Stability of Spray-Dried Tuna Oil Emulsions Encapsulated with Two-Layered Interfacial Membranes

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ω-3 Fatty acids have numerous health benefits, but their addition to foods is limited by oxidative rancidity. Spray-drying tuna oil-in-water emulsion droplets with a coating of lecithin and chitosan multilayer system could produce emulsion droplet interfacial membranes that are cationic and thick, both factors that can help control lipid oxidation. Physicochemical and oxidative stability of the spray-dried emulsions were determined as a function of storage temperature and relative humidity (RH). The combination of ethylenediaminetetraacetic acid (EDTA) and mixed tocopherols was able to increase the oxidative stability of dried emulsions. Lipid oxidation was more rapid during storage at low relative humidity (11% and 33% compared to 52% RH). At high moisture, physical modifications in the sample were observed, including reduced dispersibility and formation of brown pigments. Sugar crystallization or Maillard products produced at the higher humidities may have inhibited oxidation. Overall, spray-dried tuna oil-in-water emulsions stabilized by lecithin–chitosan membranes were more oxidatively stable than bulk oils and thus have excellent potential as an ω-3 fatty acid ingredient for functional foods.

KEYWORDS: Tuna oil; omega-3 fatty acids; spray-dry; lipid oxidation; relative humidity antioxidants; water activity; chitosan

INTRODUCTION

Tuna oils are a good source of ω-3 polyunsaturated fatty acids (ω-3 PUFAs), especially the long-chain ω-3 fatty acids EPA (eicosapentaenoic acid, C20:5 ω-3) and DHA (docosahexaenoic acid, C22:6 ω-3) (*1*). Long-chain ω-3 fatty acids are considered beneficial for growth and development throughout the life cycle and may play an important role in the prevention and treatment of coronary artery disease (arteriosclerosis), hypertension, arthritis, and immune response disorders (*2*−*4*). Moreover, DHA is important in the development of the central nervous system and eyes of infants and thus is considered to be a promising nutrient because of its beneficial effects on the retina and brain (*5*) Due to their beneficial health properties, ω-3 fatty acids have great potential as functional food ingredients.

Utilization of oils high in omega-3 fatty acids (ω-3 FA) in food is limited due to their high susceptibility to oxidation. Lipid oxidation can be reduced by addition of antioxidants and/or by microencapsulation of the oil (*6*−*9*). The oxidative stability of emulsified oil can also be increased by controlling emulsifier type, location and concentration (*10*−*13*). For example, when oil-in-water emulsion droplets are surrounded by cationic emulsifiers, prooxidant metals are repelled and lipid oxidation rates decrease (*11*, *14*, *15*).

Recently, our laboratory has shown that oil-in-water emulsions with improved stability to environmental stress can be produced by an electrostatic layer-by-layer deposition technique that produces oil droplets that are coated by multiple-layered interfacial membranes (*16*, *17*). The addition of corn syrup solids (a carbohydrate widely used in the microencapsulation of oils) increased the physicochemical stability of these emulsions system (*18*) and had no major impact on lipid oxidation rates (*15*). Spray-dried powdered tuna oil with good physicochemical properties and dispersibility was produced by use of multilayer membrane emulsions containing corn syrup solids as filler agent (*19*). This work 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.

As a constituent of most food systems, water plays a particularly important role in lipid oxidation. Water activity or relative humidity has been used to control lipid oxidation in food products (*20*). In general, lipid oxidation is lowest at water activity close to the water monolayer, which falls between 0.2 and 0.4 for most food. However, the rate of lipid oxidation increases rapidly when the water activity is either decreased below or increased above the monolayer (*20*, *21*). Numerous

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8366 *J. Agric. Food Chem.,* Vol. 53, No. 21, 2005

Klinkesorn et al.

studies have suggested that this generalized view does apply to a number of systems (*22*−*24*); however, contradicting reports also exist (*25*−*27*).

Physical and chemical properties of food powders, e.g., caking, stickiness, crystallization, dispersibility, solubility, and formation of Maillard products, can change during storage (*28*−*30*). The physical changes of the solid matrix of microencapsulated oils may affect the oil distribution by causing release of encapsulated oils, with released oil being more susceptible to oxidation (*31, 32*). Maillard reaction products can form during both drying and ambient storage of food powders (*9, 30*). Chitosan has a large number of free amino groups in its molecule, which could participate in the Maillard reaction (*33*). As a result of the Maillard reaction, the development of brown color, the formation of desirable or undesirable flavor, a change of texture, and a loss of nutritional value can occur (*30, 33*). However, the Maillard reaction products have been reported to act as antioxidants (*34, 35*).

In the present study we investigated the effects of relative humidity (RH) and temperature on dispersibility, color, and oxidative stability of spray-dried microencapsulated tuna oil.

MATERIALS AND METHODS

Materials. Powdered chitosan (medium molecular weight, deacetylation, 75%−85%) was purchased from Aldrich Chemical Co. (St. Louis, MO). Powdered lecithin (moisture, 1 wt %; phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol) was donated by ADM-Lecithin (Decatur, IL). Corn syrup solids [dextrose equivalent (DE) 36; average molecular mass ≈ 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; lipid hydroperoxides 0.35 ± 0.01 mmol/kg of oil; thiobarbituric acid-reactive substances 0.12 ± 0.01 mmol/kg of oil; no detectable tocopherols). Mixed tocopherol homologues (Covi-ox T-70, 14% α-tocopherol, 2% β-tocopherol, 60% γ-tocopherol, and 24% δ-tocopherol) was obtained from Cognis Corp. (Cincinnati, OH). Disodium ethylenediaminetetraacetic acid (EDTA) was purchased from Sigma Chemical Co. (St. Louis, MO). All other chemicals were reagent-grade or better. Distilled and deionized water was used for the preparation of all solutions.

