attributed to degradation of unsaturated fatty acids during microcapsule preparation. This is similar to the reduction of ALA from 40% to 35% previously obtained by other authors after linseed oil microencapsulation with GA/MD 3:2 (Rubilar et al., 2012).

3.3. Stability to oxidation of microencapsulated linseed oil

The accelerated Rancimat test has gained acceptance due to its ease of use and reproducibility. An oil-containing sample is heated under atmospheric pressure at a selected temperature and bubbled with oxygen at a constant flow, which can be considered an accelerated oxidation test. Under these controlled conditions, the lipoperoxidative process reaches its final steps and lipids are oxidized to short-chain volatile acids which are collected in distilled water increasing its conductivity. The IP is the time required to produce a sudden increase of conductivity, which can be defined as an indirect measure of oil stability: the higher the IP, the more stable the sample.

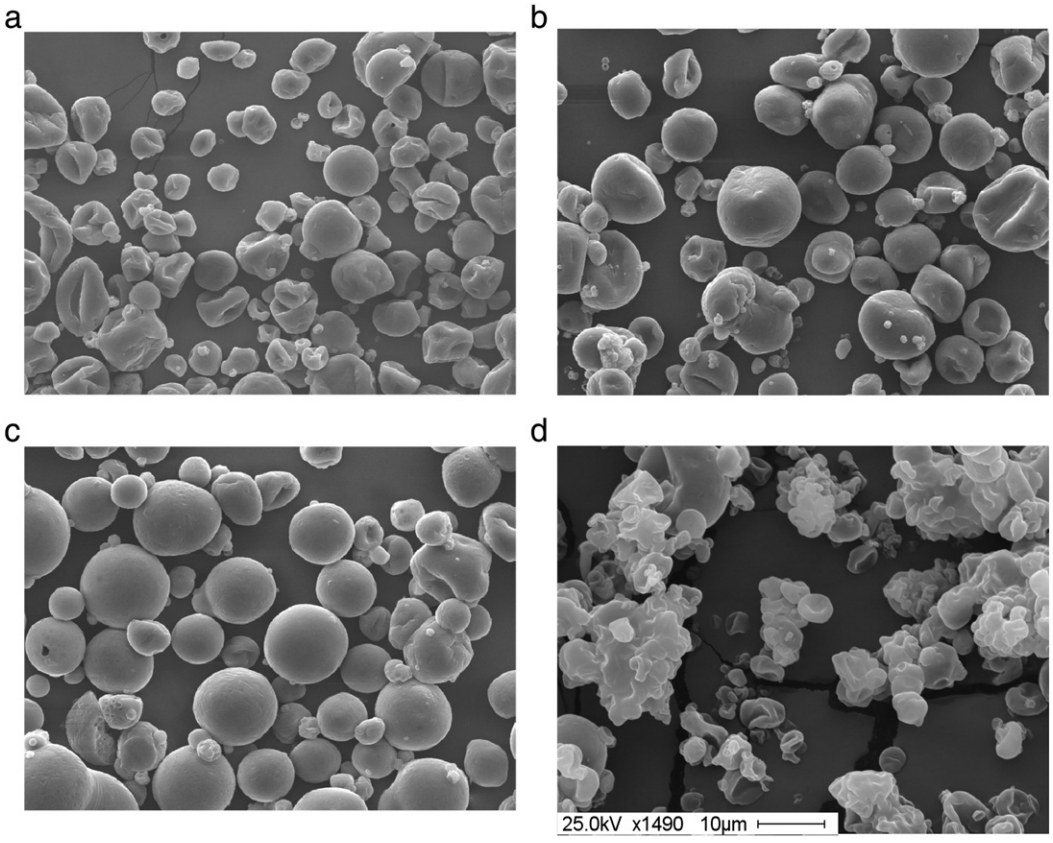

Fig. 1. SEM microphotographs of microencapsulated linseed oil a) M–GA, b)M–GA/MD, c) M–GA/MD/WPI, d) MD/MC.

