Microencapsulation of Fish Oil-Oregano Essential oil Blends by Spray Drying and its Oxidative Stability

A. Jeyakumari¹*, A.A. Zynudheen², P. K. Binsi², U. Parvathy¹, and C. N. Ravishankar²

ABSTRACT

Microencapsulation of fish oil-oregano essential oil blends were done by spray drying method. Sodium caseinate, bovine gelatin, gum Arabic and maltodextrin were used as wall material. Fish oil and wall material was used at the ratio of 1:2:2. In order to improve the oxidative stability of the fish oil encapsulates, oregano essential oil was added at 0.50% concentration. Physical, chemical and oxidative stability of fish oil microencapsulates were analyzed. Microcapsules had a moisture content of 2.56-4.2%. Encapsulation efficiency of microencapsulates was found to be 39.60-65.13%. Morphological characterization of microcapsules was done by Scanning Electron Microscopy (SEM) revealing spherical shape of particles with wrinkles. Oxidative stability studies revealed that encapsulates prepared by sodium caseinate and gum Arabic with oregano essential oil showed lower TBA (0.58 mg malonaldehyde kg⁻¹) value than control (9.92 mg malonaldehyde kg⁻¹). Results indicated that oregano essential oil can be used to improve the oxidative stability of fish oil microencapsulates.

Keywords: Microencapsulation, Omega 3 fatty acids, Oregano essential oil, Oxidative stability Spray drying.

INTRODUCTION

Fish oil represents a functional food ingredient due to the fact that they are excellent dietary sources of the important fatty acids especially PolyUnsaturated Fatty Acid (PUFA) like EPA (EicosaPentaenoic Acid) and DHA (DocosaHexaenoic Acid) (Kadam and Prabhasankar, 2010). Many researchers showed that supplemental fish oil may be beneficial for the healthy function of the heart, brain and nervous system (Wu et al., 2005). However, fish oil has a strong odor and unless protected it is easily oxidized. The oxidative deterioration of PolyUnsaturated Fatty Acids (PUFA) in fish oil results in a loss of nutritional value and the development of off flavours (Lee et al., 2006). The usual approaches to minimizing oxidation are the addition of antioxidants and microencapsulation (Adem et al., 2007). Microencapsulation of fish oil produces a dry powder from liquid fish oil, which enables its use for the enrichment of instant foods, formulae, bread etc., in order to deliver omega-3 fatty acids. Spray drying is commonly used in the food and pharmaceutical industries to transform liquid materials into dried powders (Bhandari et al., 2008) and it has been widely applied to prepare omega-3 PUFA microcapsules. Spray drying offers many advantages over other drying methods such as freeze drying, extrusion, complex-coacervation etc. which includes low operational cost, ability to handle heat-sensitive materials, readily available machinery and reliable operation and ability to control the mean particle size of the powders for spray dried emulsions (Adem et al., 2007). Typical wall materials for microencapsulation by spray-drying are

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low molecular weight carbohydrates like maltodextrins or saccharose, milk or soy proteins, gelatin and hydrocolloids like gum arabic or mesquite gum (Ronald and Shahidi, 2007). The physicochemical properties of spray dried powders are affected by process variables such as inlet air temperature, concentration of carrier agent, feed flow rate etc. (Chegini et al., 2008). Although encapsulation itself prevents lipid oxidation, additional stabilization with antioxidants is required to ensure maximum protection during processing and subsequent storage of microencapsulated bioactive ingredients. Essential oils are aromatic and volatile oily liquids extracted from plants or spices and they are rich in biologically active compounds such as terpenoids and phenolic acids (Burt, 2004). Essential oils (Eos) and their components are gaining interest because of their relatively safe status, their mode of acceptance by consumers, and also because many authors reported their antimicrobial, antifungal, antioxidant and radical-scavenging properties (Bakkali et al., 2008). Among the Eos, oregano essential oil extracted from *Origanum vulgare* L. have been shown to possess antioxidant, antibacterial, antifungal, diaphoretic, carminative, antispasmodic and analgesic activities (Botsoglou et al., 2003; Olmedo et al., 2013; Dambolena et al., 2010; De Falco et al., 2013). These activities are mainly due to the presence of two major phenolic compounds such as carvacrol and thymol and also the monoterpane hydrocarbons such as cymene and terpinene which is present at lower concentrations (Baydar et al., 2004). These phenolic compounds generally act as antioxidants by trapping free radicals and inhibiting the Fe$^{3+}$ induced oxidation (Geldof and Engeseth, 2002). Since essential oils are volatile in nature, they are easily evaporated or lost during processing and storage which can be reduced by encapsulation. Most of the studies reported the encapsulation of essential oil and its stability (Seyed et al., 2013; Ferrandiz et al., 2015; Ghaderi et al., 2016; Khoshtinat et al., 2017). However, there is a lack of data on the application of essential oil on the stability of oils rich in polyunsaturated fatty acids. In this background, the present study was undertaken to prepare the microparticle loaded with fish oil- oregano essential oil blends by spray drying and to evaluate its oxidative stability under accelerated conditions.