Methods. *Solution Preparation.* A stock buffer solution was prepared by dispersing 2 mM sodium acetate and 98 mM acetic acid in water and then adjusting the pH to 3.0. An emulsifier solution was prepared by dispersing 3.5 wt % lecithin into the 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 dismembrator, Fisher Scientific, Pittsburgh, PA) to disperse the emulsifier. The pH of the solution was adjusted to 3.0 by use of HCl and/or NaOH, and then the solution was stirred for about 1 h to ensure complete dispersion of the emulsifier. A chitosan solution was prepared by dissolving 1.5 wt % powdered chitosan into the stock buffer solution. A corn syrup solids stock solution was prepared by dispersing 50 wt % corn syrup solids into the stock buffer solution.

*Oil-in-Water Emulsion Preparation (**Figure 1**).* 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) by use of a high-speed blender (M133/1281-0, Biospec Products, Inc., ESGC, Switzerland), followed by three passes at 5000 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 (10 wt % tuna oil, 2 wt % lecithin, and 0.4 wt % chitosan). Any flocs formed in the secondary emulsion were disrupted by one passage through a high-pressure valve homogenizer at a pressure of 4000 psi (*16*). Secondary emulsions containing corn syrup solids were prepared by mixing the initial secondary emulsions with corn syrup solids solutions. For evaluation of the effectiveness of antioxidants in the
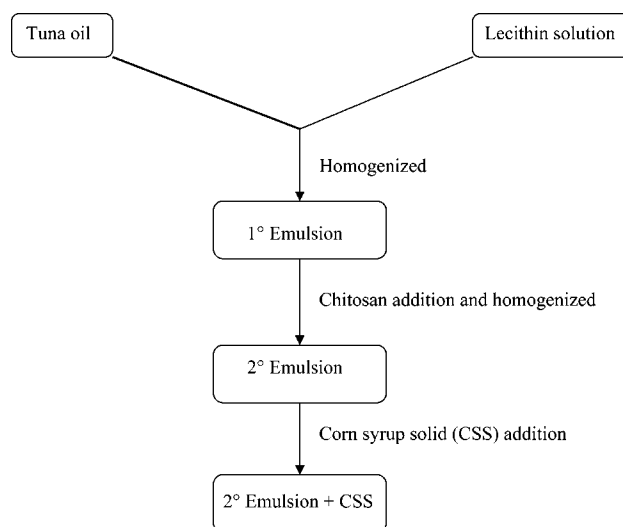

Figure 1. Preparation of tuna oil-in-water emulsions.

emulsions, the secondary (lecithin−chitosan-coated emulsion droplets) emulsions with 500 parts per million (ppm) mixed tocopherols (tocopherols were added to the tuna oil prior to emulsion preparation) and 12 μM EDTA (added directly to the secondary emulsions after emulsion preparation) were prepared. All emulsions were stored at 4 °C prior to spray-drying. Final tuna oil-in-water emulsions contained 5 wt % tuna oil, 1 wt % lecithin, 0.2 wt % chitosan, and 20 wt % corn syrup solid.

Spray-Dried Emulsion Preparation. Spray-drying was performed at a feed rate of 2.2 L/h at 180 °C inlet temperature by use of a Niro spray dryer with a centrifugal atomizer (Nerco-Niro, Nicolas & Research Engineering Corporation, Copenhagen, Denmark). The moisture content of the spray-dried powders was 1.6% ± 0.2% (grams of water/100 g of powder) in all samples. The final composition (dry basis) of the spray-dried fish oil powders was as follows: fish oil 19.1%, corn syrup solid (DE 36) 76.3%, lecithin 3.8%, and chitosan 0.8%.

Storage Studies. Spray-dried fish oil powders (3 g each) were placed in open glass beakers (20 mL, 3.5 cm diameter) and the beakers were placed in desiccators with relative humidity (RH) of 11%, 33%, or 52%, prepared from saturated LiCl, MgCl$_2$, and Mg(NO$_3$)$_2$ solutions (*36*). All desiccators were then incubated at 37 °C. For studies on the effect of storage temperature, some sample were incubated at 20 °C. Samples were withdrawn at frequent time intervals for analyses.

Characterization of Spray-Dried Powders. Spray-dried fish oil powders were reconstituted by dissolving 0.5 g of powder in 4.5 mL of acetate buffer at pH 3.0 (*37*). One day after reconstitution, the emulsion was analyzed for oil droplet diameter distribution by use of a static light scattering instrument (Malvern Mastersizer Model 3.01, Malvern Instruments, Worcestershire, U.K.). To prevent multiple scattering effects, the emulsions were diluted with pH-adjusted acetate buffer prior to analysis so the droplet concentration was less than 0.02 wt %.

Dispersibility of the spray-dried emulsions was determined by adding ∼0.3 mg of powder/mL of acetate buffer within the stirring chamber of a laser diffraction instrument (Malvern Mastersizer Model 3.01, Malvern Instruments, Worcestershire, U.K.). The dispersibility of the powdered emulsion was then assessed by measuring the change in mean particle diameter (d_{43}) and obscuration (the fraction of light lost from the main laser beam when the sample was introduced) as a function of time.

The reflectance spectra of spray-dried powders were measured on a UV−visible spectrophotometer (UV-2101PC, Shimadzu Scientific Instruments, Columbia, MD) equipped with an integrating sphere (ISR-260, Shimadzu Scientific Instruments, Columbia, MD). The dried emulsions were placed in a 0.5 cm path length measurement cell with a black background. Spectra were obtained over the wavelength range 380−780 nm at a scanning speed of 700 nm min^{-1}. The spectral reflectance of the dried emulsions was measured relative to a barium sulfate (BaSO$_4$) standard. The color of samples was reported in term

Lipid Oxidation in Spray-Dried Tuna Oil Encapsulated by Chitosan

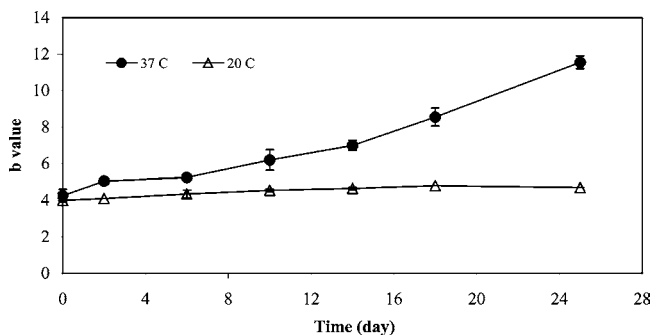*J. Agric. Food Chem.,* Vol. 53, No. 21, 2005 **8367**

Figure 2. Change in *b*-value of spray-dried tuna oil powders [19.1% fish oil, 76.3% corn syrup solid (DE 36), 3.8% lecithin, and 0.8% chitosan] during storage in 11% relative humidity (RH) at 20 or 37 °C.

of the *L*, *a*, *b* color system. The *L*, *a*, *b* values of dried emulsions were calculated from its spectral reflectance by use of color matching functions (*38*).

