CHAPTER 7

CHARACTERIZATION OF SPRAY DRIED TUNA OIL EMULSIFIED IN TWO-LAYERED INTERFACIAL MEMBRANES

7.1 Abstract

Tuna oil-in-water emulsions containing droplets stabilized by lecithin-chitosan membranes were produced using a two-stage process. Corn syrup solids were added to the emulsions and then the emulsions were spray dried, which produced a powder consisting of smooth spheroid powder microcapsules (diameter = 5-30 μm) containing small tuna oil droplets (diameter <1 μm) embedded within a carbohydrate wall matrix. The powders had relatively low moisture contents (< 3%), high oil retention levels (> 85%) and rapid water dispersibility (< 2 minute). The structure of the microcapsules was unaffected by drying temperature from 165 to 195°C ($P \geq 0.05$). This results can demonstrated that a novel interfacial engineering technology, based on production of multilayer membranes around oil droplets, is effective for producing spray dried encapsulated tuna oil. The powdered tuna oil produced by this method has good physicochemical properties, which may lead to its more widespread utilization as a food additive.

7.2 Introduction

Microencapsulation of materials susceptible to oxidation has been shown to significantly retard oxidation (Lin *et al*., 1995; Hogan *et al*., 2001a; 2001b; Keogh *et al*., 2001; Baik *et al*., 2004). Microencapsulation is a process whereby particles of sensitive or bioactive materials are covered with a thin film of a coating or wall material (Dziezak, 1988). The hydrophobic core material is usually homogenized in the presence of an aqueous solution containing an emulsifier (*e.g.,*

surfactant, phosopholipid or biopolymer) that forms a protective coating around the oil droplets, and then wall materials are mixed with the resulting emulsion. The emulsion is then dried to remove the water (*e.g.,* by spray or freeze drying), which leads to the formation of oil droplets surrounded by emulsifier molecules that are entrapped within a wall matrix (Sheu and Rosenberg, 1995; Hogan *et al*., 2001a; 2001b; Kagami *et al*., 2003).

A stable emulsion containing small oil droplets is critical for successful microencapsulation. It is therefore important to select an appropriate emulsifying system, as well as the conditions required to obtain a stable emulsion before the drying process. The results from the previous chapter (Chapter 5 and 6) has shown that oil-in-water emulsions with improved stability to environmental stresses and oxidative stability can be produced using an electrostatic layer-by-layer deposition technique that produces oil droplets that are coated by multiple-layered interfacial membranes. This technique involves forming multilayer interfacial membranes around oil droplets by depositing charged biopolymers onto the surfaces of oppositely charged surfactant-coated oil droplets. Successful microencapsulation also requires utilization of a wall material that forms a continuous matrix between the oil droplets in the powder particles. This wall material is usually composed of relatively low molecular weight carbohydrates, such as corn syrup solids and/or maltodextrin (Lin *et al*., 1995; Sheu and Rosenberg, 1995; Pauletti and Amestoy, 1999; Hardas *et al*., 2000; Heinzelmann *et al*., 2000). The previous results (Chapter 5) have shown recently that corn syrup solids (CSS, DE 36) can be added to oil-in water emulsions at fairly high concentrations ($\leq$ 25 wt%) without appreciably affecting emulsion stability and rheology. From the results, utilization of the multilayer interfacial membrane emulsion system in combination with corn syrup solids may prove to be an effective means of improving the stability of microencapsulated oils. Spray drying is the most

popular technique to prepare microcapsules of good quality (Dziezak, 1988; Pegg and Shahidi, 1999). The objective of the current study was to examine the impact of spray drying on the properties and dispersibility of encapsulated tuna oil powders.

7.3 Materials and Methods

7.3.1 Materials

Powdered chitosan ("medium molecular weight", ≈ 250 kDa) was purchased from the Sigma-Aldrich Chemical Co. (St. Louis, MO). As stated by the manufacturer the properties of this chitosan were: viscosity of 1 wt% solution in 1 wt% acetic acid = 200-800 Cps; degree of deacetylation = 75%-85%; maximum moisture content = 10 wt%; maximum ash content = 0.5 wt%. Powdered lecithin (Ultralec P; acetone insolubles, 97%; moisture, 1.0 wt%, consist primarily of phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol) was donated by ADM-Lecithin (Decatur, IL). Corn syrup solids (DRI SWEET®36, Code 335249; dextrose equivalent, 36; molecular weight, 0.5 kDa; total solids, 97.2 wt%; moisture, 2.8 wt%; ash, 0.2 wt%) was obtained from Roquette America. Inc. (Keokuk, IA). Degummed, bleached and deodorized tuna oil was obtained from Maruha Co. (Utsunomiya, Japan; 16 wt% EPA; 14.1 wt% DHA; PV, 0.35± 0.01 mmol/kg oil; TBARS, 0.12±0.01 mmol/kg oil; no tocopherol). Analytical grade sodium acetate (CH_3COONa), hydrochloric acid (HCl) and sodium hydroxide (NaOH) were purchased from the Sigma Chemical Co. (St. Louis, MO). Distilled and deionized water was used for the preparation of all solutions.

