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Oxidative Stability of Microencapsulated Fish Oil Powders Stabilized by Blends of Chitosan, Modified Starch, and Glucose

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Various indices of lipid oxidation were used to assess the oxidative stability of microencapsulated fish oil powders prepared from tuna oil-in-water emulsions (pH 4.9 or 6.0) containing chitosan, an emulsifying starch, and glucose. There were good agreements among the induction period for oxidation under accelerated conditions (80 °C, 5 bar oxygen), the development of oxidation volatile markers from fish oil (namely, propanal, 1-penten-3-ol, 1-penten-3-one, 2,4-(*Z*,*E*)-heptadienal, and 2,4-(*E*,*E*)-heptadienal), and the loss of eicosapentanoic acid (EPA) and docosahexanoic acid (DHA) over four weeks of storage at 25 °C. All indices of oxidation showed that powders prepared from emulsions at pH 6.0 were more stable to oxidation than corresponding formulations at pH 4.9. It is suggested that the increased electrostatic interactions between the chitosan and emulsifying starch at the higher pH contributed to the increased stability of the microcapsule powders.

KEYWORDS: Microencapsulation; chitosan; starch; oxidation; tuna oil

INTRODUCTION

Microencapsulation has been used to protect marine oils containing long chain polyunsaturated fatty acids (LCPUFA) against oxidation. Biopolymer composites and coacervates have been found to be useful for the stabilization of unsaturated oils and the formulation of microencapuslated oils (*1*, *2*). Among the components used for the stabilization of PUFA oils are proteins, proteins in combination with sugars or polysaccharides; maltodextrin (MD); starch and nonstarch polysaccharides such as gums, celluloses, and chitosan; and modified starch alone or in combination with gum arabic (*3*−*6*). Many patents also cover the delivery of the PUFA into food systems (*1*). In this work, we examined formulations of spray dried oil powders containing mixtures of chitosan, an emulsifying starch (*n*-octenyl succinate-derivatized starch, OSA-starch) and glucose.

Chitosan, a polysaccharide comprising mainly β (1−4)-linked glucosamine and *N*-acetylglucosamine units which are formed by deacetylation of chitin, was chosen as part of the formulation because of its antioxidative, film forming (*7*), and emulsifying properties (*8*). Previous studies have shown that spray-dried emulsions of tuna oil stabilized by chitosan−lecithin mixtures were more resistant to oxidation than the bulk oil (*9*). Encapsulants comprising mixtures of chitosan and MD or whey protein isolate (WPI) were also found to protect spray-dried emulsions containing tuna oil (*10*). Calcium alginate beads coated with chitosan have been used to encapsulate shark liver oil and control oil leakage from capsules (*11*).

The emulsifying OSA-starch is able to stabilize the emulsion droplets (*12*). This starch has been combined with glucose syrup for microencapsulation of fish oil (*13*). In our study, glucose was added to the formulation as the bulking agent. Sugars used in combination with emulsifying biopolymers can improve encapsulation properties. The free-fat content of oil powders stabilized by protein−sugar mixtures was reduced with increasing dextrose equivalence of the sugar (*14*).

A cationic polymer (chitosan, pK_a ~6.2) in combination with an anionic starch (OSA-starch) was chosen because of the potential for these biopolymers to associate and form electrostatic complexes. Complexation of oppositely charged biopolymers may be capitalized upon for encapsulation (*15*). Biopolymer complexes confer improved stability to capsules and stabilize sensitive cores against degradation (*16*). Adjustment of pH alters the charge and conformation of biopolymers and affects the formation of biopolymer complexes and properties of the biopolymer mixtures (*17*). Combinations of chitosan and OSA-starch as matrix components have not been examined, although they have been singly used to microencapsulate LCPUFA in combination with other ingredients.

The evaluation of the oxidative stability of microencapsulated oil powders has been conducted using a variety of analytical methods. These include analysis of the secondary oxidation volatiles by head space (HS) or solid phase microextraction (SPME) in conjunction with hydroperoxide values, sensory evaluations, thiobarbituric acid reactive substances (TBARS) and loss of LCPUFA (*5*, *18*−*22*). The determination of HS oxidation volatiles of microencapsulated powders provides a possible indicator of off-odors when microcapsules are exposed to the human nose. The analysis of nonvolatile oxidation products by solid phase extraction and high performance size exclusion chromatography has also been performed (*23*). Another valid rapid and reproducible method for assessing oxidation under accelerated conditions is the use of the Rancimat apparatus at high temperature (*24*). The OXIPRES instrument also may be used as an alternative for assessing the oxidative stability of dry foods under accelerated conditions. The stability is

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usually measured at a high temperature and high oxygen pressure (*25*). Because of possible changes in oxidation mechanisms at high temperature and oxygen pressure, it is useful to determine the correlation between oxidative stability measured using the OXIPRES method against more traditional markers of oxidation.

