

**MICROENCAPSULATION OF AN OMEGA-3 POLYUNSATURATED FATTY  
ACID SOURCE WITH POLYSACCHARIDES FOR FOOD APPLICATIONS**

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# MICROENCAPSULATION OF AN OMEGA-3 POLYUNSATURATED FATTY ACID SOURCE WITH POLYSACCHARIDES FOR FOOD APPLICATIONS

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## ABSTRACT

Omega-3 polyunsaturated fatty acids ( $\omega$ 3 PUFAs) provide important health benefits, but dietary consumption is low. Supplementing foods with  $\omega$ 3 PUFAs is of interest, but intervention strategies are necessary to preserve the integrity of these unstable compounds. Microencapsulation of  $\omega$ 3 PUFA sources is one means of improving their stability. In this work,  $\omega$ 3 PUFA microcapsules were prepared by spray drying with chitosan and blends of chitosan, high-amylose starch, and pullulan as wall materials. The primary objectives of this research were (1) to evaluate the effect of chitosan type and oil:wall ratio on  $\omega$ 3 PUFA microcapsule properties, (2) to evaluate the effect of blending chitosan with high-amylose starch and pullulan on  $\omega$ 3 PUFA microcapsule properties, and (3) to evaluate the oxidative stability of  $\omega$ 3 PUFA microcapsules by monitoring primary and secondary oxidation products during storage. Microcapsule encapsulation efficiencies (EE) ranged from 63% to 79% with the highest EEs observed for microcapsules prepared from chitosan with higher degree of deacetylation (DD) and lower molecular weight (MW). Median microcapsule size ranged from 3 $\mu$ m to 11 $\mu$ m. Moisture contents were all below 7% and water activities ( $a_w$ ) were below 0.27. Microcapsules prepared from blends of chitosan with starch and/or pullulan had lower  $a_w$  values than those prepared from chitosan alone. Oxidative stability was evaluated by measuring oxidation induction time (OIT) using pressure differential scanning calorimetry. OIT values ranged from 14 to 20 minutes. Microcapsules prepared from chitosan with lower DD and higher MW had longer OITs than those prepared from chitosan with higher DD and lower MW. Microcapsules prepared from blends of chitosan, starch, and pullulan had longer OITs than those prepared from chitosan alone. Oxidative stability of microcapsules during long term storage was evaluated on one microcapsule formulation by monitoring peroxide value (PV) and secondary oxidation products by HS-SPME-GC/MS. Volatiles including propanal, 1-penten-3-ol, pentanal, hexanal, and 2,4-heptadienal were detected in the headspace of the microcapsules; however, PVs did not indicate substantial oxidation of the  $\omega$ 3-PUFA source during 5 weeks of storage. Chitosan, high-amylose starch, and pullulan are effective materials for microencapsulation of  $\omega$ 3 PUFA sources.

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## LIST OF ABBREVIATIONS AND TERMS

$a_w$	water activity
CARB	carboxen
CLA	conjugated linoleic acid
DD	degree of deacetylation
DE	dextrose equivalent
DHA	docosahexanoic acid
EPA	eicosapentaenoic acid
FTIR/ATR	Fourier transform infrared spectroscopy/attenuated total reflectance
HBCD	highly branched cyclic dextrin
HS-SPME-GC/MS	headspace-solid phase microextraction-gas chromatography/mass spectrometry
MD	maltodextrin
MW	molecular weight
OIT	oxidation induction time
PDSC	pressure differential scanning calorimetry
PDMS	polydimethylsiloxane
PUFA	polyunsaturated fatty acid
PV	peroxide value
RH	relative humidity
RS	resistant starch
SC	sodium caseinate
SPI	soy protein isolate
TBARS	thiobarbituric acid reactive substances

Tg	glass transition temperature
WPC	whey protein concentrate
WPI	whey protein isolate
$\omega$ 3 PUFA	omega-3 polyunsaturated fatty acid

## CHAPTER 1 - INTRODUCTION

### Omega-3 Polyunsaturated Fatty Acids - Nutritional Values and Challenges

A growing body of research supports the idea that polyunsaturated fatty acids (PUFAs), and in particular omega-3 PUFAs, play an important role in maintaining physical and mental health. Omega-3 polyunsaturated fatty acids ( $\omega$ 3 PUFAs) are fatty acids with three or more double bonds, the first of which occurs between the third and fourth carbon from the methyl end of the structure. The most well known and widely researched  $\omega$ 3 PUFAs are eicosapentaenoic acid (EPA, 5 double bonds) and docosahexaenoic acid (DHA, 6 double bonds). EPA and DHA have been associated with health benefits including prevention of cancer, cardiovascular disease, diabetes, inflammatory diseases, autoimmune disorders and depression as well as with improved brain and visual development (reviewed by Shahidi 2008). Omega-3 fatty acids naturally occur in fatty fish (such as salmon) and also in some seeds and nuts (such as flax seed, walnuts, and almonds). Despite the well established health benefits associated with long chain  $\omega$ 3 PUFAs and the availability of natural sources, dietary consumption of these fats remains low. Supplementing food products with  $\omega$ 3 PUFAs is a growing trend in the food industry. Fish oils, algae oils, and flax products are the main sources of  $\omega$ 3 PUFAs used for supplementation of foods. Adding such products to foods presents several challenges, mainly preventing  $\omega$ 3 PUFA degradation, removing or masking undesirable flavors and odors, and overcoming matrix incompatibility issues.

The most significant challenge is preventing degradation of the long chain fatty acids. The high content of unsaturated double bonds causes PUFAs to be highly susceptible to oxidative degradation. When oxidized the long chain molecules break down eventually forming small molecules including alcohols, aldehydes, and ketones. These compounds can render a product unacceptable in terms of sensory attributes. Their presence also indicates that the  $\omega$ 3 PUFA content is not as high as anticipated. Preventing oxidation throughout ingredient storage, processing, and product storage ensures that the  $\omega$ 3 PUFA enriched product is providing the anticipated nutrients.

Oxidation of  $\omega$ 3 PUFA sources is prevented through the use of controlled storage conditions (eg. packing in an inert atmosphere and chilling), through the addition of antioxidants, and by microencapsulation. Each of these preservation methods has advantages and disadvantages.

Controlled storage can be expensive and time consuming if the atmosphere must be repeatedly modified upon opening and closing of ingredient storage containers. Selecting an effective antioxidant or blend of antioxidants is challenging. Antioxidant functioning is highly dependent on the properties of the matrix and oxidation occurs differently in bulk oils, emulsions and products of varying water activities. Throughout the stages of product development and storage it is highly possible that the  $\omega$ 3 PUFA source might see many of these different conditions. Furthermore, several commonly used antioxidants (tocopherols for example) can act as prooxidants at high levels. When the  $\omega$ 3 PUFA source ingredient is combined with other ingredients also containing antioxidants, it is possible that antioxidant content may increase to a level where prooxidant effects occur.

Microencapsulation refers to surrounding or embedding the oil in a matrix typically composed of proteins or carbohydrates and can be accomplished through a variety of processing techniques. In theory, microencapsulation protects the core material against degradation by light, heat, and oxygen; however, microencapsulation does not always produce a product that is more stable than the non-encapsulated form. Stability of encapsulated lipids depends on properties including oil distribution within the particle, particle size and surface area, particle density, wall material composition (glass transition temperatures, crystallinity, extent of interaction with the core material), moisture content, and water activity. If processing conditions and wall materials are selected appropriately, microcapsules with long term stability can be prepared. Microencapsulation also can facilitate incorporation of oily ingredients into a variety of food matrices as it transforms the lipid into a dried powder. Encapsulation also may mask undesirable flavors and odors associated with  $\omega$ 3 PUFA sources.

### **Microencapsulation of $\omega$ 3 PUFA Sources by Spray Drying**

Spray drying is commonly used in the food industry and has recently been widely applied to prepare  $\omega$ 3 PUFA microcapsules. To encapsulate lipid based materials by spray drying, an oil-in-water emulsion must first be generated. Ideally the emulsion has small oil droplet sizes and good stability. Emulsifiers are commonly added when surface active wall materials are not used. The emulsion is fed into the spray drier where it is atomized and exposed to hot air. Rapid drying occurs and dried microcapsules are collected. Properties of the spray dried particles depend on properties of the feed emulsion, properties of the wall material(s), and drying

conditions. Sources of  $\omega$ 3 PUFAs have been encapsulated by spray drying with proteins (whey, soy, casein, caseinates, gelatin), carbohydrates (derivitized starches, maltodextrins, glucose, corn syrup solids, pectin), and gums (gum arabic, alginate, carageenan). An ideal wall material would be one that forms a fine and stable emulsion, forms microcapsules with high encapsulation efficiency (low surface oil content) at high oil:wall ratios, produces a glassy shell capable of preventing diffusion of oxygen to the encapsulated material, and maintains structural integrity throughout long term storage. Taste-masking and antioxidant activity would also be beneficial for  $\omega$ 3 PUFA encapsulation. In general, the following characteristics suggest a material may be effective for encapsulation of  $\omega$ 3 PUFAs by spray drying: emulsifying capabilities, good film forming abilities, water solubility, low viscosity, bland flavor/sensory acceptability, barrier properties (water vapor and oxygen), low cost, and compatibility with regulatory and labeling requirements (Risch and Reineccius 1995; Re 1998; Madene, Jacquot et al. 2006; Gharsallaoui, Roudaut et al. 2007; Jin, Perrie et al. 2008). Despite the wide range of materials that have been evaluated, no single material stands out as ideal and there remains a desire for encapsulation materials that exhibit these pertinent properties.

### **Chitosan, High-Amylose Starch, and Pullulan – Promising Materials for $\omega$ 3 PUFA Encapsulation**

Chitosan is a  $\beta(1\rightarrow4)$  linked copolymer of D-glucosamine and N-acetyl-D-glucosamine. It is the deacetylated form of chitin, the second most abundant naturally occurring polysaccharide. Chitosan is typically generated from waste materials (e.g. shells of marine animals such as crab and shrimp). Chitosan has been studied in food applications including antimicrobials, edible films, emulsion stabilization, and texture modification. The emulsifying properties of chitosan make it particularly attractive for applications involving encapsulation of lipid ingredients. Furthermore chitosan has been shown to exhibit antioxidant effects, mainly through interactions with metals, which could aid in preserving  $\omega$ 3 PUFAs. The primary goal of this work was to evaluate chitosan as a wall material for spray dried  $\omega$ 3 PUFA microcapsules. Despite several properties that make it attractive as an encapsulating material, chitosan is limited as a spray drying component due to its high viscosity. For this reason, chitosan also was evaluated in conjunction with high-amylose starch and pullulan.

Starch is a polysaccharide comprised of D-glucose units. It has two fractions; amylose, a linear fraction formed from  $\alpha(1\rightarrow4)$  linked glucose units, and amylopectin, a branched fraction where branches are generated by  $\alpha(1\rightarrow6)$  linkages. Amylose content in starch is typically 20-30% (Bertoft 2004). High-amylose starch is a unique form of starch that contains a higher percentage of amylose. High-amylose starch has been noted to be a better film former than other forms of starch, and films prepared from high-amylose starch have shown superior oxygen barrier properties (Mehyar and Han 2004). High-amylose starch also acts as a dietary fiber and has been linked to improved gut health (Sharma, Yadav et al. 2008).

Pullulan is  $\alpha(1\rightarrow6)$  linked maltotriose. It is an excellent film former, and pullulan films demonstrate substantial oxygen barrier properties (Leathers 2005). Pullulan has also been shown to act as a dietary fiber and prebiotic (Spears, Karr-Lilienthal et al. 2005; Knapp, Parsons et al. 2008).

### **Research Objectives**

Chitosan, high-amylose starch, and pullulan are polysaccharides that have not been widely explored as lipid encapsulants, but exhibit properties that suggest they may perform well in such applications. The overall goal of this research was to evaluate chitosan alone and in conjunction with other materials as a food ingredient encapsulant. Fish oil, a good source of  $\omega3$  PUFAs was selected as the model core material due to the growing interest in supplementing foods with omega-3 fatty acids and the low stability of these nutrients. The specific research objectives of this work were:

**Objective 1:** Evaluate the effect of chitosan type (low molecular weight, high degree of deacetylation and medium molecular weight, medium degree of deacetylation) and oil:wall ratio (1:3 and 1:2) on  $\omega3$  PUFA microcapsule properties.

**Objective 2:** Evaluate the effect of blending chitosan with high-amylose starch and pullulan on  $\omega3$  PUFA microcapsule properties at a fixed oil:wall ratio.

**Objective 3:** Evaluate the oxidative stability of  $\omega3$  PUFA microcapsules by monitoring primary and secondary oxidation products throughout storage.

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## CHAPTER 2: LITERATURE REVIEW

### FISH OIL: A SOURCE OF OMEGA-3 POLYUNSATURATED FATTY ACIDS

#### Introduction

Fish oil is an important source of the omega-3 polyunsaturated fatty acids ( $\omega$ 3 PUFAs) docosahexaenoic acid (DHA, 22:6n-3) and eicosapentaenoic acid (EPA, 20:5n-3), as well as other less studied polyunsaturated fatty acids. Polyunsaturated fatty acids (PUFAs) are fatty acids that contain two or more double bonds. Omega-3 and omega-6 fatty acids are considered essential fatty acids meaning they are important for human health but cannot be synthesized in human body and instead must be acquired through the diet. The omega-3 and omega-6 terminology partially describes the location of these double bonds by indicating the position of the first double bond from the methyl or 'omega' end of the chain. The main omega-3 and omega-6 PUFAs and their relationships are shown Figure 2.1. PUFAs have been associated with numerous health benefits including prevention of cardiovascular disorders, diabetes, arthritis, inflammatory bowel disease, autoimmune diseases, cancer, and depression (Young 2001; Shahidi 2008). Furthermore, PUFAs have an important role in membrane fluidity, brain and eye development, and gene expression (Young 2001). DHA is a primary component of the lipids in the brain and is critical for brain development (Shahidi 2008)

Commercial fish oil is typically obtained from sardines, anchovies, menhaden, horse mackerel, sand lance, capelin, herring, cod liver and farmed salmon (Kulas, Olsen et al. 2006). EPA and DHA levels in fish oil depend on the source but contents generally range from 5 % (w/w) to 18% for EPA and 6% to 12 % for DHA (Kulas, Olsen et al. 2006). The remaining composition includes myristic acid (14:0), palmitic acid (16:0), stearic acid (18:0), palmitoleic acid (16:1n-7), vaccenic acid (18:1n-7), oleic acid (18:1n-9), eicosenoic acid (20:1n-9), cetoleic acid (22:1n-11), linoleic acid (18:2n-6),  $\alpha$ -linolenic acid (18:3n-3), stearidonic acid (18:4n-3), arachidonic acid (20:4n-6), and docosapentaenoic acid (22:5- $\omega$ 3) (Shahidi 2003; Ackman 2006; Kulas, Olsen et al. 2006).

## **Supplementation of Foods with Fish Oil and Other Omega-3 Lipid Sources**

Despite the growing body of research indicating numerous health benefits associated with consumption of omega-3 PUFAs, dietary intake remains below the recommended amounts (Kris-Etherton, Taylor et al. 2000; Jin, Perrie et al. 2008). The health benefits of omega-3 containing lipids combined with the growing functional foods market has led to considerable interest in supplementing a variety of food products with fish oil and other omega-3 fatty acid sources such as algae and flax oils. Omega-3 supplemented breads, cereals, milk, yogurts, juices, pastas, and cheeses can all be located in grocery stores.

## **Susceptibility to Oxidation**

The polyunsaturated nature of omega-3 and omega-6 fatty acids is critical to their functioning in terms of health benefits, but this same property also renders them highly susceptible to oxidative deterioration. Oxidation is a free radical process and can be initiated by a variety of factors including heat, light, metals and enzymes. The oxidation process occurs in three stages; initiation, propagation, and termination. During initiation, a hydrogen atom is abstracted from a lipid molecule yielding a lipid radical. This lipid radical then reacts with oxygen to form a lipid peroxide. The lipid peroxide can subsequently abstract a hydrogen atom from a different lipid molecule, producing a lipid hydroperoxide and a new lipid radical. This step propagates the oxidation process. Termination occurs when two radicals react to yield non-radical products. This can occur through a variety of mechanisms. The general oxidation process is illustrated in Figure 2.2.

