Food Chemistry 119 (2010) 1694–1703

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Analytical Methods

Physical characteristics of fish oil encapsulated by β-cyclodextrin using an aggregation method or polycaprolactone using an emulsion–diffusion method

Mi-Jung Choi [a], Uracha Ruktanonchai [a], Sang-Gi Min [b], Ji-Yoen Chun [b], Apinan Soottitantawat [c,*]

[a] National Nanotechnology Center (NANOTEC), National Science and Technology Development Agency (NSTDA), Patumthani 12120, Thailand
[b] Laboratory of Food Engineering, Department of Food Science and Biotechnology of Animal Resources, Konkuk University, Seoul 134-701, South Korea
[c] Department of Chemical Engineering, Faculty of Engineering, Chulalongkorn University, Phyathai Rd., Patumwan, Bangkok 10330, Thailand

ARTICLE INFO

Article history:
Received 29 April 2009
Received in revised form 7 July 2009
Accepted 16 September 2009

Keywords:
Fish oil
β-Cyclodextrin
PCL
Aggregation
Emulsion–diffusion method

ABSTRACT

Fish oils have many dietary benefits, but have strong odours and are easily oxidised. For these reasons, β-cyclodextrin (β-CD) a water-soluble polymer and polycaprolactone (PCL) a water-insoluble polymer were used to encapsulate fish oil in this study. In addition, the stabilities of freeze-dried fish oil (FO) in encapsulated complexes were investigated to determine fish oil release rates at different relative humidities and storage temperatures. In order to facilitate the practical applications of the water-soluble and insoluble fish oil complexes produced, release studies of fish oil were performed in de-ionised water, NaCl solution and fish sauce. Based on our studies, fish oil loaded β-CD at a mixing ratio of 10:20 (β-CD:FO (w:w)) was the best composition in terms of encapsulation efficiency (84.1%), fish oil loading (62.7%), fish oil leakage after freeze-drying (11.0%), and eicopentaenoic acid (EPA) encapsulation efficiency (6.5%). In addition, fish oil release rates from β-CD particles were slower in de-ionised water and in 15% and 25% NaCl than in fish sauce at all mixing ratios between β-CD and FO. The storage stabilities of freeze-dried β-CD–FO complexes at 10:20 (w:w) mixing ratio at various relative humidities retained 97% of fish oil within the particles during 3 days. However, the release rate of fish oil from β-CD–FO complexes of 10:20 mixing ratio was accelerated in fish sauce. In terms of the emulsion–diffusion method, PCL more efficiently retarded the release of FO in liquid or powder form, although particles were broken by freeze-drying. It is supposed that PCL better protected FO because of its water insolubility.

1. Introduction

Much work has been conducted on the diverse health advantages related to the consumption of fish oil (Castro, Tirapegui, Silva, & Cutrim, 2004; Choi, Decker, & McClements, 2009; Drusch & Berg, 2008; Fournier et al., 2007; Horn, Nielsen, & Jacobsen, 2009; Klinkesorn, Sophanodora, Chinachoti, Decker, & McClements, 2006; Serfert, Drusch, & Schwarz, 2009). These health advantages appear to be due to its high $n-3$ polyunsaturated fatty acid content (Castro et al., 2004). Furthermore, many of these studies have shown that dietary supplementation with fish oil probably benefits heart, brain, and nervous system functions. Potential mechanisms for the cardioprotective effects of omega-3 fatty acids include antiarrhythmic, anti-inflammatory, hypotriglycerideminc effects, lowered blood pressure, improved endothelial function, and dietary fat (Caggiula & Mustad, 1997; Conner, 1997). However, the development of fishy and metallic off-flavours has been encountered for various oil-bearing food emulsions due to the formation of volatiles resulting from the oxidation of fish oil. Some researchers have reported that the encapsulation of fish oil significantly retards oxidation (Klaypradit & Huang, 2007; Klinkesorn et al., 2006). Furthermore, it can mask the objectionable odours caused by volatile oxidation products and enhances the odours of fish oil-enriched products (Castro et al., 2004). Although encapsulation itself prevents lipid oxidation, some researchers have added antioxidants to fish oil encapsulation systems to ensure maximum protection during processing and subsequent storage of microencapsulated bioactive ingredients (Horn et al., 2009).

Encapsulation is a technology of packaging solid, liquid, or gaseous materials in miniature sealed capsules for release at controlled rates using desired release triggers (Klaypradit & Huang, 2007). The controlled-release of core materials and the protection of core materials from surrounding environments are highly dependent on the properties of the encapsulating materials used. In addition, the hydrophilic properties of the encapsulating materials govern the quality of final products, especially in beverage food industry. In order to use encapsulated fish oil ingredients in the beverage industry or as a food seasoning, for example, in fish sauce, we selected two different encapsulating materials; β-cyclodextrin (β-CD), which produces a water-soluble membrane, and polycaprolactone (PCL), which produces a water-insoluble membrane.

* Corresponding author. Tel./fax: +66 2 218 6899.
E-mail address: apinan.s@chula.ac.th (A. Soottitantawat).

0308-8146/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2009.09.052

The application of cyclodextrin in pharmaceutical, cosmetic, and food industries is extensive because it is inexpensive and non-toxic. Szente and Szejtli (2004) reviewed the applications of cyclodextrin as a food ingredient in association with encapsulation technology. Of the various encapsulation methods used with cyclodextrin (CD), molecular inclusion provides a means of organising molecular units, which is used in biologic systems and in chemistry and material science (Hijia, Mitsuhashi, & Miyake, 1989; Yoshii, Furuta, Yasunishi, Linko, & Linko, 1996). One interesting feature of cyclodextrin is its ability to complex organic molecules in organic structures which contain cavities. These entities are known as host–guest complexes and the process is referred to as molecular encapsulation. In particular, this complexation involving host–guest complexes involves the formation of "structures in an aqueous phase" or "molecular inclusion". Duchêne, Bothot, Yu, Pépin, and Seiller (2003) found that the natures of the cyclodextrins (α-, β-, γ-cyclodextrin, or derivatives thereof), and those of fatty acids (i.e., chain length and double-bond numbers) have significant influences on inclusion characteristics. Using the molecular inclusion method, CD can solubilise and stabilise active compounds on the molecular scale. On the other hand, CDs can form solid phases by self-assembly, though this process depends on the solubility, temperature, pH, molecular weight, and chemical natures of guest materials (Bonini et al., 2006; Bru, López-Nicolás, & García-Carmona, 1995; He, Fu, Shen, & Gao, 2007; Polarz, Smarsly, Bronstein, & Antonietti, 2001; Skiba, Wouessidjewe, Puisieux, Duchêne, & Gulik, 1996). In the present study, we elucidated the mechanisms of β-CD and fish oil self-assembly and aggregation using TEM and XRD (Choi, Ruktanonchai, Min, Chen, & Soottitantawat, 2009). We found that the mechanism involved the encapsulation of fish oil (FO) by β-CD. It involves the formation of a partial inclusion complex between FO and β-CD at the oil/water interface. And then, this β-CD–FO interaction causes CD molecules to crystallise around the oily globules by external agitation. Accordingly, we established a self-assembly method based on the use of β-CD to encapsulate FO.

Alternatively, polycaprolactone (PCL) forms a water-insoluble membrane and is a representative biodegradable polymer used for coating or encapsulation drugs, genes, and other active agents due to its biocompatibility, permeability, and biodegradability (Choi, Briançon, Andrieu, Min, & Fessi, 2004; Choi, Soottitantawat, Nuchuchua, Min, & Ruktanonchai, 2008). PCL emulsions are obtained using a partially water-miscible organic solvent containing the polymer in an aqueous phase, the subsequent addition of water to the system causes diffusion of the organic solvent from the dispersed phase to water. The advantages of this formulation are that it provides high encapsulation yields, is highly reproducible, provides better control of particle size, and allows scaling up for production (Moinard-Chécot, Chevalier, Briançon, Beney, & Fessi, 2008).

