Particle formation and characterization of mackerel reaction oil by gas saturated solution process

A. S. M. Tanbirul Haque¹ · Byung-Soo Chun¹

Revised: 30 July 2015 / Accepted: 18 August 2015 / Published online: 28 August 2015
© Association of Food Scientists & Technologists (India) 2015

Abstract Most of the health benefits of fish oil can be attributed to the presence of omega-3 fatty acids like Docosahexenoic acid (DHA) and Eicosapentaenoic acid (EPA). There are few dietary sources of EPA and DHA other than oily fish. EPA and DHA have great potential effect on human health. In this research, Supercritical carbon dioxide (scCO₂) extracted mackerel oil was reacted by enzyme at different systems to improve the EPA and DHA. Different types of immobilize enzyme TL-IM, RM-IM, Novozyme 435 were assessed for improving PUFAs. Best result was found at non-pressurized system using TL-IM. Reacted oil particle were obtained with polyethylene glycol by gas saturated solution process (PGSS). Different parameters like temperature, pressure, agitation speed and nozzle size effect on particle formulation were observed. SEM and PSA analysis showed, small size non spherical particles were obtained. It was found that after particle formation poly unsaturated fatty acids (PUFAs) were present in particle as same in oil. PUFAs release from particle was almost linear against constant time duration. Oil quality in particle not change significantly, in this contrast this

study will be helpful for food and pharmaceutical industry to provide high EPA and DHA containing powder.

Keywords Supercritical CO₂ · Omega-3 fatty acids · Immobilize enzyme · Particle · Control release

Introduction

Fish oil is recommended as part of healthy diet because it contains ω -3 polyunsaturated fatty acids (PUFAs), such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). The beneficial health effects of ω -3 PUFAs are well defined: they are essential for the normal growth and development of the brain and nervous system and are also thought to exert beneficial effects during the treatment of coronary artery disease, hypertension, arthritis, clinical depression, anxiety, inflammatory and autoimmune disorders and cancer (Cao and Hur 2005; Correa et al. 2008; Jeong et al. 2006; Lee et al. 2006; Su et al. 2003; Naliwaiko et al. 2004; Green et al. 2006; Yehuda et al. 2005; Nemets et al. 2002).

Mackerel belongs to the family *Scombridae*, and is abundant in cold and temperate shelf areas. It is a fatty species and this fat is well-distributed throughout the body (Osman et al. 2001). Mackerel also contains significant amounts of protein, essential amino acid, lipid and many other biologically active compounds.

Alcoholysis can enhance the value and the applicability of oils. Low-value oils and fats can be converted to biodiesel, trans-free fats, medium chain-length triglycerides and ω -3 fatty acids (PUFAs) rich fish oils by several methods. As an alternative of conventional method, lipases have been used as biocatalysts for alcoholysis (Gunnlaugsdottir et al. 1998; Lozano et al., 2004. Enzymatic catalysis by lipase allows modifying the properties of lipids by altering the locations of

Research Highlights

- Supercritical CO₂ extracted mackerel oil was used
- 3 different immobilized lipase enzymes were used to improve polyunsaturated fatty acid.
- Gas saturated solution (PGSS) process was used for particle formation
- Different parameters were observed for optimization in PGSS process
- Control release were observed of PUFAs from particle

✉ Byung-Soo Chun
bschun@pknu.ac.kr

¹ Department of Food Science and Technology, Pukyong National University, 45 Yongso-ro, Nam-Gu, Busan 608-737, Republic of Korea

fatty acids in glycerides or replacing one or more of the fatty acids with new one. Regiospecific lipases can be used to concentrate PUFAs in fish oil. That is because 1,3 regiospecific lipases are more likely to react with fatty acids on the sn-1 and sn-3 positions, and PUFAs usually exist on the sn-2 position of glyceride structure (Yamaguchi et al., 2004). This enables to make monoglycerides and diglycerides containing higher amounts of PUFAs.

Particles from gas saturated solutions (PGSS) process is a successful encapsulation technique based on the high solubility of supercritical carbon dioxide in molten biopolymers, oils and fats (Martín et al. 2010). The use of supercritical fluids such as supercritical carbon dioxide (scCO₂) has provided a clean and effective alternative than traditional methods of drug and polymer processing. In particular, scCO₂ has a number of unique properties those make it possible to process both bioactive molecules and amorphous polymers without using toxic organic solvents or elevated temperatures.

The formulation of natural substances together with a biocompatible or biodegradable carrier material to form composites or encapsulates has a great relevance for pharmaceutical, cosmetic and food industries (Cocero et al. 2009). Natural substances such as carotenoids, fatty acids, natural antioxidants are being extensively used on a great variety of food products (Budavari 1989). A very wide range of clinically approved pharmaceutical products take advantage of biodegradable polymers to control the rate of drug release within the body (Tracy 1998; Okada 1997). In this context, the aim of this study was to evaluate the improving of polyunsaturated fatty acid by enzymatic alcoholysis of mackerel oil and characterized of produced particle by PGSS process.

Materials and methods

Materials

Mackerel was collected from Busan Cooperative Fish Market (Seo-gu, Busan, South Korea). The muscle was separated by mechanically and washed thoroughly with cold distilled water in the laboratory. Pure carbon dioxide (99.99 %) was supplied by KOSEM (Sangbuk-myeon Yangsan, South Korea). All other chemicals used in this study were of analytical or HPLC grade.

Methods

Sample preparation

The mackerel muscle was dried in a freeze-dryer (EYELA FDV-2100, Rikakikai Co. Ltd., Tokyo, Japan) for about 72 h. The dried samples were crushed by a mechanical blender (PN, SMKA-4000, Ansan, Korea) and sieved through a sieve

(710 µm mesh size). These samples called freeze dried mackerel muscle were stored at −20 °C.

Extraction by scCO₂

A laboratory scale setup of supercritical fluid extraction (SFE) process was used. Exactly 20 g of freeze dried raw mackerel muscle was loaded into the stainless steel extraction vessel which was 200 mL in volume. A thin layer of cotton was placed at the bottom of the extraction vessel. Before plugging with cap another layer of cotton was used at the top of the sample. CO₂ was pumped at constant pressure into the extraction vessel by high pressure pump (Milroyal, Milton Roy, USA) up to the desired pressure. A back pressure regulator was used to control the pressure of CO₂. The extraction temperature was maintained by connecting the extraction vessel with water bath (MAT'L: SUS 304, Ilshin Autoclave Co. Ltd., Daejeon, Korea). Flow rates and accumulated gas volume passing through the apparatus were measured using a gas flow meter (Shinagawa, Tokyo, Japan). After scCO₂ extraction, the mackerel muscle residues remaining in the vessel and oil was stored at −20 °C until further use and analysis. Mackerel muscle was extracted at temperature 45 °C and pressure 20 MPa for 2 h. The flow rates of CO₂ were kept constant at 27 g/min.

