MICROENCAPSULATION OF FISH OIL USING HYDROXYPROPYL METHYLCELLULOSE AS A CARRIER MATERIAL BY SPRAY DRYING

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ABSTRACT

Spray drying is an important method in the food industry for the production of encapsulated oil to improve the handling and flow properties of the powder. In this study, the effect of mixture of polymers on the encapsulation of fish oil by spray drying was investigated. Fish oil powder were produced using different ratios of mixtures of hydroxypropyl methylcellulose (HPMC) 15 cps and HPMC 5 cps. Scanning electron microscopy and the amount of extracted oil from the surface revealed that the formulation containing high concentration of polymer mixture provided the highest protective and prolonged effect on the covering of fish oil. The particle sizes of less than 60 μm were obtained for all the formulations. The powder density was very suitable, which improves the flowability of the powder. Microencapsulation efficiency (69.16–74.75%) and surface morphology of encapsulated oil showed that the stability was increased and hence increased its acceptability as alternative primary polymers.

PRACTICAL APPLICATIONS

A relatively inexpensive and food-grade particles of encapsulated fish oil could be obtained, which are free from any organic solvents. Moreover, this process, parameters and technique could be facilitated in the food, pharmaceutical and nutraceutical industries.

INTRODUCTION

An increasing attention has been conceded to the human consumption of omega-3 polyunsaturated fatty acids (PUFAs) in the recent decades. Many clinical and epidemiological studies have exhibited promising roles of n-3 fatty acids (Natalie *et al.* 2009), so that daily uptake of such fatty acids can be recommended (Bao *et al.* 2011). There are numerous health benefits of omega-3 as it helps prevent hypertension, rheumatoid arthritis, coronary artery disease and some types of cancer and the rate of aging (Stone 1997; Salem *et al.* 1998; Simopoulos *et al.* 1999). However, the major limitation of omega-3 fatty acid is that it is highly susceptible to oxidation, which causes the off-flavor and degradation of color when used in the food preparation. Most importantly, hydroperoxide is considered as a major toxic element, which is the primary product of lipid oxidation.

To control this negative attribute of fish oil, microencapsulation technology can be incorporated where fish oil can be encapsulated with different carrier materials. This technology helps in masking the taste and odor and in improving the handling, storage and delivery of the powder materials. Microencapsulation technology is a recognized technology, which provides a means of converting the raw fish oil into powder form (Gibbs *et al.* 1999; Madene *et al.* 2006). Among all the microencapsulation technologies,

spray drying is the most common and recognized technology used in the food industry as well as in the pharmaceutical industry because it is efficient and cost-effective.

The selection of appropriate wall material or polymer is very important as it plays a key role in the encapsulation procedure. Sometimes, it is difficult to obtain the desired product with one polymer during the encapsulation, so the combination of two polymers can be recommended to obtain the desired products. Therefore, our study will focus on the encapsulation of fish oil with the combination of hydroxypropyl methylcellulose (HPMC) 15 cps and HPMC 5 cps as HPMC is a promising and readily available polymer. HPMC is highly viscous and used as a polymer in the encapsulation of fractionated coconut oil (Christensen *et al.* 2001).

Emulsion plays an important role in amending the oil encapsulation efficiency. By increasing the emulsion viscosity, it is possible to reduce the oil droplet size (Christensen *et al.* 2001). Previous reports showed that the oil droplet size has a noticeable effect on the encapsulation efficiency. Jafari *et al.* (2008a,b) reported the effect of the emulsion droplet size of fish oil with different carrier materials produced by spray drying. Soottitantawat *et al.* (2003) also investigated the effect of the droplet size on the encapsulation efficiency. These reports demonstrated that increasing the droplet size could decrease the encapsulation efficiency and decreasing the droplet size could increase the encapsulation efficiency (Ramakrishnan *et al.* 2013). The composition of the polymer is also an important factor as it affects the encapsulation efficiency. Jafari *et al.* (2008a,b) reported that the polymer composition affected the physicochemical properties of encapsulated fish oil. Sometimes, the different types of polymer might affect the encapsulation efficiency. From the previous reports, there are a lot of studies being conducted with different polymers (chitosan, modified starch, sugar beet pectin and sodium caseinate) to encapsulate fish oil (Shen *et al.* 2010; Bao *et al.* 2011). Still, many efforts are required for the development of new materials and formulations using spray drying process. The modified cellulose (hydroxypropyl methylcellulose, hydroxypropyl cellulose, methyl cellulose) can also be used as an alternative polymer for the encapsulation of fish oil. As the solubility of HPMC is very low at 30C and is negligible when the temperature is more than 35–40C (Kolanowski *et al.* 2004), HPMC can be easily dissolve in cold water. In the tablet coating, HPMC provides a physical barrier by generating a stable wall to the gastric mucosa against irritation (Sarkar and Walker 1995; Cerdeira *et al.* 1998). Moreover, HPMC shows the property of thermoreversible gelation in aqueous solution, which means that when it is heated, it will form gel, and when it is cooled down, it will dissolve again (Gonjari *et al.* 2009).

Hence, the aim of this study is to evaluate the different compositions of the mixture of two polymers (HPMC 15 cps and HPMC 5 cps) on the encapsulation of fish oil. The study was performed by spray drying different oil-in-water (o/w) emulsions containing fish oil dispersed homogeneously in the aqueous solution of a combination of HPMC 15 cps and HPMC 5 cps. Different reconstitution properties such as viscosity and droplet size of emulsion, surface oil, the amount of oil extracted through enzymatic digestion, wettability, density of powder particles, porosity, particle size and stability study of fish oil powder were also investigated.

MATERIALS AND METHODS

Materials

HPMC (Methocel E5 Premium LV and Methocel E15 Premium LV) was a kind gifted from Incepta Pharmaceuticals, Ltd., Dhaka, Bangladesh. The viscosities of the two polymers were 5.2 and 15.3 mPa·s (2% w/v solution) at 20C, respectively. Menhaden fish oil (20–30% omega-3) was obtained from Sigma-Aldrich, Inc., St Louis, MO. Starch, pancreatin derived from porcine pancreas and pepsin from porcine gastric mucosa (meeting the requirements of the U.S. Pharmacopeia) were obtained from Sigma-Aldrich, Inc. All other reagents used were of analytical grade. All ingredients and polymers used for the preparation of microcapsules were of food grade. Distilled water was used for the preparation of all the solutions.

Methods

Preparation of Spray-Dried Fish Oil Emulsion. A total of two series of formulations were prepared, details of which are shown in Table 1. The first series contained four formulations, which were prepared with the combination of different ratios of HPMC 15 cps and HPMC 5 cps. The second series contained five formulations, which were also prepared with different ratios of combination of HPMC 15 cps and HPMC 5 cps. Polyethylene glycol 6000 was used as a plasticizer in all the formulations. A total of 500 mL of liquid o/w emulsion was prepared for each formulation. The aqueous solution containing fish oil and HPMC 15 cps and HPMC 5 cps were homogenized in a high-speed colloid mill (Ultra-Turrax T25 basic, IKA Labortechnik, Staufen, Germany) for 12 min at 14,000 rpm.

The resultant emulsions were then spray dried using a laboratory-scale spray dryer (Lab Plant SD06A, Hunmanby Industrial Estate, North Yorkshire, U.K.) fitted with 0.5-mm atomizer, spray atomizer compressor, auto jet deblocking system with compressor, 215 mm OD × 500 mm long main chamber, cyclone and air velocity of about 4.1 m/s was set constant throughout the experiment. The inlet and outlet

TABLE 1. SERIES OF FORMULATION FOR THE PREPARATION OF SPRAY-DRIED FISH OIL EMULSION

Series	Formulations	HPMC 15 cps (g)	HPMC 5 cps (g)	PEG 6000 (g)	Fish oil (g)	Distilled water (g)	Solid content (wt %)
1	CF1	12.5	12.5	1.25	25	448.75	10.25
	CF2	9.375	9.375	1.25	25	455	9.0
	CF3	6.25	6.25	1.25	25	461.25	7.75
	CF4	3.125	3.125	1.25	25	467.5	6.5
2	DF1	–	25	1.25	25	448.75	10.25
	DF2	6.25	18.75	1.25	25	448.75	10.25
	DF3	12.5	12.5	1.25	25	448.75	10.25
	DF4	18.75	6.25	1.25	25	448.75	10.25
	DF5	25	–	1.25	25	448.75	10.25

HPMC, hydroxypropyl methylcellulose; PEG, polyethylene glycol.

temperatures were set at 180 ± 1 and 80 ± 1C, respectively. The emulsions were fed into the dryer by means of a peristaltic pump at 407.1 mL/h. Dried powder samples were collected from a Schott bottle attached at the bottom of the cyclone separator. The powder samples were then transferred in an amber glass bottle and stored at the temperature of 4C.