Lipid hydroperoxides were measured by a modified method (*11*) after an extraction step in which 0.3 mL of reconstituted emulsion (100 mg 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 Centrifuge, Fisher Scientific, Fairlawn, NJ). Next, the organic phase (0.2 mL total volume containing 0.015−0.2 mL of lipid extract depending on the oxidation state of the lipid) 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 from a cumene hydroperoxide standard curve.

Thiobarbituric acid-reactive substances (TBARS) were measured according to Mei et al. (*39*). A thiobarbituric acid (TBA) solution was prepared by mixing 15 g of trichoroacetic acid, 0.375 g of TBA, 1.76 mL of 12 N HCl, and 82.9 mL of H_2O. One hundred milliliters of TBA solution was mixed with 3 mL of 2% butylated hydroxytoluene in ethanol, and 2 mL of this solution was mixed with 1 mL of reconstituted emulsion (5 mg of emulsion powder in 1.0 mL of acetate buffer). The mixture was vortexed and heated in a boiling water bath for 15 min, cooled to room temperature, and centrifuged at 3400*g* for 25 min. Absorbance of the supernatant was measured at 532 nm. Concentrations of TBARS were determined from standard curves prepared with 1,1,3,3-tetraethoxypropane.

Statistics. All data represent the mean of six measurements of two different trials, and results are reported as the mean and standard derivation of these measurements. The data were subjected to the analysis of variance (ANOVA) univariate method. Comparison of means after the ANOVA test was performed by Duncan's multiple range test.

RESULTS AND DISCUSSION

Chemical Changes in the Spray-Dried Powders during Storage. The spray-dried powders in these experiments contain free amino groups (chitosan and phospholipid) and reducing sugars (corn syrup solids), which signifies possible participation in the Maillard reaction (*33*). Therefore, tristimulus color values *L*, *a*, and *b* were used as an index of the color changes in powders during storage at various temperature and relative humidity (RH) values. The *b*-value of the powder stored at 20 °C and 11% RH did not change significantly over 25 days (**Figure 2**). At higher temperature (37 °C), the *b*-values increased significantly (over 150%) from 0 to 25 days of storage. **Figure 3** shows the change in *b*-value as a function of relative humidity during storage at 37 °C. The powders stored at low humidity (11% and 33% RH) showed lower *b*-values than the powder stored at high humidity (52% RH) during 25 days of
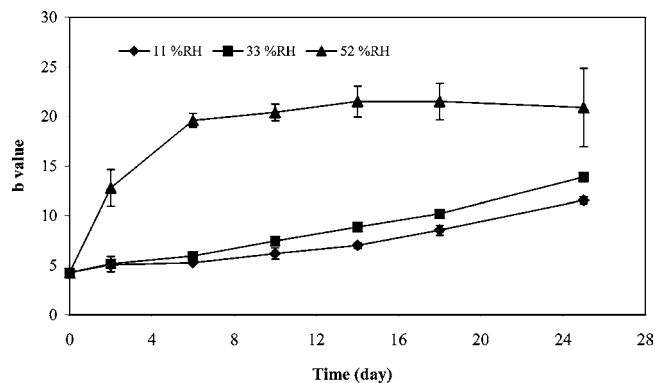

Figure 3. Change in *b*-value of spray-dried tuna oil powders [19.1% fish oil, 76.3% corn syrup solid (DE 36), 3.8% lecithin, and 0.8% chitosan] during storage at 37 °C at various relative humidity (RH) values.
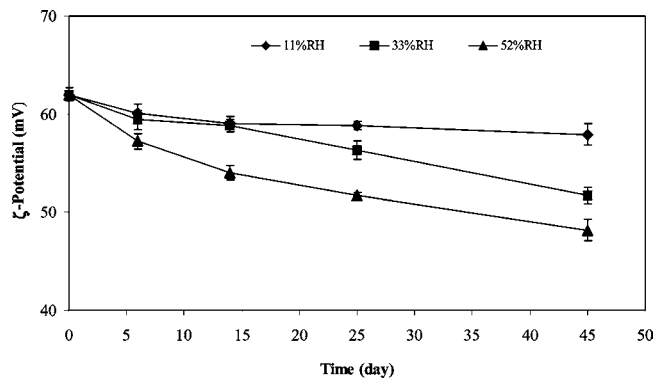

Figure 4. Influence of relative humidity (RH) on ζ-potential of reconstituted spray-dried tuna oil powders [19.1% fish oil, 76.3% corn syrup solid (DE 36), 3.8% lecithin, and 0.8% chitosan].

storage. No differences in *b*-values were observed between the samples stored at 11% RH and 33% RH ($p < 0.05$). For *L*-(lightness) values, the powders stored at low humidity (11% and 33% RH, *L*-values of 95.3 ± 0.1 and 93.8 ± 0.1, respectively) showed higher *L*-values than the powders stored at high humidity (52% RH, *L*-values of 56.8 ± 1.1) after 25 days of storage. The *a*-values of the powders stored at 11% and 33% RH did not change significantly within 25 days of storage (*a*-value ≈ 0.3 ± 0.2). At 52% RH, the *a*-value increased from 0.2 ± 0.1 at 0 days to 7.2 ± 3.2 after 25 days of storage.

If the Maillard reaction takes place between chitosan or phospholipids and reducing sugars (CSS), the amount of free amino groups at the surface of the emulsion droplets would be expected to decrease. Therefore, the electrical charge (ζ-potential) of emulsion droplets reconstituted in acetate buffer (pH 3.0) was measured (**Figure 4**). The ζ-potential of reconstituted emulsions from the powder decreased with increasing storage time and relative humidity. At high humidity (52% RH), the ζ-potential decreased from 62.0 ± 0.7 mV at day 0 to 48.2 ± 1.1 mV at 45 days of storage ($P \geq 0.05$). Similar results were reported by Tanaka et al. (*33*), who found that amount of free amino groups of chitosan decreased during Maillard reaction at 65 °C.