Enzymatic reaction

Pressurized system using scCO₂

In pressurized system for enzymatic reaction, temperature 40 °C and pressure 10 MPa were used. Reaction time was 2 h and agitation speed was 300 rpm. scCO₂ was used as an enhancer. Commercial immobilized lipases from *Thermomyces lanuginosa* (Lipozyme TL-IM), *Rhizomucor miehei* (Lipozyme RM-IM) and *Candida Antarctica* (Novozyme 435) obtained from Novozymes (Bagsvared, Denmark) were used. 20 mL of mackerel oil was placed in reaction vessel which containing 180 mL of ethanol and 5 % of water. The reaction was started by the addition of 1 g of immobilized lipase.

A laboratory scale of scCO₂ system was used for ethanolysis. The stainless steel reaction vessel which was 300 mL in internal volume (155 mm × 50 mm) consists of a stainless (SS-316) spin bar stirrer and water jacket. Samples (extracted by scCO₂) were loaded in to the stainless steel reaction vessel with ethanol and enzyme. After loading, reactor was closed with reactor's head and increased the pressure with pump. The reaction temperature was maintained by connecting the reaction vessel with water bath and temperature was measured using Fluke 52 Series II Handheld Thermometers (FLUKE, USA) continuously. Finally after finishing reaction the reactor was depressurized to atmospheric level and collects the sample.

Non-pressurized system

In the non-pressurized system alcoholysis reactions catalyzed by enzyme, typical reaction mixture consisted of 0.75 g oil, 3 g ethanol (95 %) and 0.375 g of immobilized lipase. This mixture was placed in 50 mL Erlenmeyer flask with silicon capped under inert atmosphere. The mixture was incubated at 35 °C and agitated in an orbital shaker at 300 rpm. Reaction time was 2 h.

Particle formation using PGSS process

The experiments were carried out using polymer PEG (polyethylene glycol) 8000 (g/mol) and mackerel reaction oil. PGSS experiment began by delivering scCO₂ to the precipitation chamber until the desired pressure was reached. PEG 8000 and mackerel reaction oil (5:1) in the reactor were melted using scCO₂ and mixed by a stirring wheel. During experiments, temperatures were 45 °C and 55 °C; pressures ranging from 10 to 20 MPa. In previous study shows that high solubility of CO₂ in PEG 8000 was found at range 42.85 to 99.85 °C and pressure range 2 to 30 MPa (Nalawade et al. 2006). The mixture was stirred at 200 to 400 rpm and the nozzle size was 200 and 300 µm. The duration of reactions was 1 h. When the reaction was completed, PEG conjugated materials were delivered through the nozzle and collected from a separator.

Particle characterization

Fatty acid compositions determination

Gas chromatography (GC) analysis was performed to determine the fatty acid compositions of reaction oil and after making particle. The GC–MS analysis was performed using a 6890 Agilent (Agilent Technologies, Wilmington, USA) gas chromatograph with a Fused silica capillary column (100 m length × 0.25 mm internal diameter, 0.2 µm of film) (Supelco, Bellefonte, USA). The fatty acid methyl esters were prepared firstly according to official method and recommended practices of the AOCS (AOCS 1998). Nitrogen was used as a carrier gas (1.0 mL/min). The oven temperature was programmed starting at a constant temperature of 130 °C for 3 min, and then increased to 240 °C at a rate of 4 °C/min and hold at 240 °C for 10 min. Injector and detector temperatures were both 250 °C. Fatty acid methyl esters were identified by comparison of retention time with standard fatty acid methyl esters mixture (Supelco, Bellefonte, Pa., U.S.A.).

Measurement of oil stability in particle

Several parameters may use to determine the deterioration of oil. In this study, oil and after making particle deterioration was monitored by acid value (AV), peroxide value (POV) and

free fatty acid (FFA) content test. AV and FFA value were calculated to determine the acidity of oil. POV was calculated to determine the rancidity which is result of autoxidation.

Acid value (AV)

The acid value (AV) was assessed according to the method described previously by Ping et al. (2008). 1 g of sample was dissolved in 100 mL of ether:ethanol (1:1, v/v) by shaking, after which phenolphthalein, as an indicator was added by drops. AV of oil was analyzed by titration with a 0.1 N KOH–ethanol solutions until the pink color persisted for at least 30 s, it was calculated using the following equation:

$$\text{Acid value (AV)} = 56.11 \times A \times F/S$$

Where, *A* is volume of the KOH-ethanol solution of the titration (mL), *F* is concentration of the KOH-ethanol factor, *S* is mass of oil (g), and 56.11 is molecular weight of KOH in mg.

Peroxide value (POV)

The peroxide value (POV) was determined according to the AOCS method Cd 8–53 (AOCS 1998) using modified amount of sample. 1 g of sample was dissolved in 6 mL of a 3:2 acetic acid:chloroform solution. Then, 0.1 mL of saturated potassium iodine (KI) solution was added to the mixture and allowed to stand with occasional shaking for 1 min. Distilled water (6 mL) was immediately added to the solution allowed to stand. The solution was titrated with 0.1 N of sodium thiosulfate until the yellow iodine color almost disappeared. Next, 0.4 mL of a starch indicator solution was added by shaking to extract iodine from chloroform layer, and again titrated until the blue color disappeared. A blank determination was performed with the same procedure. POVs were expressed as milliequivalents of a peroxide/1000 g sample:

$$\text{Peroxide value (POV)} = \frac{(A-B) \times N \times 1000}{W}$$

Where, *A* is volume of titrant of sample (mL), *B* is volume of titrant of blank (mL), *N* is normality of sodium thiosulfate solution, *W* is mass of sample (g).

Free fatty acid (FFA) content

FFA content was analyzed by the method of Bernardez and coworkers (Bernardez et al. 2005). Briefly, 50 mg of sample was placed into Pyrex tubes with the addition of 3 mL of cyclohexane, and then, 1 mL of cupric acetate-pyridine reagent was added. Tubes were vortexed for 30 s. After centrifugation at 2000 × g for 10 min, the upper layer was read at

710 nm. The FFA content was measured on a calibration curve constructed using oleic acid as the standard.

Solubility and PUFAs release assessment

For solubility of produced particle was determined by dissolving 0.125 g of powder in 50 mL of different solvent. For stirring magnetic stirrer was used and fixed temperature 37 °C was used. Different time interval sample was collected and check the solubility percentage, then filtered the sample to eliminate the solids, obtained solution was analyzed by gas chromatography in order to determinate the fatty acid composition encapsulated mackerel reacted oil.

Analysis of particle by scanning electron microscope analysis (SEM)

SEM is a type of electron microscope that images a sample by scanning it with a high-energy beam of **electrons** in a **raster scan** pattern. Samples of the reaction oil with PEG on the metallic frit were observed by a scanning electron microscope (S-2400, Hitachi, USA). The SEM samples were covered with gold using a sputter coater. Particles sizes were measured from SEM images using the Sigma Scan Pro image analysis software.

Analysis of particle size by particle size analyzer (PSA)

The size distributions of the PEG and reaction oil with PEG powder were measured by particle size analyzer (LS 13320, Beckman Coulter, USA). The result from the analysis is the relative distribution of volume of particles in the range of size classes. From this basic result, the data on particle size distributions are calculated. The frequency curve is useful for displaying the results to show the peaks in the graph. The peak of the frequency curve gives the modal diameter, the most commonly occurring particle diameter.