In the present study, FO complexes of β-CD were investigated to obtain additional information about complexes obtained. In addition, FO loaded PCL nanocapsules were prepared using an emulsion–diffusion based method. Finally, in order to facilitate the practical applications for both β-CD- and PCL–FO complexes, we performed release studies in NaCl solutions and in fish sauce and on freeze-dried powder forms at different relative humidities. The mechanism underlying active substance release rate was analysed using a mathematical model.

2. Materials and methods

2.1. Materials

The fish oil (FO) was purchased from Sigma–Aldrich (St. Louis, MO, USA). This product is derived from the Menhaden fish. It is composed of 14:0 Myristic acid (6–9%), 16:0 Palmitic acid (15–20%), 16:1 Palmitoleic acid (9–14%), 18:0 Stearic acid (3–4%), 18:1 Oleic acid (5–12%), 18:2 Linoleic acid (<3%), 18:3 Linolenic acid (<3%), 18:4 Octadecatetraenoic acid (2–4%), 20:4 Arachidonic acid (<3%), 20:5 Eicosapentaenoic acid (EPA) (10–15%), 22:6 Docosahexaenoic acid (DHA) (8–15%) as Sigma company reported. This sum was approximately 80% (the remaining 20% represents other unidentified fatty acids). β-CD was obtained from WACKER (Son Chemical, Bangkok) as a water-soluble material. Polycaprolactone (PCL, MW = 80,000, Sigma–Aldrich, St. Louis, MO, USA) was selected as a water-insoluble coating polymer and ethyl acetate (EA) was used as the PCL solvent. Oil red O stain was used to stain the FO, and was obtained from Sigma–Aldrich (St. Louis, MO, USA). Pluronic® F68 (Poloxamer 188, MW = 8400, Sigma, Missouri) was chosen as a surfactant. Pluronic® F68 is a poly (oxyethylene) poly (oxypropylene) block copolymer of a non-ionic polymeric surfactant. Eicosapantaenoic acid (EPA) was obtained from Sigma–Aldrich (Missouri). NaCl, KCl, and K_2CO_3 were obtained from Carloeraba, Unilab, and Sigma, respectively. All reagents were of the highest grades available and were used without further purification.

2.2. Encapsulation of fish oil

2.2.1. Self-assembly aggregation of β-cyclodextrin as a water-soluble material

Initially FO was dyed with 0.3% oil red O as a marker (Choi et al., 2007), and 0.3% (w/w) of β-cyclodextrin was added to de-ionised water. To study the effect of fish oil content on encapsulation efficiency, FO was added to the β-CD solution at various β-CD:FO (w/w) ratios (10:1, 10:5, 10:10, and 10:20). These mixtures were then placed in a shaking-incubator (Sheldon Mfg. Inc., Cornelius, OR, USA) for encapsulation by self-assembling aggregation at 250 rpm, 25 °C for 4 h.

2.2.2. Emulsion–diffusion method using PCL as a water-insoluble material

Briefly, 200 mg of PCL and 100, 200, and 300 mg of FO were dissolved in 20 ml of ethyl acetate saturated with water (PCL:FO = 2:1, 2:2, and 2:3 (w:w)). This solution was then emulsified in 60 ml of distilled water previously saturated with ethyl acetate and containing 500 mg of Pluronic® F68 as a surfactant using a high-speed homogeniser (Ultra-Turrax T25, IKA Labotechnik, Germany) at 8000 rpm for 5 min. Five-hundred millilitres of de-ionised water was then added to this emulsion to induce the diffusion of ethyl acetate from the oil phase to the water to precipitate PCL around the oil phase. Ethyl acetate and water were later removed by evaporation (Rotavapor® R210, Büchi Labortechnik AG, Postfach, Switzerland) under 30 mbar.

2.3. Extraction of free fish oil and the freeze-drying of encapsulated fish oil

To obtain the encapsulated FO, two volumes of hexane were added to β-CD–FO or PCL–FO particles to extract free oil in the continuous phase and the hexane phase was separated by centrifugation (Tomy Seiko Ltd., Tokyo, Japan) at 5000 rpm for 5 min. The encapsulated FO obtained was freeze-dried to prepare the powder form. The freezing temperature used was around −30 °C. Finally, the powder was subjected to 0.5 mbar pressure for 24 h.

2.4. Fish oil encapsulation efficiency and loading content analysis

Amounts of free FO were determined by UV–visible spectrophotometry (Perkin–Elmer Inc., MA, USA) and encapsulation efficiency and loading content were calculated. Prior to preparation, 0.3% of

oil red O was dissolved in FO as a marker to determine free FO amounts by UV–visible spectrophotometry. A wavelength of 519 nm was used to detect oil red O absorbance a surrogate of free FO amounts. FO contents in samples were calculated using a standard curve, which was prepared with known mixtures of FO and red oil O in hexane. The experiment was performed by suspending samples (10 ml) in 20 ml of hexane (Baik et al., 2004; Hardas, Danviriyakul, Foley, Nawar, & Chinachoti, 2000; Klinkesorn et al., 2006). The suspensions were then mixed and shaken in a shaking-incubator at 250 rpm for 5 min to extract the free FO. The resulting suspensions were centrifuged for 10 min at 3000 rpm and supernatants were collected for UV analysis.

2.5. Calculation of encapsulation efficiencies, loading contents, and leakages

Encapsulation efficiencies were determined from the amounts of FO actually incorporated with initial amounts of FO used, as follows:

$$\text{Actual loading fish oil content (g)} = \text{Total FO content (g)} - \text{determined free FO content (g)} \quad (1)$$

$$\text{Encapsulation efficiency (\%)} = [\text{Actual loading FO content (g)}/\text{Total FO content (g)}] \times 100 \quad (2)$$

$$\text{FO loading content (\%)} = [\text{Actual loading FO content (g)}/\text{Total mass of particles (g)}] \times 100 \quad (3)$$

In addition, we measured the percentage of FO leakage after freeze-drying using:

$$\text{FO leakage content (\%)} = [\text{Actual loading FO content (g)} - \text{determined free FO content after freeze-drying (g)}/\text{Actual loading FO content (g)}] \times 100 \quad (4)$$

2.6. Particle sizes and Z-potential determinations

The particle sizes and *Z*-potentials of prepared samples were analysed using a Nanosizer® Dynamic Lighter Scattering System (Malvern Instruments, Worcestershire, UK).

2.7. GC/MS analysis

To determine eicopentaenoic acid (EPA) contents in encapsulated fish oils, GC/MS analysis was performed with pure FO to calculate the percentage of EPA content. The standard curve of pure EPA in hexane which was obtained from Sigma Company was made by GC–MS. The free FO extracted from β-CD–FO or PCL–FO particles was calculated from the standard curve. To measure the percentage of EPA in total fish oil, the component of pure fish oil was analysed by GC–MS. The total EPA content which we used was obtained from total fish oil amount by MS. Finally, the encapsulated EPA amount could be obtained by subtracting the amount of free EPA from the total EPA amount to observe the EPA encapsulation efficiency. Analysis was carried out using an Agilent 7890A GC system equipped with a flame ionisation detector (FID) and a 5975 inert mass selective detector (MSD) (Agilent Technologies, Palo Alto, CA, USA). FO components were separated using a 50 m × 0.25 mm × 0.25 μm FFAP capillary from Quadrex (Utod Bridge, USA). Samples were injected using an automatic solid phase microextraction (SPME). The GC operating conditions used were: injector temperature 230 °C; split mode (split ratio = 20:1); carrier gas helium (with an inlet flow rate of 1.0 ml). Separation was conducted using the following oven program; starting at 80 °C for 5 min, then raised to 185 °C at 10 °C/min, and held at this temperature for 3 min. Continuously, the temperature was increased at 5 °C/min and held at this temperature for 9 min. The standard EPA curve was calculated using pure EPA in hexane. EPA encapsulation and loading efficiencies were calculated using:

$$\text{EPA encapsulation efficiency(\%)} = [(\text{Calculated EPA content from the total FO} - \text{Measured EPA content of free FO (g)})/\text{Calculated EPA content from the total FO (g)}] \times 100 \quad (5)$$

$$\text{EPA loading content (\%)} = [(\text{Calculated EPA content from the total FO} - \text{Measured EPA content of free FO (g)})/\text{Total mass of particles (g)}] \times 100 \quad (6)$$