Characterization of Fish Oil Emulsion. *Emulsion Viscosity.* The viscosities of the prepared samples were measured at 20C with the help of a viscometer (DV-III Ultra, Brookfield, MA) fitted with spindle SC4-18. Samples were placed in the measurement cell of the viscometer and allowed to equilibrate at 20C. Shear rate ranged from 150 to 300 s^{-1} for the measurement of the viscosity of all the samples. Viscosity readings were taken after subjecting the sample to shear for 1 min. The viscosity was obtained in terms of millipascal-second (mPa·s). All measurements were carried out in triplicate.

Emulsion Particle Size. Droplet size of the prepared emulsions was measured by laser diffraction using laser particle size analyzer BT-9300H (Dandong Bettersize Instruments, Dandong, China) and expressed as volume weighted mean, D[4,3]. Distilled water was used as a dispersant. Each sample was analyzed in triplicate and average data were reported.

Characterization of Encapsulated Fish Oil. *Moisture Content.* The moisture content of the spray-dried powder was determined using a moisture analyzer (A&D MS-70, A&D Company, Limited, Tokyo, Japan). Approximately 1 g of powder sample was placed on the heating pan of the moisture analyzer. The moisture content evaporates as a result of continuous heating at 105C and the experiment was stopped automatically once the mass of the sample achieved a constant value.

Particle Size Distribution. The particle size distribution of spray-dried powder was measured using laser diffraction particle size analyzer (Malvern 2000 Mastersizer, Malvern Instruments Co., Worcestershire, U.K.) equipped with an automated dry powder dispersion unit (Scirocco 2000). The particle size distribution was characterized by the volume weighted mean, D[4,3].

Particle Surface Morphology. The morphological structure of the fish oil powder particles was analyzed through field emission scanning electron microscope (JEOL JSM-7800F, Akishima, Japan). The dried powder was mounted on specimen stubs with double-sided adhesive carbon tapes. The specimen was coated with platinum and examined at 1–3 kV with magnification ranging from 500× to 10,000×.

Determination of Microencapsulation Efficiency. The procedure of determining the total oil content was followed by Anwar and Kunz (2011), with some modifications. First, a sodium phosphate buffer was prepared by mixing 80 mL of the solution of monosodium phosphate (5.6 g of NaH_2PO_4 in 200 mL of distilled water) with 420 mL of the solution of disodium hydrogen phosphate (14.2 g of Na_2HPO_4 in 500 mL of distilled water) in a 1-L Schott bottle. Porcine pancreatin (30 mg) was weighed into a clean 50-mL tube where 250 mg of microcapsule powder was added and weighed. Exactly 10 mL of sodium phosphate buffer solution was added to this mixture. The vial was vortexed (Vortex Genie 2, Scientific Industries, NY) and placed in an incubator shaker at 37C at 70 rpm for 1 h. The tube was then cooled to room temperature and 10 mL of ethyl acetate was added to the solution and weighed (W_{sol}). The tube was again vortexed for 3 min and centrifuged at 1,000 rpm for 10 min. Then approximately 3 mL of organic layer was withdrawn and added to a tared tube and the tube was weighed (W_{ext}). This layer was blanketed under nitrogen gas. The tubes were then uncapped and placed in an oven at 45C to ensure that all solvents had evaporated, which gave the final weight of the extracted oil (W_{oil}) as stated in Eq. (1). From this, the amount (g) of total oil extracted from the powder was calculated using Eq. (1):

$$Total\ oil(\mathrm{g}) = (W_{sol}/W_{ext}) \times W_{oil} \quad (1)$$

The surface oil content of encapsulated powder was determined by extraction with petroleum ether (Garcia *et al.* 2006). Spray-dried powder (2 g) were weighed and dispersed with 25 mL of petroleum ether in a volumetric flask and was shaken manually for 8 min. Then the dispersion was filtered through a Whatman No. 1 filter paper (GE Healthcare Pte Ltd., Singapore) and the collected microparticles were rinsed three times with 15 mL of petroleum ether. The filtrate solution containing the extractable oil was transferred to a tared Petri dish to allow the solvent to evaporate at room temperature. The amount (g) of surface oil on the particles was then calculated from Eq. (2):

$$Surface\ oil(g) = \frac{Amount\ of\ extracted\ oil}{Initial\ mass\ of\ the\ powder\ particles} \quad (2)$$

Microencapsulation efficiency (%) was then calculated from Eq. (3):

$$Microencapsulation\ efficiency(\%) = \frac{Total\ oil - Surface\ oil}{Total\ oil} \quad (3)$$

Bulk Density and Tapped Density of Powder. Bulk density of fish oil powder was determined according to Jinapong *et al.* (2008). The powder was gently loaded into a 50-mL tared glass cylinder up to 50 mL mark and weighed (W_m). The volume (V_{bulk}) obtained directly from the glass cylinder was used to calculate the bulk density (ρ_{bulk}) based on the following relationship shown in Eq. (4):

$$\rho_{bulk} = W_m / V_{bulk} \quad (4)$$

For tapped density (ρ_{tapped}), approximately 5 g of encapsulated powder was placed into a 50-mL glass cylinder. The powder were repeatedly tapped manually by lifting and dropping the cylinder under its own weight at a vertical distance of 10 cm until negligible difference in volume (V_{tapped}) between succeeding measurements was observed. Then the tapped density was calculated based on Eq. (5) (Goula and Adamopoulos 2008):

$$\rho_{tapped} = M / V_{tapped} \quad (5)$$

Flowability and Cohesiveness of Powder. Flowability and cohesiveness of powder were determined in terms of Carr index (*CI*) and Hausner ratio (*HR*), respectively. Both *CI* and *HR* were calculated from the value of bulk (ρ_{bulk}) density using Eq. (6) and tapped (ρ_{tapped}) density of the powder using Eq. (7):

$$CI = (\rho_{tapped} - \rho_{bulk}) / V_{tapped} \times 100 \quad (6)$$

$$HR = \rho_{tapped} / \rho_{bulk} \quad (7)$$

TABLE 2. SCALE OF FLOWABILITY AND COHESIVENESS OF POWDER

Carr index (%)	Flow character	Hausner ratio
≤10	Excellent	1.00–1.11
11–15	Good	1.12–1.18
16–20	Fair	1.19–1.25
21–25	Passable	1.26–1.34
26–31	Poor	1.35–1.45
32–37	Very poor	1.46–1.59
>38	Very, very poor	>1.60

The scale of flowability and cohesiveness of the powder particles based on the *CI* and *HR* values are shown in Table 2 (Carr 1965; Hausner 1967).

Particle Density of Powder. Particle density ($\rho_{particle}$) of the fish oil powder was determined according to A/S Niro Atomizer (1978c), with some modifications. One gram (1 g) of the powder sample was taken in a 10-mL measuring cylinder with a glass stopper. About 5 mL of petroleum ether was added to the measuring cylinder, which was then shaken until all the powder particles were suspended. Finally, the rest of the powder particles were rinsed down on the wall of the cylinder by further addition of 1 mL of petroleum ether and the total volume of petroleum ether with suspended powder was recorded. The particle density was calculated using Eq. (8) as shown below:

$$\rho_{\text{particle}} = \frac{Powder\ weight}{Total\ volume\ of\ petrolem\ ether\ with\ suspended\ powder - 6} \quad (8)$$

Bulk Porosity of Powder. Bulk porosity of powder samples was calculated from the value of tapped density (ρ_{tapped}) and particle density ($\rho_{particle}$) which is expressed in Eq. (9) as follows (Jinapong *et al.* 2008):

$$Bulk\ porosity = (\rho_{particle} - \rho_{tapped}) / V_{particle} \times 100 \quad (9)$$

In Vitro *Gastric and Intestinal Digestion of Encapsulated Oil.* The *in vitro* digestion has been carried out in two phases: first, the encapsulated oil has been exposed to simulated gastric fluid (SGF) containing pepsin and sodium chloride at low pH value (Chung *et al.* 2011), and second, an intestinal digestion was simulated by exposing gastric digestion elements to a simulated intestinal fluid (SIF) (Patten *et al.* 2009).

Preparation of SGF. The SGF was prepared according to the USP method (U.S. Pharmacopeia 2000), where 0.64 g of pepsin and 0.4 g of sodium chloride were dissolved in 180 mL of ultra-pure water. After that, hydrochloric acid

(1.4 mL, 36% w/v) was added to the solution and the final volume of the solution was made up to 200 mL with ultra-pure water. The pH of the solution was ~1.2.