**MATERIALS AND METHODS**

**Raw Materials**

Fish oil (Seacod, Universal Medicare, Mumbai, India) was used for the preparation of emulsion and encapsulates. Sodium caseinate, bovine gelatin and maltodextrin were procured from Himedia laboratory, Mumbai, India also used as wall material for encapsulation. Oregano (*Origanum vulgare* L.) essential oil was procured from Synthite Industries Ltd., Kerala, India was used as a source of natural antioxidant.

**Microencapsulation of Fish oil**

Four different emulsions formulations were made using bovine gelatin, sodium caseniate maltodextrin, gum Arabic, fish oil and oregano essential oil for encapsulation. Fish oil and wall material was used at the ratio of 1:2:2. Compositions of the emulsion used in the present study (Table 1) were selected based on our previously reported results (Jeyakumari et al., 2014; Jeyakumari et al., 2015). After the dissolution of wall material, fish oil-oregano essential oil blends were added and further it was homogenized with a tissue homogenizer (Poly system PT 2100, Kinematica, AG) at 25,000 rpm for 5 minutes. Emulsions were allowed to stabilize at room temperature for 1 hour and then spray dried using a pilot-scale spray dryer (Hemraj Enterprises, Mumbai, India) under the following experimental conditions viz., inlet temperature 160°C; outlet temperature 80°C; spray flow feed nozzle...
Table 1. Composition of emulsion for the preparation of microencapsulates.

<table>
<thead>
<tr>
<th>Sample</th>
<th>E1</th>
<th>E2</th>
<th>E3</th>
<th>E4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fish oil (ml)</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Sodium caseinate (g)</td>
<td>-</td>
<td>20</td>
<td>-</td>
<td>20</td>
</tr>
<tr>
<td>Gum Arabic (g)</td>
<td>-</td>
<td>20</td>
<td>-</td>
<td>20</td>
</tr>
<tr>
<td>Maltodextrin (g)</td>
<td>20</td>
<td>-</td>
<td>20</td>
<td>-</td>
</tr>
<tr>
<td>Bovine gelatin (g)</td>
<td>20</td>
<td>-</td>
<td>20</td>
<td>-</td>
</tr>
<tr>
<td>Oregano essential oil (ml)</td>
<td>-</td>
<td>-</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Distilled water (ml)</td>
<td>950</td>
<td>950</td>
<td>945</td>
<td>945</td>
</tr>
</tbody>
</table>

- Fish oil+Bovine gelatin+Maltodextrin;  
- Fish oil+Sodium caseinate+Gum Arabic;  
- Fish oil+Bovine gelatin+Maltodextrin+Oregano essential oil, and  
- Fish oil+Sodium caseinate+Gum Arabic+Oregano essential oil.

diameter 3 mm; air pressure 2.5 bar. Fish oil encapsulates prepared by spray drying were used for further analysis.

A known quantity of fish oil microencapsulates were filled in airtight glass test tube and incubated at 60°C for 7 days. The autoxidation reaction in fish oil was investigated continuously for 7 days at a 24 hour interval.