Result and discussion

Fatty acid composition

Sn-1,3 specific lipase from different source were used for ethanolsis of mackerel oil in order to concentrate omega-3 PUFA. It was found that after enzymatic reaction PUFAs were increased. Mainly DHA was more concentrate than EPA (Table 1). Maximum DHA is located at sn-2 position, This indicates that concentration of DHA at the sn-2 position of acylglycerols can be achieved using 1,3 specific lipases, complementing the concentration that was achieved due to the acyl-specificity of lipases by fatty acids other than DHA (Martín Valverde et al. 2012). The result showed that TL-IM

had highest activities in non-pressurized system; there PUFAs contained highest amount of EPA (8.27 ± 0.12 %) and DHA (22.93 ± 0.13 %). The hydrophilic characteristics of carbon dioxide over wide pressure ranges may affect negatively on the activity of the enzyme (Knez and Habulin, 2002; Nakaya et al. 2001). On the other hand in pressurized system Novozyme 435 shows highest activities, there EPA (3.28 ± 0.01 %), DHA (27.83 ± 0.11 %) respectively. Many reactions can be conducted in liquid or supercritical carbon dioxide and, in some cases, rates and selectivity achieved are greater than those obtained in normal liquid or gas phase reactions (Oliveira and Oliveira, 2000; Savage et al. 1995). It was found that different enzyme shows different activities at different system. Enzyme stability and activity may depend on the enzyme species, characteristics of compressed fluid, water content of the enzyme/support/reaction mixture and process variables manipulated (Oliveira et al. 2006). For particle formation, non-pressurized system TL-IM reacted oil was used. After generating the particles of reacted oil with PEG at different temperatures and pressures of ScCO_2 , the concentration of EPA and DHA not changed significantly. But little increase was observed of EPA concentration at all condition particles (Table 2), it's may be happened due to some saturated fatty acid are breakdown at experimental condition. Highest EPA was found 11.17 ± 0.07 % at 45 °C/15 MPa/200 μm and highest DHA was found in particle at 55 °C/15 MPa/200 μm , it was 24.66 ± 0.12 % (Table 2). Best result was found in 15 MPa at all condition, other parameter like temperature nozzle size was not strongly affected on EPA and DHA.

It was observed that palmitic acid (C16:0) also had considerable amount in reacted oil, highest amount was found using TL-IM at both pressurized and non-pressurized system, it was 31.95 ± 0.18 % and 21.61 ± 0.19 % respectively (Table 1). After particle formation palmitic acid was slightly decrease in all condition. In particle among the monounsaturated fatty acid, oleic acid (C18:1n7c) also found in substantial condition (Table 2) accounting for 15.28 ± 0.18 % to 20.83 ± 0.15 %.

Some fatty acid like stearic acid (C18:0), *cis*-11-eicosenoic acid (C20:1), *cis*-13, 16 docosdianoic acid (C22:2), nervonic acid (C24:1) almost disappear in particle at all condition (Table 2). Due to low oxidative stability of unsaturated fatty acid and presence of low amounts in the oil during particle formation these fatty acid were oxidize (Lanna et al. 2005).

The fatty acid compositions were changed moderately at different agitation speed (Table 3). Highest concentration of EPA was found at 400 rpm agitation speed it was 11.16 ± 0.12 % and highest DHA was found at 300 rpm, it was 24.66 ± 0.12 %. But combinedly EPA and DHA were comparatively high at 300 rpm. Mixing is an energy demanding process, poor mixing can lead to mass and/or heat transfer problems as well as inhomogeneous distribution, both which can cause possible yield reduction (Kadic et al. 2014).

Table 1 Fatty acid compositions of mackerel oil after enzymatic reaction using different enzyme at non pressurized and pressurized system

Fatty acid composition	Original oil	Amount of fatty acid (%) after Enzymatic reaction					
		Non pressurized system			Pressurized system		
		TL-IM	RM-IM	Novozyme 435	TL-IM	RM-IM	Novozyme 435
C14:0	6.31 ± 0.12 ^a	7.36 ± 0.11 ^b	7.57 ± 0.13 ^b	7.07 ± 0.09 ^b	8.57 ± 0.10 ^c	5.72 ± 0.11 ^d	5.85 ± 0.08 ^d
C16:0	23.99 ± 0.24 ^c	21.61 ± 0.19 ^a	27.10 ± 0.21 ^d	27.97 ± 0.23 ^d	31.95 ± 0.18 ^c	22.96 ± 0.19 ^b	21.45 ± 0.22 ^a
C16:1	4.46 ± 0.12 ^c	2.53 ± 0.12 ^c	1.50 ± 0.10 ^b	0.61 ± 0.13 ^a	0.86 ± 0.11 ^a	4.70 ± 0.12 ^c	2.17 ± 0.9 ^c
C18:0	5.71 ± 0.14 ^c	2.77 ± 0.11 ^b	1.06 ± 0.12 ^a	5.64 ± 0.14 ^c	0.70 ± 0.16 ^a	10.18 ± 0.14 ^d	2.32 ± 0.11 ^b
C18:1n9t	4.68 ± 0.12 ^c	3.97 ± 0.09 ^d	0.95 ± 0.11 ^a	2.4 ± 0.11 ^c	3.69 ± 0.13 ^d	1.41 ± 0.10 ^b	1.80 ± 0.12 ^b
C18:1n9c	18.1 ± 0.19 ^c	13.65 ± 0.17 ^a	18.75 ± 0.14 ^c	17.36 ± 0.19 ^c	19.79 ± 0.17 ^d	15.48 ± 0.14 ^b	15.11 ± 0.11 ^b
C18:2n6c	2.62 ± 0.07 ^b	2.70 ± 0.04 ^b	2.45 ± 0.06 ^b	2.93 ± 0.07 ^b	0.44 ± 0.05 ^a	2.22 ± 0.07 ^b	2.11 ± 0.06 ^b
C20:1	3.17 ± 0.10 ^b	1.83 ± 0.08 ^a	5.75 ± 0.05 ^d	3.35 ± 0.09 ^b	2.95 ± 0.09 ^b	3.16 ± 0.04 ^b	4.63 ± 0.08 ^c
C20:2	3.33 ± 0.12 ^c	2.78 ± 0.09 ^c	2.18 ± 0.11 ^b	2.66 ± 0.08 ^c	1.70 ± 0.12 ^a	1.71 ± 0.11 ^a	2.14 ± 0.07 ^b
C20:3n6	2.68 ± 0.08 ^c	1.96 ± 0.06 ^b	1.16 ± 0.09 ^b	0.96 ± 0.03 ^a	1.93 ± 0.06 ^b	1.60 ± 0.04 ^b	0.82 ± 0.02 ^a
C20:3n3	0.16 ± 0.01 ^a	2.94 ± 0.04 ^c	0.44 ± 0.02 ^a	4.40 ± 0.01 ^d	1.13 ± 0.05 ^b	0.76 ± 0.02 ^a	1.00 ± 0.06 ^b
C23:0	1.2 ± 0.05 ^b	1.76 ± 0.03 ^b	0.84 ± 0.06 ^a	4.02 ± 0.02 ^d	8.20 ± 0.06 ^c	2.70 ± 0.04 ^c	3.23 ± 0.07 ^c
C22:2	1.73 ± 0.04 ^b	1.92 ± 0.02 ^b	0.90 ± 0.07 ^a	0.75 ± 0.06 ^a	0.94 ± 0.02 ^a	1.19 ± 0.03 ^b	4.72 ± 0.08 ^c
C20:5n3(EPA)	7.39 ± 0.14 ^d	8.27 ± 0.12 ^d	6.52 ± 0.14 ^c	5.97 ± 0.11 ^c	0.43 ± 0.15 ^a	2.05 ± 0.14 ^b	1.54 ± 0.17 ^b
C24:1	0.73 ± 0.01 ^a	1.03 ± 0.05 ^b	0.87 ± 0.09 ^a	0.62 ± 0.11 ^a	0.44 ± 0.07 ^a	1.09 ± 0.02 ^b	3.28 ± 0.01 ^c
C22:6 (DHA)	13.66 ± 0.17 ^a	22.93 ± 0.13 ^c	21.95 ± 0.11 ^c	13.25 ± 0.17 ^a	16.27 ± 0.14 ^b	23.07 ± 0.13 ^c	27.83 ± 0.11 ^d
EPA+ DHA	21.05	31.20	28.47	19.22	16.70	25.12	29.37