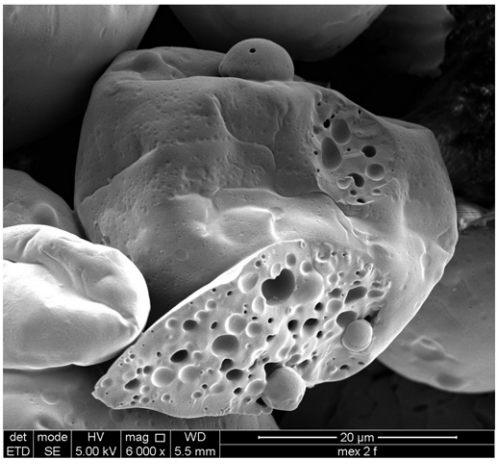

Fig. 2. SEM microphotograph of microencapsulated linseed oil M–GA.

Velasco et al. set the optimal conditions to determine IP of vegetable oils, either in bulk or in microencapsulated powders (Velasco et al., 2000, 2009). More recently, García-Moreno et al. studied the influence of operational parameters of the Rancimat test, including temperature, air flow rate and sample weight, on the determination of the oxidative oxidation index and concluded that temperature was the only statistically significant factor, confirming the robustness of this technique (García-Moreno, Pérez-Gálvez, Guadix, & Guadix, 2013). In this work, temperature (100 °C) was chosen as a compromise between the levels required for reasonable IP values (over 80 °C for vegetable oils) and the maximum temperature which does not alter peroxidation mechanisms, usually accepted as 100 °C (Méndez et al., 1996).

Table 3
Stability of microencapsulated linseed oil: ALA content obtained by gas chromatography and Induction Period (IP) obtained from Rancimat accelerated test.

	ALA %		IP (h)	
	Fresh samples	After Rancimat test[a]	Fresh samples	After 10 months[b]
M–GA	48.5 ± 0.1	44.7 ± 0.4	8.0 ± 0.7	5.9 ± 0.1[d]
M–GA/MD	48.6 ± 2.6	44.3 ± 1.2	3.8 ± 0.1[c]	2.8 ± 0.1[c,d]
M–GA/MD/WPI	47.4 ± 0.6	41.8 ± 5.2	9.5 ± 0.4	6.8 ± 0.3[d]

[a] ALA % was determined just after Rancimat test of fresh samples.
[b] Rancimat test performed after 10-month storage at 5 °C in hermetic bags.
[c] Significantly different from other values in the same column ($p<0.05$).
[d] Significantly different from values of fresh samples ($p<0.05$).

Fig. 4 shows the oxidation curves obtained for microencapsulated oil samples compared to bulk linseed oil. IP values were easily determined in all cases due to a detectable increase in conductivity (Table 3). Bulk linseed oil presented an IP of 2.04 ± 0.01 h which is comparable to the value reported for sunflower oil (1.3 h) in similar conditions (Velasco et al., 2000). Just for comparison fish oil was also analyzed in the same conditions. An IP of 1.05 ± 0.05 was obtained which is well in the reported range (0.7–1.5) of fish oils at 90 °C (Méndez et al., 1996). Conductivity curves obtained for microcapsules clearly show a protective effect of the polymer matrix against linseed oil oxidation in all cases. In fact, both M–GA and M–GA/MD/WPI presented IP values which were 3.9 and 4.7 times that of bulk oil respectively (Table 3). Regarding the mixture of GA and MD (M–GA/MD), it was surprising that the