**Determination of Moisture Content and Encapsulation Efficiency of Microencapsulated Fish oil**

Moisture content of fish oil encapsulates was determined by using standard method of AOAC (2005). Encapsulation Efficiency (EE) was derived from the relationship between total oil and surface oil or free oil. Total oil extraction was based on the soxhlet method (AOAC, 2005) and free oil fraction was extracted according to the method followed by Tan et al. (2005). A volume of 200 mL of n-hexane was added to 5 g of fish oil encapsulate. Then stirring was applied for 15 minutes at room temperature. After filtration through a filter paper, the solvent was evaporated in a rotary evaporator and the extracted oil was dried to constant weight by using a stream of nitrogen.

Encapsulation Efficiency (EE) = [(Total oil-Surface oil)/Total oil]×100

**Determination of Fatty acid Composition of Microencapsulated Fish oil**

Total lipid was extracted from the fish oil encapsulates by the method of Folch et al. (1957). The AOAC (2005) method was followed to esterify the lipid extract. Fatty acids were separated by using a Shimadzu QP2010 quadrupole gas chromatography-mass spectrometry instrument equipped with a Carbowax (30 m×0.25 mm ID; 0.25 μm film thickness) capillary column (Cromlab SA, Barcelona, Spain). Helium was used as the carrier gas. Injector and detector temperatures were set at 250°C. Injection was performed in split mode (1:15). The column temperature was programmed initially at 50°C for 2 minutes and then to increase at a rate of 10°C per minutes to a final temperature of 230°C. Fatty acid methyl esters were separated at constant pressure (23.1 kPa) and peaks were identified by comparison of their retention times with those of authentic fatty acid standard mixtures (Sigma Chemicals Co., Product No. 189-19, St. Louis, USA, 99% purity specific for gas chromatography) and by comparing the mass spectra with the mass spectral database. Fatty acid analyses were carried out in triplicate. The results were expressed as area percentage of fatty acid present in the sample by using retention time and area of fatty acids.
Determination of Flow Properties of Microencapsulated Fish oil

Bulk density (ρB) and Tapped (ρT) densities were determined according to the method of Chinta et al. (2009). Powder flowability was calculated from the Carr’s index and Hausner ratio according to the method followed by Turchiuli et al. (2005).

Determination of Microencapsulated Fish oil Colour

Colour of microencapsulated fish oil were measured using a Hunter-Lab scan XE–Spectrocolorimeter (Hunter Associates Laboratory, Reston, USA.) at D-65 illuminant and 10° observer. Results were expressed by CIE (Commission Internationale de L ‘Eclairage’s) colour values [L* (Lightness), a* (redness), b* (yellowness)]. The instrument was calibrated using white and black standard ceramic tiles and the readings were recorded in the inbuilt software.

Surface Morphology Analysis of Microencapsulated Fish oil by Scanning Electron Microscopy

Morphology of the fish oil encapsulates was analysed by scanning electron microscopy (Philips XL 30 SEM, Netherlands). Fish oil encapsulates were mounted on a bronze stub covered with carbon tap and were imaged at an acceleration voltage of 20kV, magnification at 2000X and pressure at 60 Pa by using a Large Field Detector (LFD).

Determination of Oxidative Stability of Microencapsulated Fish oil

Changes in lipid oxidation products of the fish oil encapsulate were determined by measuring the ThioBarbituric Acid Reactive Substance (TBARS) value according to the method described by McDonald and Hultin (1987). Two milliliters of thiobarbituric acid reagent was taken in test tube and 1 mL of the sample (5 mg of fish oil encapsulate in 1 mL of acetate buffer) was added. The blank was prepared with 1 mL of distilled water instead of the sample. Test tubes were closed and mixed thoroughly by vortexing twice and incubated in a boiling water bath (at 100°C) for 15 minutes, and then cooled by placing in a cool water bath for 10 minutes. Further, the sample mixture was centrifuged at 1,000 rpm for 15 minutes. The absorbance of the supernatant was measured at 532 nm. TBARS were expressed in mg of malonaldehyde kg⁻¹.

Statistical Analysis

The data obtained were analyzed by one-way Analysis Of Variance (ANOVA) using Statistical Package for Social Science (SPSS) software version 16.0 (SPSS Inc, Chicago, IL). All mean separations were carried out by Duncan’s multiple range test using the significance level of 95% (P< 0.05).