7.3.2 Methods

7.3.2.1 Solution Preparation

A stock buffer solution was prepared by dispersing 2 mM of sodium acetate and 98 mM of acetic acid in water and then adjusting the pH to 3.0. An emulsifier solution was

prepared by dissolving 3.53 wt% lecithin into stock buffer solution. The emulsifier solution was sonicated for 1 min at a frequency of 20 kHz, amplitude of 70% and duty cycle of 0.5 s (Model 500, sonic disembrator, Fisher Scientific, Pittsburgh, PA) to disperse the emulsifier. The pH of the solution was adjusted to 3.0 using HCl or NaOH, and then the solution was stirred for about 1 h to ensure complete dissolution of the emulsifier. A chitosan solution was prepared by dissolving 1.5 wt% powdered chitosan in sodium acetate-acetic acid buffer solution. A corn syrup solids solution was prepared by dispersing 50 wt% corn syrup solids in sodium acetate-acetic acid buffer solution.

7.3.2.2 Liquid Emulsion Preparation

Tuna oil-in-water emulsions were prepared containing 5 wt% tuna oil, 1 wt% lecithin, 0.2 wt% chitosan and 20 wt% corn syrup solid (DE 36). A concentrated tuna oil-in-water emulsion (15 wt% oil, 3 wt% lecithin) was made by blending 15 wt% tuna oil with 85 wt% aqueous emulsifier solution (3.53 wt% lecithin) using a high-speed blender (M133/1281-0, Biospec Products, Inc., ESGC, Switzerland), followed by three passes at 5,000 psi through a single-stage valve homogenizer (APV-Gaulin, Model Mini-Lab 8.30H, Wilmington, MA). This primary emulsion was diluted with aqueous chitosan solution to form a secondary emulsion (5 wt% tuna oil, 1 wt% lecithin and 0.2 wt% chitosan). Any flocs formed in the secondary emulsion were disrupted by passing it once through a high-pressure valve homogenizer at a pressure of 4,000 psi (Ogawa *et al*., 2003a). Secondary emulsions containing 20 wt% corn syrup solids were prepared by mixing the initial secondary emulsions with corn syrup solids solutions. The emulsions were stored at 4°C prior to spray drying.

7.3.2.3 Spray Dried Emulsion Preparation

Spray drying was performed at a feed rate of 2.2 L/h at 165, 180 and 195°C inlet temperature using Niro spray dryer with a centrifugal atomizer (Nerco-Niro, Nicolas & Research Engineering Corporation, Copenhagen, Denmark). The powders were vacuumed and stored in a hermetically sealed laminated pouch at -40°C until analysis.

7.3.3.4 Aw and Moisture Content Measurement

The water activity of samples was measured by AquaLab Water Activity Meter (Series 3, Decagon Devices, Inc., Pullman WA) at 25°C. For moisture content, duplicate samples of approximately 2 g of powder were placed in an aluminum pan and dried for 24 h at 70°C and 29 in. Hg in vacuum oven (Fisher Scientific, Fairlawn, NJ). Moisture content was calculated from the weight difference (Baik *et al*., 2004).

7.3.3.5 Extraction of Free Oil

Fifteen-mL hexane was added to 2.5 g powder. The mixture was mixed with a vortex mixer (Fisher Vertex Genie 2, Scientific Industries, Inc, Bohemia) for 2 min and then centrifuged (Sorvall RC-5B Refrigerated Superspeed Centrifuge, Du Pont Company, Wilminngton, Delaware) at 8,000 rpm for 20 min. The supernatant was filtered, the filter paper (Whatman, Maidstone, Kent, U.K.) washed twice with hexane (Baik *et al*., 2004; Hardas *et al*., 2000) and hexane was evaporated in a rotary evaporator (RE 111 Rotavapor, Type KRvr TD 65/45, BUCHI, Switzerland) at 70°C, and the solvent-free extract was dried at 105°C. The amount of encapsulated oil was determined gravimetrically.

7.3.3.6 Extraction of Encapsulated Oil

Two mL of acetate buffer (pH 3.0) was added to 0.5 g powder free of surface oil and vertexed for 1 min. The

resulting solution was then extracted with 25 mL hexane/isopropanol (3:1 v/v). The tubes were then shaken for 15 min at 160 rpm using an automatic shaker (Innova 4080 Incubator Shaker, New Brunswick Scientific Co. Inc., NJ), and centrifuged for another 15 min. The clear organic phase was collected and the aqueous phase was re-extracted with the solvent mixture (Baik *et al*., 2004; Hardas *et al*., 2000). After filtration through anhydrous Na_2SO_4 the solvent was evaporated in a rotary evaporator (RE 111 Rotavapor, Type KRvr TD 65/45, BUCHI, Switzerland) at 70°C, and the solvent-free extract was dried at 105°C. The amount of encapsulated oil was determined gravimetrically.

7.3.3.6 Extraction of total oil.

Starting from intact dried powders, 2 mL of acetate buffer (pH 3.0) was added to 0.5 g powder and vortexed for 1 min. Total oil was extracted using the same method as described above for extraction of encapsulated oil.