Oxidation reduces the nutritive quality of the lipid and produces off flavor and aroma compounds through the breakdown of lipid hydroperoxides. The structure of the fatty acid greatly affects its susceptibility to oxidation. Polyunsaturated lipids are much more susceptible to oxidation than saturated lipids due to their high content of bis-allylic methylene groups (Kulas, Olsen et al. 2006). Oleic acid, with a single double bond, reacts approximately 10 times faster than its saturated counterpart, stearic acid, while linoleic (2 double bonds) reacts more than 100 times faster, and linolenic (3 double bonds) reacts almost 200 times faster (Pokorny, Yanishlieva et al. 2001). EPA (5 double bonds) and DHA (6 double bonds) are extremely susceptible to oxidation. The highly unsaturated nature of long chain omega-3 fatty acids such as EPA and DHA not only effects the rate at which oxidation occurs, but also results in a highly

complicated outcome in terms of the products generated. Each double bond is a site for hydrogen abstraction, ultimately enabling formation of greater than 16 hydroperoxide isomers for EPA and 20 hydroperoxide isomers for DHA (Kulas, Olsen et al. 2006). This great number of isomers possible during the initiation stage results in a large quantity of possible secondary oxidation products. Secondary oxidation products reported from oxidation of omega-3 fatty acids are summarized in Table 2.1. Research also has shown that volatile compounds produced from oxidation of omega-3 fatty acids have very low flavor thresholds (Kulas, Olsen et al. 2006). Not only do these compounds render a product unacceptable in terms of sensory attributes, but some compounds produced by oxidation have negative health effects. For example, several cases of food poisoning (nausea and vomiting) in Japan in the 1960s were traced back to compounds present due to oxidized fats in instant noodles (Gotoh, Watanabe et al. 2006). Totani et al (2008) observed liver damage in rats fed oxidized oil versus those fed fresh oil. Consumption of oxidized palm oil has been associated with negative alteration of lipid profiles, reproductive toxicity, and damage to organs including the liver and heart (Edem 2002). Intervention strategies are critical to preserving the integrity of highly unsaturated fatty acids.

Fish oil and other PUFA rich oils are preserved through the addition of antioxidants, microencapsulation, and by controlling the storage conditions (temperature and atmosphere). Intervention strategies can impose additional challenges. The interactions between antioxidants are complex and some antioxidants have been shown to exhibit pro-oxidant behavior when used at high concentrations. As another example, spray drying requires that the oil be emulsified in an aqueous medium containing the intended wall materials. Oxidation occurs differently in the bulk oil, emulsified oil and dried microencapsulated oil and different intervention strategies may be necessary for each stage of processing. Oxidation in emulsified oils is affected by additive content and concentration (emulsifiers and antioxidants), additive partitioning, solution pH, and droplet size and distribution (Kulas, Olsen et al. 2006). Oxidation in dried microencapsulated oils is affected by additive content and composition, wall material, oxidation during processing, oil droplet size and distribution in the emulsion, distribution of lipid in the dried microcapsule, capsule integrity, density, porosity, moisture content and water activity. Furthermore it is difficult to compare the published research studies on oxidation in microencapsulated fish oil due to substantial differences in the oil source and antioxidant content, powder properties, storage conditions, and analytical methods.

Let et al (2007) studied oxidation in milk emulsions containing fish oil. The research showed that emulsions with smaller oil droplet size were more oxidatively stable (as evidenced by peroxide value and headspace analysis of secondary oxidation products) than emulsions with larger droplets, an unexpected result due to the larger interfacial area associated with smaller oil droplets. These researchers also found that oxidation proceeded more rapidly in milk enriched with neat fish oil as compared to milk enriched with pre-emulsified fish oil, but the opposite was true for fish oil-enriched yogurt and salad dressing (Let, Jacobsen et al. 2007). The authors also found that fish oil-enriched milk oxidized more rapidly than fish oil-enriched salad dressing and fish oil-enriched yogurt.

Iglesias, Lois and Medina (2007) applied solid phase microextraction with gas chromatography and mass spectrometry (HS-SPME-GC/MS) to the analysis of fish oil emulsions and fish oil enriched milk and mayonnaise. In this work, emulsions were intentionally oxidized in order to develop the analytical method and the method was subsequently applied to study oxidation of milk and mayonnaise during storage. These researchers identified 77 different compounds formed from oxidation of fish oil emulsions, 16 of which were discussed in the research.

The HS-SPME-GC/MS technique also was evaluated by Jimenez-Alvarez et al (2008) for monitoring oxidation in fish oil-enriched milk. These researchers induced oxidation of tri-EPA, tri-DHA, and tri-ARA for the purpose of identifying oxidation products likely to be detected in PUFA rich oils. The authors identified 18 different compounds with hexanal, 1-penten-3-ol, and 2,4-heptadienal being most abundant. During the storage study, the authors found that 2-hexenal and 4-heptenal increased the most with storage and suggest these two compounds as possible markers for monitoring oxidation.

Serfert et al (2008) compared the effectiveness of different antioxidant blends on oxidation of fish oil in bulk oil, freshly homogenized emulsions, emulsions stored for two days, and dried microencapsulated forms. These researchers found that use of the metal chelators citric acid and lecithin reduced oxidation during homogenization and subsequent storage of the emulsions. This work demonstrated that protection of the lipid is necessary during all stages of processing and the most beneficial antioxidants are those that function in the emulsion and the dried form.

Klinkersorn et al (2005) coated tuna oil droplets in an oil-in-water emulsion with layers of chitosan and lecithin as a means to preserve them in both the emulsion and dried form. Emulsions with droplets surrounded by lecithin alone were less stable (as evidenced by peroxide values and thiobarbituric acid reactive substances) than emulsions where droplets were surrounded by lecithin and chitosan. When chitosan surrounds oil droplets a positive charge can result on the oil droplet surface, this may prevent pro-oxidants such as transition metals from reaching the oil droplet. Additionally, the emulsions with both chitosan and lecithin have a thicker membrane around the oil droplets, which may also prevent pro-oxidants from reaching the oil. The authors also evaluated  $\alpha$ -tocopherol as an antioxidant in emulsions stabilized with both lecithin and chitosan and found that oxidation was not delayed by the antioxidant until after 13 days of storage (37°C). Ethylenediaminetetraacetic acid (EDTA), a metal chelator, on the other hand, had a preventative effect on oxidation that was observable on the first day of storage. This work also evaluated oxidative stability of the tuna oil after the emulsion had been lyophilized. This portion of the work is discussed later. Oxidation in dried microencapsulated PUFA rich oils has been widely evaluated. Encapsulation efforts and their effect on oxidative stability is the focus of the following section.

## **MICROENCAPSULATION AS A MEANS TO PRESERVE AND FACILITATE ADDITION OF FISH OILS TO FOODS**

### **Introduction**

Microencapsulation is a widely used technique for preserving and facilitating the use of sensitive ingredients. In microencapsulation a core material is surrounded or embedded in a protective layer of differing composition. In general, a liquid is transformed into a powder in the microencapsulation process and the powder has better stability against light, heat, and oxidation, and is easier to incorporate into a variety of food matrices. Furthermore, microencapsulation can mask undesirable flavors or odors, control the release rate and location of a compound, and impact bioavailability of the encapsulated material (Re 1998; Vilstrup 2001). Microcapsules can have a variety of structural types – core shell, multi core, single wall, multi wall, continuous matrix. The structural type depends on the processing method and materials involved in preparation (Jin, Perrie et al. 2008). Microcapsule sizes generally range from 3 to 800  $\mu\text{m}$  (Vilstrup 2001). The desired size may depend on the application. Microcapsules can be

designed with the intention of disrupting the structure during processing or with the aim of maintaining the integrity of the microcapsule throughout processing to preserve and/or target delivery of a compound during digestion. If a microencapsulated ingredient will remain intact in the final product it should be smaller than 100µm in order to not be noticed during consumption (Jin, Perrie et al. 2008).

### **Microencapsulation by Spray Drying**

Microcapsules can be prepared by a variety of techniques including extrusion, fluidized bed drying, spray chilling, spray drying, freeze drying, complex coacervation, and gelation (Vilstrup 2001). Spray drying is the most commonly used technique for producing microencapsulated food ingredients (Gharsallaoui, Roudaut et al. 2007; Venugopal 2009). In microencapsulation by spray drying, a target compound is combined with one or more ‘wall’ materials to form an emulsion or dispersion. The liquid feed is atomized by a spray nozzle or rotating disk type atomizer and the droplets come in contact with hot air (usually in the range of 150°C-250°C) in a drying chamber. Rapid evaporation of the solvent (usually water) occurs. The temperature of the particle remains low due to the large surface area of the droplets, the evaporation process, and the movement of the particle as it dries. The air carries the dried particles through a cyclone and into a collection vessel. The final powder has low moisture content and generally a multi-core type structure where several droplets of core material are embedded in a matrix of wall material(s). Spray dried particles are often spherical and can have wrinkled or smooth surfaces. It is also not uncommon for spray dried microcapsules to have air pockets within the particle. Particle characteristics depend on the materials used and the drying conditions. An overview of the spray drying process is shown in Figure 2.3.

Carbohydrates, proteins, and gums are the most commonly used wall materials for spray drying. Among these, gum arabic, modified starches including n-octyl succinate starch and maltodextrins, whey and soy protein, gelatin, sodium caseinate, alginate, carrageenan, and pectin are most commonly used (Re 1998; Gharsallaoui, Roudaut et al. 2007; Bhandari, Patel et al. 2008; Jin, Perrie et al. 2008). The following properties are important factors affecting a material’s performance as a spray drying encapsulation material: good film forming properties, water solubility, low viscosity, bland flavor/sensory acceptability, barrier properties (water vapor and oxygen), emulsifying ability (for lipid based ingredients), low cost, and compatibility with

regulatory and labeling requirements (Rishch and Reineccius 1995.; Re 1998; Madene, Jacquot et al. 2006; Gharsallaoui, Roudaut et al. 2007; Jin, Perrie et al. 2008) The most effective materials are those that will form a complete wall or shell around the core material and maintain the integrity of this shell during storage, and potentially processing, and in the food as well, depending on the product application (Jimenez, Garcia et al. 2006).

### **Microencapsulation of Fish Oil and Other PUFA Sources**

PUFA-rich oils have been encapsulated in a variety of wall materials including modified starches, glucose, trehalose, maltodextrins, hydroxypropyl methyl cellulose, lecithin, chitosan, corn syrup solids, gum Arabic, pullulan, whey protein, sodium caseinate, gelatin, alginate, and glycated proteins (Minemoto, Adachi et al. 1999; Minemoto, Hakamata et al. 2002; Jimenez, Garcia et al. 2004; Drusch, Serfert et al. 2006; Velasco, Marmesat et al. 2006; Drusch and Mannino 2009). The most commonly used processing technique for generating encapsulated PUFAs is spray drying an oil in water emulsion, but other techniques including freeze drying, ultrasonic atomization, fluidized bed coating and polymer gelation, coacervation and electrostatic self-assembly have also been used (Heinzelmann, Franke et al. 2000; Klinkesorn, Sophanodora et al. 2005; Klinkesorn, Sophanodora et al. 2006; Klaypradit and Huang 2008; Drusch and Mannino 2009). Microcapsule properties depend on wall material, lipid composition, lipid load levels, particle formation methods, drying processes, drying temperatures, and additive content and composition (Vilstrup 2001; Marquez-Ruiz, Velasco et al. 2003) Microencapsulation of PUFAs is considered an important method for preventing oxidation of the oil, but protection against oxidation varies and microencapsulated PUFAs remain quite susceptible to oxidation (Venugopal 2009).

Whey protein and maltodextrin are two of the most commonly studied materials for encapsulation of PUFA rich oils. Klaypradit and Huang (2008) encapsulated tuna oil in whey protein combined with chitosan and maltodextrin by ultrasonic atomization. The materials and process produced microcapsules with good encapsulation efficiency (~80%) and with little loss of DHA and EPA following processing; however long term stability was not evaluated.

Whey protein isolate (WPI) also has been used to encapsulate flaxseed oil, a good source of  $\alpha$ -linolenic acid (ALA, 18:3) (Partanen, Raula et al. 2008). Microcapsules were prepared by spray drying. WPI was used at a level of 10% (w,w) and flax oil was added at 40% of WPI

content. Comparing oxidation of bulk flax oil to the microencapsulated oil showed that the oxidation rates were similar for three weeks of storage at 37°C after which oxidation of bulk oil proceeded more rapidly. Storing samples at differing relative humidities (0, 11, 49, and 75%) showed that samples stored at 0% oxidized with the greatest rate over the 10 week storage period while those stored at 75% RH oxidized with the slowest rate as evidenced by peroxide value measurement.

Whey protein concentrate (WPC), alone and in combination with maltodextrin (MD) (dextrose equivalent (DE) =10), was compared to gum arabic for microencapsulation of conjugated linoleic acid (CLA) (Jimenez, Garcia et al. 2004; Jimenez, Garcia et al. 2006). Microcapsules were prepared by spray drying a 30% (w,w) polymer suspension with CLA added at a ratio of 1:4 CLA:polymer. Homogenization was done with a high speed mixer and spray drying was carried out at an inlet temperature of 200°C and outlet of 110°C. Microcapsules prepared with whey protein concentrate alone had an encapsulation efficiency of almost 90%, while those from WPC combined 1:1 with MD and those prepared with gum arabic had efficiencies of approximately 56 and 57%. The authors reported a 15% loss of CLA due to degradation from the processing conditions for the whey protein microcapsules. These microcapsules also had low moisture contents; 1.2, 1.8 and 2.5% for WPC-MD blend, gum arabic, and WPC respectively. Gum arabic microcapsules oxidized more rapidly than WPC-MD microcapsules during storage at 35°C and  $a_w=0.108$  for 60 days as evidenced by peroxide value (PV); however, WPC-MD microcapsules had better retention of CLA as evidenced by GC analysis of CLA methyl esters. This held true for water activities ranging from 0.1-0.9. The authors noted a substantial effect of water activity on oxidative stability with  $a_w=0.821$  resulting in the greatest CLA retention for WPC-MD microcapsules and  $a_w=0.515$  resulting in the greatest retention for gum arabic microcapsules. For WPC microcapsules degradation of CLA was slowest at higher water activities (up to 0.9) but water activities greater than 0.7 resulted in structural collapse. Effects of water activity on oxidative stability also were observed through measuring anisidine values and hexanal content. Moisture content and water activity affect the state and permeability of the matrix materials.

WPC with MD and modified starch (Hi-Cap 100, National Starch and Chemical Co.) with MD were used to form fish oil microcapsules. Microcapsules were prepared by spray drying with an emphasis on evaluating the effect of 'nano-particle' sized emulsions (Jafari, Assaidpoor

et al. 2008). Inlet and outlet temperatures were 180°C and 65°C respectively. Total wall components were 40% (w,w) with 10% WPC or starch and 30% MD. Emulsions were prepared by incorporating fish oil at a level of 1:4 oil:wall material while passing the suspensions through a stator equipped colloid mill, followed by either microfluidization at 60 MPa or ultrasonic homogenization at 24 KHz. The researchers found a significant effect of emulsion oil droplet size on surface oil content. Volume mean oil droplet diameters were approximately 4-6µm for pre-emulsions, 0.2-0.3µm for rotor-stator formed emulsions, and 2-4µm for ultrasonic emulsions with starch producing slightly smaller droplet sizes than WPC. Surface oil contents (measured by solvent extraction) and surface oil coverage (measured by elemental analysis by x-ray photoelectron spectroscopy) were lowest for emulsions with the smallest oil droplet sizes although no significant difference in surface oil content was observed for WPC microcapsules formed from microfluidized emulsions and ultrasonic emulsions. Reconstitution of emulsions suggested that the microfluidized emulsions were most stable as these had the smallest increases in oil droplet size. All moisture contents were between 1.3 and 1.7% and volume mean particle sizes were 25-41µm. Lack of significant differences between particle sizes led the researchers to conclude that particle size did not effect encapsulation efficiency. SEM imagery showed that WPC produced smoother, less dented particles than Hi-cap and that crust formation occurred rapidly for Hi-Cap microcapsules

Rusli, Sanguansri and Augustin (2006) compared WPI to soy protein isolate (SPI) in combination with protein-glucose syrups for microencapsulation of fish oil by spray drying. Protein:carbohydrate ratios were maintained at 1:2 while oil:protein ratios were varied from 0.75:1 to 4.5:1. These authors also evaluated effects of oil composition by encapsulating tuna oil, palm stearin oil and tuna oil blended with palm stearin oil. Drying temperatures were 180°C and 80°C. The emulsion was formed using a two stage homogenizer with different pressures evaluated. Increasing oil content did not cause an increase in oil droplet size for whey protein emulsions; however, emulsions prepared with SPI and oil:protein ratios of 3:1 and 4.5:1 displayed evidence of coalescence. Oil droplet sizes ranged from 0.25-0.5µm. Higher homogenization pressures produced emulsions with smaller oil droplet sizes. Encapsulation efficiencies of WPI microcapsules was 97% at the lowest oil:protein ratio and 86% at the highest. SPI microcapsules had encapsulation efficiencies of 93% for the lowest ratio and 81% for the highest. The better performance of WPI was attributed to better film forming properties than SPI.