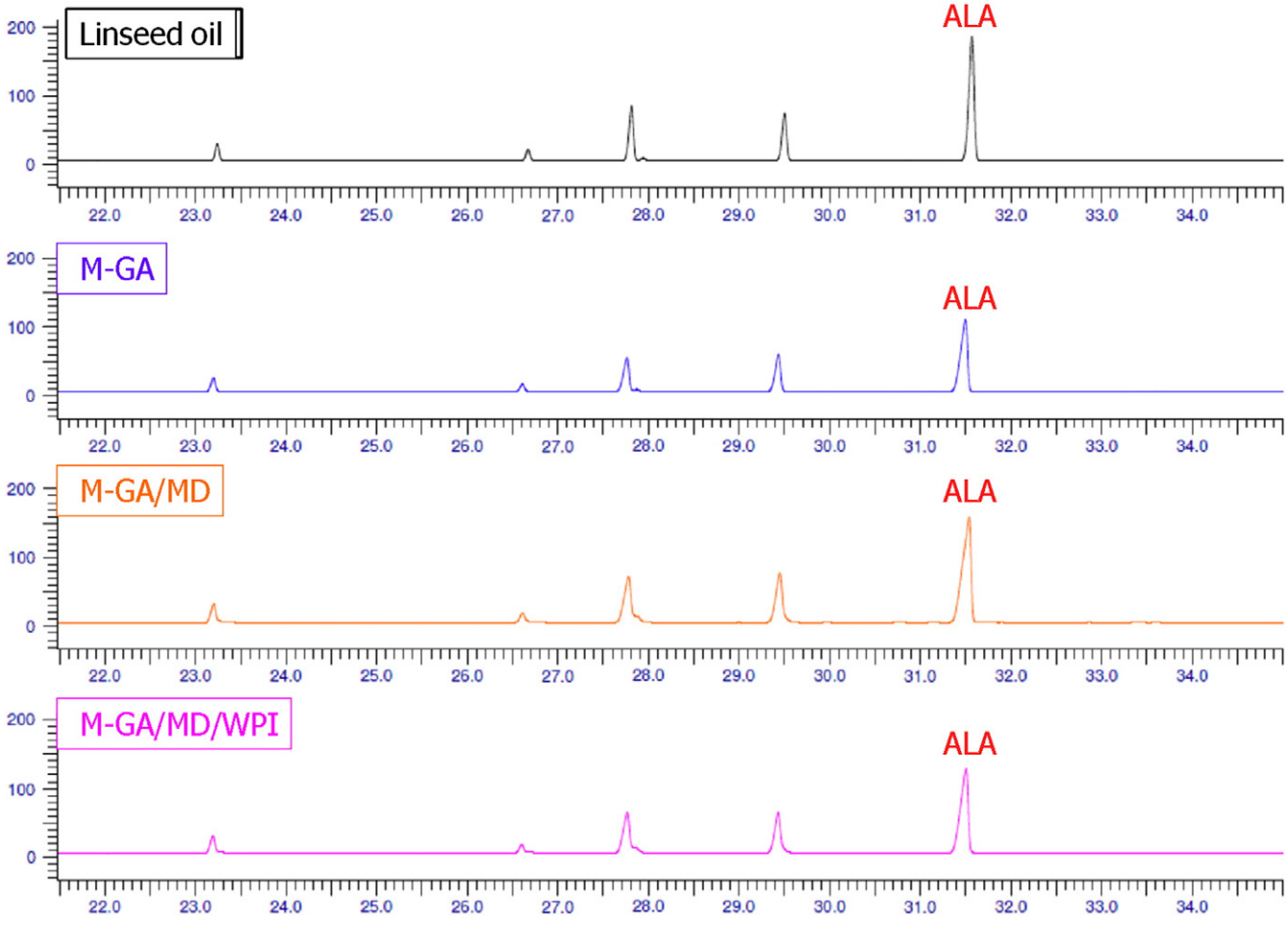

Fig. 3. Gas chromatography profiles of linseed oil extracted from microcapsules after total oil extraction. Bulk linseed oil was used as control.