2.8. Fish oil release studies in NaCl solution and fish sauce – liquid form

In order to study the stability of β-CD–FO or PCL–FO particles in fish sauce, its release was analysed in NaCl solution (0%, 15%, and 25%) and in fish sauce. Encapsulated FO particles (0.045 g/ml) were added to NaCl solutions and to fish sauce. A sample (50 ml) was then taken and mixed with 10 ml hexane to extract free FO at different time points (0–24 h after hexane addition). Hexane extracted from FO was evaporated down to ~2 ml and the amount of released FO was determined by UV/visible spectrophotometry as previously described in Section 2.6. Fish oil release rates were then calculated.

2.9. Storage stability of freeze-dried β-CD–FO or PCL–FO particles – powder form

The stability of encapsulated FO with β-CD or PCL was investigated by analysing the release rate of flavours from freeze-dried powder at a constant relative humidity (RH) and temperature (Soottitantawat et al., 2005). Freeze-dried powder (0.050 g) was weighed into a glass bottle and placed in a chamber containing a saturated salt solution of K_2CO_3 (RH = 43.18 at 20 °C, RH = 43.13 at 40 °C), NaCl (RH = 74.47 at 20 °C, RH = 74.68 at 40 °C) and KCl (RH = 85.11 at 20 °C, RH = 82.32 at 40 °C) at 20 °C and 40 °C, respectively. The chambers were sealed and kept at constant temperatures (20 or 40 °C) in an incubator. At designated times (0–24 h), samples were acquired and the amount of eugenol was measured spectrophotometrically as described above.

2.10. Morphological characterisation by atomic force microscopy (AFM)

The morphologies of samples were evaluated by AFM (Seiko, Japan). Prior to AFM imaging, samples of β-CD–FO or PCL–FO particles were diluted with de-ionised water by about 30-fold. Experiments were performed in tapping mode using a non-contact "Golgen" Silicon cantilevers (NSG 10) with 100–300 kHz resonance frequency. All images were recorded in air at room temperature at a scan speed of 1 Hz and were performed three times for each treatment and 10 fields for one single plate.

2.11. Statistical analysis

The data were analysed by ANOVA using the SAS statistical program 9.1 (SAS Institute, Cary, NC, USA). Differences among the

means were compared using Duncan's Multiple Range test, and the correlations between independent variables and measured values were calculated as Pearson's correlation coefficients. Each treatment had three replicate determinations.

3. Results

3.1. AFM observations of β-CD and PCL aggregates

In our previous experiments, we observed the morphological structure of β-CD–FO complexes and PCL–FO nanocapsules which have shown various appearances (Choi et al., 2009). According to Yang et al. (2007), AFM has several advantages such as high resolution, nanoscale, minimal sample preparation, and 3D images as compared to another microscopy method. From our present study, β-CD–FO complexes showed the largest size by the self-assembly aggregation of β-CD depending on the different mixing ratio between β-CD and FO (Fig. 1A–F). "Worm-type" β-CD–FO (10:1) aggregates did not appear. However, more "rod-type" aggregates were found (Fig. 1D–F). Fig. 1A and B shows the topology of the β-CD–FO complex. The height of particles was around 30 nm from the 3D images of topology, and furthermore, the particles were distinguished by two different phases such as fish oil in the centre of particles (Fig. 1C). These pictures are similar to the results found in other studies, which indicates that cyclodextrin contains pores (Polarz et al., 2001), or the formation of β-CD with polypseudorotex-

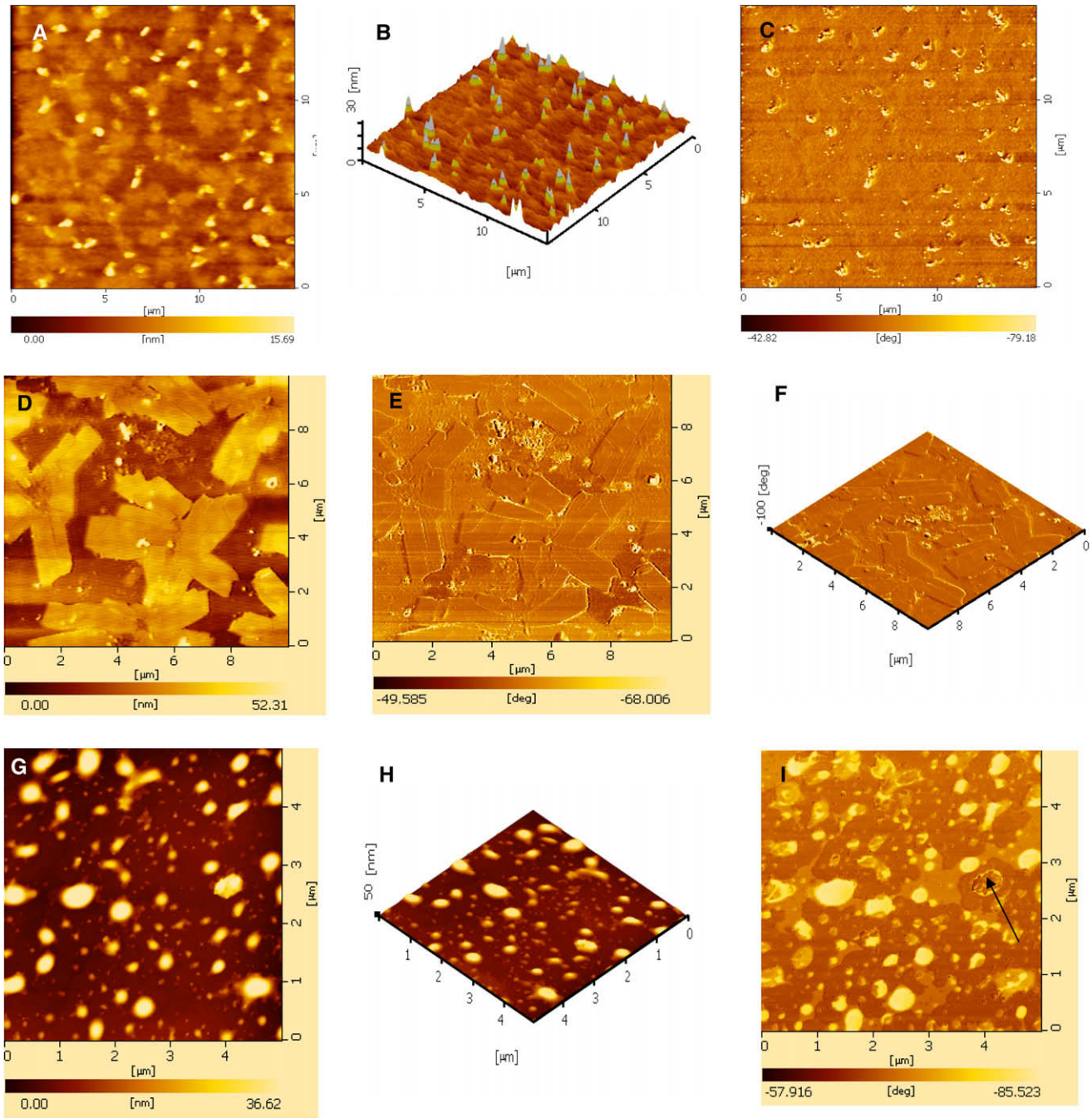

Fig. 1. AFM observation of β-CD:FO at a ratio of 10:1 (w:w) (topology (A), 3D of topology (B), and phase (C)), β-CD:FO at 10:10 (w:w) (topology (D), phase (E) and 3D of phase (F)), PCL:FO at 2:3 (w:w) (topology (G), 3D of topology (H) and phase (H)).