Oxidative Stability. Previous work has shown that lipid oxidation in spray-dried powders does not correlate well with free or extractable fat (*40*). Therefore the oxidative stability of dried powder in this study was determined on total lipids. The multilayer interfacial membrane on the dried emulsion droplets dramatically improved the oxidative stability of tuna oil when compared to bulk oil as indicated by both lipid hydroperoxide and TBARS formation (**Figure 5**, panels **a** and **b**, respectively).
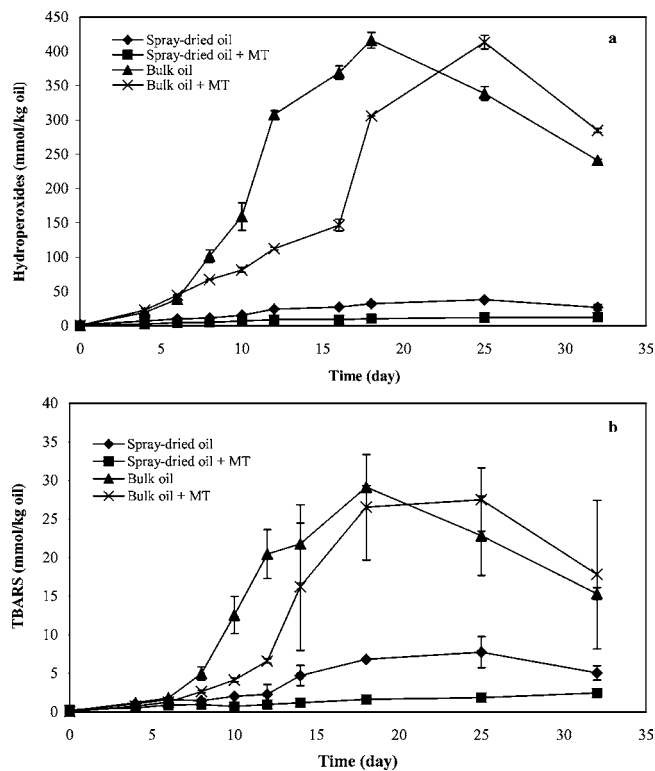
Figure 5. Formation of (**a**) lipid hydroperoxides and (**b**) TBARS in bulk oil and spray-dried tuna oil powders [19.1% fish oil, 76.3% corn syrup solid (DE 36), 3.8% lecithin, and 0.8% chitosan] with and without 500 ppm mixed tocopherol (MT) during storage at 37 °C and 33% RH.

These results are similar to those reported by Kagami et al. (*9*), who reported that a sodium caseinate—highly branched cyclic dextrin wall system on the emulsion droplets improved oxidative stability of fish oil. Mixed tocopherol (500 ppm) decreased (*P* < 0.05) lipid hydroperoxide and TBARS concentrations in both the stored bulk (8−18 days) and spray-dried (after 18 days) oils (**Figure 5**). Encapsulation of spray-dried tuna oil with the multilayer interfacial membrane was more effective at retarding oxidation than was mixed tocopherol in the bulk oil. **Figure 6** shows lipid hydroperoxide and TBARS formation as a function of RH in the spray-dried fish oil powders. Lipid hydroperoxide formation was on the order of 33% RH > 11% RH > 52% RH. (**Figure 6a**). A similar trend was observed in the development of TBARS during storage, although TBARS in the samples at 11% and 33% RH became similar after 25 days of storage (**Figure 6b**).

Several investigators have suggested that lipid oxidation in foods is lowest at water activities close to the water monolayer, which falls between 0.2 and 0.4 for many foods, due to a decrease of the prooxidant activity of transition metals, increasing quenching of free radicals and singlet oxygen and/or retardation of hydroperoxide decomposition (*21, 26*). However, other studies have suggested that this generalized view does not apply to a number of other foods. For instance, encapsulated orange oil and methyl linoleate have been found to be more oxidatively stable in very dry (0% RH) and very moist (>75% RH) states, whereas more rapid oxidation was found in the intermediate range (*20, 27*). For this study, lipid oxidation in the powders increased when the RH increased from 11% to 33%. These results are similar to the data from Minemoto et al. (*41*), who found that oxidation of encapsulated linoleic acid was higher as RH increased. The low oxidation of the spray-dried tuna oil powder at 52% RH observed in this study was potentially due to chemical and physical changes in the powders
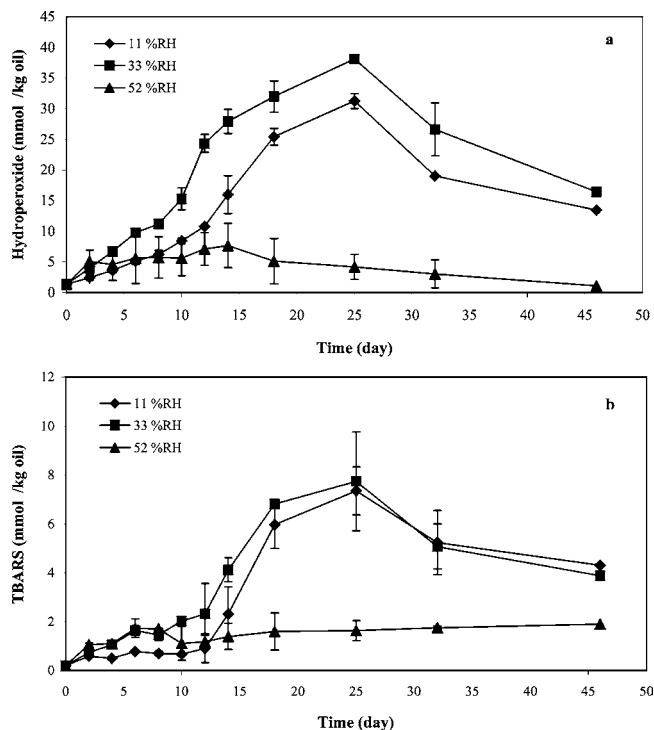