Mean ± SD ($n = 3$) different lowercase letters in each row indicate significant differences ($P < 0.05$)

Oil stability in particle

Marine fish oil contains high levels of PUFAs. The quality of oil deteriorates at different rates depending on the production and storage conditions (Kamal-Eldin and Yanishlieva 2002). Evaluation of oxidative stability of the reacted oil and oil in particle were given in Table 4. The AV was calculated to determine the acidity of oil and a low AV is indicative of a high oxidative stability (Essien et al. 2012). The AV value of reacted oil was found 20.41 ± 0.17 mg KOH/g (Table 4). After particle formation AV showed variation, value was increased with increasing temperature (Table 4) and on the other hand when the pressure was increased AV value was slightly decreased. Highest value was found in $55^\circ\text{C}/10\text{ MPa}/200\text{ }\mu\text{m}$ condition, it was 27.53 ± 0.21 mg KOH/g and lowest was found in $45^\circ\text{C}/10\text{ MPa}/200\text{ }\mu\text{m}$ condition, it was 20.46 ± 0.15 mg KOH/g (Table 4). Nozzle size and agitation speed not showed significant effect.

The POV is a measurement of rancidity of an oil or fat which is result of autoxidation. In this study POV of reacted oil was found 7.15 ± 0.07 meq/1000 g (Table 4). After particle formation POV value was not change significantly in compare to the reacted oil. But like acid value tendency was observed when temperature was increased POV value was slightly increased and with increasing pressure it showed adverse

tendency, highest and lowest value of POV in particle were 9.67 ± 0.17 meq/1000 g at $55^\circ\text{C}/10\text{ MPa}/300\text{ }\mu\text{m}$ condition respectively (Table 4). It may be accrued; at under high pressure CO_2 become more liquid and this dense CO_2 can help to prevent oxidation.

FFAs are directly responsible for the acidity of oil. Changes of FFA content are mainly related to hydrolytic reactions in the oil. FFA acid content in reacted oil particle was not found significant changed compare to reacted oil, highest value was found 4.25 ± 0.06 g/100 g at $55^\circ\text{C}/20\text{ MPa}/300\text{ }\mu\text{m}$ condition and in contrast lowest value was found 2.38 ± 0.07 g/100 g at $55^\circ\text{C}/20\text{ MPa}/300\text{ }\mu\text{m}$ condition (Table 4). It likely that mackerel reacted oil particle using PGSS process occurred less oxidation because oxygen could not penetrate into the vessel due to close chamber of whole operation period.

Solubility and PUFAs release from particle

Solubility of particles at different solvents was observed. Significant variation had been found against different solvent. Of this contrast in ethanol take 45 min to dissolve 96.8 % and in methanol it takes 3 min to dissolve 98.7 % (Fig. 1). The amount of EPA, DHA had been constantly increased with the time when the particle was dissolved in different solvent