anes or with polyethylene–glycol (PEG) in the aqueous phase (Becheri, Lo Nostro, Ninham, & Baglioni, 2003). When the mixing ratio of β-CD to FO was 10:10, a supermolecular crystal form was observed (Fig. 1D–F), which were much larger than the particles sizes measured using a Nanosizer®. According to the topology image (Fig. 1D), aggregates of β-CD were observed and the fish oil was adherent among the aggregated rod of β-CD (Fig. 1E and F). We supposed that after dropping samples in the mica plate for AFM observation, β-CD aggregation continued during the drying process. Nevertheless, β-CD–FO aggregates were observed by AFM observation, and these were irregular and had small sizes of approximately 200 nm (Fig. 1A) or supermolecules of 1–2 μm (Fig. 1C). According to Choi et al. (2009), β-CD–FO aggregates could form larger aggregates in aqueous solutions, which precipitate as a consequence of self-assembly, or due to lipophilic water-insoluble active substances through non-inclusion complexation or micelle-like structures.

In the case of encapsulation FO by PCL, we found that the FO was obviously encapsulated (Fig. 1G–F), although some PCL membranes had broken releasing the FO (Fig. 1I, arrow). The images obtained showed that the nanocapsule morphology was spherical, more homogeneous and was very low in height (around 50 nm (Fig. 1H)) when compared with the β-CD–FO aggregates. In particular, the membrane thickness was thinner than that of β-CD–FO aggregates.

3.2. Effect of fish oil content on particle size and Z-potential before and after freeze-drying

In order to investigate the influence of FO content on the properties of particles, we prepared β-CD–FO and PCL–FO particles. The results obtained showed that FO content had a significant influence on particle size for β-CD–FO particles before freeze-drying and after reconstituting the powder in water (Table 1) ($P < 0.05$). The mean sizes of β-CD–FO particles were 250–700 nm, and particle sizes increased with FO loading. The mean sizes of PCL:FO particles were less than 200 nm with a limited distribution (not presented). Mean particle sizes also increased when FO loading was increased. The largest particle size (710 nm) was encountered with β-CD–FO particles at a β-CD to FO ratio of 10:20 ($P < 0.05$). However, the distribution of β-CD–FO particles was wide, as demonstrated by a polydispersity index value of 0.5–0.7. *Z*-Potentials measured for both methods ranged from −26 to −31 mV. For PCL–FO particles, the concentration of FO did not markedly affect particle size ($P > 0.05$), but *Z*-potentials increased slightly from −1 to −4 mV on increasing the amount of the FO. Bru et al. (1995) studied the aggregation of polyunsaturated fatty acids (PUFA) in the presence of β-cyclodextrin, and reported that PUFA–CD complexes are aggregated in the presence of CDs and at high PUFA concentrations. In addition, Skiba et al. (1996) reported that the sizes of β-CD:FO particles in which β-CD was modified by attaching various chains, increased depending on carbon chain length. Therefore, it could be postulated that the larger FO loadings induced β-CD aggregation and increased particle sizes.

After freeze-dried powders were rehydrated, it was observed that the particles aggregated and were disrupted, especially the PCL–FO particles. PCL–FO particles agglomerated to over 1 μm and polydispersity index values increased to around 0.8. Interestingly, their *Z*-potentials increased in the negative direction, which was attributed to capsule breakage during the freeze-drying process. Choi et al. (2004) concluded that the freezing process can break nanoparticles due to oil solidification in the particles cores.

3.3. Effect of fish oil loading on encapsulation efficiency and fish oil loading

Higher FO loadings are advantageous for encapsulated products. Therefore, the effects of FO contents were studied (Table 2). The FO content slightly influenced the encapsulation efficiency of β-CD. The percentage encapsulation efficiency of β-CD decreased on increasing FO loadings from 87.3% to 84.1% in total used amount of fish oil using Eqs. (1) and (2). The loading of FO in β-CD–FO at a 10:20 ratio was highest at 62.7%. The PCL method showed a greater encapsulation efficiency than the β-CD method, which agrees with the findings of Choi et al. (2008) who found that emulsion–diffusion (the PCL method) has higher encapsulation efficiencies for eugenol loaded PCL nanocapsules. The FO loading content of PCL:FO particles also increased on increasing FO concentration from 33.3% to 60.1% ($P < 0.05$). Leakage rates of FO from β-CD–FO particles by freeze-drying were low at high FO concentrations, that is, at higher FO concentrations, higher encapsulation efficiencies and high loadings were achieved after the freeze-drying process. In the case of PCL:FO particles FO leakage was 59.3% at a mixing ratio of 2:3 (PCL–FO = 2:3 (w:w)). Regarding particle sizes after freeze-drying, the FO leakage was due to particle breakage after freeze-drying. For PCL, we selected only one condition, namely, a 2:3 mixing ratio, to study FO release rate in different solutions and powder storage stability in the next section owing to the severe disruption and deformation caused by the freeze-drying process.

3.4. EPA encapsulation and loading efficiency in β-cyclodextrin

Eicopentaenoic acid (EPA) or docosahexaenoic acid (DHA) is the main functional component of FO, and thus, its encapsulation is important to protect it from the oxidation. In order to establish

Table 1
Effect of fish oil content on particle size and *Z*-potential before and after freeze-drying.

	Mass ratio[A] (w/w)	Before freeze-drying				After reconstitution of freeze-dried powder			
		Z-Average (nm)	PdI	Mean count rate (kcps)	Zeta potential (mV)	*Z*-Average (nm)	PdI[C]	Mean count rate (kcps)	Zeta potential (mV)
β-	CD:FO	10:1				261.67 ± 18.07[B,a]	0.50 ± 0.03	259.20 ± 12.32	−27.82 ± 0.66[a]
		362.71 ± 5.62[a]	1.00 ± 0.00	79.03 ± 23.04	−27.13 ± 0.90[a]				
	10:5	278.67 ± 43.31[a]	0.46 ± 0.08	181.76 ± 4.10	−28.72 ± 0.67[a]	469.00 ± 134.63[a]	0.65 ± 0.10	258.65 ± 43.66	−33.70 ± 1.96[b]
	10:10	377.67 ± 32.02[b]	0.47 ± 0.03	542.45 ± 26.20	−26.67 ± 1.22[a]	973.67 ± 304.45[b]	0.88 ± 0.04	200.61 ± 5.94	−26.73 ± 3.63[a]
	10:20	714.67 ± 8.65[c]	0.73 ± 0.10	543.13 ± 6.82	−31.31 ± 1.39[b]	754.67 ± 60.90[b]	0.70 ± 0.10	142.10 ± 15.36	−32.10 ± 0.42[b]
PCL:FO	2:1	183.02 ± 1.01[a]	0.20 ± 0.03	256.31 ± 14.82	−1.45 ± 0.10[a]	1251.20 ± 450.71[a]	0.84 ± 5.12	271.70 ± 17.40	−18.58 ± 15.98[a]
	2:2	194.71 ± 2.12[a]	0.11 ± 0.03	351.00 ± 3.19	−2.81 ± 1.20[b]	1450.12 ± 501.58[a]	0.71 ± 4.20	259.20 ± 12.33	−27.77 ± 0.60[b]
	2:3	208.67 ± 4.09[a]	0.11 ± 0.03	441.61 ± 4.72	−4.60 ± 0.74[c]	1520.14 ± 324.21[a]	0.85 ± 8.10	236.6 ± 45.41	−28.43 ± 0.75[b]

[a–c] Means with different superscript within same column are significantly different ($P < 0.05$).
[A] Mixing ratio between wall material and core material.
[B] Standard deviation in triplication.
[C] Polydispersity index.