This study aimed at investigating the oxidative stability of microencapsulated tuna oil powders (30% and 40% oil in powder) formulated with mixtures of chitosan, OSA-starch, and glucose as wall materials. The ratio of chitosan/oil in all of the formulations was kept constant. The spray-dried microencapsulated powders were prepared from tuna oil-in-water emulsions made at two pH levels of 4.9 or 6.0. The properties of the emulsions and the oxidative stability of microcapsules were determined. Oxidative stability was assessed by SPME−GC/MS analyses of the secondary volatile oxidation makers of LCPUFA. The five volatile compounds measured were propanal, 1-penten-3-ol, 1-penten-3-one, 2,4-(*Z*,*E*)-heptadienal, and 2,4-(*E*,*E*)-heptadienal. These compounds have been reported as secondary oxidation products from omega-3 FAs (*21*, *26*−*29*). Levels of eicosapentanoic acid (EPA) and docosahexanoic acid (DHA) remaining in the microcapsules were also determined by GC. The induction period under accelerated oxidation conditions was tested using an OXIPRES. The oxidative stability was related to the properties of the emulsions.

MATERIALS AND METHODS

Materials. All ingredients used in the formulations were purchased from commercial suppliers: tuna oil (DHA25NFood) (Nu-Mega Ingredients Pty Ltd., Australia), water-soluble chitosan (Shandong AK Biotech Ltd., China), dextrose monohydrate (glucose) (Penford, Australia), and an OSA-starch (HiCap 100) (National Starch Food Innovation, Australia). The SPME fiber of carboxen/polydimethylsiloxane (Carboxen-PDMS), methyl tricosanoate (≥99.0%), and tetrahydrofuran (THF) were purchased from Supelco (Bellefone, PA, USA) and Sigma-Aldrich (Melbourne, Australia), respectively.

Production of Microencapsulated Tuna Oil Powders. Four powders were prepared on the basis of formulations containing 30 and 40% oil-in-powder. The ratios of chitosan/lucose/Hi-Cap/oil were 1:1:2.7:2 and 1:1:1:2 for the above-mentioned powders, respectively. Duplicate productions (trials 1 and 2) were carried out.

Chitosan dissolved in deionized water (5%, w/w) was hydrated overnight. Glucose solution (5%, w/w) was prepared by dissolving dextrose monohydrate in deionized water. Equal weights of chitosan and glucose solutions were mixed, and their pH values were adjusted to 4.9 or 6.0, respectively, using 1 M sodium hydroxide, before they were subjected to high shear homogeneous mixing (Silverson Machines, Inc., Maryland, USA). HiCap 100, dissolved in 60 °C water (20%, w/w), was added directly into the chitosan/glucose solution, and the mixture was stirred for 5 min. The aqueous dispersions used for the preparation of emulsions for 30% oil-in-powder contained 1.88% chitosan, 1.88% glucose, and 5.00% HiCap, while that for the 40% oil in powder contained 2.22% chitosan, 2.22% glucose, and 2.22% HiCap. Samples of the individual aqueous dispersions and the final aqueous mixtures were taken to measure turbidity.

The required amount of tuna oil was dispersed into the aqueous phase using the Silverson mixer to form a pre-emulsion before it was subjected to two-stage homogenizing at 350/100 bar (APV Manton-Gaulin, APV, Germany). The emulsion was spray dried at 180 °C inlet and 80 °C outlet temperatures (Drytec lab spray dryer Tonbridge, UK).

Turbidity of Encapsulant Components in the Aqueous Phase. The turbidity of aqueous dispersions of chitosan, Hi-Cap, and mixtures of these at the concentrations used for preparing the emulsions were measured. This analysis was carried out using an UV-1201 V Spectrophotometer (Shimadzu Corporation, Tokyo, Japan) in a 1 cm path length optical cell against Milli-Q water at 600 nm. Duplicate assessments were carried out for each of the prepared duplicate solutions and reported as the mean and standard deviation of the quadruplicate data.

Zeta Potential. The zeta potential of emulsion preparations was measured using Malvern Nano ZS 3600 (Malvern Instruments Ltd., Worcestershire, UK). Prior to analysis, emulsions were diluted to 0.05 wt % using buffer solution at the same pH (4.9 or 6.0).

Particle Size. The particle size of the freshly made emulsion was measured using Malvern Mastersizer Hydro 2000G (Malvern Instruments Ltd., Worcestershire, UK). A particle refractive index of 1.456 and an absorption of 0 were assumed for all emulsions. Samples were stirred and added to circulating distilled water to obtain an obscuration of ∼15%. All measurements were carried out in duplicate at room temperature (∼22 °C).

Microstructure. The emulsion was visualized using an Olympus BH2 Brightfield microscope (Anax Pty Ltd.) at 10 and 40 times magnification.