RESULTS AND DISCUSSION

Physical Properties of Microencapsulated Fish oil

Moisture content of the fish oil encapsulates ranged between 2.56 and 4.6%. This is a little higher than the moisture content (2.89–3.02%) reported for tuna oil powders by Klaypradit and Huang (2008). It may be due to wall material composition and encapsulation method used for the study. Klaypradit and Huang (2008) encapsulated tuna oil in whey protein combined with chitosan and maltodextrin by ultrasonic atomization. In the present study, the compositions of wall material used were bovine gelatin, sodium casienate...
maltodextrin and gum Arabic. The flowability of powder is an important property in handling and processing operations such as storage, transportation, formulation, mixing, compression or packaging (Kim et al., 2005). They are generally assessed by Carr’s index and Hausner ratio (Fitzpatrick and Ahrne, 2005). The higher Carr’s index and Hausner ratio means that the powder is more cohesive and less able to flow freely. Carr’s index and Hausner ratio of the powder is more than 25, 1.34 respectively which indicates the poor flowing characteristics of the dry powders (Turchiuli et al., 2005). In the present study, higher Carr index (26.57-36.27) and Hausner ratio (1.36-1.57) was observed for fish oil encapsulates which indicated poor flowability of the spray dried powder (Table 2). The microcapsules prepared in this study were comparable to other oil microcapsules prepared by spray drying as reported in the literature (Fuchs et al., 2006; Turchiuli et al., 2005). Sharma et al. (2012) reported that the oil content of the powder negatively influences the bulk density of the powder which reduces the flowability. Determination of free and encapsulated oil will provide the information of Encapsulation Efficiency (EE). EE is used to assess the quality of the dried microcapsules because it has an effect on oxidation sensitivity and flow powder properties (Baik et al., 2004). In the present study, EE of fish oil encapsulates ranged from 39.60 to 65.13% (Table 2). Jayakumari et al. (2015) observed an encapsulation efficiency of 46.83-49.34% for encapsulates containing milk protein, fish gelatin and ginger essential oil. Binsi et al. (2017) observed an encapsulation efficiency of 68.99-73.21% for encapsulates containing sodium caseinate, gum Arabic and sage poly phenols. The differences in EE can be attributed to different core and wall materials. In the present study, bovine gelatin, maltodextrin, sodium caseinate and gum Arabic were used as wall material. Gelatin is a water soluble material with wall forming ability in spray drying (Lee et al., 1999). Carbohydrates such as starches and maltodextrin are considered good encapsulating agents because they exhibit low viscosities at high solid content and good solubility, but these compounds have poor interfacial properties, and generally it can be used associated with proteins or gums (Hogan et al., 2001). In the present study, maltodextrin was used along with bovine gelatin and showed that EE ranged between 39.60-40.35%. Further, higher EE (65.13%) was observed for sample contained gum Arabic and sodium caseinate as wall material. It has been reported that the use of sodium caseinate as wall material was able to provide good encapsulating properties, especially when used in combination with carbohydrates (Hogan et al., 2001)

SEM analysis of fish oil encapsulates prepared by spray drying showed spherical shape and wrinkle appearance (Figure 1). It was observed that encapsulates produced by spray drying showed higher variation in sizes, indicating that during this process different sizes of droplets were formed. Kolanowski et al. (2006) observed the

<table>
<thead>
<tr>
<th>Sample</th>
<th>Moisture (%)</th>
<th>Encapsulation efficiency</th>
<th>Carr’s index</th>
<th>Hausner ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>E1</td>
<td>2.98 ±0.05</td>
<td>39.60 ±0.05</td>
<td>36.27 ±0.03</td>
<td>1.57 ±0.02</td>
</tr>
<tr>
<td>E2</td>
<td>4.6 ±0.02</td>
<td>65.13 ±10.04</td>
<td>35.40 ±0.01</td>
<td>1.54 ±0.01</td>
</tr>
<tr>
<td>E3</td>
<td>2.56 ±0.02</td>
<td>40.35 ±0.04</td>
<td>28.57 ±0.04</td>
<td>1.40 ±0.05</td>
</tr>
<tr>
<td>E4</td>
<td>3.46 ±0.03</td>
<td>60.47 ±0.08</td>
<td>26.57 ±0.05</td>
<td>1.36 ±0.03</td>
</tr>
</tbody>
</table>