Table 2
Influence of fish oil content on encapsulation efficiency and loading before and after freeze-drying.

	Mass ratio (w/w)	Before freeze-drying		After freeze-drying	
		Encapsulation efficiency (%)	Loading content (%)	Fish oil leakage content (%)	Loading content (%)
β-CD:FO	10:1	87.26[a]	8.03[a]	69.71[a]	2.94[a]
	10:5	86.56[a]	30.21[b]	ND[A]	ND
	10:10	85.32[a]	46.04[c]	14.67[b]	29.90[b]
	10:20	84.05[b]	62.70[d]	10.99[c]	55.69[c]
PCL:FO	2:1	99.52[a]	33.30[a]	ND	ND
	2:2	99.83[a]	50.04[b]	ND	ND
	2:3	99.86[a]	60.05[c]	12.29	61.06

[a–d]Means with different superscript within same column are significantly different ($P < 0.05$).
[A] Not determined.

EPA composition by an indirect method, free FO was extracted with hexane, and analysed by MS. We calculated EPA encapsulation efficiency and loadings into β-CD–FO particles using Eqs. (5) and (6) as shown in Fig. 2. We did not examine the EPA encapsulation rate of PCL–FO particles due to their breakdown after freeze-drying. EPA encapsulation rates were effective when the β-CD retention was augmented in the formulation method. Ultimately, a mixing ratio of β-CD–FO of 10:20 was found, efficiently encapsulating EPA at an encapsulation efficiency of 6.5% and a loading efficiency of 0.15%. This result concurs with the FO encapsulation efficiency data in Table 2. Duchêne et al. (2003) found that the terminal carbon of fatty acids is slightly twisted inside β-CD capsules and that this leads to molecular interactions with β-CD. In terms of pure EPA inclusion within β-CD, several patents describe a kneading method, which involves adding less than 50% water on as dry basis or a mixture of organic solvent and water to enhance the interaction between EPA and cyclodextrin (Hijia et al., 1989; Yoshii et al., 1996). However, Hijia et al. (1989) reported very low encapsulation contents of around 10% even when they used pure EPA.

3.5. Release study

3.5.1. Effect of the continuous phase on fish oil release – liquid form

The purpose of FO encapsulation is to protect functional components from environmental stresses, like light and oxygen. However, despite the fact that certain methods can encapsulate substances, the final product quality in terms of appearance and taste are most important. Some products demand a transparent condition without any settlement of particles, like fish sauce. For this reason, each freeze-dried particles in powder form were added to 15% and 25% sodium chloride solutions, and to fish sauce. In the case of NaCl, the concentrations used represented those in real fish sauces. The β-CD–FO and PCL–FO particles were transparent, except for PCL–FO at 2:3 (not presented). The effect of continuous phase type on the release rate of FO is shown in Fig. 3. Fish sauce had a pronounced effect on the release of FO. In particular, the release rate of FO from β-CD–FO particles at a 10:20 mixing ratio with fish sauce was faster than another mixing ratio in β-CD–FO particles although it showed higher encapsulation efficiency. For NaCl solutions and β-CD–FO particles, the release of FO remarkably increased at 25% NaCl, except at a ratio of 10:10. Interestingly, the release rate of FO in 15% NaCl was slowest for β-CD–FO particles, and for fish sauce, PCL–FO particles showed better results, although particles aggregated after freeze-drying. We supposed that the water-insoluble PCL more strongly inhibits the diffusion of FO in NaCl solution and in fish sauce than β-CD.

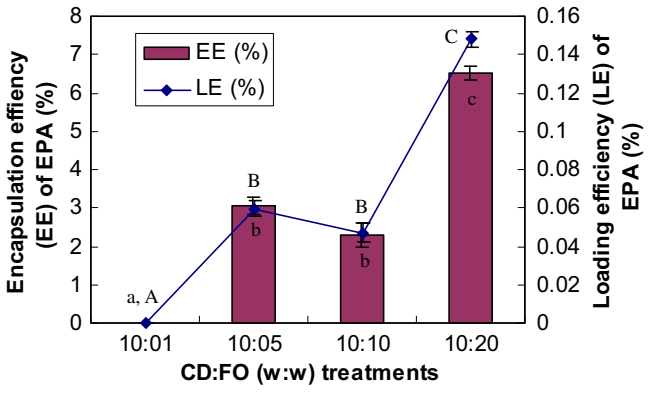

Fig. 2. The dependences of EPA encapsulation and loading efficiency on fish oil content by GC/MS analysis, (a–c) means with different letter within bar graph are significantly different ($P < 0.05$), (A–C) means with different letter within line graph are significantly different ($P < 0.05$).

A release time curve was calculated using Avrami's equation (below) to evaluate release rate constants:

$$R = \exp[(kt)^n] \tag{7}$$

where R (kg) is the retention of fish oil during release, t is the time (s), n is a parameter that represents the release mechanism, and k is the release rate constant (kg/s). Theoretically, the parameter $n = 1$ corresponds to first-order release kinetics and $n = 0.54$ to diffusion limited release (Soottitantawat et al., 2005; Yoshii, Soottitantawat, & Liu, 2001). Taking logarithms of Eq. (7), yields Eq. (8)

$$\ln(-\ln R) = n\ln k + n < t \tag{8}$$

From Eq. (8) one can calculate the parameter n from the slope by plotting ln(-ln$R$) vs. ln$t$, and the release rate constant k from the intercept at ln$t$ = 0. The release time-course of FO was analysed using Eq. (8), by plotting ln(−ln$R$) against ln$t$. Table 3 shows parameters estimated using Eq. (8). The release of FO fitted Eq. (8) well for all treatments (not presented). The values of the parameter n for a ratio of 10:20 of β-CD–FO particles in de-ionised water in 15% and 25% NaCl were 0.53, 0.51, and 0.35, respectively, which means that release followed diffusion kinetics. On the other hand, the n values of β-CD–FO particles of the other mixing ratio was around 0.9–1.4, indicating that FO was rapidly released in a first-order manner. The release rate constant k of β-CD–FO particles at a 10:1 mixing ratio was the largest, meaning that FO was released rapidly. From the k values, a 10:20 mixing ratio for β-CD–FO particles in fish sauce gave the fastest release rate for all treatments, although the n value indicated a diffusion mechanism. In terms of comparisons of n and k values at different FO contents in β-CD–FO particles, higher concentrations of FO were found to be more stable in β-CD complexes. On the other hand, the parameters n and k of PCL–FO particles represented the lowest value. Therefore, we suppose that β-CD is more easily dissolved at high concentrations of NaCl or fish sauce than PCL.