Preparation of SIF. The SIF was prepared by dissolving 0.25 g of pancreatin and 1.36 g of potassium dihydrogen phosphate in ultra-pure water. About 15.4 mL of 0.2 M sodium chloride was then added to the solution, which was stirred overnight at 4C using a magnetic stirrer. The pH of the solution was set to 6.8 with 1 M sodium hydroxide and the final volume of the solution was fixed at 200 mL with ultra-pure water.

Exposure of Sample to SGF and SIF. Abut 5 g of powder sample was added to 50 mL of SGF in a 250-mL Erlenmeyer flask. The mixture solution was then incubated at 37C for 2 h in an incubator shaker at 100 rpm and the pH was set to 6.8 using 1M NaOH. Approximately 50 mL of SIF was added to the solution and further incubated at the same conditions for another 3 h. The oil released was extracted three times with 20 mL of petroleum ether. For each extraction, petroleum ether was added into the sample solution and mixed using a flask shaker for 10 min and allowed to stand for 15 min. The extracts were mixed together and petroleum ether was removed using a rotary evaporator. The oil released from the sample was calculated as a percentage of the total oil in the sample.

Wettability. Powder wettability was determined using the modified method of Fuchs *et al*. (2006). One gram of powder sample was scattered or poured over the surface of 100 mL of distilled water in a beaker at 20C without agitation. The time taken for the powder particles to immerse and disappear from the surface of the water was measured and then compared between the formulations based on the extent of wettability.

Peroxide Value of Powder. Peroxide value (PV) was measured to determine the oxidation process during storage at 4C. The PV was determined according to the AOCS method of Cd 8-53 (2009), with chloroform and glacial acetic acid as solvents. Samples were withdrawn at 7-day interval for analysis. First, approximately 5 g of microencapsulated powder was weighed and added to a 250-mL conical flask with a plastic plug and dispersed completely into 12 mL of distilled water by magnetic stirring for 10 min. A mixture of 15 mL of chloroform and 30 mL of methanol was added to the solution. After magnetic stirring for 10 min, 15 mL of chloroform was added into the mixture and stirred for 2 min. Then, 15 mL of distilled water was added and stirred for 5 min. Mixed solution was stilled for 30 min for the formation of a layer. From the upper layer, 12 mL was pipetted into a weighed and dried 250-mL conical flask and dried at 105C for 1 h.

Exactly 12 mL of the chloroform phase, 18 mL of glacial acetic acid and 1 mL of freshly prepared saturated potassium iodide solution were pipetted into the dried 250-mL conical flask. After shaking it by hand for 10 s, the conical flask was placed with a cover in the dark for 3 min and then 30 mL of distilled water and 5 mL of 1% starch solution were added. The mixed solution was titrated under constant agitation with 0.0025 N sodium thiosulfate until the blue color disappeared completely. The PV was calculated at mEq O_2/kg oil from Eq. (10):

$$PV = [(S - B) \times N \times 1{,}000]/W \quad (10)$$

where S is the titration of the sample (in mL), B is the titration of blank (in mL), N is the normality of the sodium thiosulfate solution and W is the weight of the sample (in g).

RESULTS AND DISCUSSION

Characterization of Fish Oil Emulsion

The viscosity and droplet mean diameter of the emulsions with different solid contents are presented in Table 3. In this study, we found that there was a correlation between the solid content and viscosity of emulsion. As the total solid content increased, emulsion viscosity also increased, which was anticipated, since higher content of the mixture of HPMC 15 cps and HPMC 5 cps made the formulations more viscous. Among the C series of formulation, the highest viscosity found was 52.80 mPa·s for CF1 formulation and the lowest viscosity found was 9.59 mPa·s for CF4 formulation. Among the D series of formulations, the highest viscosity found was 63.27 mPa·s for DF1 formulation and the lowest viscosity found was 35.60 mPa·s for DF5

TABLE 3. VISCOSITY AND DROPLET SIZE OF EMULSIONS WITH DIFFERENT SOLID CONTENTS

Formulations	Total solid content (wt %)	Viscosity (mPa·s)	Droplet size, D[4.3] (μm)
CF1	10.25	52.80 ± 0.10^{a}	4.33 ± 0.02^{a}
CF2	9.0	28.83 ± 0.12^{a}	8.25 ± 0.2^{a}
CF3	7.75	24.80 ± 0.10^{a}	19.59 ± 0.02^{a}
CF4	6.5	9.59 ± 0.01^{a}	30.29 ± 0.02^{a}
DF1	10.25	63.27 ± 0.15^{b}	3.72 ± 0.03^{b}
DF2	10.25	60.23 ± 0.15^{b}	4.32 ± 0.01^{b}
DF3	10.25	52.80 ± 0.10^{b}	4.33 ± 0.02^{b}
DF4	10.25	45.20 ± 0.10^{b}	5.37 ± 0.01^{b}
DF5	10.25	35.60 ± 0.10^{b}	6.76 ± 0.03^{b}

Notes: Values are the average of triplicate ($n = 3$) analyses ± standard deviation. Different superscript letters within each column are significantly different at $P < 0.05$ when compared to CF1 with CF2, CF3 and CF4 and DF1 with DF2, DF3, DF4 and DF5 values using Tukey's HSD (honest significant difference) post hoc test.

formulation. The fish oil concentration remained constant for all the formulations. We observed that a lower content of HPMC mixture made the formulations less viscous, resulting in a slightly less pronounced thickening effect. Moreover, the effect of mixtures of different ratios of HPMC 15 cps and HPMC 5 cps on the viscosity was found to be significant ($P < 0.05$).

Droplet size of all the emulsions varied from 3.72 to 30.29 μm. At the same oil concentration, the small droplet size obtained as the total solid content was increased. This might happen due to the high viscosity of emulsions. As stated earlier, emulsions produced with higher solid content were more viscous. This higher viscosity reduces the sedimentation rate of the particles, which makes the emulsion more stable and avoid the coalescence between the particles (McClements 2005). On the contrary, emulsion with lower HPMC content led to higher droplet size, for the same oil concentration. Moreover, the effect of mixtures of different ratios of HPMC 15 cps and HPMC 5 cps on the droplet size was found to be significant ($P < 0.05$).

Characterization of Fish Oil Powder

Moisture Content. Moisture content is a key parameter for powder because high moisture content may be responsible for the formation of off-flavors in the oil by enhancing lipid oxidation as a result of the degradation of the product. In this study, moisture content of all the formulations varied from 3.85 to 6.39% (Table 4). Based on the study of Bhandari *et al.* (1992), there was a proportional relationship between the moisture content of the powder and the viscosity of the emulsion prior to spray drying. On the contrary, Hogan *et al.* (2001b) found that the types and content of polymers do not affect the moisture content. With our results, C series of formulation was consistent with the statement of Bhandari *et al.* (1992), but D series of formulation was consistent with Hogan *et al.*'s (2001b) statement. Formulation DF1 showed less moisture content of 3.85% and formulation DF4 showed high moisture content of 6.39%; moreover, all the moisture contents of different formulations were compared and found to be statistically significant ($P < 0.05$).

Determination of Microencapsulation Efficiency. Encapsulation efficiency of the encapsulated powder varied from 67.33 to 74.75% and was significantly influenced ($P < 0.05$) by the polymer composition. As the HPMC content increased, the encapsulation efficiency of fish oil also increased (Table 4). Moreover, the drying temperature was set at 180C for all formulations. This higher drying temperature increased the drying rate of the droplets, causing the formation of crust on the particle surface. This crust provided a solid membrane around the particles, preventing the leaching of oil from the droplets. The similar result was observed by Bhandari *et al.* (1992) in the encapsulation of citral and linalyl acetate by spray drying. On the contrary, Aghbashlo *et al.* (2013) found the positive effect of higher drying temperature in the encapsulation of fish oil by spray drying.