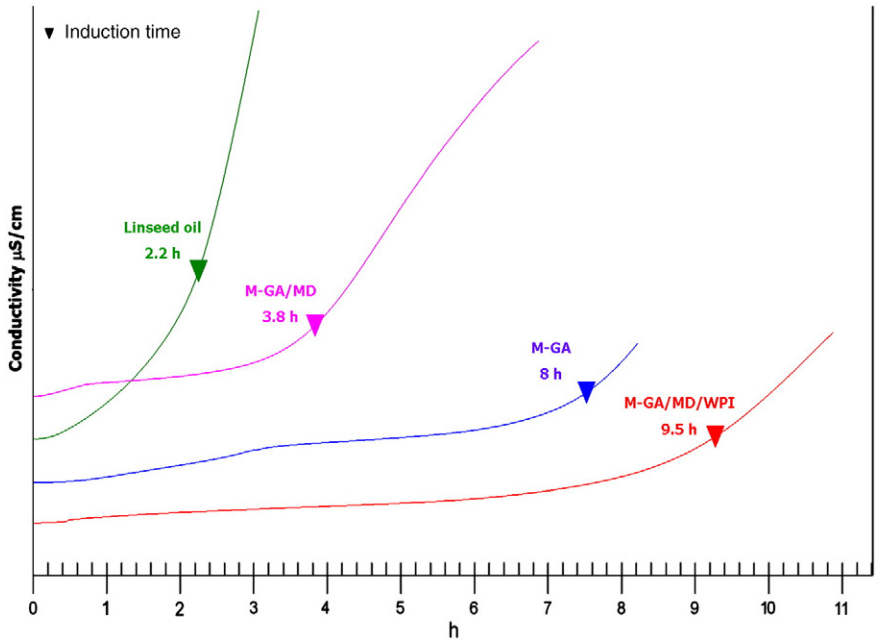

Fig. 4. Oxidation curves (Conductivity vs. time) obtained from Rancimat accelerated test of microencapsulated linseed oil. Bulk linseed oil was used as control. Inverted arrows point out the mean induction period (IP) determined for each sample.

incorporation of maltodextrin to microcapsule formulation resulted in a lower IP compared to 100% GA. However, this result is in agreement with previous works which compared GA and MD for microencapsulation of cardamom oil, an oleoresin very susceptible to oxidation (Krishnan, Kshirsagar, et al., 2005). The authors reported that microencapsulation with 100% GA offered greater protection to the oleoresin than maltodextrin or modified starch as determined during 6 weeks. Moreover, they determined that the stability of cardamom resin decreased as the percentage of GA decreases in binary mixtures with MD (Krishnan, Kshirsagar, et al., 2005). The parameter used to assess oleoresin stability ($t_{1/2}$) was reduced from 154 weeks to 99 weeks (64% reduction) when 75:25 GA/MD blend was used for encapsulation instead of 100% GA, and to 82.5 weeks for 50:50 GA/MD blend (53% reduction). In the present work, the reduction of stability due to MD incorporation in the formulation (assessed by IP value) was similar; IP decreased from 8 h to 3.8 h (48% reduction) when 100% GA was replaced by a binary blend GA/MD 56:43.

In spite of the low protection to oxidation shown by GA/MD mixture, ternary blend GA/MD/WPI had a completely different behavior. Considering the low relative GA content, the addition of WPI may be the cause of the increase in the protective effect. Indeed, WPI has been reported to possess antioxidant activity which could be beneficial to systems containing labile components as the dispersed phase (Hu et al., 2003; Kuhn & Cunha, 2012).

It is important to note that extractable oil percentage was very similar among the three formulations (around 2%) so it can be inferred that the differences in conductivity curves depend exclusively on microcapsule formulation. This finding contrasts with previous results which attributed Rancimat responses to the free oil fractions of microencapsulated powders obtained by freeze drying (Velasco et al., 2009). On the other hand, the authors could not detect well-defined IPs for encapsulated oil. In our case, IP detection was reproducible and considering our samples presented very low amounts of extractable oil (less than 0.5% w/w), we have to assume that Rancimat response comes from encapsulated linseed oil.