Storage of Microencapsulated Powders. Microencapsulated powders (15 g, trial 1; 25 g, trial 2) and fresh tuna oil (80 g, trial 1; 200 g, trial 2) were stored in 1 L clear plastic jars (air tightly closed lid) in a dark box at 25 °C. Oils and powders were sampled for SPME-GC/MS analysis of volatile compounds before and after two and four weeks of storage for the two trial powder samples. Fatty acid methyl ester (FAME) analyses were carried out for fresh oils and for stored oil and powder samples after four weeks of storage.

Determination of Total and Free Oil-in-Powder. The total oil content of the powders was determined using the Schmid−Bondzyndki−Ratzlaff method (*30*). The sample (0.5 g) was digested with 36.5% w/w hydrochloric acid in a Mojonnier tube at 100 °C for 10 min. Ethanol (96%, w/v) was added after cooling the sample to room temperature. Solvent extraction of this sample was carried out with 25 mL of diethyl ether, continued with 25 mL of petroleum ether, and then with a mixture of 5 mL of ethanol, 25 mL of diethyl ether, and 25 mL of petroleum ether. The solvent was removed using a rotary evaporator, and the residue was oven-dried at 102 °C for 1 h before weighing.

The free oil in the powders was estimated using the published method (*31*) except that petroleum ether was used in place of carbon tetrachloride. The sample (10.0 g) was agitated with petroleum ether (50 mL) for 15 min, the mixture was filtered, and the solvent was evaporated at 60 °C. The remaining oil residue was dried at 100 °C for 1 h.

Microencapsulation efficiency (ME) was calculated as follows:

$$\mathrm{ME} = [(\text{total oil} - \text{free oil})/(\text{total oil})] \times 100$$

SPME-GC/MS Analysis. One gram of microencapsulated powder was weighed (±0.01 g) into a 10 mL amber colored glass HS vial and tightly capped. Volatile compounds in the HS of sample vial were determined by SPME using a fiber of Carboxen/PDMS. The fiber was inserted into the sample HS, and the vial was incubated at 60 °C for 20 min and then withdrawn and transferred to the GC injector (operated in the splitless mode). The fiber was held in the GC injector for 7 min to desorb the volatiles into the GC column.

The analysis was performed using Combi PAL Auto Injector (CTC Analytics, Zwingen, Switzerland). Gas chromatography of the volatile compounds was performed on a HP-VOC fused silica capillary column (30 m, 0.32 mm i.d., 0.25 μm film thickness, Agilent, Melbourne), using a G1530A GC chromatograph and Hewlett-Packard 5973 Mass Selective Detector (Palo Alto, CA, USA). Helium was used as the carrier gas at a constant flow rate of 2.0 mL min^{-1}. The injector was initially operated in the splitless mode and then switched to the split mode (1:20) 2 min after sample injection. The temperatures of the injector and the MS detector (electron ionization) were held at 230 °C and 280 °C, respectively. The oven temperature was programmed from 40 °C increasing to 220 °C at the rate of 22 °C min^{-1}, held for 8 min.

Internal standard (IS) was not used to quantify the volatile compounds as it was not easy to homogenously spike liquid IS solution into the powder. The standard solutions of propanal, 1-penten-3-ol, and 2,4-(*Z*,*E*)-heptadienal with the same concentrations were analyzed at every time of analysis to correct the GC column and MS responses to the same levels. The volatile compounds were identified by MS detector scanning from 29 to 250 amu. Data analyses were performed using Chemstation software, and compounds were identified by reference to a library of spectra (Wiley 275). Volatile compounds were quantified using the abundance of specific target ions. Triplicate analyses were performed for all samples.

Table 1. Calculated and Measured Turbidity of Aqueous Components in Emulsion Formulation Intended for the Preparation of 30 and 40% Oil Powders[a]

	turbidity			
	30% oil powders [aqueous phase 1.88% chitosan/1.88% glucose/5.00% Hi-Cap]		40% oil powders [aqueous phase 2.22% chitosan/2.22% glucose/2.22% Hi-Cap]	
aqueous phase components	pH 4.9	pH 6.0	pH 4.9	pH 6.0
chitosan alone	0.024 ± 0.001	0.023 ± 0.001	0.037 ± 0.002	0.028 ± 0.001
glucose alone	0	0	0	0
Hi-Cap alone	0.585 ± 0.007	0.532 ± 0.008	0.281 ± 0.002	0.251 ± 0.004
calculated turbidity[b] (chitosan/glucose/Hi-Cap)	0.609	0.555	0.318	0.279
chitosan/glucose/Hi-Cap (measured)	0.800 ± 0.052	0.866 ± 0.054	0.409 ± 0.015	0.467 ± 0.018
difference (measured − calculated)	0.191	0.311	0.091	0.188

[a] Mean ± standard deviation of quadruplicate analyses. [b] Assuming the additivity of component contributions.