Considering the total solid content, this variable has a significant effect on the microencapsulation efficiency, i.e., higher solid content resulted in a higher microencapsulation efficiency. This result can be attributed to emulsion droplet size, which decreased in increasing the total solid content. Previously, many studies showed that emulsion droplet size have an opposite effect on the microencapsulation efficiency, i.e., lower droplet size increased the microencapsulation efficiency (Liu *et al.* 2001; Soottitantawat *et al.* 2003; Jafari *et al.* 2008a). According to Jafari *et al.* (2008b), higher surface oil in the particles with emulsion of larger droplet size attributed to the breakdown of droplets during atomization. Moreover, higher polymer concentration makes the emulsion viscosity higher and thus, resulting in a better encapsulation efficiency (Hogan *et al.* 2001a,b). The

TABLE 4. CHARACTERISTICS OF ENCAPSULATED POWDER OF DIFFERENT FORMULATIONS

Formulation	Moisture (%)	Microencapsulation efficiency (%)	Particle size, D[4.3] (μm)	Wettability (min)
CF1	6.02 ± 0.06[a]	72.27 ± 0.79[a]	31.81 ± 0.03[a]	19.00 ± 0.5[a]
CF2	5.45 ± 0.03[a]	70.91 ± 0.31	29.56 ± 0.37[a]	14.00 ± 0.50[a]
CF3	5.92 ± 0.05[a]	69.26 ± 0.67[a]	27.25 ± 0.04[a]	11.17 ± 0.29[a]
CF4	6.06 ± 0.04[a]	67.33 ± 0.15[a]	18.32 ± 0.04[a]	9.83 ± 0.29[a]
DF1	3.85 ± 0.04[b]	74.75 ± 0.39[b]	54.67 ± 0.09[b]	20.67 ± 0.58[b]
DF2	5.53 ± 0.06[b]	73.66 ± 0.34	34.35 ± 0.05[b]	19.17 ± 0.76
DF3	6.02 ± 0.06[b]	72.27 ± 0.79[b]	31.81 ± 0.03[b]	19.00 ± 0.5
DF4	6.39 ± 0.04[b]	70.37 ± 0.55[b]	30.04 ± 0.03[b]	16.83 ± 0.29[b]
DF5	4.35 ± 0.04[b]	69.16 ± 0.34[b]	27.84 ± 0.04[b]	15.67 ± 0.58[b]

Notes: Values are the average of triplicate ($n = 3$) analyses ± standard deviation. Different superscript letters within each column are significantly different at $P < 0.05$ when compared to CF1 with CF2, CF3 and CF4 and DF1 with DF2, DF3, DF4 and DF5 values using Tukey's HSD (honest significant difference) post hoc test.

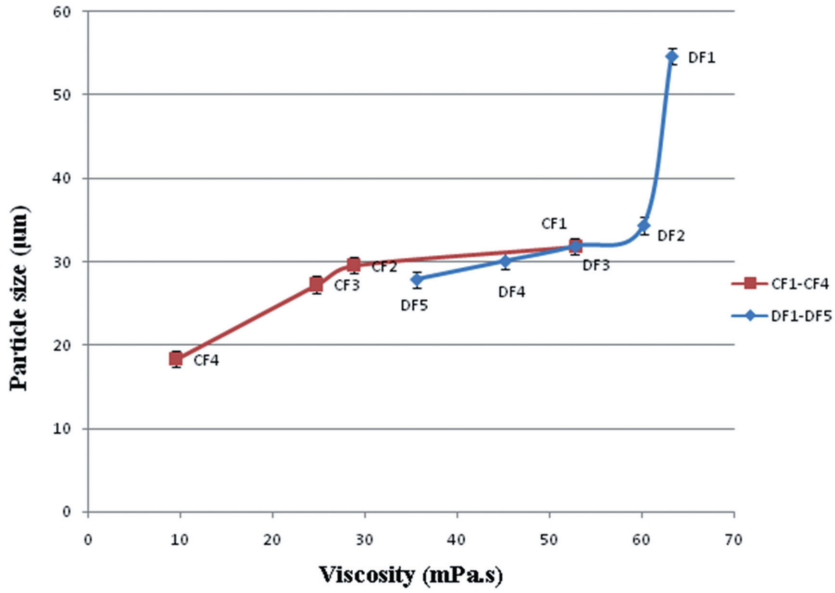

FIG. 1. EFFECT OF SOLUTION VISCOSITY ON PARTICLE SIZE

encapsulation efficiency values in our study were therefore toward the high end of previously reported encapsulation efficiency values.

Particle Size Distribution. In the study, the particle size of the fish oil powder varied from 31.81 to 18.32 µm in the C series of formulations, and in the D series of formulations, the value varied from 54.67 to 27.84 µm (Table 4). Increasing the polymer content increased the viscosity, which ultimately increased the powder particle size (Table 4) significantly ($P < 0.05$). Higher polymer content makes the solution more viscous; the rapid formation of the dried crust layer on the surface of the oil droplets hindered the shrinkage of the particles and therefore larger microcapsules were produced (Reineccius 2004; Chegini and Ghobadian 2005). Tonon *et al.* (2011) also studied the effect of the emulsion composition and inlet air temperature on the microencapsulation of flaxseed oil by spray drying. Figure 1 shows the effect of the viscosity of the mixture of HPMC 15 cps and HPMC 5 cps solutions on the particle size of the powder.

Wettability of Powder. The ability of spray-dried powder to disperse in water is one of the major physical properties related to reconstitution with water. Wettability of powder is characterized as the ability to rehydrate in water, i.e., to absorb the water (Gaiani *et al.* 2007). In this study, the degree of wettability was measured by calculating the time taken for powder to dissipate from the surface of water (Table 5). For the C series of formulations, the wetting time of CF1 formulation was 19.00 min. Gradually the wetting time was reduced to 9.83 min for formulation CF4. For the D

TABLE 5. CHARACTERISTICS OF ENCAPSULATED POWDER OF DIFFERENT FORMULATIONS

Formulation	Bulk density (g/mL)	Tapped density (g/mL)	Flowability and cohesiveness	
			Carr index (%)	Hausner ratio
CF1	0.138 ± 0.002[a]	0.154 ± 0.002[a]	10.78 ± 0.64[a]	1.12 ± 0.01[a]
CF2	0.129 ± 0.002	0.137 ± 0.003	5.56 ± 2.83	1.06 ± 0.03
CF3	0.124 ± 0.003[a]	0.136 ± 0.004[a]	9.01 ± 2.59	1.10 ± 0.03
CF4	0.118 ± 0.004[a]	0.129 ± 0.003[a]	8.72 ± 3.83	1.09 ± 0.05
DF1	0.133 ± 0.001[b]	0.149 ± 0.003[b]	10.90 ± 1.22[b]	1.12 ± 0.01[b]
DF2	0.135 ± 0.002	0.153 ± 0.001	11.74 ± 1.09	1.13 ± 0.02
DF3	0.138 ± 0.002	0.154 ± 0.002	10.78 ± 0.64	1.12 ± 0.01
DF4	0.161 ± 0.002[b]	0.176 ± 0.002[b]	8.88 ± 2.03	1.10 ± 0.03
DF5	0.173 ± 0.003[b]	0.187 ± 0.003[b]	7.83 ± 0.35	1.08 ± 0.01

Notes: Values are the average of triplicate ($n = 3$) analyses ± standard deviation. Different superscript letters within each column are significantly different at $P < 0.05$ when compared to AF1 with AF2, AF3 and AF4 and BF1 with BF2, BF3 and BF4 values using Tukey's HSD (honest significant difference) post hoc test.

TABLE 6. CHARACTERISTICS OF ENCAPSULATED POWDER OF DIFFERENT FORMULATIONS

Formulation	Particle density (g/mL)	Porosity (%)	Oil release (%)	
			SGF digestion	SGF and SIF digestion
CF1	0.643 ± 0.023[a]	76.43 ± 1.27[a]	19.93 ± 0.42[a]	48.83 ± 0.31[a]
CF2	0.520 ± 0.017	73.73 ± 0.81	37.03 ± 1.66[a]	64.80 ± 1.39[a]
CF3	0.493 ± 0.012[a]	73.17 ± 0.75	51.17 ± 1.82[a]	72.60 ± 1.73[a]
CF4	0.443 ± 0.012[a]	71.87 ± 0.74	61.20 ± 2.18[a]	81.27 ± 1.04[a]
DF1	0.697 ± 0.023[b]	78.60 ± 0.85[b]	18.27 ± 0.71[b]	46.07 ± 1.32[b]
DF2	0.683 ± 0.023	77.83 ± 1.37	18.70 ± 0.26	47.57 ± 0.42
DF3	0.643 ± 0.023	76.43 ± 1.27	19.93 ± 0.42	48.83 ± 0.31
DF4	0.500 ± 0.000[b]	68.40 ± 2.99[b]	21.43 ± 0.42	50.70 ± 0.53
DF5	0.493 ± 0.012[b]	61.91 ± 1.34[b]	22.00 ± 1.45	51.83 ± 2.18[b]

Notes: Values are the average of triplicate ($n = 3$) analyses ± standard deviation. Different superscript letters within each column are significantly different at $P < 0.05$ when compared to CF1 with CF2, CF3 and CF4 and DF1 with DF2, DF3 and DF4 values using Tukey's HSD (honest significant difference) post hoc test.SGF, simulated gastric fluid; SIF, simulated intestinal fluid.

series of formulations, the wetting time of DF1 was 20.67 min, which was reduced to 15.67 min for formulation DF5. Moreover, all values were significant ($P < 0.05$), except that formulations DF2 and DF3 with respect to DF1 were insignificant ($P > 0.05$). This indicates that decreasing the polymer content reduces the wetting time as HPMC is a hydrophilic polymer. Thus, when the polymer content was reduced, it facilitated the accessibility and penetration of water into the powder particles. Wettability of the powder also depends on the particle density, porosity, powder particle size and the surface activity of the encapsulated oil. Agglomeration of powder can increase the wettability as the liquid can easily penetrate through the pores of the powder particles (Buffo *et al.* 2002).