7.3.3.7 Calculation of Microencapsulation Efficiency

From the quantitative determinations above detailed, the encapsulation efficiency (EE) was calculated as follows:
EE = Encapsulated oil (g/100 g powder) × 100 / total oil (g/100 g powder)

7.3.3.8 Color Measurement

The reflectance spectra of spray-dried emulsions were measured using a UV-visible spectrophotometer (UV-2101PC, Shimadzu Scientific Instruments, Columbia, MD). During the measurements, the dried emulsions were contained in a 0.5 cm path length measurement cell with a black back plate. Spectra were obtained over the wavelength range 380-780 nm using a scanning speed of 700 nm min^{-1}. Spectral reflectance measurements were made using an integrating sphere arrangement (ISR-260, Shimadzu Scientific Instruments,

Columbia, MD). The spectral reflectance of the emulsions was measured relative to a barium sulfate ($BaSO_4$) standard. The color of samples was reported in term of the *L, a, b* color system (McClements *et al*., 1998).

7.3.3.9 Lipid Oxidation Measurement

Lipid hydroperoxide was measured by a modifiled method of Mancuso *et al*. (1999) after an extraction step in which 0.3 mL of reconstituted emulsion (0.1 g of emulsion powder in 0.3 mL of acetate buffer) was added to 1.5 mL of isooctane-2-propanal (3:1 v:v) followed by vortexing three times for 10 s each and centrifuging for 2 min at 3400 g (Centrific^{TM} Centrifuge, Fisher Scientific, Fairlawn, NJ). Next, the organic phase (0.2 mL total volume containing 0.015 to 0.2 mL of lipid extract) was added to 2.8 mL of methanol-butanol (2:1 v:v), followed by 15 μL of thiocyanate solution (3.94 M) and 15 μL of ferrous iron solution (prepared by mixing 0.132 M $BaCl_2$ and 0.144 M $FeSO_4$ in acidic solution). The solution was vortexed, and the absorbance at 510 nm was measured after 20 min. Lipid hydroperoxide concentrations were determined using a cumene hydroperoxide standard curve.

7.3.3.10 Reconstituted Emulsion Droplet Diameter

The powder was reconstituted to 10 g solids/100 g reconstituted emulsion by dissolving 0.5 g powder in 4.5 mL of acetate buffer (pH 3.0). One hour after reconstitution, the emulsion was analyzed for oil droplet diameter distribution using a static light scattering instrument (Malvern Mastersizer Model 3.01, Malvern Instruments, Worcs., UK). To prevent multiple scattering effects the emulsions were diluted with pH-adjusted double-distilled water prior to analysis so the droplet concentration was less than 0.02 wt%.

7.3.3.11 Dispersibility of Dried Emulsion

A small sample (~0.3 mg/mL of buffer) of the emulsion powder was added to a continuously stirred buffer solution contained within the stirring chamber of a laser diffraction instrument (Malvern Mastersizer Model 3.01, Malvern Instruments, Worcs., UK). The dispersibility of the powdered emulsion was then assessed by measuring the change in mean particle diameter and concentration as a function of time.

7.3.3.12 Influence of Medium pH on Emulsion Properties

The powder (0.5 g) was dissolved in 4.5 mL acetate buffer at the desired pH (3 to 8). The emulsions were transferred into glass test tubes (internal diameter = 15 mm, height = 125 mm), which were then stored at room temperature prior to analysis. The particle size distribution of the emulsions was measured using the same conditions as described above, but diluting the emulsion with pH-adjusted water of the same pH as the original emulsion. The electrical charge (ξ-potential) of oil droplets in the emulsions was determined using a particle electrophoresis instrument (ZEM5003, Zetamaster, Malvern Instruments, Worcs., UK). The emulsions were diluted to a droplet concentration of approximately 0.008 wt% with pH-adjusted double-distilled water prior to analysis to avoid multiple scattering effects.

7.3.3.13 Scanning Electron Microscopy

Internal and surface morphology of the powders were evaluated by Scanning Electron Microscopy (SEM) using the method of Hardas *et al*. (2000). The images were viewed by scanning electron microscope at 3.0-5.0 kV (JEOL 5400, JEOL, Japan).

7.3.3.14 Statistical Analysis

All experiments were carried out in at least duplicate using freshly prepared samples and results are reported as the mean and standard derivation of these measurements.

7.4 Results and Discussion

7.4.1 Moisture and Water Activity

The final effect of drying a product is a lower moisture content along with a lower water activity (Barbosa-Canovas and Vega-Mercado, 1996). The moisture content (1 to 3%) and water activity (0.1 to 0.25) of spray dried emulsion powders decreased with increasing air inlet temperature from 165 to 180°C ($P<0.05$), but not above that ($P\geq0.05$, Table 10). These results are consistent with the observations of Maa *et al*. (1998) and Kelly *et al*. (2002), who found that the moisture content of spray dried products was highest when operated at lowest temperature. The maximum moisture specification of dried powders such as whole milk, cocoa and whole egg, is between 3% to 4% (Masters, 1991). In this study, the desire level of moisture content could be achieved even by operating the spray drier at the lowest air inlet temperature of 165°C (feed rate = 2.2 L/h).