Table 2 Fatty acid compositions of mackerel reacted oil particle

Fatty Acid composition	Reacted oil	200 μ m				55°C			
		45°C				55°C			
		10 MPa	15 MPa	20 MPa	15 MPa	10 MPa	20 MPa	15 MPa	15 MPa
C14:0	7.36 $\pm$ 0.11 ^c	5.75 $\pm$ 0.09 ^a	7.18 $\pm$ 0.07 ^c	8.31 $\pm$ 0.11 ^c	7.10 $\pm$ 0.12 ^c	7.10 $\pm$ 0.12 ^c	8.31 $\pm$ 0.11 ^c	7.10 $\pm$ 0.12 ^c	8.43 $\pm$ 0.08 ^c
C16:0	21.61 $\pm$ 0.19 ^f	16.47 $\pm$ 0.16 ^e	12.55 $\pm$ 0.12 ^b	17.35 $\pm$ 0.19 ^e	13.50 $\pm$ 0.11 ^c	13.50 $\pm$ 0.11 ^c	17.35 $\pm$ 0.19 ^e	11.21 $\pm$ 0.12 ^a	11.21 $\pm$ 0.12 ^a
C16:1	2.53 $\pm$ 0.12 ^b	ND	ND	ND	ND	1.83 $\pm$ 0.11 ^a	ND	1.83 $\pm$ 0.11 ^a	1.71 $\pm$ 0.16 ^a
C18:0	2.77 $\pm$ 0.11 ^a	ND	ND	ND	ND	ND	ND	ND	ND
C18:1n9t	3.97 $\pm$ 0.09 ^a	5.12 $\pm$ 0.05 ^c	5.57 $\pm$ 0.07 ^c	5.60 $\pm$ 0.09 ^c	5.93 $\pm$ 0.11 ^d	5.93 $\pm$ 0.11 ^d	5.60 $\pm$ 0.09 ^c	5.93 $\pm$ 0.11 ^d	5.39 $\pm$ 0.04 ^c
C18:1n9c	13.65 $\pm$ 0.17 ^a	15.28 $\pm$ 0.18 ^b	17.81 $\pm$ 0.15 ^d	18.04 $\pm$ 0.12 ^d	19.30 $\pm$ 0.17 ^d	19.30 $\pm$ 0.17 ^d	18.04 $\pm$ 0.12 ^d	19.30 $\pm$ 0.17 ^d	17.03 $\pm$ 0.12 ^c
C18:2n6c	2.70 $\pm$ 0.04 ^b	3.78 $\pm$ 0.07 ^c	4.05 $\pm$ 0.08 ^c	3.91 $\pm$ 0.02 ^c	4.14 $\pm$ 0.06 ^c	4.14 $\pm$ 0.06 ^c	3.91 $\pm$ 0.02 ^c	4.14 $\pm$ 0.06 ^c	3.86 $\pm$ 0.11 ^c
C20:1	1.83 $\pm$ 0.08 ^a	ND	ND	ND	ND	ND	ND	ND	ND
C20:2	2.78 $\pm$ 0.09 ^a	3.41 $\pm$ 0.11 ^b	3.55 $\pm$ 0.07 ^b	3.24 $\pm$ 0.12 ^b	3.45 $\pm$ 0.09 ^b	3.45 $\pm$ 0.09 ^b	3.24 $\pm$ 0.12 ^b	3.45 $\pm$ 0.09 ^b	3.31 $\pm$ 0.07 ^b
C20:3n6	1.96 $\pm$ 0.06 ^b	2.04 $\pm$ 0.05 ^b	1.72 $\pm$ 0.08 ^b	1.57 $\pm$ 0.05 ^a	1.63 $\pm$ 0.06 ^a	1.63 $\pm$ 0.06 ^a	1.57 $\pm$ 0.05 ^a	1.63 $\pm$ 0.06 ^a	1.93 $\pm$ 0.12 ^b
C20:3n3	2.94 $\pm$ 0.04 ^a	9.59 $\pm$ 0.02 ^c	8.15 $\pm$ 0.07 ^d	7.17 $\pm$ 0.08 ^b	7.53 $\pm$ 0.02 ^c	7.53 $\pm$ 0.02 ^c	7.17 $\pm$ 0.08 ^b	7.53 $\pm$ 0.02 ^c	8.30 $\pm$ 0.07 ^d
C23:0	1.76 $\pm$ 0.03 ^a	2.55 $\pm$ 0.07 ^b	3.21 $\pm$ 0.04 ^b	3.11 $\pm$ 0.09 ^b	3.63 $\pm$ 0.03 ^c	3.63 $\pm$ 0.03 ^c	3.11 $\pm$ 0.09 ^b	3.63 $\pm$ 0.03 ^c	3.31 $\pm$ 0.02 ^b
C22:2	1.92 $\pm$ 0.02 ^b	ND	ND	ND	ND	ND	ND	ND	ND
C20:5(EPA)	8.27 $\pm$ 0.12 ^a	10.51 $\pm$ 0.11 ^c	11.17 $\pm$ 0.07 ^d	9.83 $\pm$ 0.09 ^b	10.35 $\pm$ 0.13 ^c	10.35 $\pm$ 0.13 ^c	9.83 $\pm$ 0.09 ^b	10.35 $\pm$ 0.13 ^c	10.84 $\pm$ 0.16 ^d
C24:1	1.03 $\pm$ 0.05 ^a	ND	ND	ND	ND	ND	ND	ND	ND
C22:6 (DHA)	22.93 $\pm$ 0.13 ^c	23.49 $\pm$ 0.09 ^c	23.03 $\pm$ 0.14 ^c	21.88 $\pm$ 0.18 ^b	21.63 $\pm$ 0.15 ^b	21.63 $\pm$ 0.15 ^b	21.88 $\pm$ 0.18 ^b	21.63 $\pm$ 0.15 ^b	24.66 $\pm$ 0.12 ^d

Fatty Acid composition	300 μ m				55°C			
	45°C				55°C			
	10 MPa	15 MPa	20 MPa	15 MPa	10 MPa	15 MPa	20 MPa	20 MPa
C14:0	5.75 $\pm$ 0.09 ^a	8.81 $\pm$ 0.15 ^e	7.67 $\pm$ 0.13 ^d	7.66 $\pm$ 0.11 ^d	7.67 $\pm$ 0.13 ^d	7.66 $\pm$ 0.11 ^d	5.23 $\pm$ 0.15 ^a	5.23 $\pm$ 0.15 ^a
C16:0	16.47 $\pm$ 0.16 ^e	17.16 $\pm$ 0.18 ^e	12.68 $\pm$ 0.19 ^b	11.49 $\pm$ 0.21 ^a	12.68 $\pm$ 0.19 ^b	11.49 $\pm$ 0.21 ^a	12.63 $\pm$ 0.12 ^b	12.63 $\pm$ 0.12 ^b
C16:1	ND	ND	1.83 $\pm$ 0.13 ^a	1.73 $\pm$ 0.16 ^a	1.83 $\pm$ 0.13 ^a	1.73 $\pm$ 0.16 ^a	1.90 $\pm$ 0.19 ^a	1.90 $\pm$ 0.19 ^a
C18:0	ND	ND	ND	ND	ND	ND	ND	ND
C18:1n9t	5.12 $\pm$ 0.05 ^c	5.62 $\pm$ 0.08 ^c	5.84 $\pm$ 0.07 ^d	5.39 $\pm$ 0.07 ^c	5.84 $\pm$ 0.07 ^d	5.39 $\pm$ 0.07 ^c	7.23 $\pm$ 0.04 ^e	7.23 $\pm$ 0.04 ^e
C18:1n9c	15.28 $\pm$ 0.18 ^b	18.24 $\pm$ 0.09 ^d	18.80 $\pm$ 0.14 ^e	17.28 $\pm$ 0.19 ^e	18.80 $\pm$ 0.14 ^e	17.28 $\pm$ 0.19 ^e	20.83 $\pm$ 0.15 ^f	20.83 $\pm$ 0.15 ^f
C18:2n6c	3.78 $\pm$ 0.07 ^c	3.93 $\pm$ 0.05 ^c	4.12 $\pm$ 0.06 ^c	3.94 $\pm$ 0.04 ^c	4.12 $\pm$ 0.06 ^c	3.94 $\pm$ 0.04 ^c	0.88 $\pm$ 0.09 ^a	0.88 $\pm$ 0.09 ^a
C20:1	ND	ND	ND	ND	ND	ND	4.94 $\pm$ 0.06 ^b	4.94 $\pm$ 0.06 ^b
C20:2	3.41 $\pm$ 0.11 ^b	3.31 $\pm$ 0.09 ^b	3.53 $\pm$ 0.08 ^b	3.50 $\pm$ 0.05 ^b	3.53 $\pm$ 0.08 ^b	3.50 $\pm$ 0.05 ^b	4.08 $\pm$ 0.12 ^b	4.08 $\pm$ 0.12 ^b
C20:3n6	2.04 $\pm$ 0.05 ^b	1.56 $\pm$ 0.05 ^a	1.66 $\pm$ 0.02 ^a	1.96 $\pm$ 0.08 ^b	1.66 $\pm$ 0.02 ^a	1.96 $\pm$ 0.08 ^b	1.64 $\pm$ 0.03 ^a	1.64 $\pm$ 0.03 ^a
C20:3n3	9.59 $\pm$ 0.02 ^c	7.21 $\pm$ 0.011 ^b	7.93 $\pm$ 0.14 ^d	8.72 $\pm$ 0.011 ^e	7.93 $\pm$ 0.14 ^d	8.72 $\pm$ 0.011 ^e	6.63 $\pm$ 0.04 ^a	6.63 $\pm$ 0.04 ^a
C23:0	2.55 $\pm$ 0.07 ^b	3.26 $\pm$ 0.08 ^b	3.51 $\pm$ 0.11 ^c	3.13 $\pm$ 0.13 ^b	3.51 $\pm$ 0.11 ^c	3.13 $\pm$ 0.13 ^b	4.79 $\pm$ 0.08 ^d	4.79 $\pm$ 0.08 ^d
C22:2	ND	ND	ND	ND	ND	ND	1.15 $\pm$ 0.07 ^a	1.15 $\pm$ 0.07 ^a
C20:5(EPA)	10.51 $\pm$ 0.11 ^c	9.74 $\pm$ 0.13 ^b	10.28 $\pm$ 0.17 ^c	10.94 $\pm$ 0.11 ^d	10.28 $\pm$ 0.17 ^c	10.94 $\pm$ 0.11 ^d	10.11 $\pm$ 0.15 ^b	10.11 $\pm$ 0.15 ^b
C24:1	ND	ND	ND	ND	ND	ND	ND	ND
C22:6 (DHA)	23.49 $\pm$ 0.09 ^c	21.15 $\pm$ 0.17 ^b	22.14 $\pm$ 0.12 ^b	24.26 $\pm$ 0.11 ^d	22.14 $\pm$ 0.12 ^b	24.26 $\pm$ 0.11 ^d	17.96 $\pm$ 0.21 ^a	17.96 $\pm$ 0.21 ^a