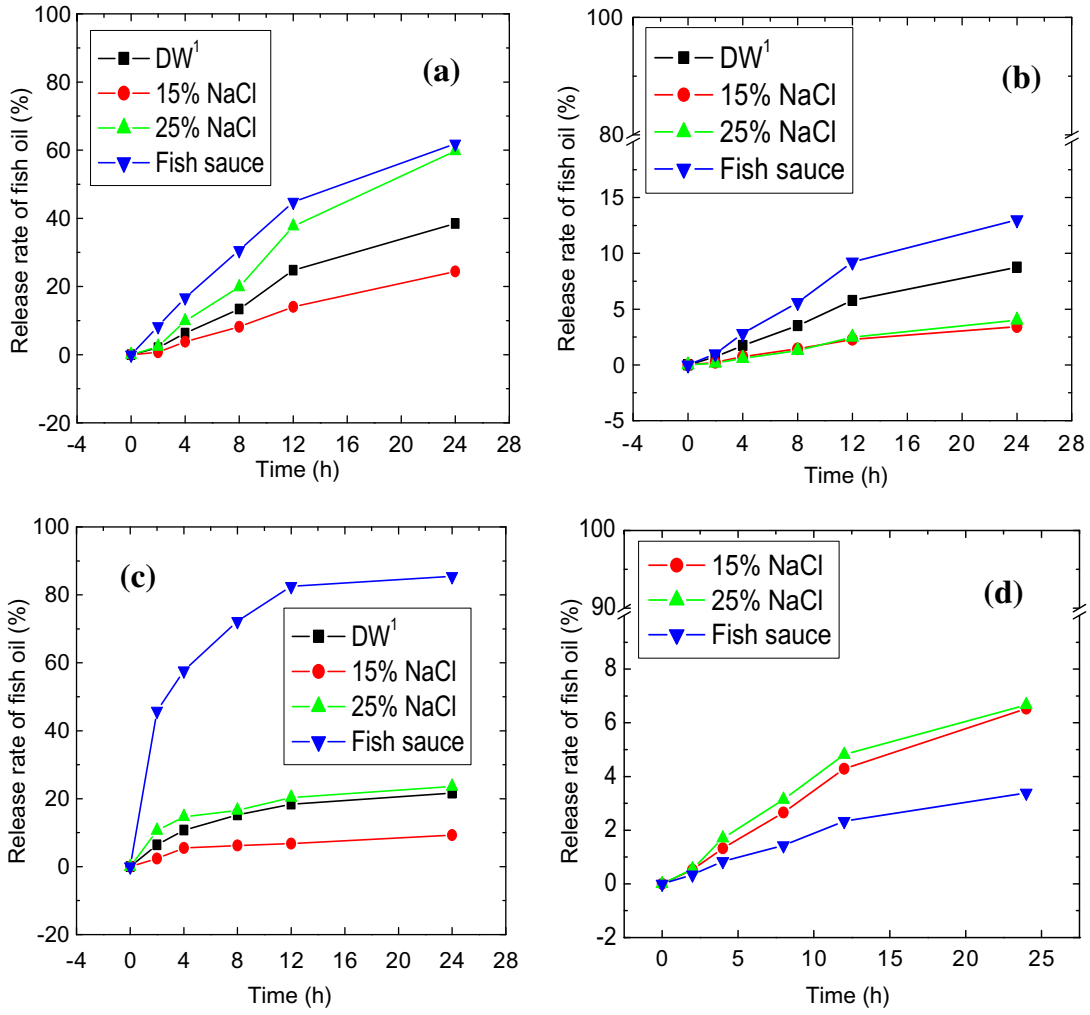

Fig. 3. Effect of continuous phase type (de-ionised water (DW), 15% and 25% NaCl, and fish sauce) on fish oil release, (a) β-CD:FO at 10:1 (w:w), (b) β-CD:FO at 10:10 (w:w), (c) β-CD:FO at 10:20 (w:w), and (d) PCL:FO at 200:300 (w:w).

Table 3
Release rate constant k and release mechanism as determine by n of Avrami's equation in various continuous phases.

Mass ratio (w/w)	(a) De-ionised water			15% NaCl			(b) 25% NaCl			Fish sauce		
	n	$k \times 10^{-3}$ (1/h)	r^2	n	$k \times 10^{-3}$ (1/h)	r^2	n	$k \times 10^{-3}$ (1/h)	r^2	n	$k \times 10^{-3}$ (1/h)	r^2
β-CD:FO = 10:1	1.29	27.26	0.99	1.45	20.78	0.96	1.45	44.37	0.96	0.99	44.14	0.99
β-CD:FO = 10:10	1.04	4.81	0.99	1.09	2.34	0.96	1.30	4.17	0.97	1.08	8.12	0.96
β-CD:FO = 10:20	0.53	3.55	0.97	0.51	0.50	0.99	0.35	1.01	0.98	0.49	195.82	0.96
PCL = 200:300	ND[a]	ND	ND	1.01	3.73	0.99	1.04	3.59	0.98	0.93	1.37	0.98

[a] Not determined.

3.5.2. Effect of relative humidity and storage temperature on release rates – powder form

The effects of relative humidity (RH) and storage temperature on FO release rates from the powder form of freeze-dried samples are shown in Fig. 4. The FO release rates of β-CD–FO at 10:20 and PCL–FO at 200:300 were well-controlled in all RH values and storage temperatures examined. However, the retention rate of β-CD–FO at 10:1 quickly reduced with time as compared to 10:10 or 10:20 at all RH values, and FO was almost lost within 72 h. This trend was similar to the FO retention rate in the liquid condition, as described previously by the release study in various continuous phase, but the retention rate of FO in β-CD freeze-dried powder was not significantly dependent on the relative humidity or storage temperature. Therefore, Avrami's equation was used to calculate kinetic parameters representing the release mechanism n and the release rate constant k. The release time-courses of FO under different conditions were analysed by plotting $\ln(-\ln R)$ against $\ln t$ (not presented). Higher k values were obtained at lower FO concentrations in β-CD, as displayed in Table 4. For β-CD–FO particles at 10:1, the k value increased between RH 43.18% and 85.11% at a storage temperature of 20 °C, excluding a RH of 74.47%. This result was similar at mixing ratios of 10:10 and 10:20 in the β-CD–FO complexes. On the other hand, the k value of the release rate constant during the storage at 40 °C differed. For example, the k value of β-CD at a 10:20 mixing ratio decreased depending on increasing RH, and the n value was higher than at β-CD–FO at 10:1. This indicates that the retention of fish oil at a 10:1 mixing ratio was unstable and that it was rapidly released from particles at certain RH