7.4.2 Lipid Oxidation

Oxidation of oils is a major cause of their deterioration, and hydroperoxides formed by the reaction between oxygen and the unsaturated fatty acids are the primary products of this reaction (O'Brien, 2004). The hydroperoxide concentrations of the spray dried emulsified tuna oil at different drying temperatures are shown in Table 10. There was no effect of drying temperature on the hydroperoxides of the tuna oil powders ($P\geq0.05$). The concentration of hydroperoxides of tuna oil emulsion increased from 0.86 ± 0.13 mmol/kg oil in the original liquid emulsion to 2.79 ± 0.48 mmol/kg oil in the spray dried powder. During processing, tuna oil is exposed to air, high pressure and high temperature, which leads to an increase in lipid oxidation (Baik *et al*., 2004). For soybean oil, a

hydroperoxide concentration less than 5 mmol/kg oil has previously been shown to indicate a low degree of lipid oxidation (O'Brien, 2004). The relatively low hydroperoxide level in our fresh powder would therefore seem to indicate that the tuna oil was relatively stable to oxidation during the spray drying process.

7.4.3 Free Oil and Encapsulation Efficiency

The amount of "free oil" in powdered emulsions is usually defined as that part of the oil that can be extracted with organic solvents (Buma, 1971a). Nevertheless, it should be noted that the amount of free oil measured in an analytical test is highly dependent on the precise extraction conditions used. In a recent study, the "free oil" of powdered emulsions was considered to be equivalent to the hexane extractable oil (Danviriyakul *et al*., 2002). The amount of free oil in the powders (3.0-3.5 g/100 g powder) was found to be independent of air inlet temperature ($P \geq 0.05$, Table 1). This result is similar to those of Danviriyakul *et al*. (2002) who found that the matrix system, but not the drying temperature, affected the amount of free oil. The encapsulation efficiency (EE) reflects the presence of free oil on the surface of the particles within the powder and the degree to which the wall matrix can prevent extraction of internal oil through a leaching process (Hogan *et al*., 2001a). In this study, the EE values (85% to 87%) were unaffected by air inlet temperature ($P \geq 0.05$, Table 10). Previous workers have reported EE values from 0% to 95% depending on the type and composition of wall material, the ratio of core material to wall material, the drying process used, and the stability and physicochemical properties of the emulsions (Lin *et al*., 1995; Hardas *et al*., 2000; Heinzelmann *et al*., 2000; Velasco *et al*., 2000; Hogan *et al*., 2001a; 2001b; Baik *et al*., 2004). The EE value for the multilayer emulsion system used in our system was therefore towards the high end of previously reported EE values.

Table 10 Effect of inlet temperature on properties of spray dried tuna oil emulsion.

Measured Properties	Inlet Temperature (°C)		
	165	180	195
Moisture content (g water/100 g powder)	2.84± 0.05[a*]	1.63± 0.24[b]	1.68± 0.39[b]
Water activity (Aw)	0.24± 0.01[a]	0.19± 0.01[b]	0.19± 0.02[b]
Hydroperoxide (mmol / kg oil)	2.27± 0.64[a]	3.22± 0.44[a]	2.89±0.32[a]
Total oil (g/100 g powder)	21.36± 0.79[a]	21.21± 0.34[a]	21.69± 0.63[a]
Hexane-extractable oil (g/100 g powder)	3.21± 0.74[a]	2.94± 0.18[a]	3.31± 0.28[a]
Encapsulation efficiency (%)	18.77± 0.68[a]	17.92± 0.77[a]	18.41± 0.82[a]
Droplet mean diameter ($d_{3,2}$, μm)**	86.94± 3.89[a]	84.49± 3.64[a]	86.94± 3.80[a]
	0.36± 0.02[a]	0.38± 0.02[a]	0.37± 0.01[a]

*Within rows, means followed by different superscript letters differ significantly ($P<0.05$)