Table 2. Zeta Potential and Particle Size for Emulsion Formulations Intended for the Preparation of 30 and 40% Oil Powders[a]

	30% oil powders [emulsion formulation 1.88% chitosan/1.88% glucose/5.00% Hi-Cap/1.88% oil]		40% oil powders [emulsion formulation 2.22% chitosan/2.22% glucose/2.22% Hi-Cap/4.44% oil]	
parameter	pH 4.9	pH 6.0	pH 4.9	pH 6.0
zeta potential (mV)	52.93 ± 1.50	48.38 ± 1.67	53.47 ± 0.86	48.23 ± 1.36
particle size D [0.5] (μm)	0.36(±0.00)	0.43 (±0.00)	0.87 (±0.00)	0.52 (±0.00)

[a] Mean ± STDEV of duplicate measurements.

FAME Analysis by GC. About 200 mg of methyl tricosanoate as internal standard (IS) weighed accurately (±0.1 mg) into a 100 mL volumetric flask was made to volume with isooctane (stored at 4 °C before using). Accurately weighed tuna oil (80 ± 0.1 mg) or microencapsulated powder (80 ± 0.1 mg) was added into a Teflon-lined screw-cap Pyrex tube (dimensions 15 cm × 2.5 cm i.d.). Two milliliter aliquots of IS, THF, and freshly made 2 N sulphuric acid in methanol were pipetted into the tube containing the powder. Five milliliter aliquots of IS, THF, and 2 N sulphuric acid in methanol were added into the tube containing the oil. The mixtures were vortexed for 0.5 min at 2000 rpm on Heidolph Multi Reax (John Morris Scientific, NSW, Australia). The solution containing pure fish oil was divided into three tubes for triplicate methylations.

The above mixtures were blanketed under argon, capped tightly, and agitated (200 rpm) at 70 °C for two and a half hours in a water bath shaker (Julabo, SW23, John Morris Scientific, NSW, Australia). During the shaking period, the tubes were taken out from the water bath and vortexed for 30 s every half hour. Five milliliters of 5% (w/v) sodium chloride in water was added to stop the methylation, and the methyl esters were extracted with 4 mL of heptane by gently inverting the tube (a few drops of ethanol was added to separate any that were forming emulsions). The upper organic phase was withdrawn and washed with 4 mL of aqueous potassium bicarbonate (5% w/v) and dried over anhydrous sodium sulfate. The methyl ester solution was subjected to GC injection without further concentration.

A Shimadzu GC-17A and AOC-17 autoinjector (Shimadzu, Japan) were used for the GC analysis. The GC was fitted with a BPX 70 fused silica column (30 m, 0.25 mm i.d. and 0.25 μm films, SGE, Australia) operating with a constant helium pressure of 110 kpa. For EPA and DHA analyses, the GC column was programmed from 170 °C, first increasing at a rate of 1 °C /min to 200 °C and then increasing at 10 °C /min to a final temperature of 220 °C. A 1-μL aliquot of sample solution was injected with a split ratio of 1:50. The injector and detector (FID) were held at 220 and 250 °C, respectively.

Calculations. The peak area counts were integrated using Ezchrom Chromatography Data System (Scientific Software, USA). EPA and DHA concentrations were calculated as in AOCS Official Method (*21*). The remaining percent of EPA and DHA were calculated as described below:

$$\text{remaining \% of EPA and DHA} = [\text{ratio of EPA or DHA/palmitic acid (GC area at oxidation time)}]/[\text{ratio of EPA or DHA/palmitic acid (GC area at time 0)}] \times 100$$

Induction Period Using OXIPRES. OXIPRES (MIKROLAB, Aarhus, Denmark) was used to determine the induction period. Samples containing 4 g of oil were tested at 80 °C for the induction period. The initial oxygen pressure was 5 bar at ambient temperature. All samples were stored at −18 °C prior to analysis.

Statistical Analysis. Analysis of variance (ANOVA) was performed on the full factorial design analysis data using the statistical package MINITAB, release 14.

RESULTS AND DISCUSSION

Turbidity of the Aqueous Phase. Turbidity measurements of aqueous phase components were used to provide information about the complexes formed at different pH values. The turbidity measurements of the aqueous dispersions of the individual components and the mixtures used for emulsification of the oil are shown in **Table 1**.