*a* Fish oil+Bovine gelatin+Maltodextrin; *b* Fish oil+Sodium caseinate+Gum Arabic; *c* Fish oil+Bovine gelatin+Maltodextrin+Oregano essential oil, and *d* Fish oil+Sodium caseinate+Gum Arabic+Oregano essential oil. Results are mean±SD (n= 3), values within a column with different superscript letters are significantly (P< 0.05) different.
spherical structure of microencapsulates with different sizes by spray drying of fish oil. Kagami et al. (2003) also reported different degrees of formation of surface indentations for fish oil containing microcapsules produced from protein and dextrin wall materials.

**Fatty acid Composition of Microencapsulated Fish oil**

The fatty acid composition of cod liver oil had a higher percentage (51.80%) of total PUFAs, 29.97% Mono Unsaturated Fatty Acid (MUFA) and lower percentage (18.25%) of Saturated Fatty Acids (SAFAs). PUFAs are susceptible to oxidation, a degradative pathway that is affected by light, temperature and humidity (Wan et al., 2011). In the present study, fatty acid profile of microencapsulated fish oil showed that the sample containing oregano essential had the highest percentage of PUFA retention (45.53-45.93%) than encapsulates prepared without oregano essential oil (36.79-37.71%) (Table 3). Jeyakumari et al. (2015) reported that encapsulation of fish oil along with ginger essential oil could improve the oxidative stability of fish oil encapsulates. Klaypradit and Huang (2008) observed the protection of EPA and DHA by encapsulation process.

**Changes in Colour Value of Microencapsulated Fish oil**

Color is a very important quality factor for any product because it influences consumer acceptability. There are many reactions that take place during thermal processing and affect color. Among them, the most common are pigment degradation and browning reactions such as the Maillard reaction. Tristimulus color values of $L^*$, $a^*$ and $b^*$ were used as indices for the color changes in fish oil encapsulates during storage. It was observed that freshly encapsulated E3 and E4 were slightly yellow-greenish in colour than E1 and E2 which may due to the presence of oregano.
Table 3. Fatty acid composition (%) of microencapsulated fish oil.

<table>
<thead>
<tr>
<th>Sample/ Fatty acids</th>
<th>Cod liver oil</th>
<th>E1</th>
<th>E2</th>
<th>E3</th>
<th>E4</th>
</tr>
</thead>
<tbody>
<tr>
<td>SAFA (%)</td>
<td>18.25±0.03</td>
<td>16.72±0.02</td>
<td>16.51±0.01</td>
<td>16.09±0.03</td>
<td>16.57±0.02</td>
</tr>
<tr>
<td>MUFA (%)</td>
<td>29.97±0.02</td>
<td>46.49±0.10</td>
<td>45.78±0.20</td>
<td>37.98±0.02</td>
<td>37.90±0.09</td>
</tr>
<tr>
<td>PUFA (%)</td>
<td>51.80±0.14</td>
<td>36.79±0.05</td>
<td>37.71±0.05</td>
<td>45.93±0.05</td>
<td>45.53±0.05</td>
</tr>
</tbody>
</table>

**a** Fish oil+Bovine gelatin+Maltodextrin; **b** Fish oil+Sodium caseinate+Gum Arabic; **c** Fish oil+Bovine gelatin+Maltodextrin+Oregano essential oil; **d** Fish oil+Sodium caseinate+Gum Arabic+Oregano essential oil; **e** Saturated Fatty Acid; **f** Mono Unsaturated Fatty Acid, and **g** Poly Unsaturated Fatty Acid. Results are mean±SD (n= 3), values within a row with different superscript letters are significantly (P< 0.05) different.