** Reconstituted emulsion, for original emulsion $d_{3,2} = 0.26 \pm 0.01$ μm.

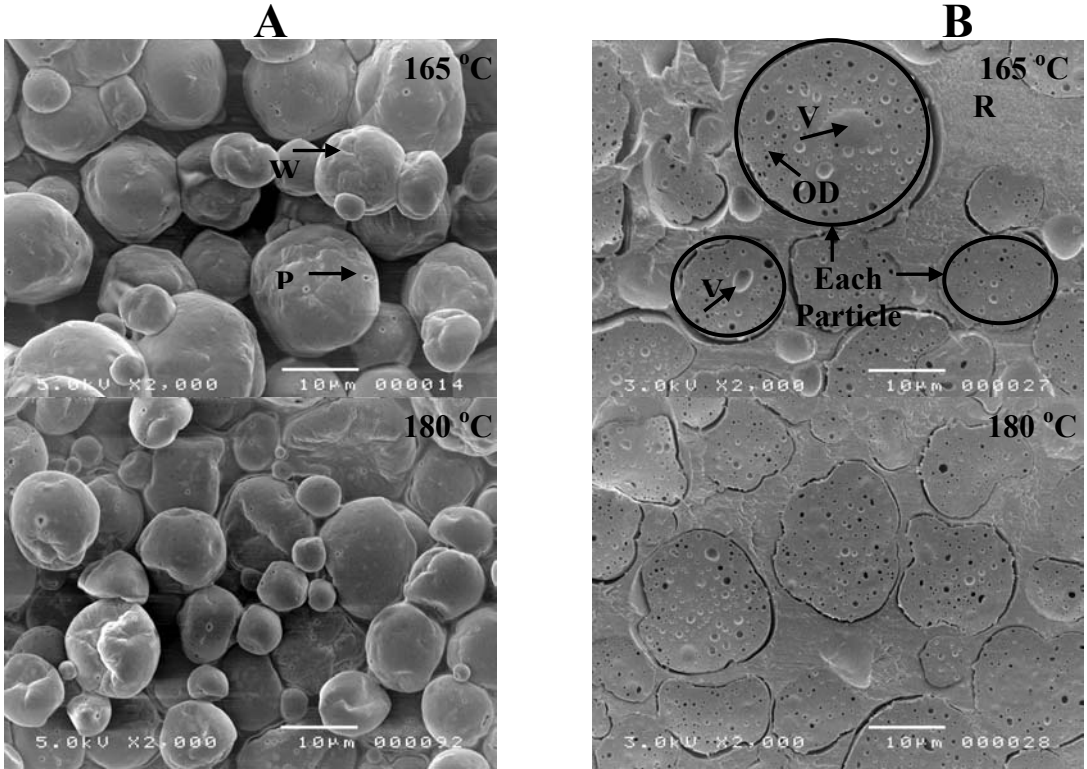
7.4.4 Powder Morphology

Many properties of microencapsulated systems, such as the retention of core materials, flow properties and the protection of core materials from the environment, depend on their internal microstructure (Rosenberg *et al*., 1985). It is therefore important to characterize the internal structure of the powder. Drying temperature had no effect on the structure of the powder based on our scanning electron microscopy study (Figure 27). All powder samples contained approximately spherical particles with a diameter in the range 5-30 μm (Figure

27A). Some wrinkles or dimples on the surface were observed. These results are consistent with Hardas *et al*. (2000) and Danviriyakul *et al*. (2002), who detected wrinkles or scars on the surface of the particles in spray dried anhydrous milk fat powders consisting of sodium caseinate and maltodextrin. Wrinkles on powder particle surfaces have also been reported for other carbohydrate based microcapsules (Moreau and Rosenberg, 1993; Sheu and Rosenberg, 1998; Kagami *et al*., 2003) and were attributed to the results of mechanical stresses induced by uneven drying at different parts of the liquid droplets produced during the early stages of drying (Moreau and Rosenberg, 1993; Sheu and Rosenberg, 1998), to the movement of the moisture during the nonsaturated surface drying period (Walton, 2000), and to the effect of a surface tension-driven viscous flow (Sheu and Rosenberg, 1998). The powder particles appeared to be largely free of cracks but some the presence of pores was observed. These pores probably arise in the last phase of the drying process due to uneven shrinkage of the material (Buma, 1971b). Porosity has been suggested to affect the extractability of fat from spray-dried milk powders through its effect on solvent penetration into the dry particles (Moreau and Rosenberg, 1993). The "free oil" measured using the solvent extraction procedure mentioned above may therefore have been due to the presence of these pores in the powder particles. A considerable part of the free oil consists of surface fat or of fat globules from the interior of the microcapsules (Buma, 1971b). Previous studies suggest that it may be possible to reduce the level of pore formation and free oil by using amorphous lactose in the wall material to act as a barrier that limits the diffusion of the apolar solvent into the particles (Moreau and Rosenberg, 1993).

To study the inner structure of spray-dried microcapsules and how the core material is organized within the dry matrix, the capsules must be opened. This procedure was carried out by dispersing powders in LR-White resin and then

incubating under UV-light to polymerize the resin. The blocks containing embedded powder were then sectioned using a microtome (Poter Blum Ultra-Microtome MT-2, Ivan Sorvall, Inc., Norwalk, CT). The inner structure of the capsules (Figure 27B) indicated that in all cases the core material was in the form of small droplets embedded in the wall matrix. The mean diameter of the droplets was between 0.2 and 1.0 μm, which was very similar to the dispersed phased droplets in the liquid emulsions prior to drying. The inner structure of the capsules, including void, was similar to that reported for other spray dried emulsions using carbohydrates-based wall materials (Moreau and Rosenberg, 1993; Hardas *et al*., 2000; Danviriyakul *et al*., 2002; Kagami *et al*., 2003). The formation of voids may be related to several mechanisms connected with atomization and spray drying, *e.g.* desorption of dissolved gases from the emulsion during drying and subsequent expansion associated with the temperature increase that occurs in the particles during the latter stages of drying, and the formation of steam bubbles within the drying atomization (Rosenberg *et al*., 1985).