The researchers found that neither oil composition nor total solids content of the emulsion affected the encapsulation efficiency, but higher homogenization pressures (smaller oil droplet sizes) resulted in higher encapsulation efficiencies. The authors point out that higher pressures also may affect the conformation of the proteins in the emulsion. Oxidative stability was evaluated during storage at 23°C for 4 weeks without controlling humidity. WPI microcapsules had lower PV and headspace propanal contents than SPI microcapsules. Furthermore, microcapsules prepared at lower oil:protein ratios exhibited greater oxidative stability. Higher total solids content seemed to have a favorable effect on oxidative stability of the microcapsules and this was attributed to lower contents of occluded air in microcapsules generated from higher solids content emulsions.

Minemoto et al (1999) found that the oil:wall ratio was very important for oxidative stability of linoleic acid encapsulated in pullulan (MW=200,000 g/mol), MD (DE=2-5), and gum arabic (individually, not blended) by hot air drying. Wall materials were dissolved at a level of 15% (w,v) and lipid:wall ratios evaluated were 0.2, 0.5, 0.75, 1.0, and 2.0 by weight. Particles formed had diameters on the order of 2-3µm. These particles were stored at 37°C and 75% RH for 15 days. Oxidation was monitored by measuring the concentration of the methyl ester of linoleic acid by gas chromatography. The researchers observed rapid oxidation during the first few days of storage followed by a leveling off. Gum arabic provided the best protection against oxidation and MD the least, with lower oil:wall ratios generally oxidizing more slowly than higher ratios for all wall materials. Surface lipid contents were not reported.

Kagami et al (2003) investigated the performance of highly branched cyclic dextrin (HBCD) and two MDs (DE=4 (MD18) and DE=8 (MD8)) as fish oil encapsulants in combination with sodium caseinate. Oil loads of 46%, 69% and 83% were prepared. Emulsions were formed by homogenizing at 14.7 MPa. Microcapsules were formed by spray drying at 150°C/90-100°C. Particle sizes were on the order of 20µm for all microcapsules. Increasing the oil load resulted in greater amounts of surface oil as evidenced by FTIR/ATR measurements. Formulations with DE18 MD generally had lower surface oil levels than those with DE4 MD or HBCD; HBCD containing formulations had lower surfaced oil levels than DE4 MD formulations. Oxidative stability of SC, HBCD/SC, DE4MD/SC, DE11MD/SC, DE18MD/SC and HBCD/WPI microcapsules with 69% oil load were evaluated during storage at 37°C and 70% RH by measuring peroxide value. HBCD/SC and DE18MD/SC microcapsules oxidized much more

slowly and to a dramatically lower extent than the other formulations. PV of HBCD/SC and DE18MD/SC microcapsules stayed below 10meq peroxide/kg oil throughout storage for 100 days while the other formulations except DE11MD/SC microcapsules all exceeded 10meq/kg oil by day 40 (DE11MD/SC microcapsules exceeded 10meq/kg by day 60). The fish oil contained  $\alpha$ -tocopherol (6000 ppm) and ascorbyl palmitate (500 ppm).

Kolanowski et al (2006) encapsulated fish oil in modified cellulose (METHOCEL A15, Dow) and MD (DE not specified, Crystal Tex 626, National Starch & Chemical). Additional agents were included in the formulation – soy lecithin for emulsion formation and  $\alpha$ -tocopherol and lycopene as antioxidants. The researchers compared the oxidative stability of bulk and microencapsulated oil with and without antioxidants at different storage conditions. Emulsions for microcapsules were formed using a laboratory homogenizer operated at 10,000 rpm for 5 minutes; producing emulsions with large oil droplet sizes (10-40 $\mu$ m). Microcapsules were formed by spray drying at 160/65°C and inlet pressure of 350kPa. Methylcellulose:MD ratio was 2:1 and wall content was approximately 40% (w,w). Wall:core ratios of 3:1 and 1.5:1 were evaluated. Microcapsules all had mean particle diameters close to 30 $\mu$ m. The researchers reported encapsulation efficiencies of 86.5% for 3:1 wall:core microcapsules and 84.8% for 1.5:1 wall:core microcapsules, however these efficiencies were reported based on total oil extracted from the microcapsules as no attempt to measure surface oil was made. This encapsulation efficiency is therefore different from those typically reported that are based on surface oil content not merely total oil content. The authors reported that the spray drying process resulted in an increase in PV of the fish oil from 1.05 meq/kg oil to 4.06 meq/kg oil for 3:1 wall:core microcapsule and to 2.10 meq/kg oil for 1.5:1 wall:core microcapsules but that no PV increase was observed following the homogenization step. Microcapsules prepared at 3:1 wall:core ratio reached a PV of 172 meq/kg after 14 days of storage in air at room temperature while those prepared at 1.5:1 wall:core ratios had a PV of 50 after 14 days of storage, but reached a PV of 211 by day 23 when stored in air at room temperature. Storing samples in air but at lower temperature (5°C) slowed oxidation substantially and on day 32 3:1 microcapsules had a PV close to 30 meq/kg and 1.5:1 microcapsules had a PV close to 20 meq/kg. Storing samples at room temperature but under vacuum provided the best protection against oxidation of the conditions evaluated. After 30 days of storage under vacuum, PV was less than 10 for 3:1 microcapsules and less than 14 meq/kg for 1.5:1 wall:core microcapsules. These researchers

also considered oxidation of bulk fish oil and found that microencapsulated samples oxidized more rapidly than bulk oil, a factor attributed to surface area.

Partanen et al (2002; 2008) encapsulated sea buckthorn seed oil, a good source of linoleic and  $\alpha$ -linolenic acids, in sodium octenyl succinate derivitized starch (Hi-Cap 100, DE=32-37, National Starch and Chemical Co.), gum arabic, MD (DE=18.5) and with a 1:2 blend of gum arabic and MD (DE=18.5) and a 1:7 blend of gum arabic and MD. Wall content was maintained at 40 % (w,w) and oil was added at levels of 10-40% based on wall component weight. Emulsions were formed using a shear homogenizer operated at 24,000 rpm for 3 minutes. 1:7 GA/MD emulsions had median oil droplet sizes of approximately 4  $\mu\text{m}$  while 1:2 GA:MD emulsions had median oil droplet sizes less than 2 $\mu\text{m}$ . Starch emulsions had oil droplet sizes between 0.3-0.8 $\mu\text{m}$ . Emulsions were dried at an inlet temperature of 200°C and an outlet of 80°C on a spray drier equipped with a rotary atomizer type nozzle. Starch microcapsules had very low surface oil (less than 2%) for all oil contents (15, 20, 30, and 40%). GA microcapsules prepared at 20% oil load had the same surface oil content as 20% oil starch microcapsules. MD microcapsules prepared at 10 and 15% oil load had approximately 8% surface oil while those at 20% oil load had 10% and those at 30% oil load had 17% surface oil. 1:7 GA:MD microcapsules had 33% surface oil. Microcapsules exhibited median particle sizes on the order of 30-50 $\mu\text{m}$ . Oxidative stability of bulk oil, oil encapsulated in starch (oil load 20% and 30%), and oil encapsulated in MD (20%) was monitored during storage at 20°C and 50% RH. The starch microencapsulated samples prepared at 30% oil load had the best oxidative stability maintaining a PV less than 20 meq/kg oil over 9 weeks of storage. The oxidative stability of the other microencapsulated samples was quite similar; however, they exhibited PVs just slightly above 20 after 9 weeks of storage. The oil encapsulated for the storage study contained sage extract as an antioxidant. Bulk oil with sage extract had greater stability than bulk oil without sage extract. Without sage extract the bulk oil had a PV of 60 meq/kg oil after 3 weeks of storage while oil with the extract did not reach PV of 60meq/kg oil until 8 weeks of storage. The authors evaluated the effect of their extraction procedure on the PV and found that the value increased from 5.7 meq/kg to 11.0 meq/kg due to the extraction procedure. Microcapsule had greater oxidative stability when stored at 20°C and 50% RH then when stored at ambient conditions. This was attributed to the additional moisture at ambient conditions that reduced glass transition temperatures of the wall materials.

Tan, Chan, and Heng (2005) encapsulated fish oil in a chemically modified starch product, (Capsul®, National Starch and Chemical Company) by spray drying at 150/80°C using a rotary wheel atomizer. The starch content was 15% by weight and oil:starch ratios of 0.5:1, 1:1 and 1.5:1 were evaluated. Emulsions were homogenized for 3 minutes at 4,500rpm and 2 minutes at 5,000 rpm. Median oil droplet diameters were between 3 and 5 µm and were higher for emulsions with higher oil contents. The researchers found that size and encapsulation efficiency varied with oil:starch ratio. Sizes ranged from ~14µm to ~20µm and the greatest efficiency (92%) was achieved with a ratio of 0.5:1. Oxidative stability of the encapsulated oil was not investigated.

Drusch, Serfert, Schwarz and colleagues have done extensive research on *n*-octenylsuccinate-derivitized (*n*-OSA) starch for microencapsulation of fish oil. Two types of *n*-OSA differing in viscosity were compared for encapsulating fish oil (Drusch and Schwarz 2006). Factorial experiments were used to evaluate emulsion conditions (homogenization pressure, number of passes, starch type, starch content, and oil content.) and spray drying conditions (starch type, matrix composition, spray drying temperatures). Four different formulations were spray dried. Dried particles had median particle sizes in the range of 22 to 29 µm, moisture contents between 1.10 and 3.23% and water activities between 0.097 and 0.273. Higher drying temperatures (210/90°C) resulted in larger particles with greater incorporation of air whereas lower temperatures (160/60°C) resulted in wrinkled, misshapen particles. Higher spray drying temperatures also resulted in higher levels of conjugated dienes and propanal. Overall, the lower viscosity starch produced microcapsules with more favorable properties.

Subsequently, *n*-OSA starch was evaluated in conjunction with glucose for generating fish oil microcapsules (Drusch, Serfert et al. 2006). Starch content in the feed emulsion was 45% (w,w), glucose syrup (DE=38) content was 4.5% and fish oil content was 18%. The emulsion was homogenized with 2 passes through a two stage pressure homogenizer at 200/50 bar producing an emulsion with a median oil droplet size of approximately 1 µm. Spray drying was carried out at 170°C inlet temperature and 70°C outlet temperature on a drier equipped with a rotary disk style atomizer. Particles had approximately 3-3.5% surface fat and a moisture content of approximately 1.5%. The authors reported an increase in peroxide value from 5mmol/kg prior to emulsifying and drying to 65 mmol/kg oil after drying. The peroxide value of the encapsulated fat (surface oil removed) was only 30 mmol/kg oil. Microcapsules were stored

at 11, 33 and 48% RH at 40°C. After 17 days of storage PV increased to 98 mmol/kg oil for the driest conditions, 124 mmol/kg oil for the medium humidity condition and 400 mmol/kg oil for the highest humidity condition. Propanal content was also lowest for microcapsules stored at 11% RH and highest for those stored at 48% RH. Different storage temperatures were also considered, and it was noted that humidity had a much more pronounced effect on oxidative stability than storage temperature. As part of this work flow agents (fumed silica, precipitated silica and tricalciumphosphate) also were evaluated. These were applied to the microcapsules after drying and all three improved microcapsules flow properties without negatively impacting oxidative stability.

The performance of *n*-OSA starch in conjunction with trehalose also was evaluated and compared to a blend of *n*-OSA starch and glucose syrup (Drusch, Serfert et al. 2006). In this work the *n*-OSA starch was used at a level of only 10% (w,w) and trehalose or glucose syrup (DE=38) were used at 35%. Fish oil was added at either 10% or 40% of the total solids content. Emulsions and microcapsules were prepared as above. Median oil droplet size ranged from 0.99  $\mu\text{m}$  to 1.17  $\mu\text{m}$ . Median particle size ranged from 19 $\mu\text{m}$  to 23 $\mu\text{m}$ , moisture contents ranged from 2.03 to 3.65% and water activities ranged from 0.093 to 0.151. For microcapsules prepared with 10% fish oil, surface fat was 3.4% when trehalose was used and 3.5% when glucose was used. For the higher level of fish oil (40%) surface fat was 4.1% when trehalose was used and 7.9 % when glucose was used. Microcapsules again were stored at 20°C and either 0% RH or 54% RH. Peroxide values were higher for glucose containing microcapsules than for trehalose containing microcapsules at both oil load levels. For storage at 0% RH, PV reached 71 mmol/kg oil for low oil load glucose microcapsules and 91 mmol/kg for high oil load glucose microcapsules after 7 days of storage; trehalose values were 34 and 41 mmol/kg oil. Peroxide values were higher for storage at 54% RH in all cases. At 54% RH crystallization of trehalose occurred and oxidative stability was quite poor. The researchers indicated that the protection afforded by trehalose could be due to its ability to bind dienes.

Drusch (2007) also evaluated sugar beet pectin, a pectin with a high content of acetyl groups, and therefore the ability to act as an emulsifying agent, as a microencapsulant for fish oil. The sugar beet pectin was used in conjunction with glucose syrup (DE=38) and different emulsification and spray drying parameters were evaluated. For spray drying, pectin was used at either 1.1% (w,w) or 2.2% (w,w) based on glucose syrup content with glucose syrup added to

achieve a total solids level of 45% (w,w). Fish oil was added at either 20% or 50% based on wall component content. Median oil droplet diameters ranged from 1.58 $\mu$ m for the high level of pectin with low oil content to 2.87 $\mu$ m for the low level of pectin with high oil content. Particle sizes ranged from 14.2 $\mu$ m to 18.1 $\mu$ m. The lowest surface oil (0.4%) was achieved with 2.2% pectin and 20% oil while 1.1% pectin and 50% oil produced microcapsules with the highest level of surface oil (10.3%). The oxidative stability of the microcapsules was not evaluated in this work.

Drusch and colleagues (2007) also applied principal component analysis to identify key properties determining oxidative stability of microcapsules. Microcapsules were prepared from *n*-OSA starch, *n*-OSA starch and glucose syrup, *n*-OSA starch and gum arabic and glucose syrup, sugar beet pectin and glucose syrup, and caseinate and glucose syrup. The authors highlight the importance of interfacial composition and properties in both the feed emulsion and dried particles in effecting oxidative stability.

Klinkesorn et al (2005) used electrostatic assembly techniques to surround tuna oil with lecithin and chitosan (degree of deacetylation = 75-85%) in an oil-in-water emulsion. Emulsions were prepared by combining tuna oil and lecithin and sonicating to homogenize. A chitosan suspension was then added to this emulsion and the mixture was sonicated again. Final composition was 5% (w,w) tuna oil, 1% (w,w) lecithin, and 0.2% (w,w) chitosan. Dried particles were prepared by adding corn syrup solids and freeze drying. Measurement of lipid hydroperoxide and thiobarbituric acid reactive substances indicated that a layer of lecithin + chitosan provided more protection than a layer of lecithin alone in both liquid and dried systems. Incorporating EDTA and  $\alpha$ -tocopherol in the emulsions prior to drying resulted in microcapsules with greater stability. General microcapsules properties were not evaluated in this work. The authors believe that chitosan is able to inhibit initiation of oxidation by metals such as iron due to charge repulsion effects.

In later work, the microcapsule properties and oxidative stability of tuna oil, lecithin, chitosan microcapsules prepared with corn syrup solids at a level of 20% (w,w) prepared at different inlet temperatures (165°C, 180°C, and 195°C) were compared (Klinkesorn, Sophanodora et al. 2005). Microcapsules had encapsulation efficiencies between 85 and 87% and particle diameters of about 5-30 $\mu$ m. The drying temperature did not have a significant effect on surface oil content, encapsulation efficiency or oxidative stability as evidenced by PV

measurement. The spray drying process caused PV to increase from less than 1 mmol/kg oil to approximately 3 mmol/kg oil. Microcapsules had moisture contents between 1 and 3% and water activities between 0.1 and 0.25. SEM imaging of the microcapsule interiors indicated that air pockets were present in the particles.