It has been reported that the oxygen permeability of the wall matrix is affected by the porosity of the wall and determines the oxidative stability of the core substance (Moreau & Rosemberg, 1999). Considering the similar morphology of the microcapsules, with no apparent pores, the differences observed may be due to biopolymer composition. Kagami et al. encapsulated fish oil with different blends of maltodextrins and WPI and reported that their microcapsules exhibited a molecular-sieve-type porosity that was affected by the composition. This type of morphology describes the characteristics of materials with pores small enough to resist the permeation of molecules (Keogh et al., 2001). In this work, the binary mixture GA/MD might have led to a wall matrix more permeable to oxygen than 100% GA and GA/MD/WPI.

There are two additional parameters which may affect the accessibility of oxygen to the oil, namely moisture content and glass transition temperature of biopolymers. Experiments are being performed to determine Oxidation Onset Temperature by differential scanning calorimetry (DSC) in an oxygen environment, which would resemble Rancimat test. Preliminary data have shown no endothermic transitions for the selected wall materials and their blends from 30 to 270 °C, which means that they would be in a glassy state throughout Rancimat test (data not shown). Considering this fact and the low water content in microencapsulated samples it can be inferred that the whole encapsulating system has minimized lipid oxidation by interfering with oxygen permeation. Further assays will be performed with bulk oil and microencapsulated oil formulations to evaluate if the protection of polymer matrix from oxidation can be assessed by DSC.

In order to evaluate the loss of ALA after Rancimat test, treated samples were extracted as described for total oil and analyzed by GC to register the oil fatty acid profiles. It has to be pointed out that samples were extracted only after the equipment finished all measurements, i.e. 11–12 h after, and not immediately after each sample IP. Therefore, even though they were exposed to oxidative conditions for the same time, their differential protection from oxidation evidenced by their different IP values might lead to different ALA contents. In fact, we expected a very low ALA content, particularly after M–GA/MD treatment. On the other hand, ALA contents were similar and higher than expected as shown in Table 3 (ALA % After Rancimat Test). ALA content after the oxidative accelerated treatment was 91% with respect to freshly prepared samples and 81% with respect to bulk oil, being M–GA/MD/WPI barely more affected than GA and GA/MD formulations.

Microcapsules were also analyzed by the accelerated Rancimat test 10 months after preparation (Table 3). Even though they were

kept at 5 °C in hermetic bags, the test detected the aging of the samples. In fact, IP values after 10-month storage decreased in all formulations to 71–74% of the initial IP values, evidencing a decrease in the protective effect of the microencapsulation system on linseed oil. SEM microphotographs were obtained from aged samples to evaluate any visual deterioration such as the emergence of pores, cracks or rupture of the capsules. Fig. 5a, b and c shows examples of individual microcapsules M–GA, M–GA/MD and M–GA/MD/WPI respectively after 10-month storage. After comparing several images to freshly prepared samples and contrarily to what expected, no signs of physical damage can be observed in the aged samples. These images obtained at a high magnification clearly show that the differences among formulations remain over time. Although the three of them present non-porous surfaces, M–GA are wrinkled structures (Fig. 5a), M–GA/MD microcapsules present some irregularities that appear like surface cracks (Fig. 5b), while M–GA/MD/WPI microcapsules are the most spherical and smooth (Fig. 5c). Therefore it has to be concluded that the decrease in stability to oxidation after storage inferred from lower IP values is not due to evident morphology changes. However, this cracked surface observed in M–GA/MD after 10 months (Fig. 5c) might be one of the causes of the reported IP differences. In fact, Rancimat has been included as an accelerated aging test. Even though it would not be adequate to study the morphology of microcapsules after Rancimat test, because it is a drastic treatment, it is possible that cracking does occur in this formulation upon incubation at high temperature and oxygen flow. Therefore, oxygen permeability would be increased by a change in microcapsule structure. Further assays will have to be done to determine the cause of the increased susceptibility to oxidation of M–GA/MD and the significant decrease of IP values for all formulations.