Figure 6. Formation of (**a**) lipid hydroperoxides and (**b**) TBARS in spray-dried tuna oil powders during storage at 37 °C at various relative humidity (RH) values.

during storage. First, lipid oxidation products may polymerize to produce brown-colored oxypolymers in the presence of other compounds such as amines, amino acids, and antioxidants. These polymerization reactions can lead to inactivation of free radicals, a pathway that can influence oxidation rates and produce significant color formation in powders (*42*). Second, Maillard reaction products are well documented to reduce lipid oxidation (*34, 43, 44*). Wijewickreme et al. (*43*) reacted L-lysine with D-glucose, D-fructose, and D-ribose under different conditions and produced Maillard reaction products with potent antioxidant activity in the linoleic acid emulsions. For the present work, brown color formation (as seen from increase in *b*-value, **Figure 3**) was observed in the powder stored at 52% RH. The formation of these brown pigments by one or both of the above pathways could explain why lipid oxidation was slower in powders stored at high relative humidity. In addition, when the spray-dried tuna oil powder was stored at 52% relative humidity, the corn syrup solids were observed to crystallize (the glass transition temperature of corn syrup solids with a dextrose equivalent of 36 at 52% RH is ∼ 6 °C; *36*), causing the powder particles to collapse. Collapse of spray-dried powders is linked to a decrease in porosity; thus, lipid oxidation could be lower due to a decrease in the diffusivity of O_2 (*30*).

Previous work (*15*) found that the combination of mixed tocopherol and EDTA is effective at inhibiting lipid oxidation in oil-in-water emulsions stabilized by lecithin—chitosan membrane. The physical nature of dried emulsions could affect the antioxidant activity of tocopherols and EDTA in a different manner than the oil-in-water emulsions (*45*); therefore, the effectiveness of mixed tocopherols and EDTA on lipid oxidation in the spay-dried powders was reevaluated. Both lipid hydroperoxide (**Figure 7a**) and TBARS (**Figure 7b**) formation in the spray-dried emulsions containing 500 ppm mixed tocopherols and 12 µM EDTA were lower than in samples with no added antioxidants (shown in **Figure 6**) at all RH values studied
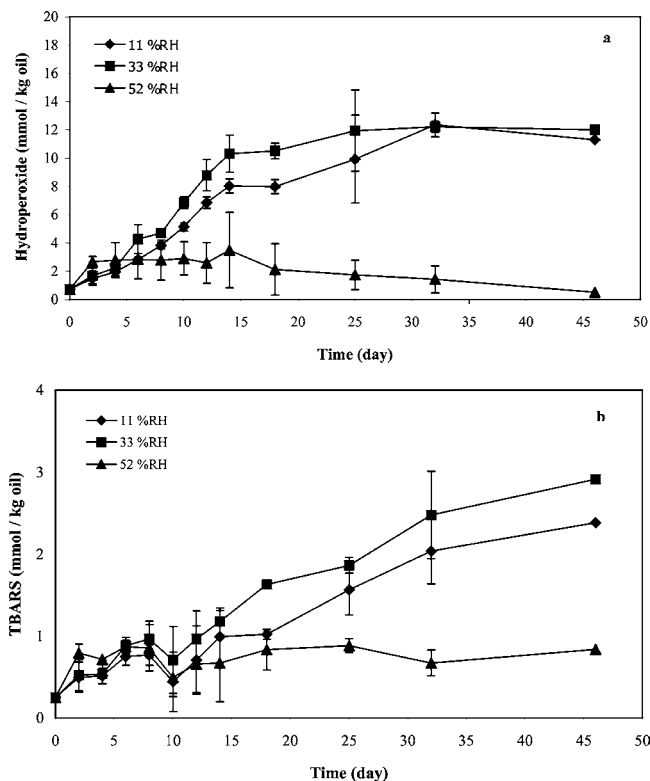
Lipid Oxidation in Spray-Dried Tuna Oil Encapsulated by Chitosan

J. Agric. Food Chem., Vol. 53, No. 21, 2005 **8369**

Figure 7. Formation of (**a**) lipid hydroperoxides and (**b**) TBARS in spray-dried tuna oil powders containing 500 ppm mixed tocopherol and 12 μM EDTA during storage at 37 °C at various relative humidity (RH) values.
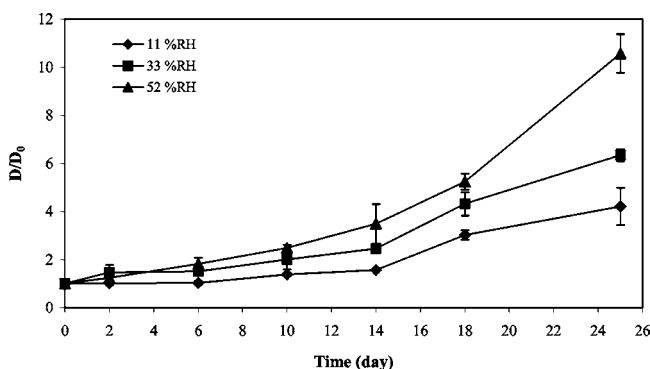

Figure 8. Mean droplet diameter of reconstituted spray-dried tuna oil powders (5 wt % oil, 1 wt % lecithin, 0.2 wt % chitosan, and 20 wt % corn syrup solid) during storage at 37 °C at various relative humidity (RH) values.

after 46 days of storage, indicating that the antioxidants were also effective in spray-dried emulsions.