ND-Not detected. Data are the mean value of three replicates $\pm$ S.D. (standard deviation)

Table 3 Fatty acid compositions of mackerel reacted oil particle using different agitation speed

Fatty Acid composition	Reacted oil	55°C/15 MPa		
		200 rpm	300 rpm	400 rpm
C14:0	7.36 ± 0.11 ^a	17.81 ± 0.15 ^c	8.43 ± 0.08 ^e	12.37 ± 0.12 ^b
C16:0	21.61 ± 0.19 ^d	8.16 ± 0.18 ^c	11.21 ± 0.12 ^a	1.86 ± 0.15 ^a
C16:1	2.53 ± 0.12 ^b	ND	1.71 ± 0.16 ^a	1.37 ± 0.11 ^a
C18:0	2.77 ± 0.11 ^b	ND	ND	0.78 ± 0.09 ^a
C18:1n9t	3.97 ± 0.09 ^a	5.51 ± 0.06 ^b	5.39 ± 0.04 ^c	5.73 ± 0.05 ^b
C18:1n9c	13.65 ± 0.17 ^a	16.24 ± 0.04 ^b	17.03 ± 0.12 ^c	18.42 ± 0.13 ^b
C18:2n6c	2.70 ± 0.04 ^b	3.81 ± 0.07 ^c	3.86 ± 0.11 ^c	4.03 ± 0.11 ^c
C20:1	1.83 ± 0.08 ^a	ND	ND	ND
C20:2	2.78 ± 0.09 ^a	4.26 ± 0.05 ^a	3.31 ± 0.07 ^b	3.48 ± 0.14 ^b
C20:3n6	1.96 ± 0.06 ^a	1.47 ± 0.07 ^a	1.93 ± 0.12 ^b	1.79 ± 0.06 ^a
C20:3n3	2.94 ± 0.04 ^a	8.42 ± 0.12 ^b	8.30 ± 0.07 ^d	8.25 ± 0.07 ^c
C23:0	1.76 ± 0.03 ^a	3.38 ± 0.06 ^b	3.31 ± 0.02 ^b	ND
C22:2	1.92 ± 0.02 ^a	ND	ND	1.17 ± 0.04 ^a
C20:5(EPA)	8.27 ± 0.12 ^a	9.74 ± 0.13 ^b	10.84 ± 0.16 ^d	11.16 ± 0.12 ^c
C24:1	1.03 ± 0.05 ^a	ND	ND	ND
C22:6 (DHA)	22.93 ± 0.13 ^b	21.15 ± 0.17 ^b	24.66 ± 0.12 ^d	23.59 ± 0.22 ^b

ND-Not detected. Mean ± SD ($n = 3$) different lowercase letters in each row indicate significant differences ($P < 0.05$)

(Table 5). In ethanol release of EPA and DHA before 30 min almost linear and in methanol linear release showed for 1 min.

Morphological analysis of particle by SEM

Several parameters like different pressure, temperature, nozzle size and agitation speed were used in supercritical states. The

particles produced by PGSS process were characterized by SEM. Fig. 2a-m shows SEM images of micro particles of mackerel reaction oil with PEG obtained by PGSS process using scCO₂. According to the SEM image original shape of PEG was large spherical but after particle formation spherical shape was denatured. When temperature was 45 °C and nozzle size was 200 µm at different pressure particle was rough shape and in fixed temperature at low pressure higher degree

Table 4 Acid value, peroxide value and free fatty acid content of mackerel reacted oil particle obtained by PGSS process

Agitation speed(rpm)	Nozzle size (µm)	Temperature (°C)	Pressure (MPa)	Free fatty acid determination (g/100 g)	AV (mg KOH/g)	POV (meq/1000 g)
300	200	45	10	3.27 ± 0.06 ^b	25.32 ± 0.16 ^b	8.54 ± 0.04 ^b
			15	3.01 ± 0.03 ^b	23.85 ± 0.12 ^b	7.82 ± 0.07 ^a
			20	2.38 ± 0.07 ^a	20.46 ± 0.15 ^a	7.17 ± 0.09 ^a
		55	10	3.42 ± 0.03 ^a	27.53 ± 0.21 ^c	9.3 ± 0.11 ^b
			15	3.67 ± 0.06 ^a	24.75 ± 0.19 ^b	8.62 ± 0.07 ^b
			20	4.11 ± 0.05 ^b	22.38 ± 0.18 ^a	7.23 ± 0.05 ^a
	300	45	10	3.35 ± 0.02 ^b	25.67 ± 0.13 ^c	9.21 ± 0.13 ^b
			15	3.19 ± 0.07 ^b	23.27 ± 0.18 ^b	7.55 ± 0.11 ^a
			20	2.78 ± 0.05 ^a	21.42 ± 0.17 ^a	7.16 ± 0.14 ^a
		55	10	3.48 ± 0.04 ^a	27.21 ± 0.19 ^c	9.67 ± 0.17 ^b
			15	3.89 ± 0.06 ^b	25.46 ± 0.23 ^b	7.92 ± 0.12 ^a
			20	4.25 ± 0.06 ^b	21.86 ± 0.19 ^a	7.29 ± 0.08 ^a
Reacted oil		35	Ambient pressure	2.24 ± 0.07	20.41 ± 0.17	7.15 ± 0.07

Mean ± SD ($n = 3$) different lowercase letters in each column indicate significant differences ($P < 0.05$)

Fig. 1 Solubility of particle in **a** ethanol, **b** methanol

of aggregation is observed but at 55 °C fibers shape was showed, its may be occurred for well melting of polymer due to high temperature. At 300 μm nozzle size, different temperature and pressure particle shape showed some agglomeration. Due to large nozzle size it can help to agglomeration. This SEM figure suggests that all oil is not encapsulated and therefore decreasing of encapsulation efficiency at higher pre-expansion pressure that making the particles sticky.