Bulk Density and Tapped Density of Powder. Both bulk density and tapped density of powder are affected by the type and composition of polymer, moisture content and particle size as well as by powder processing and storage conditions (Beristain *et al.* 2001). In our study, the bulk density was in the range of 0.118–0.138 g/mL for the C series of formulations and 0.133–0.173 g/mL for the D series of formulations. It was observed in the C series of formulations that increasing the polymer content increased the bulk density of the powder. Tonon *et al.* (2011) also reported that the heavier material may accommodate itself more easily in the spaces among the powder particles, resulting in higher density. The same bulk density values were also obtained by Botrel *et al.* (2014) in the encapsulation of fish oil by spray drying.

Tapped density is an important parameter associated with the packaging, transport and commercialization of powder, and thus, this value can be useful in terms of weight and the amount of material that will fit into a container (Finney *et al.* 2002). The value of tapped density was between 0.129 and 0.154 g/mL for the C series of formulations and between 0.149 and 0.187 g/mL for the D series of formulations. At higher temperatures, the drying process is accomplished very fast, resulting in larger expansion of the droplets and therefore lower powder density (Walton 2000). In the study, the inlet air temperature was maintained at high temperature (180C) for all the formulations. Bae and Lee (2008) found the tapped density value of 0.25–0.28 g/mL for the spray drying of avocado oil, i.e., the lowest density was obtained with high inlet air temperature.

Flowability and Cohesiveness of Powder. Quality control parameters like *CI* and *HR* are an important factor for evaluating the flow properties of the microcapsules (Fitzpatrick *et al.* 2004). The *CI* of the powder produced in this study was influenced by the particle size, but the *CI* value among each of the series (C and D) was insignificant ($P > 0.05$). As the polymer is hydrophilic in nature, the powder obtained showed very good flow characteristics. In our study, *CI* value varied from 8.72 to 10.78% for the C series of formulations and from 7.83 to 11.74% for the D series of formulations, which is very much consistent with the values in Table 2. The compositions of the surface of the powder particles have a significant role on this property because flowability involves overcoming the surface interactions among the particles (Fitzpatrick 2005). A low *HR* means that the powder is less cohesive and more capable of flowing freely. The value obtained from the C and D series of formulations was in the range of 1.06–1.13, which can be classified as an excellent and good flow property based on Table 2. Jinapong *et al.* (2008) conducted the spray drying of instant soymilk powder where they found *HR* values between 1.47 and 1.67.

Particle Density of Powder. Particle density is a key factor that was influenced by the composition of the mixture of HPMC 15 cps and HPMC 5 cps (Table 6). In our

study, the value of particle density ranged from 0.443 to 0.697 g/mL. Botrel *et al*. (2012) studied the encapsulation of oregano essential oil where they found particle density in the range of 0.74–0.92 g/mL. Because of the formation of steam in the droplets, the particle density can decrease, which causes the expansion of the particle even with the continuity of the drying process (Finney *et al*. 2002). Abadio *et al*. (2004) found that the particle density of pineapple juice powder particle was reduced with the increase of the polymer concentration.

Bulk Porosity. Bulk porosity plays an important role in the reconstitution of dry powder products controlling the rehydration speed (Krokida *et al*. 1997). In the C series of formulations, the porosity of the powder was influenced insignificantly by the polymer concentration ($P > 0.05$). However, in the D series of formulations, the porosity of the powder was significant ($P > 0.05$) with each other. The porosity value of the powder was in the range of 71.87–76.43% in the C series of formulations and in the D series of formulations, the value was in the range of 61.91–78.60%. Souza *et al*. (2009) found the porosity ranging from 50 to 59% for the encapsulation of tomato pulp. A study conducted by Jinapong *et al*. (2008) found porosity value of 70.02–74.47% for soy milk powder obtained by atomization.

Determination of *In Vitro* Gastric and Intestinal Digestion of Encapsulated Oil. *In vitro* gastric and intestinal digestion of the encapsulated oil was significantly influenced by the concentration of the mixture of HPMC 15 cps and HPMC 5 cps ($P < 0.05$) in the C series of formulations. In the C series of formulations, the percentage of oil released after SGF treatment was in the range of 19.93–61.20% (Table 6), whereas in the D series of formulations, the oil release was in the range of 18.27–22.00% (Table 6). In the study, it was found that increasing the polymer concentration reduces the percentage of oil from the encapsulated powder, i.e., formulations CF1 and DF1 released the lowest amount of oil (19.93 and 18.27% respectively) among the C series and D series of formulations. As HPMC 5 cps is less viscous than HPMC 15 cps, it was found that the formulation CF4 containing less concentration of a mixture of HPMC 5 cps and HPMC 15 cps released the maximum percentage of oil (61.20%) from the powder among the C series of formulations.

The percentage of oil released after the exposure to SGF and SIF was higher than the SGF alone (Table 6). Formulations CF1 and DF1 released the less percentage of oil (48.83 and 46.07%, respectively), whereas CF4 and DF4 released the high percentage of oil (81.27 and 51.83%, respectively). Formulations CF4 and DF4 showed the maximum percentage of the oil released from the fish oil powder due to increased polymer concentration.

PV of Powder. Another advantage of microencapsulation technology of the fish oil is to protect the PUFAs from oxidation. The primary product of lipid oxidation is hydroperoxides, which are formed by the reaction between oxygen and the unsaturated fatty acids and these peroxides have been considered to be toxic and thus should be avoided (Verardo *et al*. 2009). During processing, fish oil was exposed to air, high pressure and high temperature, which led to an increase in lipid oxidation (Baik *et al*. 2004). Tonon *et al*. (2011) also reported that the application of a higher inlet drying air temperature increased the PV. In our study, PV was measured at 7-day interval until 1 month for all the formulations. PV of formulation CF4 increased from 18.61 to 29.67 mEq O_2/kg oil in 28 days, whereas formulations CF3 and CF2 increased to 27.96 and 24.71 mEq O_2/kg oil, respectively, in 28 days (Fig. 2). Formulation CF1 containing higher concentration HPMC mixture possessed the lower PV (23.66 mEq O_2/kg oil) in 28 days. It is argued that the early formation of particle crust and the lower surface oil content could most probably shield the oil from oxidation attacks. On the contrary, formulations DF5, DF4 and DF3 initially possessed higher PV of 16.51, 16.52 and 16.49 mEq O_2/kg oil, respectively, at high inlet drying air temperature (Fig. 3) which was increased to 25.57, 22.81 and 23.66 mEq O_2/kg oil, respectively, in 28 days. Initially, DF1 possessed lower PV but in 28 days, PV increased to 21.55 mEq O_2/kg oil. Tonon *et al*. (2011) reported the lowest PV of 0.017 mEq O_2/kg oil for the highest encapsulation efficiency when investigating the effects of emulsion composition and inlet drying air temperature on the microencapsulation characteristics of flaxseed oil. Hogan *et al*. (2003) found the PV of fish oil powder in the range of 18–70 mEq O_2/kg oil.

Particle Surface Morphology. Spray-dried fish oil powder containing HPMC 15 cps and HPMC 5 cps were observed to have a more granular structure using field emission scanning electron microscopy (Figs. 4 and 5). It was observed that the type and concentration of polymer have a significant influence on the particle surface morphology. Figure 4 shows that fish oil powder encapsulate with the mixture of HPMC 15 cps and HPMC 5 cps, which is the C series of formulations, are more deformed than those of D series of formulations (Fig. 5). D series of formulations have more wrinkled and dented surface than the C series of formulations. Formation of wrinkled surfaces of spray-dried particles is attributed to the shrinkage of the particles during the drying process (Rosenberg *et al*. 1985). This increased the expansion of the gases within the particles during drying without rupturing the particles and the surface appears to be flexible enough to allow the particles to collapse after the maximum expansion point was reached (Wang and Langrish 2010). At