Shaw, McClements and Decker (2007) also investigated the stability of spray dried tuna oil-in water emulsions prepared with lecithin, chitosan, and corn syrup solids. Emulsions were prepared above and corn syrup solids were added at varying levels for spray drying. Spray drying was carried out at an inlet temperature of 180°C on a spray drier with a rotary disk atomizer. Oxidative stability was evaluated during storage at 37°C and 33% RH. The authors found that powders encapsulated with 5% (w,w) corn syrup solids had the greatest oxidative stability relative to those prepared with 1%, 2%, 10%, and 20%, but that the physical stability of the powders was better for those prepared with 10% or 20% corn syrup solids. Particles prepared with only 1 or 2% corn syrup solids exhibited undesirable agglomeration of particles suggesting these levels of corn syrup solids were not sufficient to produce usable microcapsules.

Chitosan (degree of deacetylation=80%) also has been used as an encapsulant for tuna oil in combination with MD (DE=5) and whey protein. Microcapsules were prepared by ultrasonic atomization followed by precipitation with sodium hydroxide and freeze drying (Klaypradit and Huang 2008). These researchers found that chitosan was unable to produce stable emulsions when used at levels of 0.5% and 1.0% (w,w) in tuna oil emulsions containing 10, 20, and 30% (w,w) tuna oil as evidenced by creaming (Tween 80 was added as an additional emulsifier). No creaming was observed when the emulsion contained 1% chitosan and 10% MD and 10 and 20% tuna oil; an effect most likely due to increased viscosity of these formulations. Oil droplet size of these emulsions was between 0.8 and 1.4µm. Emulsions with 1% or 1.5% chitosan and 1% whey protein were slightly less stable and had slightly higher oil droplet sizes. Emulsions with only 0.5% chitosan had significant increases in oil droplet size when stored for 30 days. These authors saw slight decreases in EPA content of the tuna oil from 28.3g/100g prior to encapsulation to 24.9g/100g post encapsulation and from 28.3g/100g to 24.3g/100g for DHA. The generated microcapsule had encapsulation efficiencies close to 80%, particle sizes in the range of 8-10µm, low moisture contents (~3%) and water activities close to 0.3.

It is generally believed that higher microencapsulation efficiencies (i.e. lower levels of surface oil) will correspond to greater oxidative stability due to the expectation that oil on the

surface has greater exposure to air (Marquez-Ruiz, Velasco et al. 2003). It is most common in the literature to monitor oxidation of microencapsulated lipid as a single extract, in other words not to consider surface oil separately from encapsulated oil. There has however been some research conducted where surface oil and encapsulated oil are analyzed separately. For example, Marquez-Ruiz et al (2003) found that surface and encapsulated oil oxidized at approximately the same rate for microencapsulated sunflower oil prepared without antioxidants, but that surface oil oxidized more rapidly for microencapsulated infant formula, and that surface oil oxidized more slowly for microencapsulated sunflower with tocopherol.

Baik et al (2004) monitored oxidation of surface lipid and encapsulated lipid separately for fish oil microcapsules. The microcapsules were prepared with a blend of corn syrup solids and sodium caseinate as wall materials by spray drying at 210/95°C. Fish oil was added at a 0.7:1 oil:wall ratio. Homogenization conditions were selected to produce emulsions with oil droplet diameters on the order of 0.5µm and lecithin was included in the formulation for emulsion stabilization. Microcapsules had low moisture content (1.1%). Encapsulation efficiency was close to 88%. Peroxide value was measured using a spectrophotometric method. Spray drying resulted in an increase in PV from 6 mmol/kg fat in fresh oil to approximately 9.5 mmol/kg fat post homogenization to 17.4 mmol/kg fat post spray drying. Surface oil PVs were ten times higher than those for encapsulated oil when stored at 30°C and 11% RH. The effect of  $\alpha$ -tocopherol and ascorbyl palmitate at 250 ppm and 1000 ppm was also evaluated and the authors report that  $\alpha$ -tocopherol at both levels was effective in delaying oxidation based on PV measurements made over 7 days of storage, but that only the 1000 ppm level made a difference after 14 days of storage according to sensory testing. PVs of surface fat for microcapsules with no antioxidant reached about 600 mmol/kg oil by day 2 and peaked at about 1300 mmol/kg oil on days 3 and 4. Encapsulated fat for the same microcapsules had a PV close to 60 mmol/kg on day 2 and peaked at about 150 mmol/kg on day 4. The effect of relative humidity was evaluated based on measurement of thiobarbituric acid reactive substances (TBARS). Samples were stored at 0, 11, 33, and 43% RH. No significant effect on lag time or peak TBARS value was observed for different humidities.

While surface oil is often viewed as a key factor for oxidative stability, it has been pointed out that encapsulated oil can still be quite susceptible to oxidation, particularly when air is incorporated in the particle during processing. It is not uncommon for spray dried particles to

contain a large central vacuole or several small vacuoles embedded within the particle matrix. Keogh et al (2001) evaluated relationships between vacuole volume, homogenization conditions (pressure and number of passes), and packaging conditions on free and surface oil content and oxidative stability of microencapsulated fish oil. Microcapsules were prepared from skim milk powder (38% protein content, casein:whey ratio of 76:24 and 50% lactose) or sodium caseinate and lactose or calcium caseinate and lactose. Skim milk powder produced particles with a vacuole volume of 7ml/100g powder, calcium caseinate, 14ml/100g and sodium caseinate 21ml/100g. Wall materials were used at approximately 20% and oil:wall ratios were approximately 1:2. Emulsions were prepared using a single stage pressure homogenizer at 15, 30, and 50 MPa for 1, 3, or 5 passes. Increasing pressure and number of passes decreased oil droplet size with oil droplet sizes ranging from 2.73 $\mu$ m to 0.66 $\mu$ m. Emulsions prepared with sodium caseinate had smaller oil droplet sizes than those prepared with calcium caseinate and skim milk powder produced the largest oil droplet sizes. These trends held true for reconstituted oil droplet sizes. Emulsions were spray dried using a two-fluid nozzle at an inlet temperature of 177°C and outlet temperature of 75°C.

Jonsdottir, Bragadottir, and Arnarson (2005) evaluated oxidative stability of fish oil microencapsulated in blends of sodium caseinate (SC) and lactose, SC and sucrose, and SC and MD (DE=20) and fish gelatin, gum arabic, and MD (DE-20). Fish oil was added at a level of 20% (w,w) based on total emulsion content and wall content was maintained at 80% (w,w) of the total composition. Span 80 and lecithin were used as additional emulsifiers. Emulsions were homogenized for 5 minutes at 20,000rpm and spray dried at an inlet temperature of 175°C and outlet temperature of 100-110°C. Microcapsules had mean particle diameters between 9 and 46  $\mu$ m with gelatin microcapsules being the largest. Gelatin microcapsules also had the highest ratio of microcapsules with visible vacuoles using light microscopy (37%). Interestingly, SC-lactose particles prepared with Span 80 had a particle size of approximately 10 $\mu$ m while SC – lactose particles prepared with lecithin had a mean diameter of 32 $\mu$ m (emulsion properties—oil droplet size, and viscosity —were not reported). All particles had surface fat levels close to 3%. Oxidation was monitored by using an Oxipres apparatus, as well as by measuring TBARS, headspace solid phase microextraction with gas chromatography-mass spectrometry (HS-SPME-GC/MS), gas chromatography-olfactometry (GC-O), and by hedonic ranking by a trained sensory panel. Oxipres analysis suggested that the caseinate-sucrose microcapsules were less

stable than caseinate-maltodextrin and caseinate-lactose microcapsules. These researchers also suggest that hexanal, 2-nonenal, and 2,4-decadienal may be useful markers for monitoring oxidation of microencapsulated fish oils.

Clearly, microencapsulation is viewed as an important technique for improving the stability of fish oil and other PUFA rich oils. Despite the range of work already completed, there remains a need for information regarding the interplay of various wall materials and the relationship between microcapsules properties and oxidative stability.

## **POLYSACCHARIDES FOR MICROENCAPSULATION OF FISH OILS AND OTHER PUFA SOURCES BY SPRAY DRYING**

### **Introduction**

In this work, polysaccharides were evaluated as wall materials for generation of fish oil microcapsules by spray drying. The primary emphasis was on chitosan, a naturally occurring polysaccharide with protein-like qualities that provide unique properties suggesting it may be well suited for microencapsulation applications. Two other polysaccharides, high-amylose starch and pullulan, which also exhibit promising qualities in terms of preserving oxidatively unstable materials by microencapsulation, also were considered in conjunction with chitosan. The following section discusses some general properties of these materials in reference to their use as food ingredients and microencapsulants.

### **Chitosan: General Properties**

Chitosan is a naturally occurring polysaccharide of D-glucosamine and N-acetyl-D-glucosamine joined through a  $\beta(1\rightarrow4)$  linkage. It is derived from chitin, the second most abundant naturally occurring polymer; the first being cellulose. The main sources of chitin and chitosan are crustacean exoskeletons, but these polysaccharides are also structural components in fungal cell walls (Agullo, Rodriquez et al. 2003). The structures of both chitin and chitosan are shown in Figure 2.4. Chitosan is classified by the quantity of amine groups present on the chain. This property is referred to as either the degree of acetylation (DA) or the degree of deacetylation (DD). Generally, a material is considered chitosan rather than chitin if the degree of deacetylation is greater than 60% (Muzzarelli and Muzzarelli 2005). The amine groups along

the chitosan backbone contribute to unique and beneficial properties including antimicrobial properties, the ability to chelate metal ions, bioadhesion, and immunostimulatory activity (Agnihotri, Mallikarjuna et al. 2004). The amine groups have a  $pK_a$  in the range of 6.2 to 6.8 such that the material acts as a cationic polyelectrolyte under acidic conditions (Agullo, Rodriquez et al. 2003). Chitosan is nontoxic, biocompatible, biodegradable, and can be recovered from waste materials.

### **Chitosan as a Food Additive or Preservative**

The use of chitosan in food applications is growing, and food preservation is a primary interest. Chitosan has been evaluated as an antimicrobial agent in products including fruits and vegetables, pre-pizza, meats, and fish products. The microorganisms it has been shown to inhibit include *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli*, *Proteus vulgaris* and various fungi including *Alternaria sp.*, *Penicillium sp.*, and *Cladosporium sp* (Shahidi, Arachchi et al. 1999). When applied as a coating to fruits and vegetables chitosan not only reduces microbial growth but also extends product shelf-life by delaying ripening, controlling moisture transfer, limiting oxygen transmission, and preventing browning reactions (Shahidi, Arachchi et al. 1999; Agullo, Rodriquez et al. 2003). Pears, peaches, kiwi, lichi, cucumbers, squash, bell peppers, strawberries, and tomatoes all have exhibited improved properties when coated in chitosan films (Shahidi, Arachchi et al. 1999; Agullo, Rodriquez et al. 2003). Edible chitosan films also have been shown to improve the quality of frozen salmon fillets and beef products (Darmadji and Izumimoto 1994; Spagna, Pifferi et al. 1996; Georgantelis, Blekas et al. 2007; Sathivel, Liu et al. 2007). Darmadji and Izumimoto (1994) demonstrated that chitosan (DD and MW not reported) could prevent oxidation (as indicated by TBARS values) in beef when stored at refrigeration temperatures. Georgantelis et al (2007) found that chitosan (DD=88.2% and MW= $4.9 \times 10^5$  Da) applied as a coating prevented oxidation (as indicated by conjugated diene values, peroxide values, and malondialdehyde content) and additionally improved color retention in frozen beef patties. Oxidation prevention also was observed in cooked herring treated with chitosan (Kamil, Jeon et al. 2002). These researchers evaluated three different chitosan samples, DD=86.4%,  $M_v=1.8 \times 10^6$  Da, DD=89.3%,  $M_v=9.6 \times 10^5$  Da, and DD=91.3%,  $M_v=6.6 \times 10^5$  Da, and found that all were effective at preventing oxidation. This was evidenced by peroxide values, TBARS and volatile aldehyde content. Chitosan also has demonstrated value in the juice and wine industry.

Spagna et al (1996) found that chitosan added to white wines prevented oxidative browning reactions by binding phenolics to the same degree as some conventionally used adsorbents. Clarification of grapefruit and apple juice using chitosan is possible and the additional benefit of acidity control is achieved (Shahidi, Arachchi et al. 1999). Chitosan was observed to have a beneficial effect on viability of probiotic bacteria in yogurt. Iyer and Kailasapathy (2005) found that when probiotics were contained in capsules that had been coated in chitosan the survival of the probiotics in *in vitro* acidic conditions was almost 0.6 log higher than for uncoated capsules and for capsules coated in alginate or lysine. When the chitosan coated capsules were incorporated into a yogurt product the decrease in cell viability over a 6 week storage period was half of what it was for free cultures. This study did not evaluate the effects of chitosan on yogurt properties.

### **Chitosan as a Functional Food Component (Health Related Properties of Chitosan)**

In this work, the intention is for the encapsulated lipid to act as the main therapeutic agent; however, chitosan has been found to exhibit some health-beneficial properties that also may be relevant. Chitosan has been shown to have both weight loss and cholesterol lowering effects. The cationic nature of chitosan results in interactions between chitosan and fatty acids, bile acids, and phospholipids (Shahidi, Arachchi et al. 1999; Agullo, Rodriguez et al. 2003). The binding of bile acids results in secretion of lipids and is related to weight loss properties of chitosan (Muzzarelli and Muzzarelli 2005). In an *in vitro* study Agulla et al (2003) found that in the stomach, chitosan binds fat, forming micelles. In the environment of the intestines the structure of the droplets changes such that the fat is entrapped and cannot be attacked by lipase, resulting in fat excretion. In rat models, dietary supplementation with chitosan has been shown to prevent increases in serum cholesterol, reduce cholesterol, and increase fat excretion (Muzzarelli 1996). In human studies similar results were obtained. A feeding trial where males were fed 3-6 g of chitosan per day over 4 weeks revealed that total serum cholesterol levels decreased and the ratio of high density lipoprotein (good cholesterol) to low density lipoprotein (bad cholesterol) was improved (Maesaki, Tsuji et al. 1993). Dietary supplementation with chitosan (at a level of 240 mg/day) has been shown to result in weight loss, reduce triglyceride levels, and reduce total and LDL cholesterol levels (Muzzarelli 1996).

### **Chitosan as an Emulsifier**

The amine groups along the chitosan chain contribute protein-like behavior to chitosan and enable chitosan to behave well as an emulsifying agent. Considering the necessity of forming a stable oil-in-water emulsion for spray drying of lipid based foods, the emulsifying properties of chitosan are very relevant to its use as an encapsulating medium. Del Blanco et al (1999) and Rodriguez et al (2002) evaluated the ability of chitosan to act as an emulsifier in sunflower oil emulsions. Emulsion stability and viscosity depended on chitosan concentrations, with higher concentrations producing better emulsions, and on chitosan DD. Of chitosan samples with DD ranging from 73% to 95%, the authors found that those with DD of 81% and 88% were most effective as emulsifiers. In this regard, chitosan is believed to have great applicability because it can stabilize emulsions through both an electrostatic method and through viscosity effects. It also has been suggested that chitosan may be useful as a gelling agent and for water binding (Shahidi, Arachchi et al. 1999; Agullo, Rodriguez et al. 2003).

### **Chitosan as an Encapsulant**

Chitosan has been used as an encapsulant for food ingredients, but has been explored much more widely as a pharmaceutical encapsulant (Agnihotri, Mallikarjuna et al. 2004; Uragami and Tokura 2006). Food applications evaluated include pigments, antioxidants, and PUFAs.

Parize and colleagues (2008) used chitosan (DD=90%, MW not reported) to encapsulate urucum pigment by spray drying. Urucum was combined with chitosan in a ratio of 1:6 by weight and this mixture was dissolved in either 5% acetic acid, 5% lactic acid, or 5% citric acid. Spray drying was carried out with a two fluid spray nozzle at an inlet temperature of 180°C and an outlet temperature of 100°C. Particles were in the range of 2-20µm. Thermal stability was evaluated using thermogravimetric analysis (TGA). Microcapsules prepared in acetic acid degraded at the lowest temperature (302°C) while those prepared with citric acid degraded at the highest (392°C).