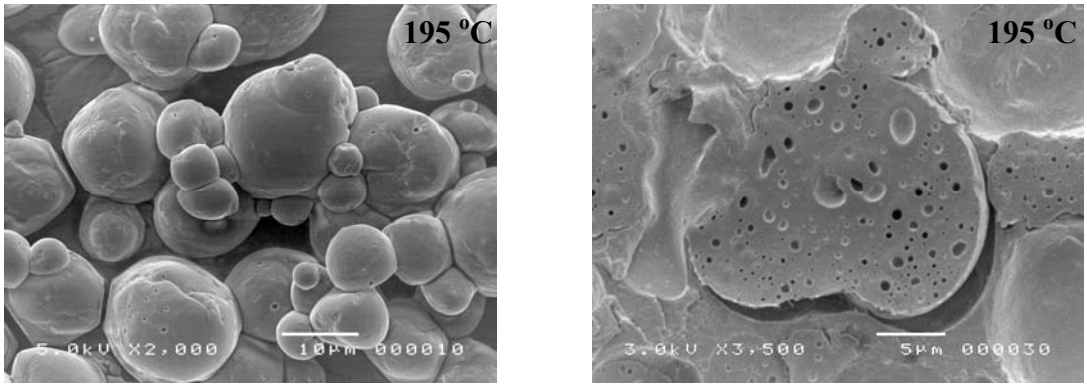


Figure 27 Representative micrographs showing the outer morphology (A) and inner structure (B) of tuna oil-containing capsules. W = wrinkle, P = pore, V = void, R = resin, OD = oil droplet or air cell.

7.4.5 Powder Color

Thermal treatments during the preservative processing can affect the quality of food product containing sugars through non-enzymatic browning reactions. Changes in the color of powders can be quantified by colorimetric measurements of tristimulus coordinates, such as *L*- (lightness), *a*- (redness and greenness) and *b*- (yellowness and blueness) values (Rittanathanalerk *et al*., 2005). Corn solid syrup (CSS) powder (DE 36) was used as a color control sample. There was no significant effect of drying temperature on the color (*L, a, b* values) of the spray-dried emulsions ($P \geq 0.05$, Table 11). Nevertheless, the *L*-value of the powdered emulsions was smaller (less light) and the *b*-value was higher (more yellow) than the CSS control, probably due to some non-enzymatic

browning reaction products occurring in the spray dried emulsions. For example, the chitosan is known to have a small protein fraction, which may have reacted with the sugar molecules in the CSS.

Table 11 Effect of inlet temperature on color of spray dried tuna oil emulsion

Inlet Temperature (°C)	Color Index		
	L	a	b
165	$97.7 \pm 0.3^{ab*}$	0.3 ± 0.1^{ab}	3.1 ± 0.4^{ab}
180	96.9 ± 0.6^{b}	-0.3 ± 0.5^{b}	5.2 ± 3.1^{b}
195	96.7 ± 0.8^{b}	0.2 ± 0.2^{ab}	5.3 ± 3.0^{b}
CSS**	99.1 ± 0.1^{a}	0.8 ± 0.1^{a}	1.7 ± 0.1^{a}

* Within columns, means followed by different superscript letters differ significantly ($P<0.05$)
** CSS was used for control

7.4.6 Reconstitution of Emulsions Powder

The rate and efficiency of powder dispersion is particularly important in the application of powdered food ingredients (Freudig *et al*., 1999; Hogekamp and Schubert, 2003). For this reason, we used a laser diffraction technique to provide information about the rate and efficiency of the dispersion of the spray dried emulsions. No significant increase in the mean oil droplet was observed after drying and reconstitution at all drying temperatures ($P\geq0.05$, Table 10). A bimodal distribution occurred in reconstituted emulsions (Figure 28), indicating the formation of some large particles. For studying the dispersibility, a small sample (~0.3 mg/mL of buffer) of the emulsion powder was added to a continuously stirred buffer solution contained within the stirring chamber of a laser diffraction instrument (Malvern Mastersizer Model 3.01, Malvern Instruments, Worcs., UK). The dispersibility of the powdered emulsion was then assessed by measuring the change

in mean particle diameter and droplet concentration of the system as a function of time (Figure 29). The droplet concentration increased with agitation time up to 3 min (0.016 %Vol) after which it reached a constant value. On the other hand, the mean particle diameter decreased from 0.5 ± 0.1 μm at the beginning to 0.3 ± 0.01 μm after 3 min stirring. The droplet concentration and mean particle size remained relatively constant at agitation times longer than 3 min. The large particles size and the lower droplets concentration observed at the beginning of the measurement indicates considerable clumping of the emulsion powder (Freudig *et al*., 1999). The rapid decrease in particle size and increase in droplet concentration indicated that the majority of the powder dissolved rather quickly giving a homogeneous suspension (Raphael and Rohani, 1996).