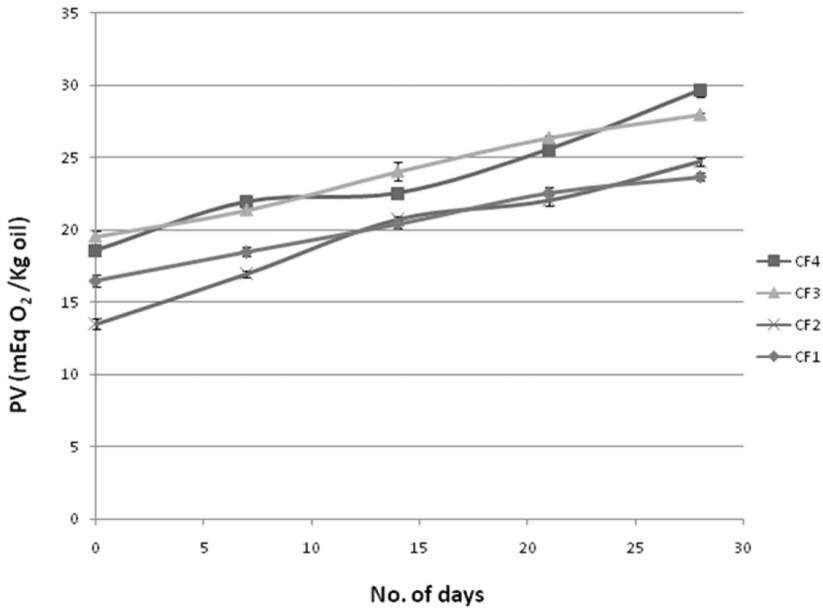

FIG. 2. EFFECT OF STORAGE TIME ON THE PEROXIDE VALUE (PV) OF FORMULATIONS CF1, CF2, CF3 AND CF4

lower concentrations of HPMC 15 cps and HPMC 5 cps, the particles seem to have more wrinkled surface than the ones at higher concentration, which was observed to have a spherical structure. However, for D series of formulations, the HPMC concentration was the same with respect to fish oil (1:1) and some wrinkled surface was also observed in every formulations. This is due to the fact that the higher concentration of a mixture of HPMC 15 cps and HPMC 5 cps tends to have higher viscosity and hence restraining the elasticity of the droplet during drying. For instance, the viscosity of the C series of formulations increased from 9.59 to 52.8 mPa·s, whereas the solid content also increased from 6.5 to 10.25%. However, for D series of formulations, formulation DF1 contains high content of HPMC 15 cps, whereas DF5 contains high content of HPMC 5 cps. As the ratio of HPMC mixture is the same with respect to fish oil, the wrinkled surface was observed in almost all the formulations. Bubble inflation may become more prevalent in a mixture of lower concentration of HPMC 15 cps and HPMC 5 cps, which results in more dented surface formed in the final stage of the drying process. Regardless of their morphology, the particle surface irregularity did not cast much effect during the microencapsulation of fish oil.

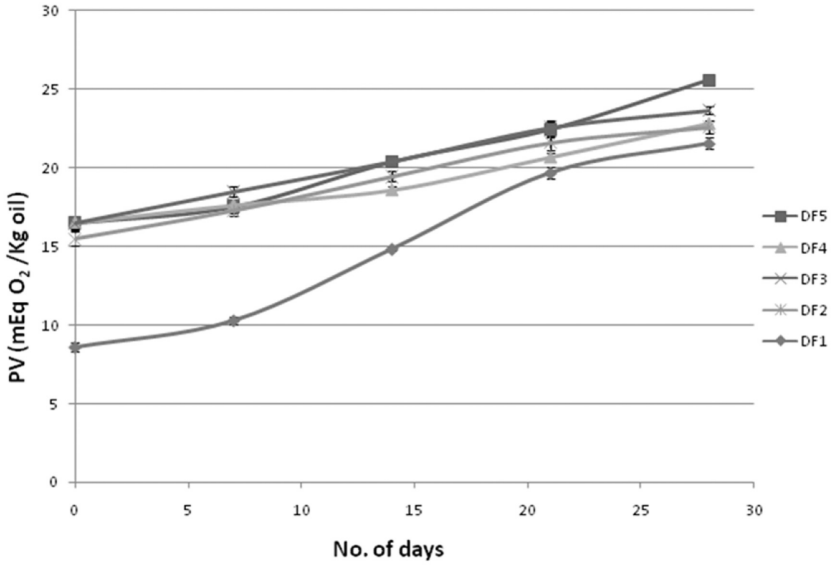

FIG. 3. EFFECT OF STORAGE TIME ON THE PEROXIDE VALUE (PV) OF FORMULATIONS DF1, DF2, DF3, DF4 AND DF5

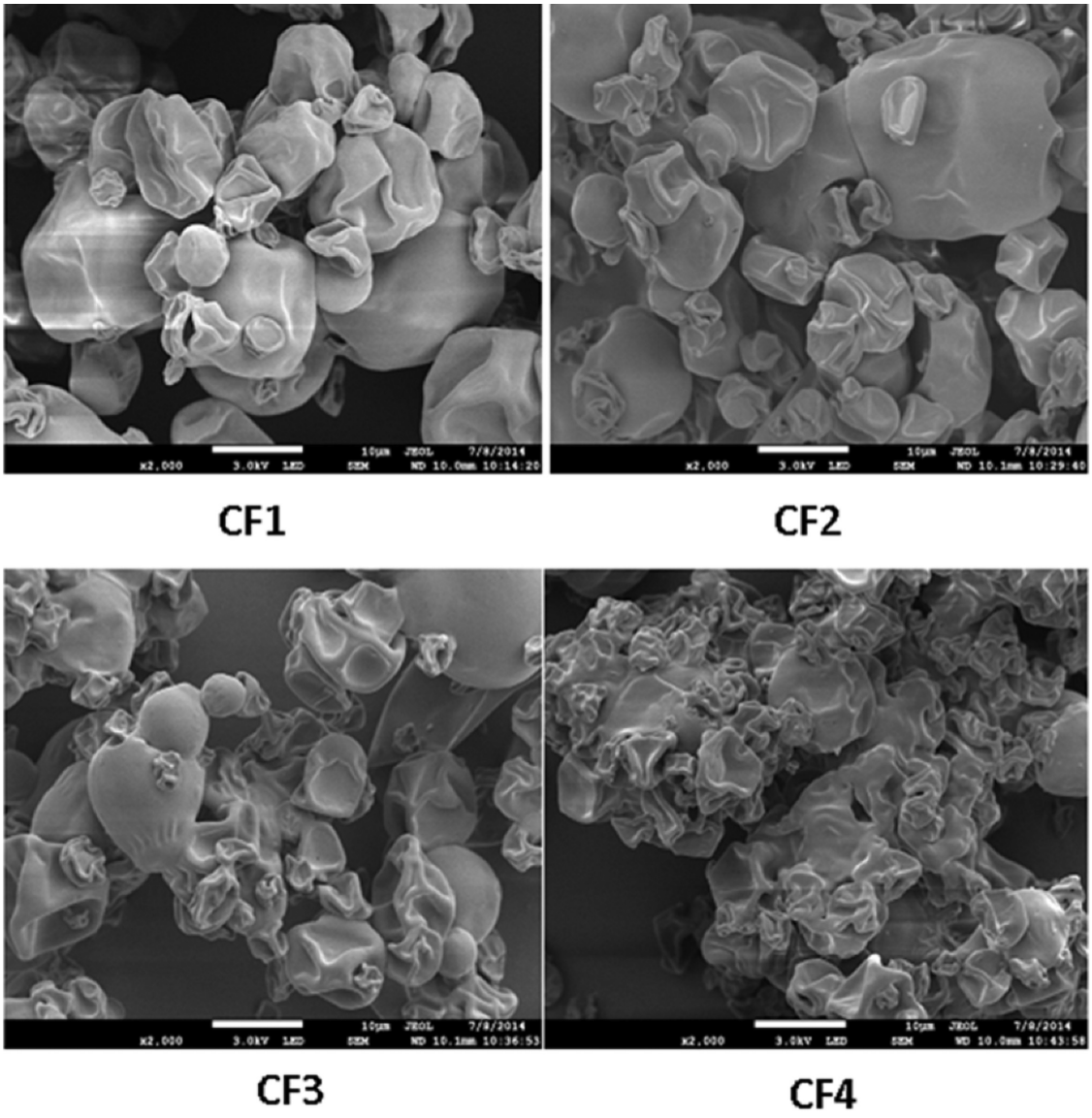

FIG. 4. MORPHOLOGY OF ENCAPSULATED POWDER OF C SERIES OF FORMULATIONS

CONCLUSION

The different concentrations of the mixture of HPMC 15 cps and HPMC 5 cps as polymers were found to have positive effects in producing the fine fish oil emulsion and encapsulated powder. Overall, the results showed that every selected response has been influenced significantly by the studied independent variables. Our previous study showed that HPMC 15 cps and HPMC 5 cps can be used as a single carrier material for the successful encapsulation of fish oil. In this study, our focus was to find out the effect of the mixture of HPMC 15 cps and HPMC 5 cps on the encapsulation of fish oil. So we found that higher solid concentration leads to bigger particle size, lower moisture content and fewer dented surface which may improve particle flowability, which is consistent with our previous study. Most importantly, PV of formulations containing higher concentration of a mixture of HPMC 15 cps and HPMC 5 cps was improved compared with formulations containing lower concentration of the mixture of HPMC 15 cps and HPMC 5 cps. However, the use of a mixture of HPMC 15 cps and HPMC 5 cps is also a good alternative for the microencapsulation of fish oil using spray drying. Because of the cost-effectiveness and easy availability of this polymer and current research findings, the formulation of new materials can be possible.