Kosaraju, D'ath, and Lawrence (2006) encapsulated olive leaf extract in chitosan (DD and MW not reported). Chitosan was dissolved at a level 1% (w/w) in 5% (v/v) acetic acid. The ratio of olive leaf extract to chitosan was 1.25:1 by weight. Microcapsules were spray dried at an inlet temperature of 180°C and an outlet temperature of 74°C. Median particle diameter of the

microcapsules was approximately 10 $\mu$ m. The authors reported a loading percent of polyphenolic compounds in the microcapsules of 27%.

Shi and Tan (2002) used chitosan to encapsulate vitamin D. Two different types of chitosan were evaluated, chitosan with DD= 91% and MW=1.26 x 10<sup>3</sup> kDa, and chitosan with DD=83.5% and MW=1.85 x 10<sup>3</sup> kDa. Chitosan was used at 2-4% (w,v) in 1-2% (w,v) acetic acid and vitamin D was added at 20-40mg/g chitosan. Spray drying was done at an inlet temperature of 168°C. Microcapsule had encapsulation efficiencies higher than 95% and median particle size of 2-20  $\mu$ m. The researchers observed a slower release rate for the higher molecular weight chitosan and for more concentrated chitosan solutions. Higher acetic acid concentrations appeared to produce less compact particles.

Chitosan also has been used to encapsulate fish oil through electrostatic layer by layer assembly and ultrasonic atomization followed by freeze drying (Klinkesorn, Sophanodora et al. 2005; Klinkesorn, Sophanodora et al. 2005; Klinkesorn, Sophanodora et al. 2006; Shaw, McClements et al. 2007; Klaypradit and Huang 2008). This research was discussed in a previous section.

The mucoadhesive and immunostimulatory properties and potential for controlled/targeted delivery of chitosan make it attractive for pharmaceutical applications. Desai and Park (2005) encapsulated acetaminophen with chitosan by spray drying and subsequent cross-linking with tripolyphosphate. The prepared microspheres were positively charged and ranged in size from ~3 $\mu$ m to ~10 $\mu$ m. Drug release occurred by a diffusion mechanism and was affected by chitosan molecular weight and degree of crosslinking by tripolyphosphate. Similarly, work by Liu et al (2006) showed diffusion controlled release of acetaminophen from crosslinked spray-dried microspheres of mean particles size 3.8 to 4.2 $\mu$ m. These researchers considered tripolyphosphate, formaldehyde, and glutaraldehyde as crosslinking agents and found that surface morphology and drug release was affected by crosslinker type and concentration. Lower crosslinker concentration (1% w/w versus 2% w/w) produced more spherical particles and greater drug release. Crosslinking with formaldehyde or glutaraldehyde produced a more rigid matrix therefore a lower extent of drug release than that for tripolyphosphate crosslinked microspheres. These systems showed benefits including sustained release, improved drug solubility, and improved drug activity (Muzzarelli and Muzzarelli 2005).

## **Resistant Starch: General Properties**

Starch, a polysaccharide comprised of glucose units, is the most common carbohydrate in the diet. Starch consists of two major fractions; amylose, a linear region formed from  $\alpha(1\rightarrow4)$  linkages, and amylopectin, a branched structure that results from  $\alpha(1\rightarrow6)$  linkages. These structures are illustrated in Figure 2.5. In general, starch is digested in the stomach and small intestine by the action of enzymes ( $\alpha$ -amylase, glucoamylase, and sucrase-isomaltase). Some starch, however, passes through the small intestine undigested and is partially fermented in the large intestine, acting as a dietary fiber (Nugent 2005). This starch is referred to as resistant starch (RS) and occurs in four forms, RS1 – RS4. RS1 is the form found in whole grains and is resistant due to physical inaccessibility to enzymes. RS2 describes native starch granules that have a highly compact structure, resulting in inaccessibility to enzymes. RS3 describes starch that has been retrograded, forming highly crystalline regions that resist enzyme attack. RS4 describes chemically modified starches such as crosslinked or esterified starch. Starch generally has 20-30% amylose content (Bertoft 2004). Amylose contents in resistant starch are generally higher. The type of resistant starch used in this work was a high-amylose maize starch that was amorphous and had 70% amylose content. High amylose maize is a form of RS2 that remains resistant throughout processing (Sajilata, Singhal et al. 2006).

## **Resistant Starch as a Food Additive/Food Ingredient**

Resistant starch exhibits functional properties that make it attractive as a food ingredient. It has bland flavor, making it easy to incorporate without negative flavor effects (Sajilata, Singhal et al. 2006). Incorporating RS into bread improved loaf volume and cell structure as compared to cellulose, oat fiber, and wheat fiber (Sajilata, Singhal et al. 2006). RS has also been shown to improve tenderness in cakes when added to increase fiber content, act as a crisping agent in products such as frozen waffles, and produce greater expansion during extrusion of products such as cereals. RS also has been shown to increase viscosity and aid in formation of gels. RS can be added to foods to increase health benefit, while also possibly improving food properties.

## **Resistant Starch as a Functional Food Component**

As previously mentioned, resistant starch acts as a dietary fiber. In relation to this, it has been shown to have a variety of health benefits. RS consumption has been shown to significantly

increase the production of short chain fatty acids, especially butyrate, in the colon. This effect has been linked to prevention of colon cancer (Nugent 2005; Sajilata, Singhal et al. 2006). Toden et al (2006) found that damage to DNA and reduction of the colonic mucus layer from high protein diets could be avoided if RS was included in the high protein diet. RS has also shown benefits in relation to diabetes, for example Robertson et al (2005) found that dietary supplementation with RS increased insulin sensitivity and glucose clearance in human subjects when incorporated in the diet. Such an effect could be quite beneficial in dairy products. Morais et al (1996) found that absorption of both calcium and iron was increased in pigs when fed a meal containing resistant starch.

### **Pullulan: General Properties**

Pullulan is a water soluble linear polysaccharide formed from  $\alpha(1\rightarrow6)$  linked maltotriose units. The structure of pullulan is shown in Figure 2.6. Extensive research has shown that pullulan is an excellent film former (Kshirsagar, Yenge et al. 2009). Furthermore, pullulan films have been shown to exhibit substantial oxygen barrier properties (Leathers 2005). Pullulan has also been shown to act as a dietary fiber and prebiotic (Spears, Karr-Lilienthal et al. 2005; Knapp, Parsons et al. 2008)

### **Pullulan and Emulsions**

Kshirsager et al (2009) found that pullulan had a stabilizing effect on turmeric oleoresin emulsions prepared with gum arabic. These researchers found that incorporating pullulan at a level of 1% (w,w) resulted in an emulsion index (ESI) of 1, indicating no oil separation after 24 hours of storage in a cylinder. The emulsion contained 30% (w,w) gum arabic and turmeric oleoresin at 5% (w,w) based on gum arabic content. ESI of the emulsion without pullulan was 0.4. The enhanced stability was attributed to increased viscosity and polymer-polymer interactions.

Including pullulan in cottonseed oil emulsions relying on cottonseed protein isolates as emulsifier reduced initial average oil droplet diameter and improved emulsion stability as monitored by coalescence (Tsaliki, Pegiadou et al. 2004). In this case, positive effects on emulsions were attributed to the increase in viscosity.

Matsumura et al (2000) evaluated pullulan as an emulsifier in comparison to gum arabic and maltodextrin and reported that only gum arabic was able to produce stable emulsions under the conditions evaluated. Emulsions were prepared using a high speed blender followed by ultrasonic homogenization. In emulsions containing 20% (w,w) polysaccharide and 18% (w,w) lipid (methyl linoleate), gum arabic produced an emulsion with oil droplet size in the range of 1-4 $\mu$ m whereas oil droplet size in the pullulan containing emulsion were widely distributed with most in the range of 10 to 200 $\mu$ m. Furthermore, the pullulan emulsion had signs of separation within a few hours of being prepared. Rheological assessment led the researchers to conclude that the concentration of pullulan was likely not high enough to support entanglement of the pullulan chains.

### **Pullulan as an Encapsulant**

Kshirasagar et al (2009) incorporated pullulan in gum Arabic and turmeric oleoresin emulsions to generate turmeric oleoresin microcapsules. The microcapsules were prepared by spray drying at an inlet temperature of 140°C and an outlet temperature of 88°C. The authors found that spherical microcapsules formed when pullulan was incorporated at 1% (w/w) for emulsions containing 30% (w/w) gum arabic and 5% oleoresin (w/w, based on gum Arabic weight), but that at higher levels fiber-like structures started to form with cobweb structures resulting for pullulan concentrations of 3-5% (w,w). The authors evaluated blends of gum arabic and MD all of which contained 1% pullulan and found that gum Arabic and pullulan alone produced microcapsules with the highest encapsulation efficiency (72%). This was also the most stable emulsion of those evaluated.

Pullulan (MW= 2 x 10<sup>5</sup> Da) in combination with gum arabic and MD (DE=2-5) were also considered for encapsulation of linoleic acid (Minemoto, Adachi et al. 1999; Minemoto, Adachi et al. 1999). The researchers used a hot air droplet drying method that produces particles approximately 2-3 mm in size. Wall materials were used at levels of 15% (w,v) and lipid was added to yield lipid:wall ratios of 0.2, 0.5, 1.0 or 2.0. In work comparing oxidative stability of the encapsulated linoleic acid, the researchers found that gum arabic performed best, followed by pullulan, and MD performed worst (Minemoto, Adachi et al. 1999). In work focused on lipid extraction from the microcapsules the authors found that the fraction of lipid extracted gradually increased over time with exposure to organic solvent and that lipid was extracted more rapidly

from MD microcapsules than pullulan or gum arabic microcapsules (Minemoto, Adachi et al. 1999). The maximum fraction of lipid extractable was greater for microcapsules with higher lipid:wall ratios.

## **SUMMARY**

Increasing dietary consumption of  $\omega$ 3 PUFAs may improve overall health and well-being and formulating food products to contain recommended levels of  $\omega$ 3 PUFAs is of interest to the food industry. Fish oil is a good source of EPA and DHA and has value as a food ingredient but low stability of PUFAs and off flavors and odors associated with fish oil limit its use as an ingredient. PUFAs are highly susceptible to oxidation and intervention strategies are necessary to preserve the integrity of PUFAs and PUFA enriched products throughout processing and storage. Microencapsulation by spray drying is one method for preserving PUFAs that may also mask undesirable flavors and odors and reduce food matrix incompatibility issues. Although much research has been conducted on materials for preparing fish oil microcapsules by spray drying, there is still an interest in alternative materials and a need for a improved understanding of the relationships between emulsion characteristics, drying conditions, and wall materials, and their impact on microcapsules properties, in particular oxidative stability.

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## TABLES

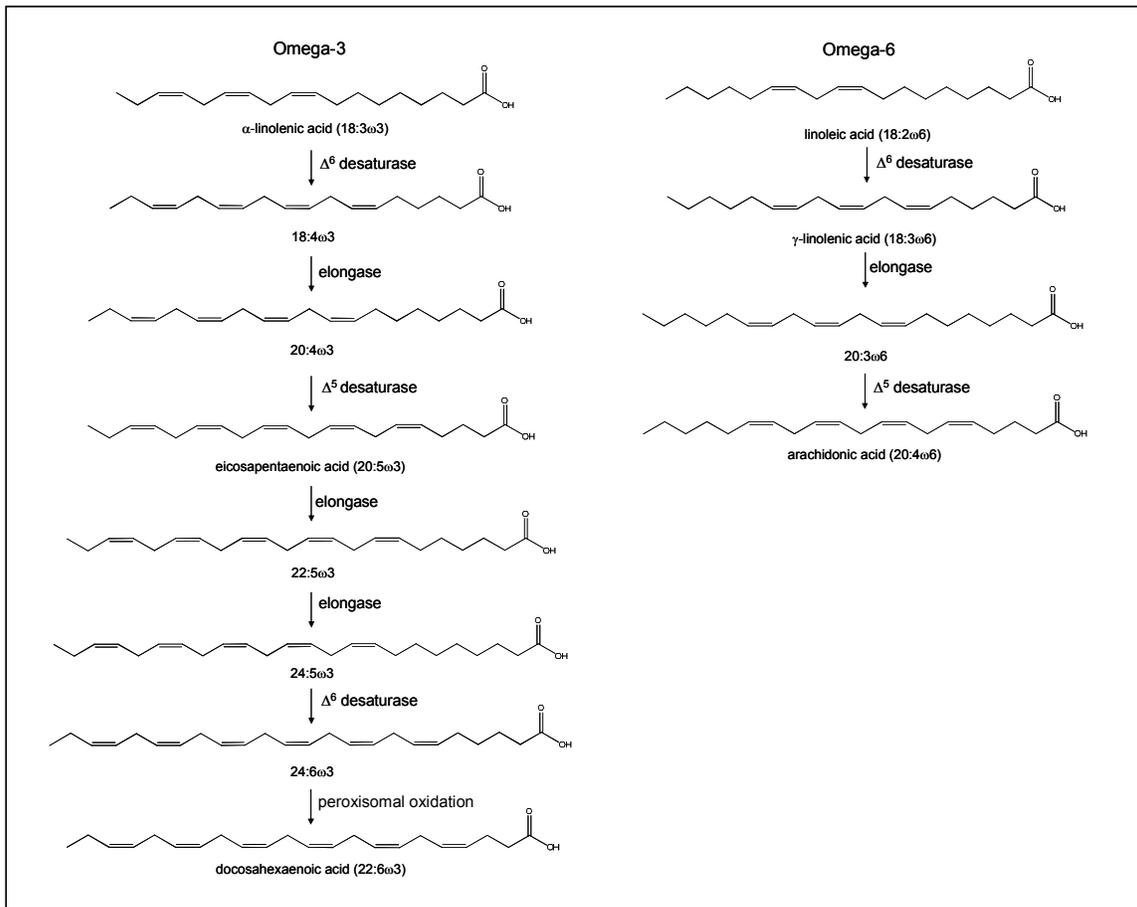
**Table 2.1. Secondary oxidation products identified in bulk fish oil, fish oil emulsions, and microencapsulated fish oil.**

Compound	Odor Description	Reference(s)
acetic acid		5
acetaldehyde		1
pentane		1
propanal	sharp, irritating	1, 2, 6
2-propenal		3, 6
1-butanol	caramel, vanilla	5
butanal		1,5
2-butenal	caramel, vanilla	3, 5
<i>t</i> -2-butenal	old cheese	6
3-methylbutanal	caramel, vanilla	5
3-hydroxy-2-butanone		5
1-pentanol		5
pentanal	caramel, vanilla	1, 3, 5
<i>t</i> -2-pentenal	pungent, glue, green, grassy, paint, apple	6
<i>c</i> -2-pentenal	fruity	6
2-pentenal		1, 3, 5
1-penten-3-one	sharp, fishy, pungent, rancid green, glue	1, 2, 4, 6
3-penten-2-one		5
2-penten-1-ol		3
<i>c</i> -2-penten-1-ol	musty, compost-like	6
<i>t</i> -2-penten-1-ol	green	6
1-penten-3-ol	caramel, vanilla, sweet	1,3, 4, 5, 6
2-penten-3-ol		5
hexanal	grassy	1, 3, 5
2-hexenal		3, 4, 5
<i>t</i> -2-hexenal	sour, green	6
<i>c</i> -3-hexenal	fresh green leaves, sour, old cheese	2, 6
1-hexen-3-ol		5
2,4-hexadienal		6
heptanal	rancid	1, 3, 5
2-heptenal		1, 3
<i>c</i> -4-heptenal	rancid, potato, putty, stale, burnt, fishy	2, 5
4-pentanal, 2-methyl		3
<i>t,t</i> -2,4-heptadienal	fatty, oily, nasty, green, rancid, hazelnuts	1, 2, 3, 4, 5, 6
<i>t,cis</i> -2,4-heptadienal	frying odor, fishy, fatty, burnt, rotten apples	2, 6
<i>E, E</i> 2,4-heptadienal	citrus, fresh	5
2-ethyl-1-hexanol		5
2-ethylfuran	flower	1,6
2-pentylfuran		1
benzaldehyde		5
octane		1
1-octanol		5