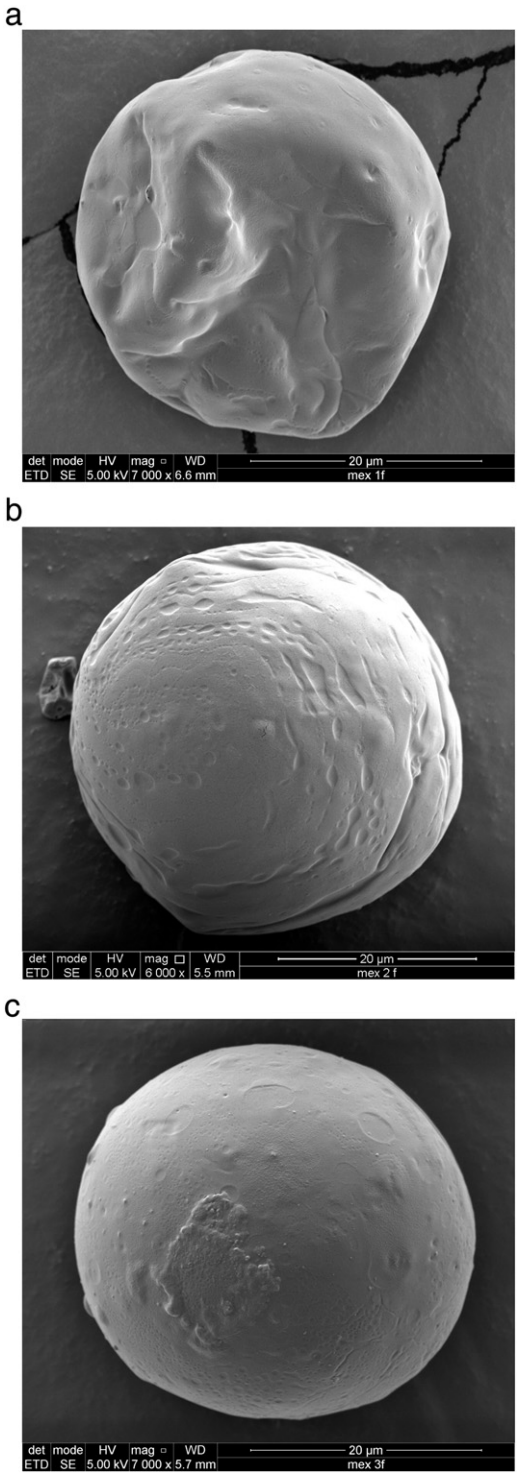

Fig. 5. SEM microphotographs of individual microencapsulated linseed oil obtained at a high magnification a) M–GA, b) M–GA/MD, c) M–GA/MD/WPI.

3.4. Bread fortification with microencapsulated linseed oil

M–GA microcapsules were first chosen for bread fortification because it is the simplest formulation with a high encapsulation efficiency and IP value, comparable to WPI containing microcapsules. The amount of microencapsulated linseed oil was calculated to fulfill with one portion (around 32 g) 40% of the recommendation of the International Society for the Study of Fatty Acids and Lipids (0.5 g ω-3/day). In fact, adding 20 g of microcapsules (4.4 g linseed oil) to 330 g of dough would represent 0.2 g ALA per portion, considering 10% ALA degradation due to microcapsule preparation. Fig. 6 is a photograph of bread containing microencapsulated linseed oil. This fortified bread resulted similar in appearance to control bread without microcapsules. However, the analysis of ALA content by oil extraction and subsequent gas chromatography evidenced a great loss of ω-3. Actually, $16.3 \pm 3.4\%$ ALA was detected in the extracted oil after bread manufacturing, which means that only 33% of ALA remained in baked bread with respect to freshly prepared microcapsules. Therefore, one portion would only contain 64 mg ALA instead of the theoretical 200 mg. It should be noticed that the oxidation of this highly unsaturated lipid might trigger the spoilage of the whole system, which could alter its organoleptic properties and even lead to toxic by-products.