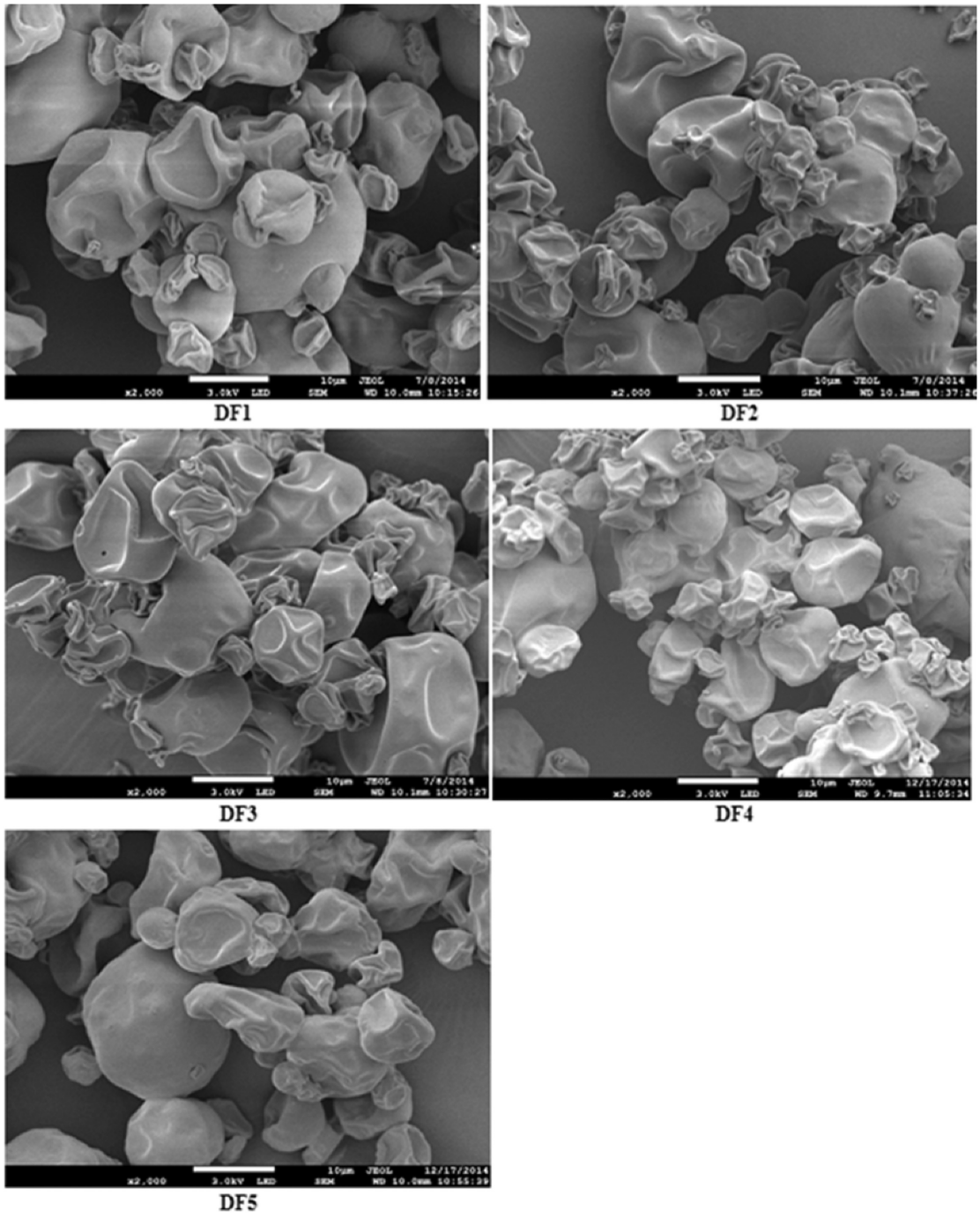

FIG. 5. MORPHOLOGY OF ENCAPSULATED POWDER OF D SERIES OF FORMULATIONS

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REFERENCES

ABADIO, F.D.B., DOMINGUES, A.M., BORGES, S.V. and OLIVEIRA, V.M. 2004. Physical properties of powdered pineapple (*Ananas comosus*) juice-effect of malt dextrin concentration and atomization speed. J. Food Eng. *64*, 285–287.

AGHBASHLO, M., MOBLI, H., MADADLOU, A. and RAFIEE, S. 2013. Influence of wall material and inlet drying air temperature on the microencapsulation of fish oil by spray drying. Food Bioprocess Technol. *6*, 1561–1569.

ANWAR, S.H. and KUNZ, B. 2011. The influence of drying methods on the stabilization of fish oil microcapsules: Comparison of spray granulation, spray drying, and freeze drying. J. Food Eng. *105*, 367–378.

AOCS (AMERICAN OIL CHEMISTS' SOCIETY). 2009. *Official Methods and Recommended Practices of the American Oil Chemists' Society. Method Cd 8–53*, AOCS Press, Champaign, III.

A/S NIRO ATOMIZER, COPENHAGEN, DENMARK. 1978. Determination of particle density, content of occluded air and interstitial air. In *Analytical Methods for Dry Milk Products*, 4th Ed. (I.H. Sørensen, J. Krag, J. Pisecky and V. Westergaard, eds.) pp. 52–53, De Forenede Trykkerier A/S, Copenhagen.

BAE, E.K. and LEE, S.J. 2008. Microencapsulation of avocado oil by spray drying using whey protein and maltodextrin. J. Microencapsul. *25*, 549–560.

BAIK, M.Y., SUHENDRO, E.L., NAWAR, W.W., MCCLEMENTS, D.J., DECKER, E.A. and CHINACHOTI, P. 2004. Effects of antioxidants and humidity on the oxidative stability of microencapsulated fish oil. J. Am. Oil Chem. Soc. *81*, 355–360.

BAO, S.S., HU, X.C., ZHANG, K., XU, X.K., ZHANG, H.M. and HUANG, H. 2011. Characterization of spray-dried microalgal oil encapsulated in cross-linked sodium caseinate matrix induced by microbial transglutaminase. J. Food Sci. *76*, 112–118.

BERISTAIN, C.I., GARCIA, H.S. and VERNON-CARTER, E.J. 2001. Vernon-carter spray-dried encapsulation of cardamom (*Elettaria cardamomum*) essential oil with mesquite (*Prosopis juliflora*) gum. LWT – Food Sci. Technol. *34*, 398–401.

BHANDARI, B.R., DUMOULIN, E.D., RICHARD, H.M.J., NOLEAU, I. and LEBERT, A.M. 1992. Flavor encapsulation by spray drying: Application to citral and linalyl acetate. J. Food Sci. *57*, 217–221.

BOTREL, A.D., VILELA BORGES, S., VICTORIA DE BARROS FERNANDES, R., DANTAS VIANA, A., GOMES DA COSTA, J.M. and MARQUES, G.R. 2012. Evaluation of spray drying conditions on properties of microencapsulated oregano essential oil. Int. J. Food Sci. Technol. *47*, 2289–2296.

BOTREL, D.A., BORGES, S.V., FERNANDES, R.V.D.B. and CARMO, E.L.D. 2014. Optimization of fish oil spray drying using a protein: Inulin system. Dry. Technol. *32*, 279–290.

BUFFO, R.A., PROBST, K., ZEHENTBAUER, G., LUO, Z. and REINECCIUS, G.A. 2002. Effects of agglomeration on the properties of spray-dried encapsulated flavours. Flavour Fragrance J. *17*, 292–299.

CARR, R.L. 1965. Evaluating flow properties of solids. Chem. Eng. *72*, 163–168.

CERDEIRA, A.M., GOUCHA, P. and ALMEID, A.J. 1998. Hydroxypropyl methylcellulose phthalate beads containing a model non-steroid anti-inflammatory drug. Int. J. Pharm. *164*, 147–154.

CHEGINI, G.R. and GHOBADIAN, B. 2005. Effect of spray-drying conditions on physical properties of orange juice powder. Dry. Technol. *23*, 657–668.

CHRISTENSEN, K.L., PEDERSEN, G.P. and KRISTENSEN, H.G. 2001. Preparation of redispersible dry emulsions by spray drying. Int. J. Pharm. *212*, 187–194.