Reconstituted Emulsion Droplet Size and Dispersion. The mean particle diameter of the emulsion droplets in spray-dried powders was measured during storage at 37 °C at various relative humidities after they were reconstituted in acetate buffer (pH 3.0) (**Figure 8**). The mean particle diameter measured at a particular storage time (D) was normalized relative to the initial mean particle diameter (D_0) for that sample to highlight differences between the samples. The mean particle diameter increased for all samples with the order of increase being 52% > 33% > 11% RH. One of most important factors that determines the stability of dried powders is the temperature. **Figure 9** shows that mean particle diameter of the reconstituted spray dried powders increased during storage at 11% RH and 37 °C, while no change in mean droplet diameter was observed at 20 °C. At a storage temperature of 37 °C, mean droplet
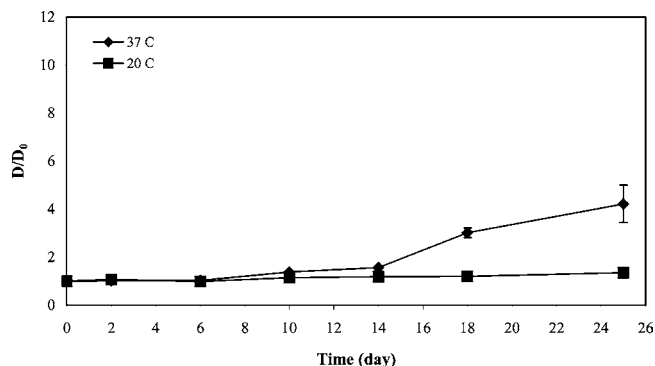

Figure 9. Mean droplet diameter of reconstituted spray-dried tuna oil powders (5 wt % oil, 1 wt % lecithin, 0.2 wt % chitosan and 20 wt % corn syrup solid) during storage in 11% relative humidity (RH) at 20 and 37 °C.
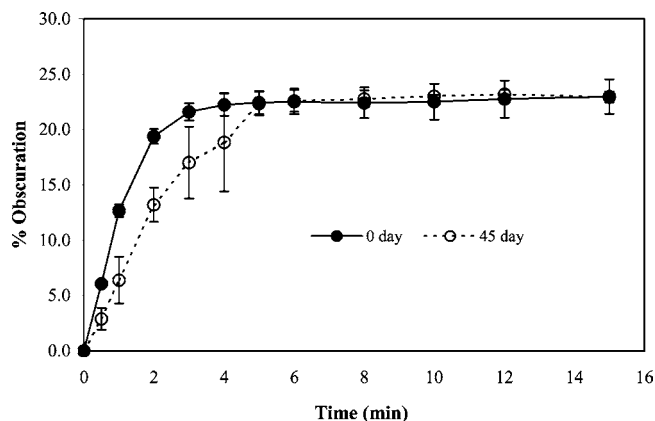

Figure 10. Influence of stirring time on obscuration of spray-dried tuna oil powders stored at 20 °C and 11% relative humidity for 0 or 45 days. Obscuration was measured as a function of time after powders were added to the stirring cell of laser diffraction instrument.

diameter began to increase after 18 days of storage and increased by more than 200% after 25 days of storage. The increase in mean particle diameter observed during storage at 37 °C could be attributed to the Maillard reaction, which would lead to the formation of polymers between carbohydrates and amine groups at the emulsion droplet interface.

The rate and efficiency of powder dispersion is also an important property of powdered food ingredients (*46, 47*). The rate and efficiency of powder dispersion was determined by a laser diffraction technique. A small sample ($\sim$0.3 g/mL of buffer) of the spray-dried emulsion powder was added to a continuously stirred buffer solution contained within the measurement chamber of a laser diffraction instrument (Malvern Mastersizer model 3.01, Malvern Instruments, Worcestershire, U.K.). The dispersibility of the powdered emulsion was then assessed by measuring the change in droplet obscuration (**Figure 10**) and mean particle diameter (d_{43}) (**Figure 11**) of the system as a function of time. The d_{43} value is particularly sensitive to the presence of large particles in a sample, while the obscuration is sensitive to the total amount of material dispersed in the fluid. When samples were tested on the same day that they were spray-dried (0 day), the droplet obscuration (**Figure 10**) increased steeply with agitation time up to 3 min, after which it reached a fairly constant value (21.6% $\pm$ 0.8%). In addition, d_{43} (**Figure 11**) (*1*) decreased from over 10 μm at the beginning to less than 1 μm after 1 min of stirring. The droplet obscuration and mean particle size remained relatively constant at agitation times longer than 3 min. The large particles size and the lower droplet
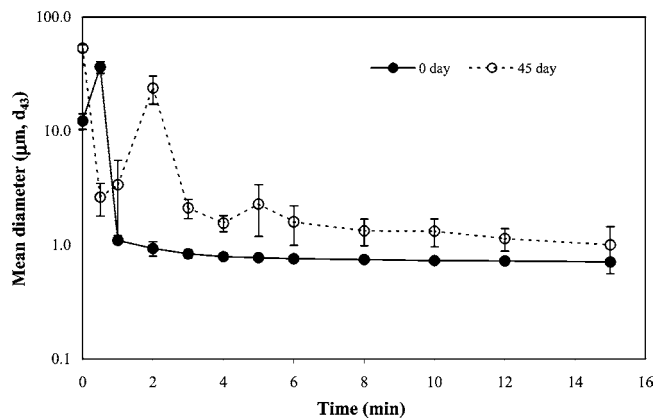
Figure 11. Influence of stirring time on mean particle diameter of spray-dried tuna oil powders stored at 20 °C and 11% relative humidity for 0 or 45 days. Particle diameter (d_{43}) was measured as a function of time after powders were added to the stirring cell of the laser diffraction instrument.

concentration observed at the beginning of the measurement indicates considerable clumping of the emulsion powder (*46*). The rapid decrease in particle size and increase in droplet obscuration indicated that the majority of the powder dispersed rather quickly, producing a homogeneous suspension (*48*). For the powders stored at 20 °C in 11% RH for 45 days, the droplet obscuration took slightly longer (5 min) to reach a constant value (22.4% ± 1.1%) than for the fresh powders (3 min). In addition, the large particles took longer to fully disappear (3 min) in the 45-day-old sample than in the 0-day-old sample (1 min). Nevertheless, the final mean particle diameter of the fully redispersed emulsions was similar to the droplet diameter of the original liquid emulsions ($d_{32} = 0.34 \pm 0.01$ μm) for both the 0- and 45-day-old samples stored at 11% RH. The increase in dispersion time for the 45-day-old sample may have been caused by the collapse of the particle structure during storage. A decrease in the surface area of powder particles exposed to the surrounding water phase is known to decrease dispersion rates. Moreover, a decrease in particle porosity would limit the access of water to the interior of the powder particles (*30*).