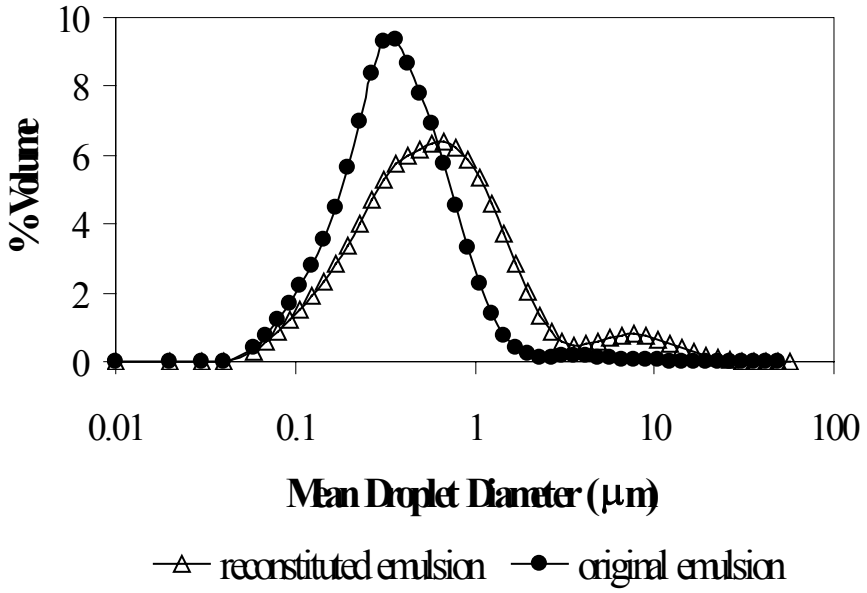


Figure 28 Mean droplet distribution of original and reconstituted tuna oil emulsion (5 wt% oil, 1 wt% lecithin, 0.2 wt% chitosan and 20 wt% corn syrup solid).

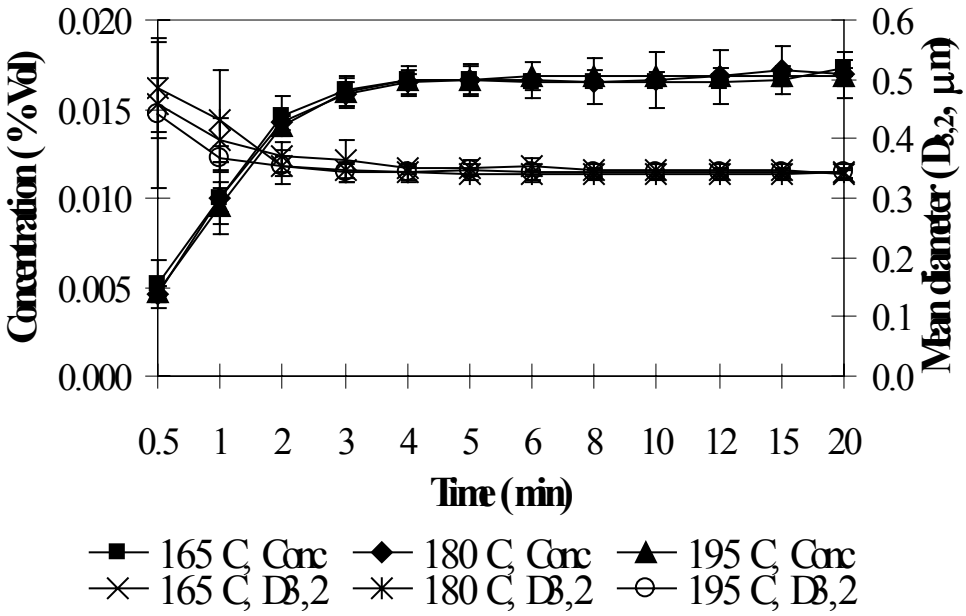


Figure 29 Influence of stirring time on mean particle diameter and concentration of emulsion after powdered was added to the stirring cell of laser diffraction instrument.

The influence of pH on the stability of reconstituted emulsions was examined. A series of dilute emulsions (10g solid/100 g emulsion) was prepared by dispersing powdered emulsions in a variety of aqueous solutions with different pH values (3 to 8). The emulsions were stored at room temperature for 24 h and then the electrical charge (ζ-potential) and mean particle diameter were measured (Figures 30 and 31). The ζ-potential of the reconstituted emulsions was positive at low pH values (<pH 8) but became negative at higher values (Figure 30). The cationic groups on chitosan typically have pK_a values around 6.3-7 (Schulz *et al*., 1998), hence the chitosan begins to lose some of its charge around this pH. Consequently, there may have been a weakening in the electrostatic attraction between the chitosan and the lecithin-coated droplets, which may have led to the release of some of the adsorbed chitosan. The reconstituted emulsions were stable to droplet aggregation

at pH <5.0, but highly unstable at higher pH values (Figure 31), as deduced from the large increase in mean particle diameter. The instability of the emulsions at higher pH values was probably because the magnitude of the ζ-potential was relatively low (Figure 30), which reduced the electrostatic repulsion between the droplets, leading to extensive droplet flocculation (Ogawa *et al*., 2003b). In addition, partial desorption of chitosan molecules from the droplet surfaces may have led to some bridging flocculation.