Analysis of particle size by particle size analyzer (PSA)

The size distributions of original PEG and mackerel oil particles with PEG 8000 obtained by PGSS using scCO_2 under different conditions are shown in Fig. 3a-c. In this study, the

average particle size of PEG before PGSS process was 178.688 μm . Average particle size was decrease in all condition. The average size of mackerel oil particles with PEG was found to be ranged from 102.527 μm to 136.58 μm . Temperature and pressure was moderately affected the size of mackerel oil particles with PEG. It was found that nozzle size was not affected on particle size. In 45 °C the particle size was smaller than 55 °C. At 45 °C and 15 MPa highest smaller particle was found in different nozzle size. In a previous work, an analysis of experimental results of micronization of polyethylene glycol by PGSS-drying showed a relationship between the saturation concentration of carbon dioxide in the solution and particle size indicating that if the concentration of carbon dioxide in the gas saturated solution is increased, the

Table 5 Fatty acid release from reaction oil particle in different solvent at different time interval

Fatty Acid composition	Ethanol			Methanol		
	15 min	30 min	45 min	1 min	2 min	3 min
Amount of fatty acid (%)						
C14:0	23.32 $\pm$ 0.16 ^d	18.91 $\pm$ 0.11 ^c	19.70 $\pm$ 0.13 ^c	16.19 $\pm$ 0.09 ^b	14.36 $\pm$ 0.12 ^a	16.82 $\pm$ 0.13 ^b
C16:0	4.32 $\pm$ 0.09 ^b	3.96 $\pm$ 0.07 ^a	4.08 $\pm$ 0.11 ^a	4.96 $\pm$ 0.14 ^b	6.82 $\pm$ 0.13 ^c	5.85 $\pm$ 0.08 ^c
C16:1	7.92 $\pm$ 0.08 ^d	6.81 $\pm$ 0.11 ^c	1.65 $\pm$ 0.07 ^a	8.32 $\pm$ 0.09 ^d	2.90 $\pm$ 0.05 ^b	2.42 $\pm$ 0.09 ^b
C18:0	1.32 $\pm$ 0.17 ^a	1.31 $\pm$ 0.13 ^a	1.54 $\pm$ 0.09 ^b	ND	ND	2.04 $\pm$ 0.14 ^b
C18:1n9t	10.03 $\pm$ 0.02 ^d	7.80 $\pm$ 0.06 ^b	5.99 $\pm$ 0.08 ^a	9.22 $\pm$ 0.11	8.71 $\pm$ 0.015 ^c	7.09 $\pm$ 0.07 ^b
C18:1n9c	13.95 $\pm$ 0.06 ^d	12.65 $\pm$ 0.09 ^c	7.57 $\pm$ 0.13 ^a	8.72 $\pm$ 0.21 ^a	10.61 $\pm$ 0.19 ^b	9.29 $\pm$ 0.17 ^b
C18:2n6c	5.14 $\pm$ 0.15 ^a	4.90 $\pm$ 0.07 ^a	5.81 $\pm$ 0.11 ^b	5.89 $\pm$ 0.17 ^b	6.78 $\pm$ 0.14 ^c	5.73 $\pm$ 0.06 ^b
C20:1	ND	ND	ND	ND	ND	ND
C20:2	4.38 $\pm$ 0.23 ^a	4.70 $\pm$ 0.18 ^a	6.56 $\pm$ 0.09 ^c	5.88 $\pm$ 0.13 ^b	5.77 $\pm$ 0.17 ^b	5.91 $\pm$ 0.21 ^b
C20:3n6	2.16 $\pm$ 0.07 ^b	1.67 $\pm$ 0.03 ^a	2.61 $\pm$ 0.04 ^b	3.95 $\pm$ 0.09 ^c	ND	ND
C20:3n3	7.17 $\pm$ 0.14 ^b	6.51 $\pm$ 0.12 ^a	8.85 $\pm$ 0.08 ^c	9.60 $\pm$ 0.11 ^d	9.97 $\pm$ 0.05 ^d	8.38 $\pm$ 0.19 ^c
C23:0	4.02 $\pm$ 0.15 ^b	3.56 $\pm$ 0.12 ^a	3.62 $\pm$ 0.18 ^a	4.69 $\pm$ 0.21 ^b	4.78 $\pm$ 0.11 ^b	3.81 $\pm$ 0.14 ^a
C22:2	ND	ND	ND	ND	ND	ND
C20:5(EPA)	4.98 $\pm$ 0.23 ^a	7.88 $\pm$ 0.18 ^b	9.59 $\pm$ 0.17 ^d	7.98 $\pm$ 0.11 ^b	8.26 $\pm$ 0.09 ^c	9.68 $\pm$ 0.15 ^d
C24:1	ND	ND	ND	ND	ND	ND
C22:6 (DHA)	11.29 $\pm$ 0.07 ^a	19.34 $\pm$ 0.13 ^c	22.42 $\pm$ 0.11 ^d	14.59 $\pm$ 0.17 ^b	21.05 $\pm$ 0.22 ^d	22.99 $\pm$ 0.26 ^d
EPA + DHA	16.27	27.22	32.01	22.57	29.30	32.67

ND-Not detected. Mean $\pm$ SD ($n = 3$) different lowercase letters in each row indicate significant differences ($P < 0.05$)

Fig. 2 SEM images of the makarel oil particles obtained by PGSS process using SC-CO₂. **a** 45 °C, 10 MPa, 200 µm; **b** 45 °C, 15 MPa, 200 µm; **c** 45 °C, 20 MPa, 200 µm; **d** 55 °C, 10 MPa, 200 µm; **e** 55 °C, 15 MPa, 200 µm; **f** 55 °C,

20 MPa, 200 µm; **g** 45 °C, 10 MPa, 300 µm; **h** 45 °C, 15 MPa, 300 µm; **i** 45 °C, 20 MPa, 300 µm; **j** 55 °C, 10 MPa, 300 µm; **k** 55 °C, 15 MPa, 300 µm; **l** 55 °C, 20 MPa, 300 µm and **m** Original PEG (8000 g/mol).

Fig. 3 Mackerel oil particle size and distribution volume formed by PGSS. **a** using 200 μm nozzle; **b** using 300 μm nozzle **c** PEG 800

atomization is more effective leading to the production of smaller particle (Martin and Cocero 2008).

Conclusion

There is a wide range of benefits on offer when using supercritical fluids (SCFs) such as more controllable particle size and high levels of diffusivity in both synthetic polymers and drugs. What is more, the use of supercritical fluids such as scCO_2 has provided a clean alternative to traditional techniques that employ toxic organic solvents or elevated temperatures. In this study, the feasibility of the PGSS process for the micronization of PEG and mackerel reacted oil was assessed. After enzymatic reaction of mackerel oil contained high amount PUFAs were obtained. TL-IM and Novozyme 435 enzyme will be the good for improving PUFAs. After particle formation among different parameters, it was found that comparatively low temperature and medium pressure produce high containing PUFAs particle. The oil quality was not changed significantly after particle formation. Constantly

release of PUFAs from particle was showed. And result shows it also can possible to maintain oil quality. By PGSS process smaller size of particle were obtain, this powder containing EPA and DHA can be easily use in food and pharmaceuticals industry.