In conclusion, this study has shown that the oxidative stability of dried tuna oil-in-water emulsion droplets coated by a lecithin−chitosan multilayer system is higher than that of bulk tuna oil. The combination of mixed tocopherol and EDTA was effective at increasing the oxidative stability of spray-dried multilayer emulsion. In the present study, spray-dried tuna oil emulsion did not follow classical oxidation patterns of minimum oxidation at intermediate water activity but instead has minimal oxidation at high water activity. Decreasing oxidation rates at high water activity could be due to physical and chemical changes in the powder, including sugar crystallization that could decrease oxygen permeability to the lipid and/or the formation of Maillard reaction products that can act as antioxidants. Overall, these data suggest that spray-dried tuna oil-in-water emulsions stabilized by lecithin−chitosan membranes may be used to produce ω-3 fatty acids that are more oxidatively stable than bulk oils.

LITERATURE CITED

(1) Klinkesorn, U.; H-Kittikun, A.; Chinachoti, P.; Sophanodora, P. Chemical transesterification of tuna oil to enriched omega-3 polyunsaturated fatty acids. *Food Chem.* **2004**, *87*, 415−421.

(2) Harris, W. S. Omega-3 long-chain PUFA and triglyceride lowering: minimum effective intakes. *Eur. Heart J. Suppl.* **2001**, *3*, D59−D61.

(3) Harris, W. S. Fish oil supplementation: evidence for health benefits. *Cleveland Clin. J. Med.* **2004**, *71*, 209−221.

(4) Uauy, R.; Valenzuela, A. Marine oils: the health benefits of n-3 fatty acids. *Nutrition* **2000**, *16*, 680−684.

(5) Shibasaki, A.; Irimoto, Y.; Kim, M.; Saito, K.; Sugita, K.; Baba, T.; Honjyo, I.; Moriyama, S.; Sugo, T. Selective binding of docosahexaenoic acid ethyl ester to a silver ion loaded porous hollow-fiber membrane. *J. Am. Oil Chem. Soc* **1999**, *76*, 771−775.

(6) Lin, C.-C.; Lin, S.-Y.; Hwang, L. S. Microencapsulation of squid oil with hydrophilic macromolecules for oxidative and thermal stabilization. *J. Food Sci.* **1995**, *60*, 36−39.

(7) Heinzelmann, K.; Franke, K.; Velasco, J.; Marquez-Ruiz, G. Microencapsulation of fish oil by freeze-drying techniques and influence of process parameters on oxidative stability during storage. *Eur. Food Res. Technol.* **2000**, *211*, 234−239.

(8) Velasco, J.; Dobarganes, C.; Marquez-Ruiz, G. Oxidation of free and encapsulated oil fractions in dried microencapsulated fish oils. *Grasas Aceites (Sevilla)* **2000**, *51*, 439−446.

(9) Kagami, Y.; Sugimura, S.; Fujishima, N.; Matsuda, K.; Kometani, T.; Matsumura, Y. Oxidative stability, structure, and physical characteristics of microcapsules formed by spray drying of fish oil with protein and dextrin wall materials. *J. Food Sci.* **2003**, *68*, 2248−2255.

(10) Fomuso, L. B.; Corredig, M.; Akoh, C. C. Effect of emulsifier on oxidation properties of fish oil-based structured lipid emulsions. *J. Agric. Food Chem.* **2002**, *50*, 2957−2961.

(11) Mancuso, J. R.; McClements, D. J.; Decker, E. A. The effects of Surfactant type, pH, and chelators on the oxidation of salmon oil-in-water emulsions. *J. Agric. Food Chem.* **1999**, *47*, 4112−4116.

(12) Donnelly, J. L.; Decker, E. A.; McClements, D. J. Iron-catalyzed oxidation of menhaden oil as affected by emulsifiers. *J. Food Sci.* **1998**, *63*, 997−1000.

(13) Hu, M.; McClements, D. J.; Decker, E. A. Lipid oxidation in corn oil-in-water emulsions stabilized by casein, whey protein isolate, and soy protein isolate. *J. Agric. Food Chem.* **2003**, *51*, 1696−1700.

(14) Mei, L.; Decker, E. A.; McClements, D. J. Evidence of iron association with emulsion droplets and its impact on lipid oxidation. *J. Agric. Food Chem.* **1998**, *46*, 5072−5077.

(15) Klinkesorn, U.; Sophanodora, P.; Chinachoti, P.; McClements, D. J.; Decker, E. A. Increasing the oxidative stability of liquid and dried tuna oil-in water emulsions with electrostatic layer-by-layer deposition technology. *J. Agric. Food Chem.* **2005** (in press).

(16) Ogawa, S.; Decker, E. A.; McClements, D. J. Production and characterization of O/W emulsions containing cationic droplets stabilized by lecithin−chitosan membranes. *J. Agric. Food Chem.* **2003**, *51*, 2806−2812.

(17) Ogawa, S.; Decker, E. A.; McClements, D. J. Influence of environmental conditions on the stability of oil in water emulsions containing droplets stabilized by lecithin-chitosan membranes. *J. Agric. Food Chem.* **2003**, *51*, 5522−5527.

(18) Klinkesorn, U.; Sophanodora, P.; Chinachoti, P.; Decker, E. A.; McClements, D. J. Encapsulation of emulsified tuna oil in two-layered interfacial membranes prepared using electrostatic layer-by-layer deposition. *Food Hydrocolloids* **2005**, *19*, 1044−1053.

(19) Klinkesorn, U.; Sophanodora, P.; Chinachoti, P.; Decker, E. A.; McClements, D. J. Characterization of spray-dried tuna oil emulsified in two-layered interfacial membranes prepared using electrostatic layer-by-layer deposition. *Food Res. Int.* **2005** (in press).

(20) Nelson, K. A.; Labuza, T. P. *ACS Symposium Series 500*; American Chemical Society: Washington, DC; pp 93−103.