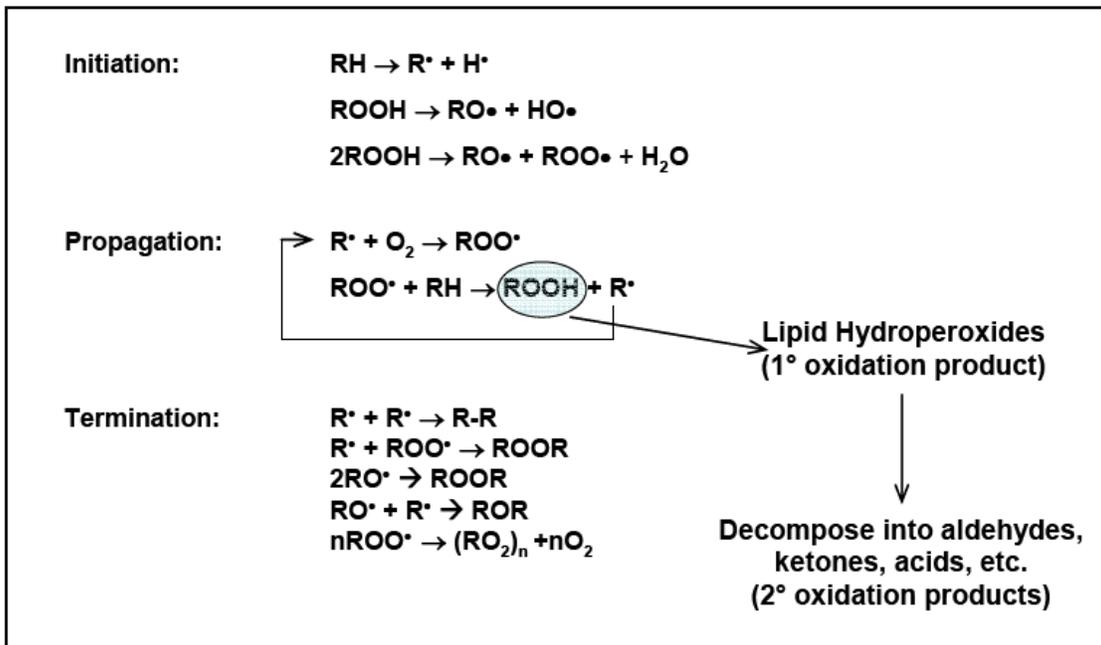
octanoic acid		5
octanal		5
2-octenal		3
1-octen-3-ol	mushroom	3, 5
2,5-octanediene		3
3,5-octadiene-2-one		5
nonanal		5
2-nonenal	cucumber	3, 5
2-nonanone		5
<i>t,t</i> -2,6-nonadienal	cucumber, tallowy	4
<i>t,c</i> -2,6-nonadienal	fresh cucumbers	2, 6
decane		5
decanal	rancid	5
2-decenal	rancid	5
undecanal	tallow	5
hexadecane		5
hexadecanal	tallow	5
2-decanone		5
2,4-decadienal	rancid	3, 5
2,4,7-decatrienal		6
<i>t,t,c</i> -2,4,7-decatrienal	burnt, fishy	2
<i>t,c,c</i> -2,4,7-decatrienal	butnt, fishy	2
1,5-octadien-3-ol	citrus, green	6
1,5-octadien-3-one	geranium, metallic	6
1- <i>c</i> -5-octadien-3-one	metallic, geranium-like	2

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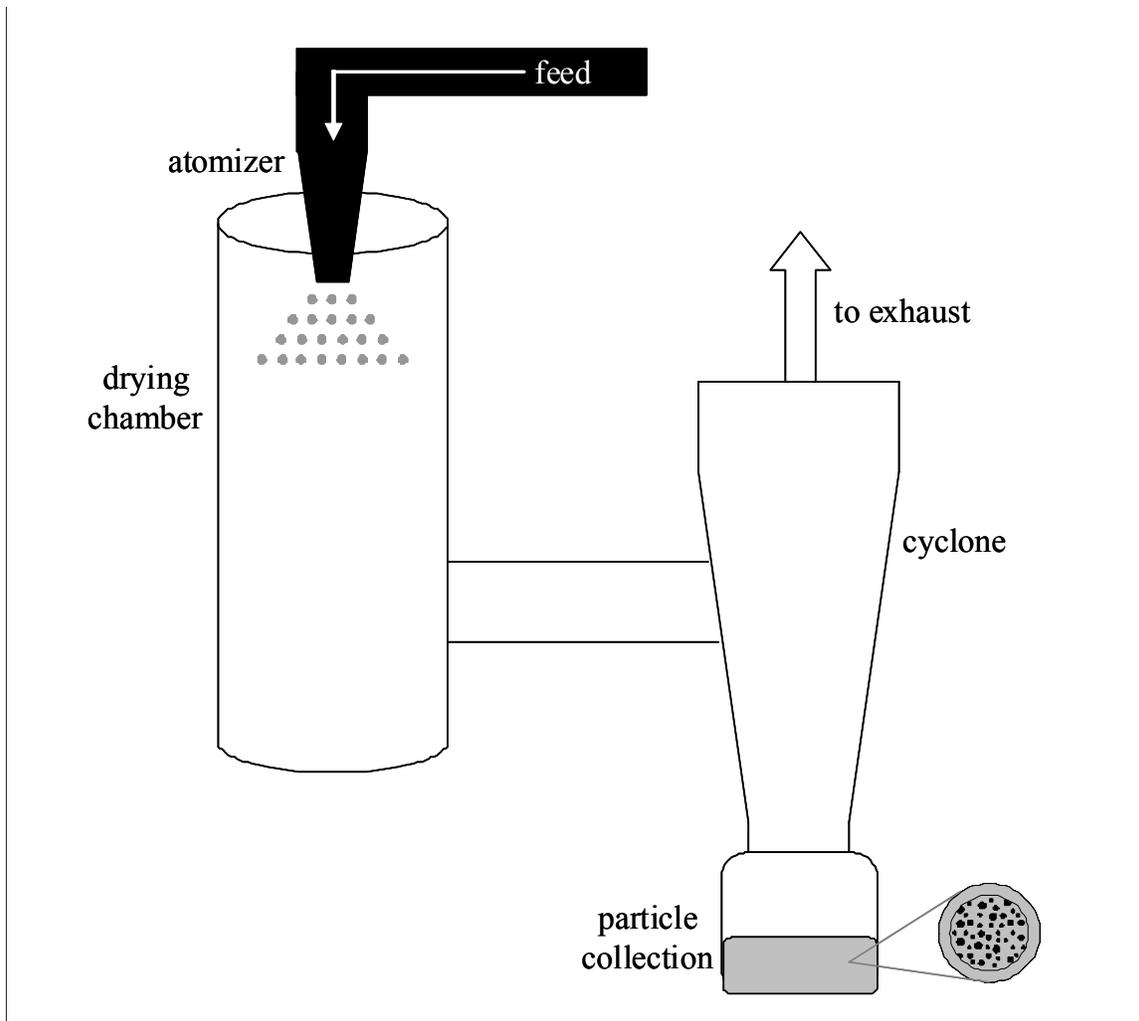
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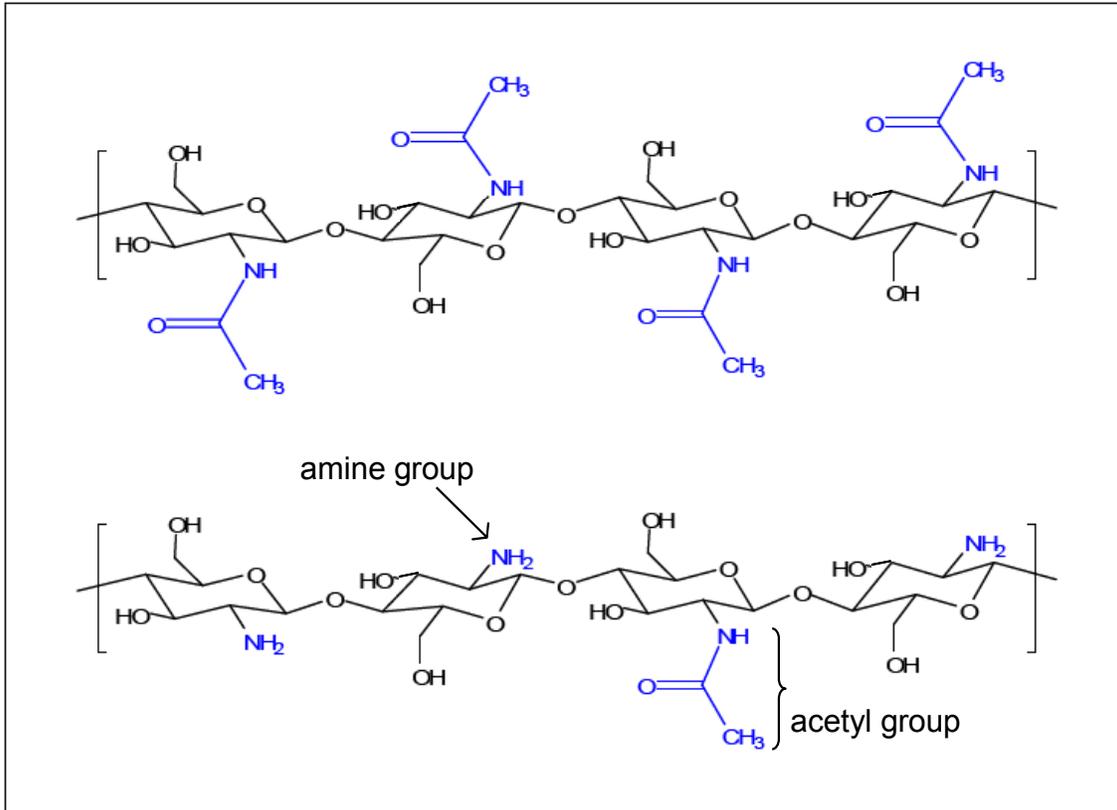
**Figure 2.1. Structures of and relationships between important omega-3 and omega-6 fatty acids.**



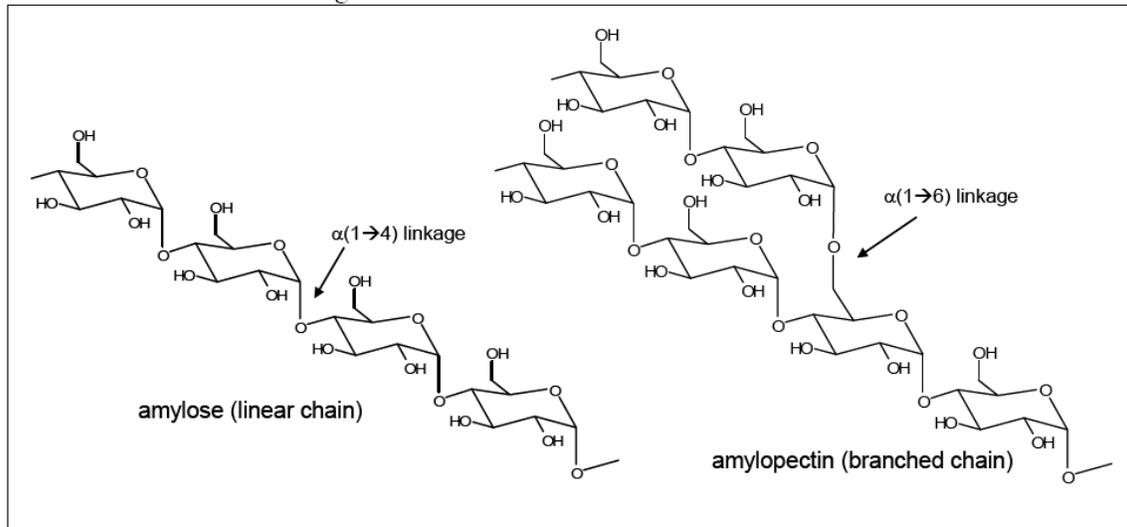
**Figure 2.2. General reactions involved in oxidation of lipids. Secondary oxidation products impact flavor and aroma. Adapted from Kamel-Eldin, 2003.**



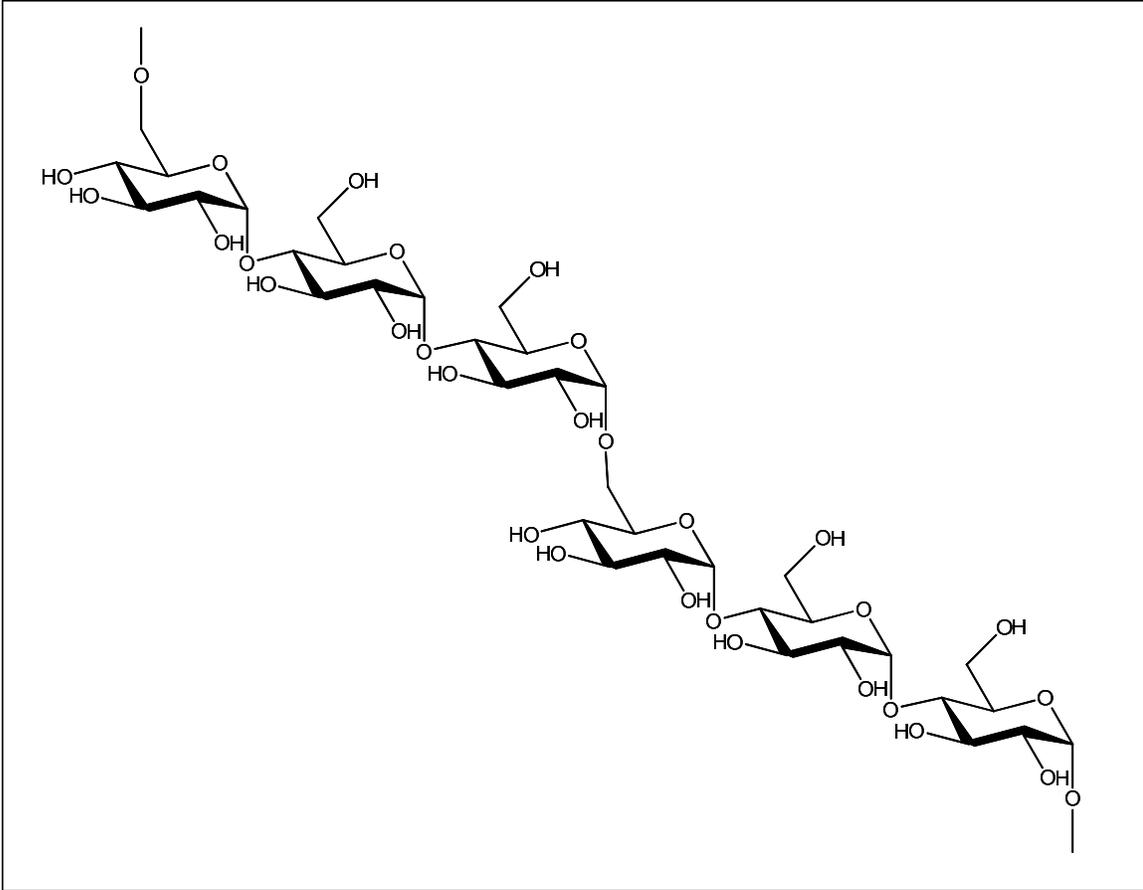
**Figure 2.3. Schematic overview of the spray drying process. The liquid feed is pumped into the atomizer generating small droplets. The droplets rapidly dry upon contact with hot air in the drying chamber. Dried particles are separated in the cyclone. Spray dried microcapsules are generally comprised of a target material embedded in a carbohydrate matrix and surrounded by a carbohydrate shell.**



**Figure 2.4. Structures of chitin (upper) and chitosan (lower). In general a material is considered chitosan rather than chitin if the degree of deacetylation (DD) is greater than 60%. As illustrated in this image the chitosan would have a DD of 75%.**



**Figure 2.5. Structures of the primary components of starch, amylose and amylopectin.**



**Figure 2.6. Basic structure of pullulan,  $\alpha(1\rightarrow6)$  maltotriose.**

## **CHAPTER 3: MICROENCAPSULATION OF FISH OIL WITH TWO DIFFERENT TYPES OF CHITOSAN BY SPRAY DRYING**

### **ABSTRACT**

Two types of chitosan differing in molecular weight and degree of deacetylation (DD) were used to encapsulate fish oil by spray drying at two different oil load levels. Oxidative stability of the microcapsules was evaluated by measuring oxidation induction time (OIT) using pressure differential scanning calorimetry. The interaction between chitosan type and oil load level had a significant effect on median oil droplet size, encapsulation efficiency, surface lipid content, and moisture content. Chitosan with DD=95% and low molecular weight (chitosan95) outperformed chitosan with DD=78% and medium molecular weight (chitosan78) by having lower surface lipid content and higher encapsulation efficiency. Both types of chitosan produced emulsions with median oil droplet sizes below 1.5 $\mu$ m. Microcapsules prepared from chitosan78 exhibited OITs significantly longer (17 minutes) OITs than those prepared from chitosan95 (14 minutes). Oil load level did not have a significant effect on OIT.