The turbidities of the Hi-Cap dispersion were higher than those for the chitosan dispersions. The measured turbidities of the mixed aqueous dispersions were markedly higher than the turbidity calculated as a sum of the turbidity of the individual components (i.e., turbidity additivity). The higher measured turbidity compared with the turbidity additivity can be ascribed to the formation of a complex between the chitosan and the Hi-Cap in the aqueous phase. This is because the formation of associative complexes leads to increased scattering. The formation of an electrostatic complex may be expected as the chitosan carries a positive charge, while the Hi-Cap has a negative charge at the examined pH values. Others have also observed increases in turbidity of mixtures containing *ss*-lactoglobulin and sodium alginate at pH 3, 4, and 5. This was attributed to the formation of electrostatic complexes between these polymers (*32*). In the present study, there was a larger difference between the measured and calculated turbidity at pH 6.0 compared to that for the corresponding dispersions at pH 4.9 (**Table 1**), which suggests a higher degree of cross-linking at the higher pH.

Properties of Emulsions. The results of the zeta potential, particle size, and microstructure of the emulsion formulations intended for the manufacture of 30 and 40% oil powders were shown in **Table 2** and **Figure 1a−d**.

Zeta Potential. The zeta potential of all emulsions, both at pH 4.9 and 6.0, were positive. This result is expected and confirms that the isoelectric point of the emulsion system used in this study was > pH 6.0. The zeta potential was slightly and consistently

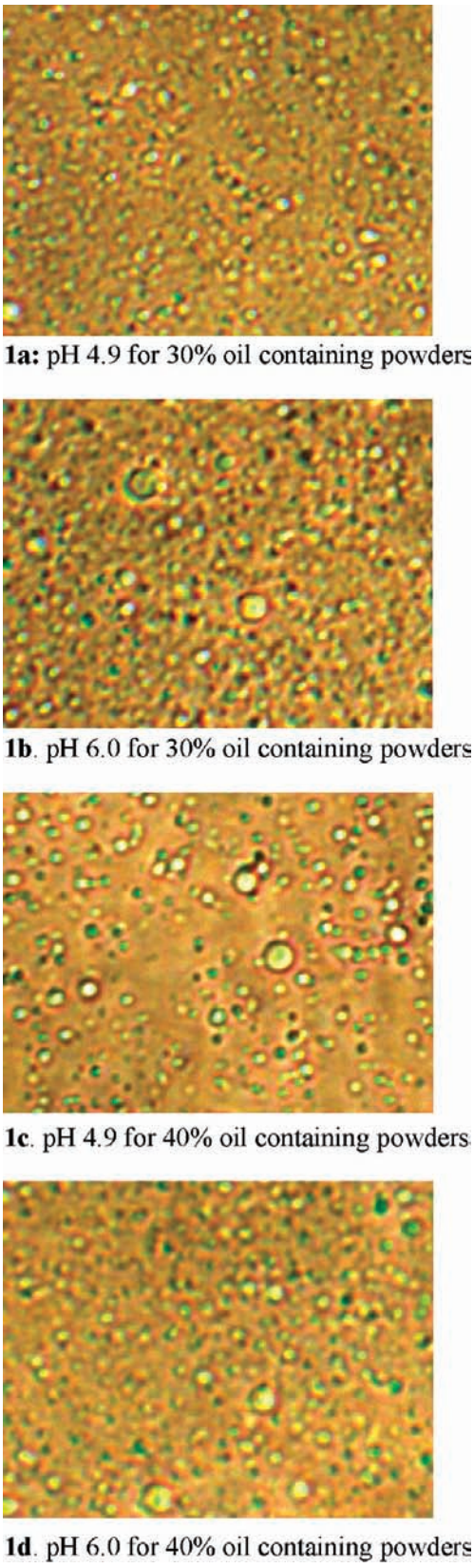

Figure 1. Micrographs of emulsions intended for the preparation of 30 and 40% oil containing powders (duplicate micrographs were produced and were similar). (**a**) pH 4.9 for 30% oil containing powders; (**b**) pH 6.0 for 30% oil containing powders; (**c**) pH 4.9 for 40% oil containing powders; (**d**) pH 6.0 for 40% oil containing powders.

higher for emulsions made at pH 4.9 than that of the emulsions made at pH 6.0 (**Table 2**). The lower zeta potentials at pH 6.0 (i.e., less positive) are due to the increased ratio of COO^-/COOH groups of OSA starch (Hi-Cap) and also the increased degree of complexation between the anionic COO^- group and the cationic NH_3^+ group of chitosan. These zeta potential measurements of emulsions corroborate the data on the turbidity of aqueous components.

Particle Size. The particle size of emulsions intended for the manufacture of 40% oil powders was larger than that used for the preparation of 30% oil powders (**Table 2**). The increased particle size of emulsion droplets in systems intended for the production of 40% oil powders compared to those for 30% oil powders may be rationalized in terms of the availability of surface-active material. These results suggest that the emulsifying components were limiting in emulsions with higher oil content, as one would have expected that the particle size would not be altered if there was an excess of surface active materials.