CHUNG, C., SANGUANSRI, L. and AUGUSTIN, M.A. 2011. In vitro lipolysis of fish oil microcapsules containing protein and resistant starch. Food Chem. *124*, 1480–1489.

FINNEY, J., BUFFO, R. and REINECCIUS, G.A. 2002. Effects of type of atomization and processing temperatures on the physical properties and stability of spray-dried flavors. J. Food Sci. *67*, 1108–1114.

FITZPATRICK, J.J. 2005. Food powder flowability. In *Encapsulated and Powdered Foods* (C. Onwulata, ed.) pp. 247–260, CRC Press, Boca Raton, FL.

FITZPATRICK, J.J., IQBAL, T., DELANEY, C., TWOMEY, T. and KEOGH, M.K. 2004. Effect of powder properties and storage conditions on the flowability of milk powders with different fat contents. J. Food Eng. *64*, 435–444.

FUCHS, M., TURCHIULI, C., BOHIN, M., CUVELIER, M.E., ORDONNAUD, C., PEYRAT-MAILLARD, M.N. and DUMOULIN, E. 2006. Encapsulation of oil in powder using spray drying and fluidised bed agglomeration. J. Food Eng. *75*, 27–35.

GAIANI, C., SCHER, J., EHRHARDT, J.J., LINDER, M., SCHUCK, P., DESOBRY, S. and BANON, S. 2007. Relationships between dairy powder surface composition and wetting properties during storage: Importance of residual lipids. J. Agric. Food Chem. *55*, 6561–6567.

GARCIA, E., GUTIERREZ, S., NOLASCO, H., CARREON, L. and ARJONA, O. 2006. Lipid composition of shark liver oil: Effects of emulsifying and microencapsulation processes. Eur. Food Res. Technol. *222*, 697–701.

GIBBS, B.F., KERMASHA, S., ALLI, I. and MULLIGAN, C.N. 1999. Encapsulation in the food industry: A review. Int. J. Food Sci. Nutr. *50*, 213–224.

GONJARI, I.D., HOSAMANI, A.H., KARMARKAR, A.B., GODAGE, A.S., KADAM, S.B. and DHABALE, P.N. 2009. Formulation and evaluation of in situ gelling thermoreversible mucoadhesive gel of fluconazole. Drug Discov. Ther. *3*, 6–9.

GOULA, A.M. and ADAMOPOULOS, K.G. 2008. Effect of maltodextrin addition during spray drying of tomato pulp in

dehumidified air: I. Powder properties. Dry. Technol. *26*, 726–737.
HAUSNER, H.H. 1967. Friction conditions in a mass of metal powder. Int. J. Powder Metall. *3*, 7–13.
HOGAN, S.A., MCNAMEE, B.F., O'RIORDAN, E.D. and O'SULLIVAN, M. 2001a. Emulsification and microencapsulation properties of sodium caseinate/carbohydrate blends. Int. Dairy J. *11*, 137–144.
HOGAN, S.A., MCNAMEE, B.F., O'RIORDAN, E.D. and O'SULLIVAN, M. 2001b. Microencapsulating properties of sodium caseinate. J. Agric. Food Chem. *49*, 1934–1938.
HOGAN, S.A., O'RIORDAN, E.D. and O'SULLIVAN, M. 2003. Microencapsulation and oxidative stability of spray-dried fish oil emulsions. J. Microencapsul. *20*, 675–688.
JAFARI, S.M., ASSADPOOR, E., BHANDARI, B. and HE, Y. 2008a. Nano-particle encapsulation of fish oil by spray drying. Food Res. Int. *41*, 172–183.
JAFARI, S.M., ASSADPOOR, E., HE, Y. and BHANDARI, B. 2008b. Encapsulation efficiency of food flavours and oils during spray drying. Dry. Technol. *26*, 816–835.
JINAPONG, N., SUPHANTHARIKA, M. and JAMNONG, P. 2008. Production of instant soymilk powders by ultrafiltration, spray drying and fluidized bed agglomeration. J. Food Eng. *84*, 194–205.
KOLANOWSKI, W., LAUFENBERG, G. and KUNZ, B. 2004. Fish oil stabilisation by microencapsulation with modified cellulose. Int. J. Food Sci. Nutr. *55*, 333–343.
KROKIDA, M.K., ZOGZAS, N.P. and MAROULIS, Z.B. 1997. Modelling shrinkage and porosity during vacuum dehydration. Int. J. Food Sci. Technol. *32*, 445–458.
LIU, X.D., ATARASHI, T., FURUTA, T., YOSHII, H., AISHIMA, S. and OHKAWARA, M. 2001. Microencapsulation of emulsified hydrophobic flavors by spray drying. Dry. Technol. *19*, 1361–1374.
MADENE, A., JACQUOT, M., SCHER, J. and DESOBRY, S. 2006. Flavour encapsulation and controlled release – a review. Int. J. Food Sci. Technol. *41*, 1–21.
MCCLEMENTS, D.J. 2005. *Food Emulsions: Principles, Practice, And Techniques*, 2nd Ed., CRC Press, Boca Raton, FL.
NATALIE, D.R., RGIA, A.O., MIYOUNG, S. and MOHAMMED, H.M. 2009. A systemic review of the roles of N-3 fatty acids in health and disease. J. Am. Diet. Assoc. *109*, 668–679.
PATTEN, G.S., AUGUSTIN, M.A., SANGUANSRI, L., HEAD, R.J. and ABEYWARDENA, M.Y. 2009. Site specific delivery of microencapsulated fish oil to the gastrointestinal tract of the rat. Dig. Dis. Sci. *54*, 511–521.
RAMAKRISHNAN, S., FERRANDO, M., ACENA-MUNOZ, L., DE LAMO-CASTELLVI, S. and GUELL, C. 2013. Fish oil microcapsules from o/w emulsions produced by premix membrane emulsification. Food Bioprocess Technol. *6*, 3088–3101.
REINECCIUS, G.A. 2004. The spray drying of food flavors. Dry. Technol. *22*, 1289–1324.
ROSENBERG, M., KOPELMAN, I.J. and TALMON, Y. 1985. A scanning electron microscopy study of microencapsulation. J. Food Sci. *50*, 139–144.
SALEM, N., SIMOPOULOS, A.P., GALLI, C., LAGARDE, M. and KNAPP, H. 1998. Proc. 2nd Congr ISSFAL on Fatty acids and lipids from cell biology to human disease. Lipids *31*(Suppl. 1), 11–32.
SARKAR, N. and WALKER, L.C. 1995. Hydration – dehydration properties of methylcellulose and hydroxypropylmethylcellulose. Carbohydr. Polym. *27*, 177–185.
SHEN, Z., AUGUSTIN, M.A., SANGUANGSRI, L. and CHENG, L.J. 2010. Oxidative stability of microencapsulated fish oil powders stabilized by blends of chitosan, modified starch, and glucose. J. Agric. Food Chem. *58*, 4487–4493.
SIMOPOULOS, A.P., LEAF, A. and SALEM, N., JR. 1999. Essentiality of and recommended dietary intakes for omega-6 and omega-3 fatty acids. Ann. Nutr. Metab. *43*, 127–130.
SOOTTITANTAWAT, A., YOSHII, H., FURUTA, T., OHKAWARA, M. and LINKO, P. 2003. Microencapsulation by spray drying: Influence of emulsion size on the retention of volatile compounds. J. Food Sci. *68*, 2256–2262.
SOUZA, A.S., BORGES, S.V., MAGALHAES, N.F., RICARDO, H.V., CEREDA, M.P. and DAIUTO, E.R. 2009. Influence of spray drying conditions on the physical properties of dried pulp tomato. Food Science and Technology *29*, 291–294.
STONE, N.J. 1997. Fish consumption, fish oil, lipids, and coronary heart disease. Am. J. Clin. Nutr. *65*, 1083–1086.
TONON, R.V., GROSSO, C.R.F. and HUBINGER, M.D. 2011. Influence of emulsion composition and inlet air temperature on the microencapsulation of flaxseed oil by spray drying. Food Res. Int. *44*, 282–289.
U.S. PHARMACOPEIA. 2000. National formulatory (USP 24 NF 19), Rockville, MD.
VERARDO, V., FERIOLI, F., RICIPUTI, Y., IAFELICE, G., MARCONI, E. and CABONI, M.F. 2009. Evaluation of lipid oxidation in spaghetti pasta enriched with long chain n-3 polyunsaturated fatty acids under different storage conditions. Food Chem. *114*, 472–477.
WALTON, D.E. 2000. The morphology of spray-dried particles a qualitative view. Dry. Technol. *18*, 1943–1986.
WANG, S. and LANGRISH, T. 2010. The use of surface active compounds as additives in spray drying. Dry. Technol. *28*, 341–348.