(21) Velasco, J.; Dobarganes, C.; Marquez-Ruiz, G. Variables affecting lipid oxidation in dried microencapsulated oils. *Grasas Aceites (Sevilla)* **2003**, *54*, 304−314.

(22) Rockland, L. B.; Swarthout, D. M.; Johnson, R. A. Studies on English (Persian) walnuts, *Juglans regia*. 3. Stabilization of kernels. *Food Technol.* **1961**, *15*, 112−116.

Lipid Oxidation in Spray-Dried Tuna Oil Encapsulated by Chitosan

J. Agric. Food Chem., Vol. 53, No. 21, 2005 **8371**

(23) Maloney, J. F.; Labuza, T. P.; Wallace, D. H.; Karel, M. Autoxidation of methyl linoleate in freeze-dried model systems. I. Effect of water on autocatalyzed oxidation. *J. Food Sci.* **1966**, *31*, 878−884.

(24) Quast, D. G.; Karel, M. Effects of environmental factors on oxidation of potato chips. *J. Food Sci.* **1972**, *37*, 584−588.

(25) Baik, M.-Y.; Suhendro, E. L.; Nawar, W. W.; McClements, D. J.; Decker, E. A.; Chinachoti, P. Effects of antioxidants and humidity on the oxidative stability of microencapsulated fish oil. *J. Am. Oil Chem. Soc.* **2004**, *81*, 355−360.

(26) Ponginebbi, L.; Nawar, W. W.; Chinochoti, P. Effect of relative humidity on lipid oxidation in freeze-dried emulsions. *Grasas Aceites (Sevilla)* **2000**, *51*, 348−354.

(27) Kahl, J. L.; Arta, W. E.; Schanus, E. G. Effects of relative humidity on lipid oxidation in a model system. *Lipids* **1988**, *23*, 275−279.

(28) Roos, Y. H. Importance of glass transition and water activity to spray drying and stability of dairy powders. *Lait* **2002**, *82*, 475−484.

(29) Beristain, C.; Azuara, E.; Tamayo, T.-T.; Vernon-Carter, E. J. Effect of caking and stickiness on the retention of spray-dried encapsulated orange peel oil. *J. Sci. Food Agric.* **2003**, *83*, 1613−1616.

(30) Thomas, M. E. C.; Scher, J.; Desobry-Banon, S. Milk powders aging: effect on physical and functional properties. *Crit. Rev. Food Sci.* **2004**, *44*, 297−322.

(31) Shimada, Y.; Roos, Y.; Karel, M. Oxidation of methyl linoleate encapsulated in amorphous lactose-based food model. *J. Agric. Food Chem.* **1991**, *39*, 637−641.

(32) Rosenberg, M.; Kopelman, I. J.; Talmon, Y. Factors affecting retention in spray-drying microencapsulation of volatile materials. *J. Agric. Food Chem.* **1990**, *38*, 1288−1294.

(33) Tanaka, M.; Huang, J. R.; Chiu, W. K.; Ishizaki, S.; Taguchi, T. Effect of the Maillard reaction on functional properties of chitosan. *Nippon Sulsan Gakkaishi* **1993**, *59*, 1915−1921.

(34) Elizalde, B. E.; Dalla Rosa, M.; Lerici, C. R. Effect of maillard reaction volatile products on lipid oxidation. *J. Am. Oil Chem. Soc.* **1991**, *68*, 758−762.

(35) Friedman, M. Food browning and its prevention: An overview. *J. Agric. Food Chem.* **1996**, *4*, 631−653.

(36) Rahman, S. *Food Properties Handbook*; CRC Press: Boca Raton, FL, 1995; p 500.

(37) Hardas, N.; Danviriyakul, S.; Foley, J. L.; Nawar, W. W.; Chinachoti, P. Accelerated stability studies of microencapsulated anhydrous milk fat. *Lebensm.-Wiss. Technol.* **2000**, *33*, 506−513.

(38) McClements, D. J.; Chantrapornchai, W.; Clydesdale, F. Prediction of food emulsion color using light scattering theory. *J. Food Sci.* **1998**, *63*, 935−939.

(39) Mei, L.; McClements, D. J.; Wu, J.; Decker, E. A. Iron-catalyzed lipid oxidation in emulsion as affected by surfactant, pH and NaCl. *Food Chem.* **1998**, *61*, 307−312.

(40) Hogan, S. A.; O'Riordan, E. D.; O'Sullivan, M. Microencapsulation and oxidative stability of spray-dried fish oil emulsions. *J. Microencapsulation* **2003**, *20*, 675−688.

(41) Minemoto, Y.; Adachi, S.; Matsuno, R. Comparison of oxidation of methyl linoleate encapsulated with gum arabic by hot-air-drying and freeze-drying. *J. Agric. Food Chem.* **1997**, *45*, 4530−4534.

(42) Zamora, R.; Hidalgo, F. J. Coordinate contribution of lipid oxidation and maillard reaction to the nonenzymatic food browning. *Crit. Rev. Food Sci.* **2005**, *45*, 49−59.

(43) Wijewickreme, A. N.; Krejpcio, Z.; Kitts, D. D. Hydroxyl scavenging activity of glucose, fructose, and ribose-lysine model maillard products. *J. Food Sci.* **1999**, *64*, 457−461.

(44) McGookin, B. J.; Augustin, M. Antioxidant activity of casein and Maillard reaction products from casein-sugar mixtures. *J. Dairy Res.* **1991**, *58*, 313−320.

(45) McClements, D. J.; Decker, E. A. Lipid Oxidation in oil-in-water emulsions: impact of molecular environment on chemical reactions in heterogeneous food systems. *J. Food Sci.* **2000**, *65*, 1270−1282.

(46) Freudig, B.; Hogekamp, S.; Schubert, H. Dispersion of powders in liquids in a stirred vessel. *Chem. Eng. Process.* **1999**, *38*, 525−532.

(47) Hogekamp, S.; Schubert, H. Rehydration of food powders. *Food Sci. Technol. Int.* **2003**, *9*, 223−235.

(48) Raphael, M.; Rohani, S. On-line estimation of solids concentrations and mean particle size using a turbidimetry method. *Powder Technol.* **1996**, *89*, 157−163.