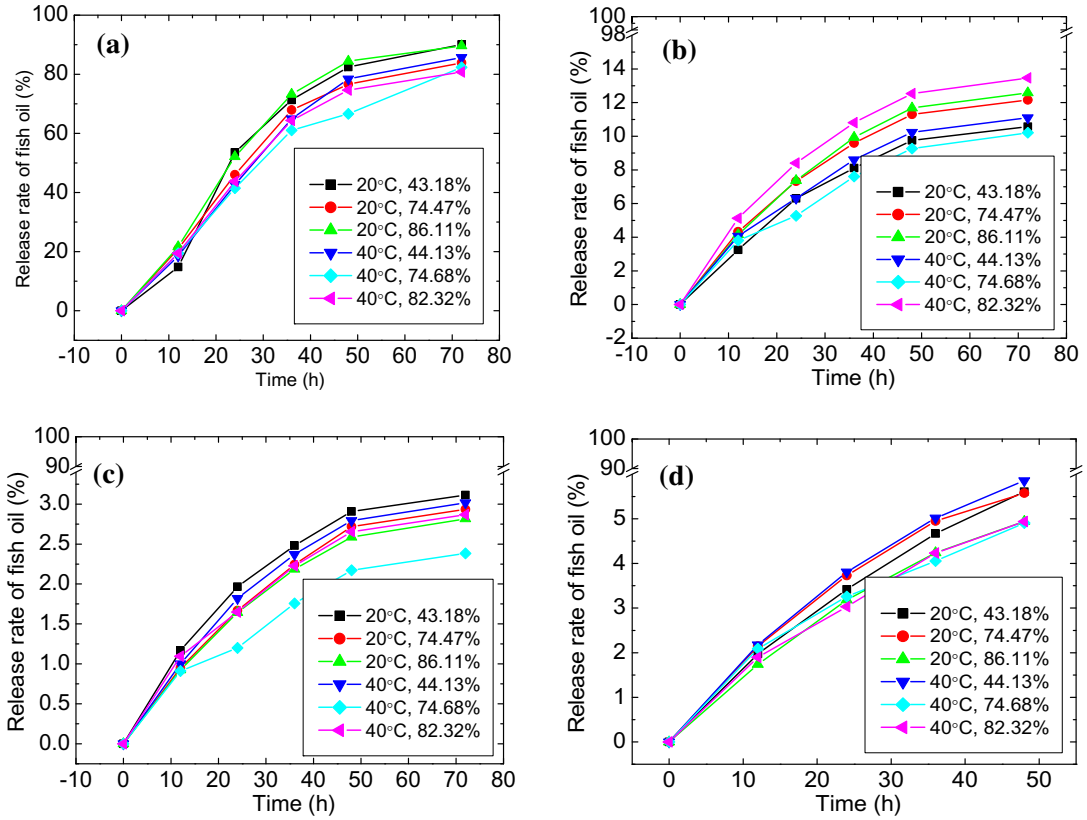

Fig. 4. Effect of relative humidity and storage temperature on fish oil release from oil and power forms at β-CD/fish oil mixing ratios of (a) 10:1 (w:w), (b) 10:10 (w:w), and (c) 10:20 (w:w).

values and storage temperature. For PCL treatment, k values decreased on increasing RH.

According to Whorton and Reineccius (1995), the release of entrapped flavouring materials is closely related with the adsorption of water on the walls of materials and with the hydration of powder. Water begins to penetrate the surface walls of particles, and cracks appear near surfaces and subsequently release flavours. However, our results did match with this theory for all treatments. Yoshii et al. (2001) concluded that the reasons for these complex observations are not well understood. In terms of this disagreement of our findings, we supposed that the weight percentage of composition (water or solid weight) was not accurate when taken at certain times since we did not measure the total weight causing the absorbing water into the particles during storage, especially at high water activities. Nonetheless, the thin layer of wall material can be easily dissolved when particles are made with low β-CD levels, which would enhance release rates at all RH values. On the other hand, PCL as a water-insoluble material can inhibit the release rate of fish oil, although these particles were damaged by freeze-drying. Regarding the oxidation stability during storage at various conditions, the stability of fish oil oxidation could be retarded at 74% of relative humidity, 40 °C owing to the relative slow release rate of fish oil from the previous work (Soottitantawat et al., 2004). It was reported that the release rate could be related to the oxidation rate, for example, the release rate of limonene-oxide or carvone as an oxidative marker was accelerated at 51% relative humidity.

4. Discussion

In general, the cyclodextrin inclusion method is the better-known technique for encapsulating active substances (He et al., 2007). This molecular inclusion method cannot be applied to include fish oil as a core material, because fish oil has a complicated make-up composing of different components such as linoleic acid, oleic acid and so on. However, the self-assembly of β-CD was used successfully to encapsulate FO with a water-soluble wall material in the present study. Our experiments indicate that β-CD encapsulation is dependent on the FO/β-CD mixing ratio, and that this determines particle size, encapsulation efficiency, morphological shape, and membrane thickness. Remarkably, for β-CD–FO the 10:20 (w:w) ratio had the greatest encapsulation efficiency (84.1%), highest loading content (46.0%), lowest leakage rate after freeze-drying (11.0%), and highest EPA encapsulation efficiency (6.5%). Moreover, self-assembly aggregation were accelerated according to the increment of fish oil amount forming the largest particle size from the measurement by the Nanosizer® and the thick layer of β-CD around fish oil from the AFM observation.

In terms of the PCL emulsion–diffusion method, FO encapsulation efficiency was reasonable at around 98%. PCL:FO particles were small (~200 nm), homogeneous, and spherical. However, the particles produced were broken and aggregated (to 1.5 μm) by freeze-drying. In terms of visible observations of dispersibility in various continuous media, β-CD–FO particles were transparent in fish sauce and NaCl solutions after being added in a freeze-dried powered form. However, PCL:FO particles were opaque in all media.

Based on release studies in de-ionised water, 15% and 25% NaCl, and fish sauce, PCL:FO particles released FO slower than β-CD–FO particles. In particular, the release rate of FO from PCL nanoparticles was retarded in fish sauce. Moreover, release rates from FO–PCL particles in their power forms at various humidities were slow because the release rate constant k was low according to Avrami's equation. For β-CD–FO particles, release rates at a 10:20 ratio were

Table 4
Release rate constants (k) and release mechanisms as determined by n of Avrami's equation at different RH values and storage temperatures.

Mass ratio (w:w)	Relative humidity (%)/storage temperature (°C)																	
	43.18/20			74.47/20			85.11/20			43.13/40			74.68/40			82.32/40		
	$k \times 10^{-4}$ (1/h)	n	r^2	$k \times 10^{-4}$ (1/h)	n	r^2	$k \times 10^{-4}$ (1/h)	n	r^2	$k \times 10^{-4}$ (1/h)	n	r^2	$k \times 10^{-4}$ (1/h)	n	r^2	$k \times 10^{-4}$ (1/h)	n	r^2
β-CD:FO = 10:1	289.00	1.49	99.12	268.19	1.18	99.89	308.16	1.29	99.05	263.31	1.31	99.32	235.15	1.16	99.84	250.02	1.18	98.91
β-CD:FO = 10:10	7.25	0.70	99.05	6.24	0.62	99.77	8.37	0.67	99.65	4.89	0.61	99.00	4.01	0.61	99.98	6.09	0.59	99.53
β-CD:FO = 10:20	0.39	0.57	98.89	0.82	0.66	98.52	0.69	0.65	97.51	0.73	0.64	98.15	0.27	0.59	98.75	0.30	0.57	99.42
PCL = 200:300	5.35	0.77	97.31	0.03	0.31	97.00	0.04	0.34	97.97	0.06	0.34	98.34	0.04	0.32	99.01	0.09	0.38	98.13

low and increased in ascending order from de-ionised water, to 15% and 25% NaCl, except fish sauce. In fish sauce, the release rate of FO from β-CD–FO particles was accelerated for the 10:20 ratio. In contrast, FO was released quickly from β-CD–FO 10:1 particles in the liquid phase and in the powder form.

To improve food quality, the combination of particle morphology and membrane thickness are important factors that control the release kinetics of an active substance, that is, they protect an active substance from the environment (e.g., oxidation of lipids) and mask undesirable odours (Guinebretière, Briançon, Fessi, Teodorescu, & Blanchin, 2002). However, care must be taken when these are applied to real food systems, e.g. fish sauces, which can produce unexpected results. In order to apply some designed ingredients to real food systems, a further study should be undertaken on the chemical composition of real food, physicochemical properties related to such ingredients, and any possible side effects. Further research is required to elucidate release kinetics in NaCl solution from the internal to continuous phase.

5. Conclusions

We concluded that high amounts of core material in β-CD–FO particles could enhance the aggregation of β-CD and retard FO release in liquid or in powder form although β-CD concentrations were the same. On the other hand, PCL more efficiently retarded the release of FO in liquid or powder form, although particles were broken by freeze-drying. It was supposed that PCL better protected FO because of its water insolubility.

Acknowledgements

This work was supported by the Thailand Research Fund (TRF) and a National Nanotechnology Center (NANOTEC) Grant funded by National Science and Technology Development Agency (NSTDA) (Nano-Encapsulation Program (C1-18), Grant No. B-21-CR0174), Fiscal 2009, Thailand.

References

Baik, M.-Y., Suhendro, L., Nawar, W. W., McClements, D. J., Decker, E. A., & Chinachoti, P. (2004). Effects of antioxidants and humidity on the oxidative stability of microencapsulated fish oil. *Journal of the American Oil Chemists Society, 81*(4), 355–360.