The particle size was larger at pH 6.0 than that at pH 4.9 for emulsions (1.88% chitosan/1.88% glucose/5.00%Hi-Cap/3.76% oil) intended for the preparation of 30% oil powders, but was smaller at pH 6.0 than that at pH 4.9 for emulsions (2.22% chitosan/2.22% glucose/2.22% Hi-Cap/4.44% oil) intended for the preparation of 40% oil powders (**Table 2**). Except for glucose which is not surface active, all of the components present in the aqueous phase of the emulsions (i.e., Hi-Cap, chitosan, and the complex between Hi-Cap and chitosan) can stabilize the air−water interface of the oil droplets. As the ratio of chitosan/oil was constant in the emulsion formulations intended for the manufacture of 30 and 40% oil powders, all that may be inferred is that there is the involvement of components other than (uncomplexed) chitosan in stabilizing the interface. It is further postulated that the complex will be preferentially adsorbed during homogenization due to the shear forces encountered. However, whether this remains at the interface or to some extent is replaced by the other surface active components (i.e., chitosan or starch) after homogenization is not known.

Microstructure. Micrographs of emulsions intended for the manufacture of 30 and 40% oil powders are given in **Figure 1a−d**. The microstructure images confirmed the data on particle size obtained by light scattering.

Total and Free Oil Content of Powders. The total oil and free oil contents of the powders are given in **Table 3**. The main finding was that the microencapsulation efficiencies were higher in samples made from the higher pH emulsions (**Table 3**).

Oxidative Stability Index Using OXIPRES. The oxidative stability of the powders under accelerated conditions is given in **Table 4**. The results clearly demonstrated the longer induction period for powders prepared from emulsions at pH 6.0 compared to those at pH 4.9. The induction periods were not markedly different between the two oil loadings (**Table 4**). However, at the end of the induction period, the uptake of oxygen was greater in powders containing the higher oil content (40.7 and 43.9%) at the same pH. In these experiments, the amount of oil in the sample oxidized under the accelerated conditions in OXIPRES was the same (4.0 g for each sample irrespective of whether 30% or 40% oil powders was used). Therefore, the difference in the uptake of oxygen between samples will be related to the protection afforded to the oil by the microencapsulation system. Oxidation occurs at an interface of the oil droplet, and hence, differences in the interfacial properties of the oil droplets in powders with different oil content contribute to the stability of the encapsulated oils. The different proportions of the encapsulants in the powders with different oil content can themselves also affect the stability of the encapsulated oil.

Oxidative Stability of Powders Stored at 25 °C. A number of markers of oxidative stability were used.

Table 3. Target Powder Formulation and Measured Total and Free Oil Contents[a]

target powder formulation	pH of emulsion prior to drying	total oil (% in powder)	free oil (% in powder)	microencapsulation efficiency (%)
15% chitosan/15% glucose/40% Hi-Cap/30% oil	4.9	32.70 ± 0.44	1.21 ± 0.03	96.3
	6.0	32.86 ± 0.09	0.47 ± 0.02	98.6
20% chitosan/20% glucose/20% Hi-Cap/40% oil	4.9	40.66 ± 0.28	2.68 ± 0.07	93.4
	6.0	43.93 ± 0.50	1.70 ± 0.02	96.1

[a] Mean ± STDEV of duplicate measurements.

Table 4. Assessment of Oxidative Stability by OXIPRES[a]

target powder formulation	pH of emulsion prior to drying	induction period	slope (mbar/h)
15% chitosan/15% glucose/40% Hi-Cap/30% oil	4.9	1.2 ± 0.1	−3148 ± 10
	6.0	18.2 ± 0.1	−888 ± 1
20% chitosan/20% glucose/20% Hi Cap/40% oil	4.9	1.3 ± 0.1	−6553 ± 149
	6.0	20.5 ± 0.1	−2807 ± 28

[a] Mean ± standard deviation of triplicate analyses.

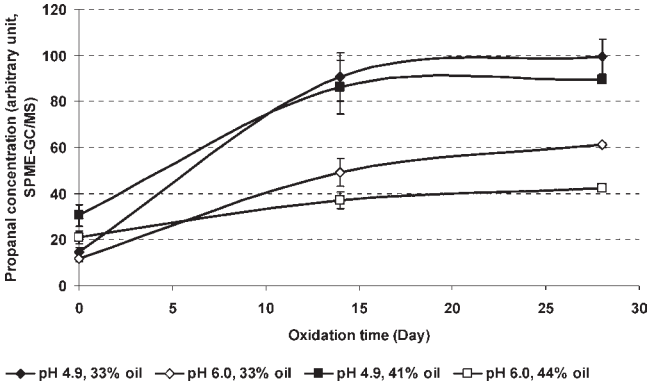

Figure 2. Development of propanal in microencapsulated tuna oils during storage at 25 °C. Concentration of propanal is expressed in arbitrary units as abundance of ion 58 amu. The error bars represent standard deviations of triplicate analyses for trial 2.