This was an unexpected result because Rancimat test predicted a very high resistance to oxidation by temperature. In the case of M–GA, used for bread manufacturing, ALA content was only reduced to 81%

Fig. 6. Photograph of bred fortified with microencapsulated linseed oil M–GA.

of the initial value after 8 h (IP) at 100 °C. Consequently, this dramatic reduction in ALA content has to be attributed to other factors related to bread manufacturing conditions, such as water addition, dough kneading and incubation at 80% RH, and finally baking at 220 °C which could promote an accelerated oxidation of the unsaturated oil. Another drawback related to ALA determination in bread fortified with microcapsules was the high standard deviation of data. This may be due to inefficient blend of flour with microcapsule powder which has to be especially evaluated.

Although there is some previous research related to food fortification with microencapsulated linseed oil, the remaining ω-3 content has hardly been reported. Gökmen et al. demonstrated that the fortification of bread with nanoencapsulated flaxseed oil decreased lipid oxidation after baking compared to free oil, but only reported a decrease in hexanal and nonanal formation (Gokmen et al., 2011). It is interesting to notice that these authors included nanoencapsulated oil after dough preparation, which might help to prevent or reduce ω-3 oxidation. Rubilar et al. developed a soup fortified with linseed oil by adding 14% of dried microencapsuled oil (oil content: 14–20%) to formulation, which increased oil stability with respect to free oil (Rubilar et al., 2012). Anyway, this case involves a procedure which is not comparable to bread manufacturing as the soup is homogenized with water and boiled for 15 min upon stirring. Serna-Saldivar et al. have prepared bread fortified with commercial microencapsulated algae and fish oils and flax oil (apparently non-encapsulated). As inferred from fatty acid analysis by GC, 150 and 300 mg ALA were theoretically added per bread portion (32 g), while 93 and 172 mg respectively were determined after bread preparation (Serna-Saldivar, Zorrilla, De La Parra, Stagnitti, & Abril, 2006). This would mean a reduction of 62 and 57% of the initial value due to manufacturing, which is a better result than ours. However, it is not clear if the ω-3 source has been added to the formulation together with flour or after dough preparation, as reported by Gökmen et al.

Further assays will have to be performed to elucidate the cause for ALA loss and to test other microcapsule formulations. In the first case, it would be interesting to monitor ALA content along the process of bread manufacturing to determine which the critical step is and optimize manufacturing process and/or select the suitable wall materials combination. Secondly, M–GA/MD/WPI formulation will be used for bread fortification to evaluate if it offers an increased protection against linseed oil oxidation. Once selected a formulation which can minimize ALA loss after to bread preparation, quality assessment and sensory tests will be performed.

4. Conclusion

Microencapsulated linseed oil was obtained using different wall materials with a mean oil content of 22% w/w and similar microencapsulation efficiencies around 90%. Microcapsules presented spherical shapes and smooth, non-porous surfaces, except for those composed of MC and MD. In this case, mostly agglomerates were obtained instead of well-formed microcapsules, which is coincident with the low results for yield and EE%. The formulations composed of GA and GA/MD/WPI protected oil from oxygen and high temperature compared to bulk oil, as evidenced by a huge increase in the induction period determined by the accelerated Rancimat test. GA, GA/MD and GA/MD/WPI microcapsules presented similar surface oil contents, so it has to be assumed that Rancimat response is due to encapsulated oil. Moreover, considering the low water content in all powders and the glassy state of all wall materials, the lower protection observed for GA/MD formulation may be due to (i) an increased permeability to oxygen of that specific biopolymer combination and/or (ii) structural changes of morphology, as suggested from microphotographs of GA/MD aged samples. ALA content in linseed oil extracted from microcapsules was hardly affected by microencapsulation process. However, the addition of one of the formulations (M–GA) during bread manufacturing produced a dramatic decrease in ALA content as determined in oil extracted from fortified bread. This was an unexpected result as we had verified the stability of this formulation to the drastic oxidative conditions in Rancimat test. Further assays will be performed to evaluate the cause of ALA % reduction, including those which can determine the wall matrix resistance to kneading. Other formulations such as WPI containing microcapsules will also be tested and eventually optimized to achieve an efficient bread fortification with ω-3 from a vegetable source.

Acknowledgments

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