### **KEYWORDS**

chitosan, microencapsulation, spray-drying, fish oil, omega-3, oxidation

## INTRODUCTION

Spray drying is commonly used in the pharmaceutical and food industries to transform liquid materials into dried powders (Bhandari, Patel et al. 2008). A spray dried product can offer several advantages over a liquid form, particularly when the spray drying process is used to encapsulate a core material. Microencapsulation can convert a material into an easier-to-handle form, may provide protection against degradation, mask undesirable flavors or odors, control the release rate and location of a compound, and facilitate processing (Re 1998). In microencapsulation by spray drying, a target material is combined with one or more “wall” material(s) to form an emulsion or dispersion. During drying, the target material becomes embedded in a protective matrix composed of the wall material(s). Commonly used wall materials include gums (gum arabic, carageenan, alginate), modified starches (maltodextrins, n-octylsuccinate derivitized starch), and proteins (whey, soy, caseinate, gelatin) (Re 1998; Gharsallaoui, Roudaut et al. 2007; Bhandari, Patel et al. 2008). Despite the availability of several wall materials, there remains interest in evaluating new materials that could afford better protection to sensitive materials and to expand the range functional properties of microcapsules in end use products.

Chitosan, a copolymer of D-glucosamine and *N*-acetyl-D-glucosamine, has several properties that make it attractive as a spray drying wall material. The structure of chitosan is shown in Figure 3.1. These attractive properties include good film forming properties, emulsifying capabilities, and taste masking functions (Peniche, Arguelles-Monal et al. 2003; Bora, Borude et al. 2008). Furthermore, chitosan is nontoxic, biocompatible, biodegradable, and can be recovered from waste materials (Muzzarelli and Muzzarelli 2007). Chitosan is derived from chitin, which is the second most abundant naturally occurring polymer, after cellulose (Uragami and Tokura 2006). Chitosan is differentiated from chitin by a larger percentage of deacetylated (glucosamine) residues along the polymer chain. In general, a material is considered chitosan rather than chitin if it has a degree of deacetylation (DD) greater than 60% (Muzzarelli and Muzzarelli 2007). The amine groups along the chitosan backbone contribute to unique and beneficial properties for food and pharmaceutical applications, including antimicrobial activity, emulsion stabilization, metal chelation, bioadhesion, immunostimulatory activity and the potential for controlled release (Agnihotri, Mallikarjuna et al. 2004; Uragami and Tokura 2006)).

Chitosan has been widely explored as a pharmaceutical encapsulant (Agnihotri, Mallikarjuna et al. 2004; Uragami and Tokura 2006), but is less widely studied as a food ingredient encapsulant. Parize et al (2008) used chitosan (DD=90%, MW not reported) to encapsulate urucum, a natural pigment, by spray drying. Kosaraju, D'ath, and Lawrence (2006) encapsulated olive leaf extract in chitosan (DD and MW not reported). Shi and Tan (2002) generated vitamin D microcapsules two different types of chitosan (DD= 91%, MW=1.26 x 10<sup>3</sup> kDa and DD=83.5%, MW=1.85 x 10<sup>3</sup> kDa). Microcapsules in all three cases were prepared by spray drying. Klinkesorn et al (2005; 2005; 2006) and Shaw, McClements and Decker (2007) generated tuna oil microcapsules from lecithin and chitosan (DD=75-85%, medium MW) by electrostatic layer-by-layer assembly and subsequent spray or freeze drying. Spray dried particles were prepared with the addition of corn syrup solids. Klaypradit and Huang (2008) encapsulated tuna oil with mixtures of chitosan (DD=80%, MW not reported), maltodextrin, and whey protein isolate using ultrasonic atomization followed by freeze drying.

Marine oil is a good source of omega-3 fatty acids and therefore has value as a food ingredient. It is technically challenging to incorporate into foods due to high susceptibility to oxidative deterioration, off flavors and odors associated with the source, and food matrix compatibility issues. Microencapsulation can alleviate some of these challenges. Fish oil has previously been encapsulated by spray drying with sugar beet pectin (Drusch 2007), n-octenylsuccinate-derivatized starch (Drusch and Schwarz 2006), corn syrup solids, sodium caseinate and lecithin (Baik, Suhendro et al. 2004), lecithin, chitosan, and corn syrup solids (Shaw, McClements et al. 2007), modified starch (Hi-Cap 100), whey protein (Jafari, Assaidpoor et al. 2008), maltodextrin and highly branched cyclic dextrin together with sodium caseinate (Kagami, Sugimura et al. 2003), and modified cellulose, (Kolanowski, Ziolkowski et al. 2006). In this work, two types of chitosan were used to encapsulate fish oil by spray drying. Chitosan with DD=78% and medium molecular weight (chitosan78) was compared to chitosan with DD=95% and low molecular weight (chitosan95).

## **MATERIALS AND METHODS**

### **Materials**

Chitosan with DD=78% and medium molecular weight (viscosity 200-800 mPA·s as a 1% solution in 1% acetic acid as reported by supplier), denoted as chitosan78, was purchased from

Sigma-Aldrich (St. Louis, MO). Chitosan with DD=95% and low molecular weight (15 mPA·s as a 1% solution as reported by supplier), denoted as chitosan95, was donated by Primex, Inc. (Siglufjordur, Iceland). Fish oil (from anchovies and sardines) with total omega-3 content of 300mg/g (as reported by the supplier, triglyceride basis) was donated by Ocean Nutrition (Nova Scotia, Canada). All reagents were purchased from Sigma-Aldrich (St. Louis, MO) and were ACS reagent grade or higher.

### **Microcapsule Preparation**

Chitosan was dissolved in 1% (v/v) aqueous acetic acid. Medium molecular weight chitosan was dissolved at 1.5% by weight and low molecular weight chitosan was dissolved at 5% by weight. Chitosan concentrations were selected based on preliminary analysis to determine maximum concentrations that resulted in viscosities suitable for processing. Fish oil was added at a ratio of either 33% or 50% by weight, based on chitosan weight and rapidly stirred with a non-aerating overhead stirrer with propeller style blade (Kraft Apparatus, New York, NY) to prepare a coarse emulsion. Coarse emulsions then were passed twice through a two-stage pressure homogenizer (Model 15MR, APV, Everett, MA) at 20/5 MPa (200/50 Bar). Emulsions were spray dried within 24 hours of preparation on a Buchi 190 mini spray drier (Flawil, Switzerland) equipped with a 0.5mm two fluid nozzle. Air pressure was set at 0.55 MPa. Inlet and outlet temperatures were maintained at 160°C and 90°C respectively. Microcapsules for general characterization were stored in glass jars at room temperature, while those for characterization of oxidative stability were packaged in oxygen barrier bags, nitrogen flushed, and stored at -80°C.

### **Feed Emulsion Characterization**

**Viscosity.** Viscosity of the feed emulsion was measured in duplicate on a rotational viscometer with an LV2 spindle (Brookfield, Middleboro, MA). Emulsions were kept in a 25°C water bath prior to measuring viscosity.

**Oil Droplet Size.** Median oil droplet diameter was measured in triplicate using a laser diffraction particle size analyzer (Horiba LA-700, Horiba Instruments Inc, Irvine, CA) with water as a dispersant.

## **Microcapsule Characterization**

**Water Activity.** Water activity was measured at 25°C on an Aqualab Series 3TE water activity meter (Decagon Devices, Pullman, WA). Reported values are the mean of three measurements.

**Moisture Content.** Moisture content was measured by drying overnight at 100°C in a vacuum oven with admission of dried air. Measurements were made in duplicate.

**Surface Lipid, Inner Free Lipid and Encapsulation Efficiency.** Surface lipid content was measured using a solvent wash. Microcapsule samples (~1g) were rinsed three times with 10ml of hexanes through a fine sintered glass filter. The solvent was dried to constant weight and percent surface lipid was determined gravimetrically based on theoretical lipid content. Microcapsules were briefly dried and subsequently transferred to a glass jar. Twenty milliliters of hexane were added and the jars were shaken for 48 hours at 250rpm. Solvent was collected by filtering through the sintered glass filter and dried to constant weight. Inner free lipid was determined gravimetrically based on theoretical lipid content. Measurements were carried out in duplicate. Encapsulation efficiency was calculated based on theoretical total lipid content and measured surface lipid content.

**Oxidation Induction Time.** A TA Instruments (New Castle, Delaware) differential scanning calorimeter (DSC) equipped with a pressure cell was used to evaluate relative oxidative stability by measuring oxidation induction time (OIT) according to ASTM D6186-08 (ASTM 2008). A sample of microcapsules (8-12mg) was placed in an open pan in the pressure cell and heated to 120°C. After equilibrating for two minutes at 120°C, oxygen (3.45MPa) was introduced into the cell. The sample was held at 120°C and 3.45MPa until an oxidation exotherm was observed. OIT was reported as the time in minutes from the time oxygen was introduced to the cell to the peak onset time. Measurements were made in duplicate.

**Morphology.** Microcapsule morphology was characterized by scanning electron microscopy (SEM) on a Leo 1550 field emission SEM (Zeiss, Peabody, MA). Microcapsules were adhered to the stub with sticky tape and sputter coated with gold (~5nm).

**Particle Size.** Median particle size was measured in triplicate using a laser diffraction particle size analyzer (Horiba LA-700, Horiba Instruments Inc, Irvine, CA) with ethanol as a dispersant.

## **Statistical Analysis**

The effects of chitosan type and oil load level on emulsion and microcapsules properties was evaluated using two way ANOVA. Significance was set at the  $\alpha=0.05$  level. Means comparisons were carried out using the Tukey-Kramer HSD test. Statistical analyses were performed with JMP Statistical Software, version 7.0 (Cary, NC).

## **RESULTS AND DISCUSSION**

### **Feed Emulsion Characteristics**

One advantage of chitosan over other commonly used spray drying materials is its emulsifying capabilities. Emulsion formation and stability are critical factors for encapsulation of lipid-based ingredients that, for the food industry, not only includes fats, but also many flavor and color compounds. For spray dried microcapsules, oil droplet size should be less than  $2\mu\text{m}$ ; smaller oil droplet sizes are related to better emulsion stability and lower levels of surface oil (Re 1998; Drusch 2007). In order to form emulsions with small oil droplet sizes, and acceptable stability, it is often necessary to add a separate emulsifying agent. Sugar beet pectin has recently been investigated as a promising polymer for microencapsulation of lipid ingredients because of its high content of emulsion stabilizing acetyl groups (Drusch 2007). Chitosan has both acetyl groups and protonated amine groups. The protonated amine groups are capable of providing additional stabilization due to electrostatic effects. Furthermore, chitosan has the ability to stabilize emulsions through steric interactions and through viscosity effects (Rodriquez, Albertengo et al. 2002). Both types of chitosan evaluated in this work, at both oil load levels (33% and 50% based on chitosan weight) exhibited median oil droplet diameters below  $2\mu\text{m}$  without addition of a separate emulsifying agent (Table 3.1). Droplet size distributions were unimodal for all formulations. The interaction between chitosan type and oil load level had a significant effect on median oil droplet diameter ( $p=0.0082$ ) with chitosan95 at 50% fish oil load exhibiting a significantly higher median oil droplet diameter than the other formulations evaluated. Larger median oil droplet diameters for chitosan95 formulations are partially due to the higher viscosities of these emulsions; feed emulsion viscosity and oil droplet diameter were highly correlated ( $r=0.84$ ).

The viscosity of the feed emulsion is also an important parameter in spray dried microcapsules. Generally, for encapsulation by spray drying it is desirable to have a high

dissolved solids content in the emulsion such that the efficiency of the process is high. Higher viscosities have been related to increased retention of core compounds, but viscosity must be low enough for proper atomization of droplets (Re 1998; Gharsallaoui, Roudaut et al. 2007). The viscosity of a chitosan solution increases dramatically with increases in concentration. Chitosan78 is considered a medium molecular weight chitosan and was used at a concentration of 1.5 wt%. Increasing the concentration from 1.5% to 2.0% resulted in a ten-fold increase in viscosity (for the 50% oil load emulsion) and the 2.0% suspension proved impractical to spray dry on our laboratory equipment. The lower molecular weight of chitosan95 allowed a higher solids content in the original chitosan suspension (5.0% was selected in this work) while maintaining a processable viscosity. Higher viscosity feeds have been shown to produce irregularly shaped particles and can lead to inclusion of air in the particle forming a central vacuole in the microcapsule interior (ballooning) which is undesirable for oxidatively unstable core materials (Re 1998; Drusch 2007; Gharsallaoui, Roudaut et al. 2007). Vacuole formation also is related to high drying temperatures and very rapid drying rates (Bhandari, Patel et al. 2008). Based on particle size comparisons between chitosan78 and chitosan95, and SEM imagery (Figure 3.2), it does not appear that the high viscosity caused ballooning in the chitosan95 microcapsules. Furthermore SEM images indicate microcapsules from both types of chitosan were regularly shaped, implying the viscosity of the 5% chitosan95 emulsion was acceptable.

## **Microcapsule Properties**

### **Size and Morphology**

All formulations produced spherically shaped microcapsules as shown in Figure 3.2. Particle surfaces were slightly dimpled for all formulations, but chitosan95 microcapsules appeared smoother than those prepared from chitosan78. Dimpling and wrinkling on the particle surface are generally attributed to uneven drying throughout the droplet and shrinkage during drying (Klinkesorn, Sophanodora et al. 2006). Particle agglomeration was noted in all formulations. Agglomeration can have favorable effects on powder flow properties and reconstitution (Bhandari, Patel et al. 2008). Particle size ranged from  $3.1 \pm 0.3 \mu\text{m}$  to  $7.8 \pm 1.6 \mu\text{m}$  (Table 3.1). Chitosan type and oil load had a significant effect ( $p=0.0012$ ) on median particle size with chitosan95 at 50% oil load exhibiting significantly larger median particle sizes than other

microcapsules. This formulation also exhibited significantly larger median oil droplet sizes. The larger oil droplet sizes and higher viscosity of the feed emulsion are the most likely causes of the larger particle size.

### **Encapsulation Efficiency**

Encapsulation efficiency is an important parameter for evaluating performance of materials as encapsulation agents. Furthermore, for ingredients susceptible to oxidation, the portion of core material that remains on the surface is particularly important — materials on the surface are believed to be more susceptible to oxidation as they do not have the benefit of a carbohydrate barrier film. Particle flow and wetting characteristics also are impacted by surface fat content (Drusch and Berg 2008). Encapsulation efficiency (EE) was calculated based on surface lipid content. EEs ranged from 66% -77% and were affected only by chitosan type ( $p < 0.001$ ). The surface lipid content of the chitosan fish oil microcapsules ranged from 21% (chitosan95 at 33% oil load) to 34% (chitosan78 at 33% oil load). Chitosan95 produced microcapsules with significantly lower levels of surface oil at both oil load levels when compared to chitosan78 ( $p < 0.0001$ ).

Encapsulated lipid can refer to lipid that is contained within the surface layer of the particle, lipid that is embedded deeper in the particle but is accessible to solvent, and lipid in the interior of the particle that is only accessible to solvent once holes have been generated by extracting other lipid fractions (Buma 1971). In this work “inner free fat” of the microcapsules also was analyzed. The inner free fat was determined by exposing samples that had surface fat previously removed to hexane for 48 hours with shaking. Chitosan type had a significant effect on inner free lipid ( $P = 0.001$ ) but oil load level did not. Chitosan78 microcapsules had higher levels of surface oil, but lower levels of inner free oil compared to chitosan95 microcapsules. As chitosan78 microcapsules had smaller median particle diameters, the surface to volume ratio plays a role in the higher levels of surface oil. Despite lower levels of inner free oil, chitosan78 microcapsules still had higher overall levels of extractable oil than chitosan95 microcapsules. SEM imagery suggests that microcapsules from both types of chitosan had continuous carbohydrate shells on the surface so it is unclear why chitosan95 microcapsules had higher

levels of inner free oil. It is possible that the chitosan95 microcapsules had a less dense carbohydrate shell allowing access and easier removal of lipid from the particle by solvent.