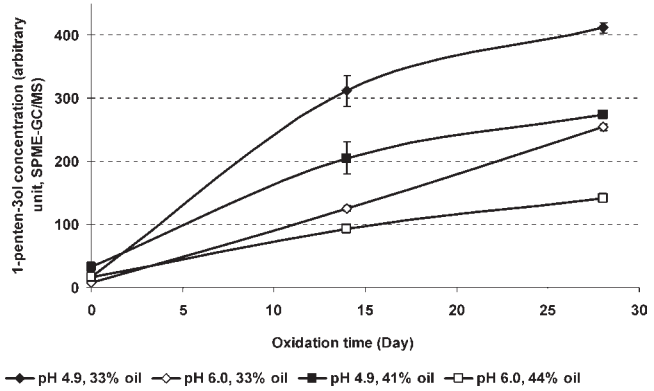

Figure 3. Development of 1-penten-3-ol in microencapsulated tuna oil during storage at 25 °C. Concentration of 1-penten-3-ol is expressed in arbitrary units as abundance of ion 57 amu. The error bars represent standard deviations of triplicate analyses for trial 2.

Oxidative Volatiles. The SPME/GC-MS responses for propanal, 1-penten-3-ol, 1-penten-3-one, 2,4-(*Z*,*E*)-heptadienal, and 2,4-(*E*,*E*)-heptadienal generated from the microencapsulated powders during the storage at 25 °C for a period of up to four weeks are given in **Figures 2**−**6**. The volatiles produced in the powders increased significantly ($p < 0.001$) during storage for the duplicate trials. The SPME-GC/MS analysis results of the five volatile compounds from the duplicate trial showed similar trends (data not shown for trial 1).

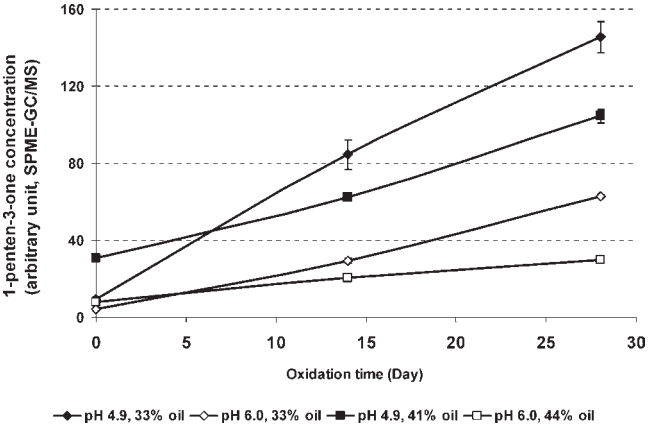

Figure 4. Development of 1-penten-3-one in microencapsulated tuna oils during storage at 25 °C. Concentration of 1-penten-3-one is expressed in arbitrary units as abundance of ion 55 amu. The error bars represent standard deviations of triplicate analyses for trial 2.

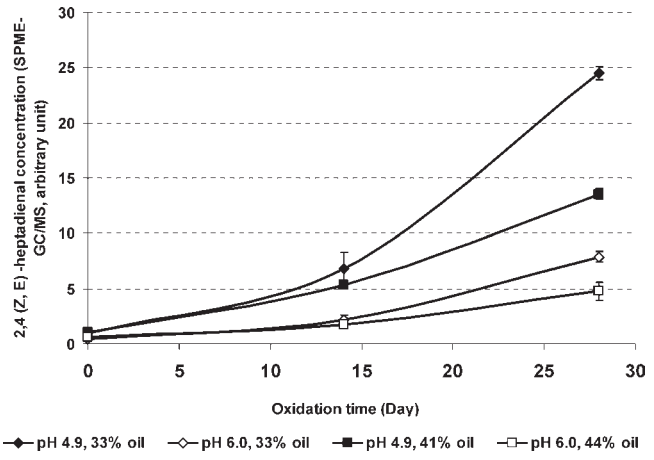

Figure 5. Development of 2,4-(*Z*,*E*)-heptadienal of microencapsulated tuna oils, during storage at 25 °C. Concentration of 2,4-(*Z*,*E*)-heptadienal is expressed in arbitrary units as abundance of ion 81 amu. The error bars represent standard deviations of triplicate analyses for trial 2.