Changes in Thiobarbituric Acid Reactive Substances

Lipid oxidation of microencapsulated oils is affected by microcapsule characteristics. Oxidative stability was evaluated by measuring ThioBarbituric Acid Reactive

Figure 2. Changes in $L^*$ value of microencapsulated fish oil during storage. Results are mean±SD (n= 3), values with different small, capital superscript letters are significantly (P< 0.05) different respect to between treatment, storage days, respectively.
Substances (TBARS). TBARS are secondary oxidation products formed from the breakdown of oxidized PUFAs (Shahidi and Wanasundara, 2002). TBARS assay is a useful tool in monitoring lipid peroxidation owing to its sensitivity and simplicity. Measuring secondary oxidation products is important in the determination of lipid oxidation in food products for human consumption because they are generally odor active, whereas primary oxidation products are colorless and flavorless. In the present study, initial TBARS values of the encapsulates varied from 0.30 to 0.99 mg malonaldehyde kg\(^{-1}\) and it showed an increased trend during storage. Results indicated that encapsulates prepared by sodium caseinate and gum Arabic with oregano Essential oil (E4) showed lower TBA (0.58 mg malonaldehyde kg\(^{-1}\)) value than E1 (9.92 mg malonaldehyde kg\(^{-1}\)) and E2 (6.2 mg malonaldehyde kg\(^{-1}\)) samples on the 7\(^{th}\) day. However, the levels of secondary lipid oxidation products were lower in the fish oil encapsulates prepared with addition of oregano essential oil than others (Figure 3). It might be due to the protective effect of oregano essential oil incorporated in the fish oil. Oregano essential oils are rich in polyphenols such as carvacrol and thymol. They react as effective protein cross-linkers, at the same time serve as a potent antioxidant for fish oil by acting as reducing agents or singlet oxygen scavengers. Binsi et al. (2017) found the lowest rate of increase in TBARS value in fish oil encapsulates containing sage polyphenols. Jeyakumari et al. (2014) observed lower TBARS value in fish oil encapsulates prepared with fish gelatin and 0.25% ginger essential oil under room temperature. Lauren et al. (2007) also reported that combination of tocopherol and EDTA was effective at increasing oxidative stability of spray dried multilayer emulsion. Although oil/fat is encapsulated, the oxidative stability may be due to the presence of surface oil and air inclusion which influences the shelf life of microencapsulated fish oil (Keogh et al., 2001). Ahn et al. (2008) also observed microencapsulated high oleic sunflower oil stabilized by rosemary extract. The low levels of TBARS in fish oil encapsulates prepared with 0.50% oregano essential oil samples indicated the protective effect of oregano essential oil as a natural antioxidant.

Figure 3. Changes in TBARS value of microencapsulated fish oil during storage. Results are mean±SD (n=3), values with different small, capital superscript letters are significantly (P<0.05) different respect to between treatment, storage days, respectively.
CONCLUSIONS

The present study was aimed to improve the oxidative stability of fish oil microencapsulates by incorporating polyphenol rich oregano essential oil as antioxidant. Fatty acid profiles revealed that microencapsulates containing oregano essential had the highest percentage of PUFA retention than control. Surface morphology of fish oil encapsulates prepared by spray drying had spherical and wrinkle appearance. Higher encapsulation efficiency was observed for samples containing gum arabic and sodium caseinate as wall material. Oxidative stability of the microencapsulates during accelerated storage revealed that fish oil microencapsulates prepared with a combination of sodium caseinate, gum Arabic and oregano essential oil had improved oxidative stability compared to control. Results suggest that incorporation of polyphenols rich oregano essential oil in fish oil before spray drying may be advocated for protecting highly susceptible omega-3 fatty acids from lipid oxidation.

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REFERENCES


رطوبت میکروکپسول ها 2/56 تا 3/60 درصد بود. بازده کپسول کردن میکروکپسول ها 13/65 تا 13/65 درصد بود. شناسایی خصوصیات مورفولوژیکی میکروکپسول ها که توسط میکروسکوب الکترونی (SEM) انجام شد نشان داد که شکل ذرات کروی و همراه با چین و چرک می باشد. مطالعات پایداری اکسایشی نشان داد که کپسول های تهیه شده با کازئین های سدیم و صمغ عربی و malonaldehyde/kg (TBA) اساس بیو مقدار 56/58 میلیگرم (malonaldehyde/kg) دارند. نتایج نشان داد که اساس بیوهای می توانند در برای بهبود پایداری اکسایشی میکروکپسول های روانگ ماهی استفاده شود.