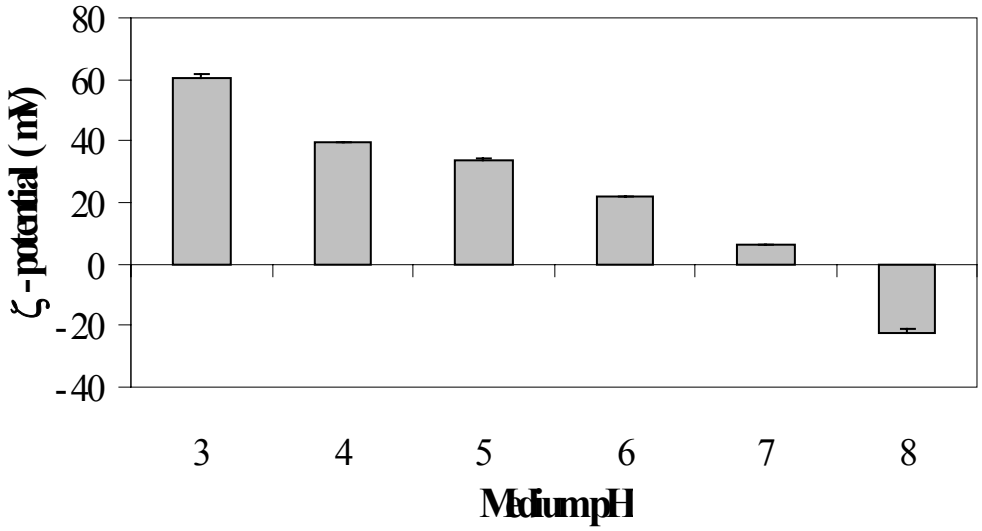


Figure 30 Influence of medium pH on electrical charge (ζ-potential) of reconstituted emulsion of spray dried powdered.

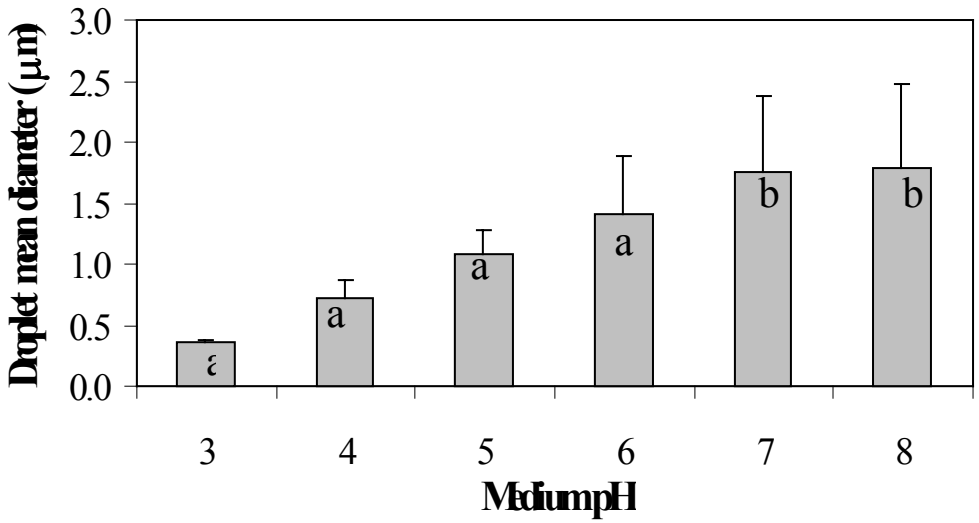


Figure 31 Influence of medium pH on mean particle diameter of reconstituted emulsion of spray dried powdered. For each column, means followed by different letters differ significantly ($P<0.05$).

7.5 Conclusion

This study has shown that high quality microencapsulated tuna oil can be produced by spray drying oil-in-water emulsions containing corn syrup solids and oil droplets surrounded by multilayer interfacial membranes (lecithin-chitosan). Spray drying produced powdered emulsions consisting of smooth spheroid powder particles (diameter = 5-30 μm) containing small tuna oil droplets (diameter <1 μm) embedded within a carbohydrate wall matrix. The structure of the microcapsules was unaffected by drying temperature (165 to 195°C). The powders had relatively low moisture contents (<3 %), high oil retention levels (>85 %) and rapid water dispersibility (<2 minute). This work has demonstrated that a novel interfacial engineering technology, based on production of multilayer membranes around oil droplets, is effective for producing spray-dried encapsulated tuna oil. The powdered tuna oil produced by this method has good physicochemical properties, which may lead to its more widespread utilization as a food additive.

7.6 Concision of Further Study

Spray dried powdered tuna oil with good physicochemical properties and dispersibility could be produced using multilayer membrane emulsions containing corn syrup solids as filler agent. The results demonstrated that a novel interfacial engineering technology, based on production of multilayer membranes around oil droplets, is effective for producing spray dried encapsulated tuna oil. However, physical properties of food powders, e.g. caking, stickiness, crystallization, dispersibility and solubility, can dramatically change upon storage and influence quality depending on temperature and moisture. The physical changes of the solid matrix of microencapsulated oils may affect the lipid oxidation of microencapsulated oils. Thus, the effects of relative humidity (RH) and temperature on dispersibility, color, and oxidative stability of spray dried microencapsulated tuna oil during storage were investigated.