Becheri, A., Lo Nostro, P., Ninham, B. W., & Baglioni, P. (2003). The curious world of polypseudorotaxanes: Cyclodextrins as probes of water structure. *Journal of Physical Chemistry B, 107*(16), 3979–3987.

Bonini, M., Rossi, S., Karlsson, G., Almgren, M., Lo Nostro, P., & Baglioni, P. (2006). Self-assembly of beta-cyclodextrin in water. Part 1. Cryo-TEM and dynamic and static light scattering. *Langmuir, 22*(4), 1478–1484.

Bru, R., López-Nicolás, J. M., & García-Carmona, F. (1995). Aggregation of polyunsaturated fatty acids in the presence of cyclodextrins. *Colloids and Surface A: Physicochemical and Engineering Aspects, 97*, 263–269.

Caggiula, A. W., & Mustad, V. A. (1997). Effects of dietary fat and fatty acids on coronary artery disease risk and total and lipoprotein cholesterol concentrations: Epidemiologic studies. *American Journal of Clinical Nutrition, 65*(5), 1597S–1610S.

Castro, I. A., Tirapegui, J., Silva, R. S. S. F., & Cutrim, A. J. S. (2004). Sensory evaluation of a milk formulation supplemented with n3 polyunsaturated fatty acid and soluble fibres. *Food Chemistry, 85*, 503–512.

Choi, M. J., Briançon, S., Andrieu, J., Min, S. G., & Fessi, H. (2004). Effect of freeze-drying process conditions on the stability of nanoparticles. *Drying Technology, 22*, 335–346.

Choi, M. J., Briançon, S., Bazile, D., Royere, A., Min, S. G., & Fessi, H. (2007). Effect of cryoprotectant and freeze-dried process on the stability of W/O/W emulsions. *Drying Technology, 25*(5), 809–819.

Choi, S. J., Decker, E. A., & McClements, D. J. (in press). Impact of iron encapsulation within the interior aqueous phase of water-in-oil-in-water emulsions on lipid oxidation. *Food Chemistry*.

Choi, M. J., Ruktanonchai, U., Min, S. G., Chen, J. Y., & Soottitantawat, A. (2009). Morphological characterization of encapsulated fish oil with β-cyclodextrin and polycaprolactone. *Food Research International, 42*, 989–997.

Choi, M. J., Soottitantawat, A., Nuchuchua, O., Min, S. G., & Ruktanonchai, U. (2008). Physical and light oxidative properties of eugenol encapsulated by molecular

inclusion and emulsion–diffusion method. *Food Research International, 42*, 148–156.

Conner, W. E. (1997). The beneficial effects of omega-3 fatty acids: Cardiovascular disease and neurodevelopment. *Current Opinion in Lipidology, 8*(1), 1–3.

Drusch, S., & Berg, S. (2008). Extractable oil in microcapsules prepared by spray-drying: Localisation, determination and impact on oxidative stability. *Food Chemistry, 109*, 17–24.

Duchêne, D., Bothot, A., Yu, S.-P., Pépin, C., & Seiller, M. (2003). Cyclodextrins and emulsions. *International Journal o Pharmaceutics, 266*, 85–90.

Fournier, V., Destaillats, F., Hug, B., Golay, P.-A., Joffre, F., Juanéda, P., et al. (2007). Quantification of eicosapentaenoic and docosahexaenoic acid geometrical isomers formed during fish oil deodorization by gas–liquid chromatography. *Journal of Chromatography A, 1154*, 353–359.

Guinebretière, S., Briançon, S., Fessi, H., Teodorescu, V. S., & Blanchin, M. G. (2002). Nanocapsules of biodegradable polymers: Preparation and characterization by direct high resolution electron microscopy. *Materials Science and Engineering: C, 21*(1–2), 137–142.

Hardas, N., Danviriyakul, S., Foley, J. L., Nawar, W. W., & Chinachoti, P. (2000). Accelerated stability studies of microencapsulated anhydrous milk fat. *Lebensmittel-Wissenshaft and Technologie, 33*, 506–513.

He, Y., Fu, P., Shen, X., & Gao, H. (2007). Cyclodextrin-based aggregates and characterization by microscopy. *Micron, 39*(5), 495–516.

Hijia, H., Mitsuhashi, M., & Miyake, T. (1989). Inclusion compound of eicosapentaenoic acid and food product containing the same. US Patent 4831022.

Horn, A. F., Nielsen, N. S., & Jacobsen, C. (2009). Additions of caffeic acid ascorbyl palmitate or γ-tocopherol to fish oil-enriched energy bars affect lipid oxidation differently. *Food Chemistry, 112*, 412–420.

Klaypradit, W., & Huang, Y.-W. (2007). Fish oil encapsulation with chitosan using ultrasound atomizer. *LWT – Food Science and Technology, 41(6),* 1133–1139.

Klinkesorn, U., Sophanodora, P., Chinachoti, P., Decker, E. A., & McClements, D. J. (2006). Characterization of spray-dried tuna oil emulsified in two-layered interfacial membrane prepared using electrostatic layer-by-layer deposition. *Food Research International, 39*, 449–457.

Moinard-Chécot, D., Chevalier, Y., Briançon, S., Beney, L., & Fessi, H. (2008). Mechanism of nanocapsules formation by the emulsion–diffusion process. *Journal of Colloid and Interface Science, 317*, 458–468.

Polarz, S., Smarsly, B., Bronstein, L., & Antonietti, M. (2001). From cyclodextrin assemblies to porous materials by silica templating. *Angewandte Chemie International Edition, 40*, 4417–4421.

Serfert, Y., Drusch, S., & Schwarz, K. (2009). Chemical stabilisation of oils rich in long-chain polyunsaturated fatty acids during homogenisation, microencapsulation and storage. *Food Chemistry, 113*, 1106–1112.

Skiba, M., Wouessidjewe, D., Puisieux, F., Duchêne, D., & Gulik, A. (1996). Characterization of amphiphilic β-cyclodextrin nanospheres. *Intentional Journal of Pharmaceutics, 142*, 121–124.

Soottitantawat, A., Takayama, K., Okamura, K., Muranaka, D., Yoshii, H., Furuta, H., et al. (2005). Microencapsulation of l-menthol by spray drying and its release characteristics. *Innovative Food Science and Emerging Technologies, 6(2),* 163–170.

Soottitantawat, A., Yoshi, H., Furuta, T., Ohgawara, M., Forssell, P., Paratanen, R., et al. (2004). Effect of water activity on the release characteristics and oxidative stability of D-limonene encapsulated spray drying. *Journal of Agricultural and Food Chemistry, 52*, 1269–1276.

Szente, L., & Szejtli, J. (2004). Cyclodextrins as food ingredients. *Trends in Food Science and Technology, 15*(3–4), 137–142.

Whorton, C., & Reineccius, G. A. (1995). Encapsulation and controlled release of food ingredients. In S. J. Rish & G. A. Reineccius (Eds.), *Evaluation of the mechanisms associated with the release of encapsulated flavour from maltodextrin matrices* (pp. 143–160). Washington, DC: American Chemical Society.

Yang, H., Wang, Y., Lai, S., An, H., Li, Y., & Chen, F. (2007). Application of atomic force microscopy as a nanotechnology tool in food science. *Journal of Food Science, 72*, R65–75.

Yoshii, H., Furuta, T., Yasunishi, A., Linko, Y.-Y., & Linko, P. (1996). Oxidation stability of eicosapentaenoic and docohexaenoic acid included in cyclodexrins. *Journal of Inclusion Phenomena and Molecular Recognition in Chemistry, 25*, 217–220.

Yoshii, H., Soottitantawat, A., & Liu, X. D. (2001). Flavor release from spray-dried maltodextrin/gum Arabic or soy matrices as a function of storage relative humidity. *Innovative Food Science and Emerging Technologies, 2*, 55–61.