Acknowledgments This work was supported by a Research Grant of Pukyong National University (2015).

References

- American Oil Chemists' Society (AOCS) (1998) Official methods of analysis. 6th edn. American Oil Chemists' Society, Champaign.
- Bernardez M, Pastoriza L, Sampedro G, Herrera JJR, Cabo ML (2005) Modified method for the analysis of free fatty acids in fish. *Agric Food Chem* 53(6):1903–1906
- Budavari S (1989) An encyclopedia of chemicals, drugs and biological, 11th edn. The Merck Index, Merck and Co. Inc, Rahway, New Jersey

- Cao XJ, Hur BK (2005) Separation of EPA and DHA from fish oil using modified zeolite 13X and supercritical CO₂. *J Ind Eng Chem* 11: 762–768
- Cocero MJ, Martin A, Mattea F, Varona S (2009) Encapsulation and coprecipitation processes with supercritical fluids: fundamentals and applications. *J Supercrit Fluids* 47:546–555
- Correa APA, Peixoto CA, Goncalves LAG, Cabral FA (2008) Fractionation of fish oil with supercritical carbon dioxide. *J Food Eng* 88:381–387
- Essien EA, Umoren SA, Essien EE, Udoh AP (2012) Preparation and evaluation of cucumeropsis mannii seed oil metallic soaps as driers in gloss paint. *J Mater Environ Sci* 3(3): 477–484
- Green P, Hermesh H, Monselise A, Marom S, Presburger G, Weizman A (2006) Red cell membrane omega-3 fatty acids are decreased in nondepressed patients with social anxiety disorder. *Eur Neuropsychopharmacol* 16(2):107–113
- Gunnlaugsdottir H, Wannerberger K, Sivik B (1998) Alcoholysis and glyceride synthesis with immobilized lipase on controlled-pore glass of varying hydrophobicity in supercritical carbon dioxide. *Enzym Microb Technol* 22:360–367
- Jeong YS, Song SK, Lee SJ, Hur BK (2006) The growth and EPA synthesis of shewanella oneidensis MR-1 and expectation of EPA biosynthetic pathway. *Biotechnol Bioprocess Eng* 11:127–133
- Kadic A, Palmqvist B, Liden G (2014) Effects of agitation on particle-size distribution and enzymatic hydrolysis of pretreated spruce and giant reed. *Biotechnol Biofuels* 7:77. doi:10.1186/1754-6834-7-77
- Kamal-Eldin A, Yanishlieva NV (2002) ω -3 fatty acid for human nutrition: stability considerations. *Eur J Lipid Sci Technol* 104(12):825–836
- Knez Z, Habulin M (2002) Compressed gases as alternative enzymatic-reaction solvents: a short review. *J Supercrit Fluids* 23:29–42
- Lanna AC, Jose IC, Oliveira MGA, Barros EG, Moreira MA (2005) Effect of temperature on polyunsaturated fatty acid accumulation in soybean seeds. *Braz J Plant Physiol* 17(2):213–222
- Lee SJ, Jeong YS, Kim DU, Seo JW, Hur BK (2006) Eicosapentaenoic acid (EPA) biosynthetic gene cluster of shewanella oneidensis MR-1: cloning, heterologous expression and effects of temperature and glucose on the production of EPA in Escherichia coli. *Biotechnol Bioprocess Eng* 11:510–515
- Lozano P, Villora G, Gomez D, Gayo AB, Sanchez-Conesa JA, Rubio M, Iborra JL (2004) Membrane reactor with immobilised *Candida antarctica* lipase B for ester synthesis in supercritical carbon dioxide. *J Supercrit Fluids* 29:121–128
- Martín Valverde L, González Moreno PA, Rodríguez Quevedo A, Hita Peña E, Jiménez Callejo N, MJ, Esteban Cerda N, Molina Grima E, Robles Medina A (2012) Concentration of docosahexaenoic acid (DHA) by selective alcoholysis catalyzed by lipases. *J Am Oil Chem Soc* 89: 1633–1645
- Martin A, Cocero MJ (2008) Micronization process with supercritical fluids: fundamentals and mechanisms. *Adv Drug Deliv Rev* 60: 339–350
- Martín Á, Pham HM, Kilzer A, Kareth S, Weidner E (2010) Micronization of polyethylene glycol by PGSS (particle from gas saturated solutions)-drying of aqueous solutions. *Chem Eng Process Process Intensif* 49:1259–1266
- Nakaya H, Miyawaki O, Nakamura K (2001) Determination of log P for pressurized carbon dioxide and its characterization as a medium for enzyme reaction. *Enzym Microb Technol* 28(2–3):176–182
- Nalawade SP, Picchioni F, Janssen LPBM (2006) Supercritical carbon dioxide as a green solvent for processing polymer melts: processing aspects and applications. *Prog Polym Sci* 31:19–43
- Naliwaiko K, Araújo RLF, Da Fonseca RV, Castilho JC, Andreatini R, Bellissimo MI, Oliveira BH, Martins EF, Curi R, Fernandes LC, Ferraz AC (2004) Effects of fish oil on the central nervous system: a new potential antidepressant. *Nutr Neurosci* 7(2):91–99
- Nemets B, Stahl Z, Belmaker RH (2002) Addition of omega-3 fatty acid to maintenance medication treatment for recurrent unipolar depressive disorder. *Am J Psychiatry* 159(3):477–479
- Okada H (1997) One- and three-month release injectable microspheres of the LH-RH superagonist leuporelin acetate. *Adv Drug Deliv Rev* 28(1):43–70
- Oliveira JV, Oliveira D (2000) Kinetics of enzymatic alcoholysis of palm kernel oil in SC-CO₂. *Ind Eng Chem Res* 39:4450–4454
- Oliveira D, Feihmann AC, Rubira AF, Kunita MH, Dariva C, Oliveira JV (2006) Assessment of two immobilized lipases activity treated in compressed fluids. *J Supercrit Fluids* 38:373–382
- Osman H, Suriah AR, Law EC (2001) Fatty acid composition and cholesterol content of selected marine fish in Malaysian waters. *J Food Chem* 73:55–60
- Savage PE, Gopalan S, Mizan TL, Martino CJ, Brock EE (1995) Reactions at supercritical conditions—applications and fundamentals. *J AIChE* 41(7):1723–1778
- Su KP, Huang SY, Chiu CC, Shen WW (2003) Omega-3 fatty acids in major depressive disorder. *Eur Neuropsychopharmacol* 13(4):267–271
- Tracy MA (1998) Development and scale-up of a microsphere protein delivery system. *Biotechnol Prog* 14(1):108–115
- Yamaguchi I, Akoh CC, Lai OM (2004) Modification of fish oil by lipozyme TL IM to produce structured lipid. *J Food Lipids* 11:65–73
- Yehuda S, Rabinovitz S, Mostofsky DI (2005) Mixture of essential fatty acids lowers test anxiety. *Nutr Neurosci* 8(4):265–267