Powders made from the emulsions adjusted to pH 4.9 all had significantly higher levels of the five oxidation volatiles in HS than the corresponding powders from the emulsions adjusted to pH 6.0 at the beginning of storage and after 14 and 28 days of storage ($p < 0.001$) for all samples in the duplicate trials (**Figures 2**−**6**,

Table 5. LCPUFA in Microencapsulated Powders after 4 Weeks of Storage at 25 °C

target powder formulation	pH of emulsion prior to drying	amount of EPA (mg /g oil)[a]	EPA remaining[b] (%)	amount of DHA (mg /g oil)[a]	DHA remaining[b] (%)
15% chitosan/15% glucose/40% Hi-Cap/30% oil	4.9	54.21 ± 0.5	90.8	239.2 ± 4.7	89.4
	6.0	55.29 ± 0.3	92.6	241.9 ± 3.4	90.4
20% chitosan/20% glucose/20% Hi-Cap/40% oil	4.9	53.97 ± 0.1	90.4	237.6 ± 4.1	88.8
	6.0	55.94 ± 0.6	93.7	249.1 ± 1.4	93.1

[a] Mean ± standard deviation of triplicate analyses for trial 2. [b] Expressed as the percentage of the initial LCPUFA contents for the fresh tuna oil, which were 59.70 ± 0.9 and 267.58 ± 2.3 for EPA and DHA, respectively, assuming that there was no oxidation during the manufacture of powders.

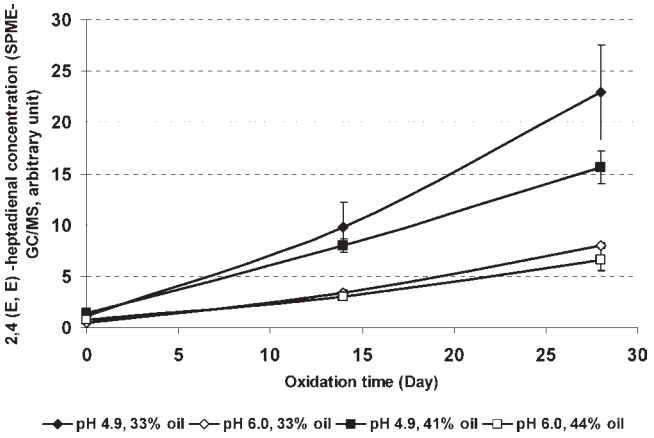

Figure 6. Development of 2,4-(*E*,*E*)-heptadienal in microencapsulated tuna oils, during storage at 25 °C. Concentration of 2,4-(*E*,*E*)-heptadienal is expressed in arbitrary units as abundance of ion 81 amu. The error bars represent standard deviations of triplicate analyses for trial 2.

data not shown for trial 1). The increase in oxidative volatiles in HS was markedly higher after 14 days of storage for the powders made from emulsions at pH 4.9 compared to that of corresponding powders from emulsions at pH 6.0.

FAME Analysis. The FAME analysis results for the powders stored for four weeks are given in **Table 5**. The data showed that powders made from emulsions at pH 6.0 had 1.1−4.6% higher ($p < 0.001$) EPA and DHA remaining, compared to that of their corresponding powders made from emulsions at pH 4.9 (powder samples for trial 2). Analyses were also performed on fresh bulk oil and oil stored for four weeks. The EPA and DHA contents for the fresh tuna oil were 59.70 ± 0.9 and 267.58 ± 2.3 mg/g oil, respectively, and for the stored oils after four weeks 52.60 ± 0.8 and 234.48 ± 2.3 mg/g oil, respectively. The remaining percentages of EPA and DHA in the powders (**Table 5**) were all slightly and consistently higher (from 0.8 to 8.0%) than those of the bulk oil stored at the same time ($p < 0.005$). This observation indicates that in this study microencapsulation protected the LCPUFA from oxidation.

This trend of the data for the remaining LCPUFA agreed with the oxidative volatile analyses and the induction period from the OXIPRES assessment. The higher remaining levels of LCPUFA matched with the lower levels of oxidative volatiles and longer induction periods, which indicated that microencapsulated omega-3 FAs were more protected from oxidation when the emulsions were made at the higher pH.

Effect of pH on Oxidative Stability. The results clearly demonstrated that increasing the pH of the emulsion prior to drying improved the oxidative stability of the microcapsules (**Tables 4−5**; **Figures 2−6**). As evidenced by turbidity measurements of aqueous phase components and zeta potential data, there is increased interaction between OSA-starch (Hi-Cap) with the positively charged chitosan biopolymer as pH is increased from 4.9 to 6.0. The increased electrostatic interactions between chitosan and emulsifying starch at the higher pH contribute to the increased stability of the microcapsule powders. The electrostatic cross-linking between two opposite charged biopolymers has previously been capitalized upon for the stabilization of microencapsulated polyunsaturated oils where different biopolymer combinations were used (*1*). Another possible contributory factor to improved stability at the higher pH (6.0) is the increased chelating effect of OSA-starch for oxidation catalyst metals (e.g., iron) due to the deprotonation of the carboxylic group as pH is increased.

This work demonstrates that judicious adjustment of pH of the emulsions containing chitosan and OSA-starch prior to drying can be used to improve the stability of microencapsulated oil powders.

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