### **Water Activity and Moisture Content**

Neither chitosan type ( $p=0.64$ ) nor oil load level ( $p=0.65$ ) had a significant effect on water activity. All formulations exhibited water activities considered acceptable for use as food ingredients. The interaction between chitosan type and oil load level had a significant effect on moisture content ( $p=0.022$ ). Moisture content and water activity affect the glass transition temperature of carbohydrates and higher moisture contents and water activities have been noted to increase oxygen permeability of carbohydrate wall materials (Jin, Perrie et al. 2008). Low water activity and moisture contents at the completion of drying and maintained throughout storage of microcapsules can help ensure integrity of the particles. The moisture content (but not the water activity) of the chitosan95 microcapsules prepared at 50% oil load level was significantly lower than the other formulations; however, this did not have a positive effect on oxidative stability as measured by oxidation induction time.

### **Oxidative Stability**

Oxidative stability was evaluated using pressure differential scanning calorimetry (PDSC). In this method a sample is held at a fixed temperature ( $120^{\circ}\text{C}$ ) in a pressure cell at 500psi of oxygen until an exotherm associated with oxidation is observed. Tan et al (Tan, Che Man et al. 2002) found that measurement of oxidation induction time of various edible oils using DSC at atmospheric conditions correlated well to those measured using the commercially available Oxidative Stability Instrument from Omnion, Inc. (Rockland, MA) and recommend DSC as a means of evaluating oxidative stability. Use of the pressure cell improves the technique in that pressure prevents loss of volatile compounds that could impact oxidation. Kodali (2005) used the ASTM method used herein to evaluate oxidative stability of high-stability oils and found the method to be reliable, reproducible, and repeatable.

A typical DSC thermogram is shown in Figure 3.3. A single exotherm corresponding to oxidation of fish oil is observed. Microcapsules prepared from chitosan78 had an OIT of approximately 17 minutes, while those prepared from chitosan95 had an OIT of approximately 14 minutes. Despite the lower levels of surface oil in the chitosan95 microcapsules relative to

chitosan78 microcapsules, these formulations exhibited significantly ( $p=0.0088$ ) lower oxidative induction times as measured by PDSC. Oil load level did not significantly effect oxidative induction time ( $p=0.98$ ). The OIT method was applied to microcapsules that had the surface oil removed to determine whether the method could detect differences between surface oil and encapsulated oil. The exotherms for surface oil-free microcapsules and unwashed microcapsules occurred at the same times suggesting that the observed exotherm represents oxidation in the encapsulated fraction and that the method is perhaps not sensitive enough to detect oxidation of the surface fraction. The OIT results indicate that chitosan78 affords more protection against oxidation than chitosan95, although chitosan95 seems to be a more efficient encapsulation material. Differences in oxidative induction time could be due to differences in membrane permeability as a result of DD and MW differences. The OIT method is an accelerated shelf life evaluation tool and was used to rapidly compare performance of the different matrix materials rather than to provide a comprehensive view of microcapsule shelf life.

It is important to consider the possibility that the OIT results may be impacted by differences in glass transition temperatures ( $T_g$ ) of the two chitosans. Dong et al (2004) reported that DD did not affect chitosan  $T_g$ ; however, chitosan78 would be expected to have a higher  $T_g$  than chitosan95 due to its higher molecular weight. The hygroscopic nature of chitosan makes it difficult to measure  $T_g$  and conflicting values have been reported in the literature. The  $T_g$  of chitosan has been reported to be 140-150°C, 150°C, 203°C, and 220°C (Ogura, Kanamoto et al. 1980; Pizzoli, Ceccorulli et al. 1991; Sakurai, Maegawa et al. 2000; Dong, Ruan et al. 2004) Despite the lack of agreement in  $T_g$  values for chitosan, all reported values are well above the temperature used for OIT measurements (120°C) such that morphological changes in the polymer structure are not expected to have artificially impacted the results.

## CONCLUSIONS

Both types of chitosan evaluated allowed successful encapsulation of fish oil at both oil load levels evaluated. Microcapsules prepared from chitosan95 had higher encapsulation efficiencies than those prepared with chitosan78, whereas those prepared from chitosan78 had higher oxidative induction times. Reducing oil load level from 50% to 33% did not improve encapsulation efficiency. Chitosan appears to be a suitable wall material for encapsulation of lipid materials and can be used without separate emulsifiers. The high viscosity of chitosan at

dilute concentrations is somewhat limiting for spray drying applications and blending chitosan with other materials should be considered in order to take advantage of the favorable properties of chitosan while improving the efficiency of the process.

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## TABLES

**Table 3.1. Properties<sup>1</sup> of chitosan-fish oil feed emulsions and dried microcapsules**

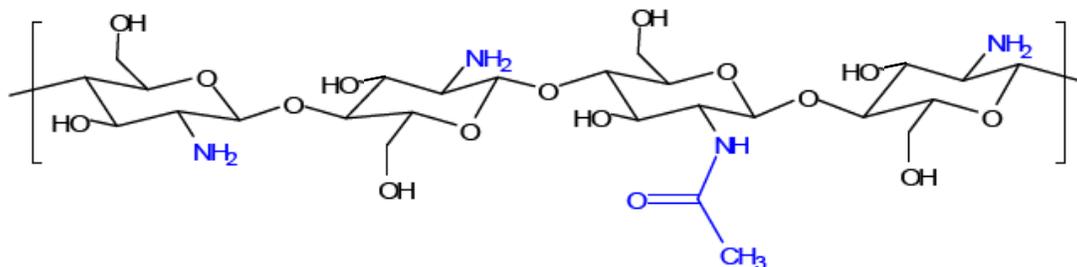
wall material oil load level	chitosan78 <sup>2</sup>		chitosan95 <sup>2</sup>	
	33% $\bar{x} \pm se$	50% $\bar{x} \pm se$	33% $\bar{x} \pm se$	50% $\bar{x} \pm se$
feed emulsion viscosity (mPa s)	380 ± 1 <sup>a</sup>	390 ± 6 <sup>a</sup>	2430 ± 120 <sup>b</sup>	2860 ± 280 <sup>b</sup>
feed emulsion median oil droplet size (µm)	1.20 ± 0.02 <sup>a</sup>	1.22 ± 0.02 <sup>a</sup>	1.26 ± 0.02 <sup>a</sup>	1.41 ± 0.02 <sup>b</sup>
encapsulation efficiency (%)	66.3 ± 0.6 <sup>a</sup>	67.4 ± 1.0 <sup>a</sup>	79.1 ± 1.3 <sup>b</sup>	77.4 ± 0.5 <sup>b</sup>
surface oil (%)	33.7 ± 0.6 <sup>a</sup>	32.6 ± 1.0 <sup>a</sup>	21.0 ± 1.3 <sup>b</sup>	22.6 ± 0.5 <sup>b</sup>
inner free oil (%)	1.8 ± 0.2 <sup>a</sup>	1.8 ± 0.2 <sup>a</sup>	3.9 ± 0.3 <sup>ab</sup>	5.3 ± 1.2 <sup>b</sup>
median particle size (µm)	3.10 ± 0.26 <sup>a</sup>	3.07 ± 0.26 <sup>a</sup>	4.42 ± 0.40 <sup>a</sup>	7.77 ± 1.58 <sup>b</sup>
water activity (25°C)	0.220 ± 0.006 <sup>a</sup>	0.266 ± 0.020 <sup>a</sup>	0.238 ± 0.051 <sup>a</sup>	0.220 ± 0.044 <sup>a</sup>
moisture content (%)	6.23 ± 0.26 <sup>ab</sup>	5.54 ± 0.21 <sup>ab</sup>	7.40 ± 1.25 <sup>a</sup>	4.00 ± 0.27 <sup>b</sup>
oxidative induction time (min)	16.5 ± 0.2 <sup>a</sup>	16.9 ± 0.2 <sup>a</sup>	14.0 ± 0.2 <sup>b</sup>	13.7 ± 0.8 <sup>b</sup>

<sup>a,b</sup> Means within row followed by the same superscript are not significantly different at the  $\alpha=0.05$  level using the Tukey-Kramer HSD test

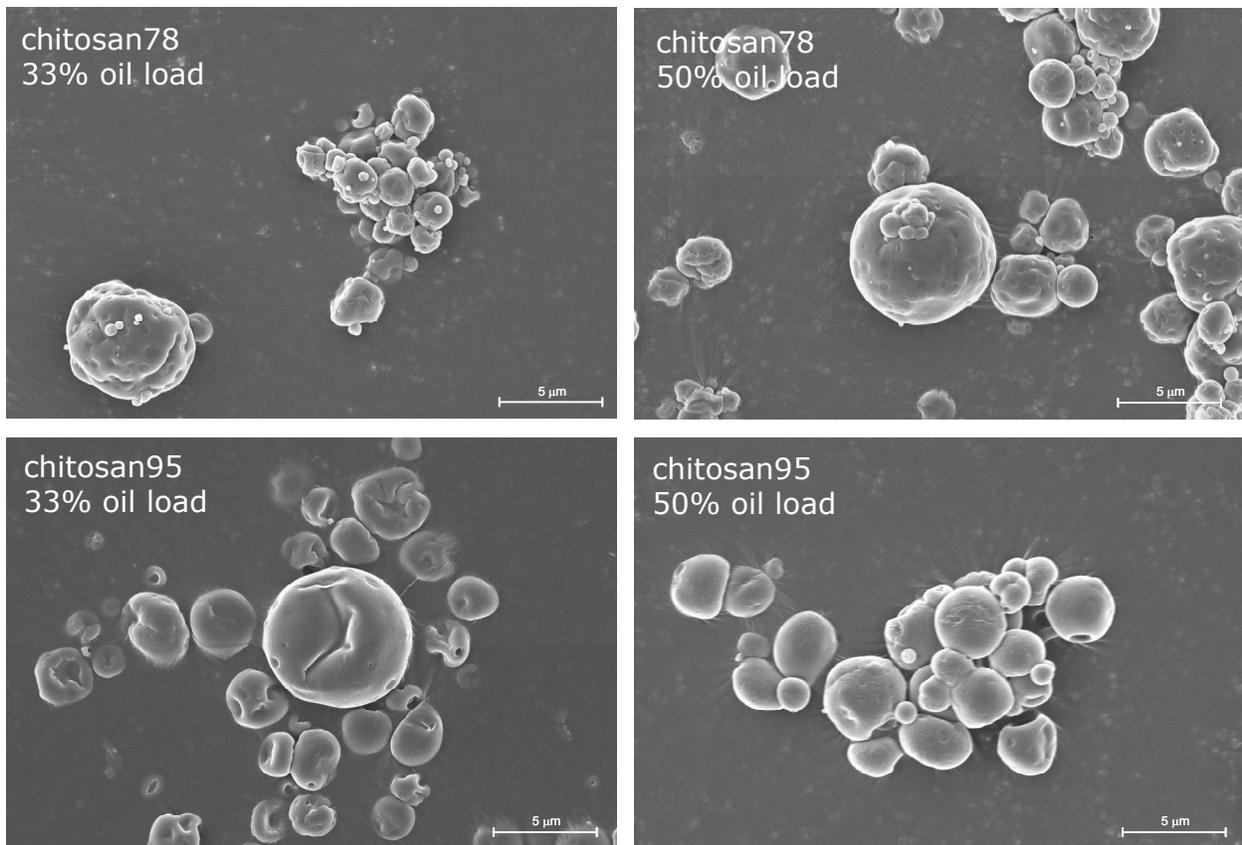
<sup>1</sup> Values are reported as mean ± standard error, n=4 for viscosity, encapsulation efficiency, surface oil, inner free oil, moisture content, and oxidative induction time, n=6 for median oil droplet size, median particle size, and water activity

<sup>2</sup> Chitosan95 refers to chitosan with DD=95% and low molecular weight, chitosan78 refers to chitosan with DD=78% and medium molecular weight. Oil load level is based on chitosan weight.

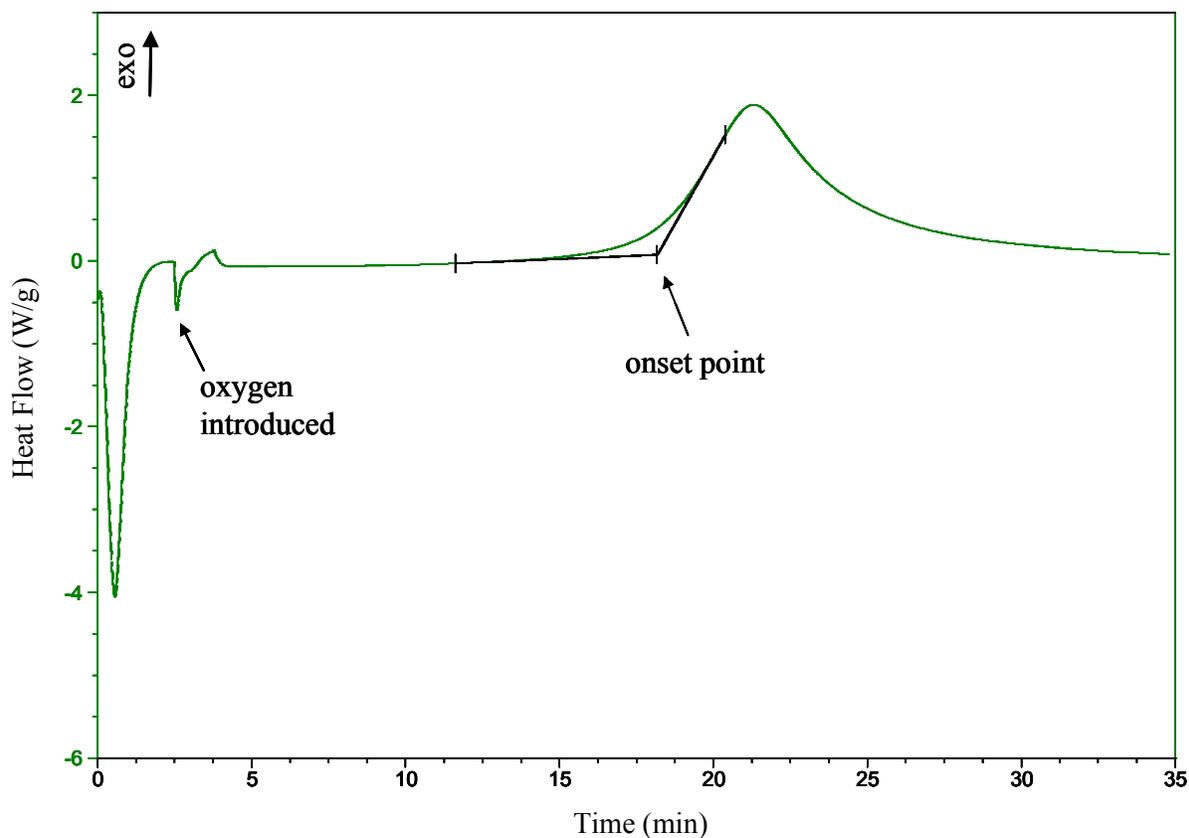
## FIGURES



**Figure 3.1.** Structure of chitosan,  $\beta(1\rightarrow4)$  linked D-glucosamine and N-acetyl-D-glucosamine. The amine groups in chitosan have a  $\text{pK}_a$  of approximately 6.5 and therefore carry a positive charge under mildly acidic conditions. Partially deacetylated chitosans such as those used in this work contain varying percentages of acetyl residues along the backbone.



**Figure 3.2. SEM images of chitosan-fish oil microcapsules prepared with chitosan78 (top) and chitosan95 (bottom) at 33% oil load level (left) and 50% oil load level (right). Images were recorded at 5,000 times magnification.**



**Figure 3.3.** Typical DSC thermogram obtained when measuring oxidative induction time of chitosan-fish oil microcapsules. Sample was heated to 120°C. Oxygen was introduced to the cell two minutes into the run and oxygen pressure is maintained at 500psi for the remainder of the run. Two minutes is subtracted from the onset point and this is reported as the oxidation induction time (OIT). The OIT in this example is 18.5 minutes – 2 minutes = 16.5 minutes. Data shown is for chitosan78 at a 33% oil load.

## **CHAPTER 4: PROPERTIES OF FISH OIL EMULSIONS AND SPRAY DRIED MICROCAPSULES PREPARED WITH CHITOSAN, HIGH-AMYLOSE STARCH, AND PULLULAN**

### **ABSTRACT**

Fish oil was microencapsulated by spray drying with blends of chitosan and high-amylose starch, chitosan and pullulan, and chitosan, high-amylose starch and pullulan. Median oil droplet sizes of all feed emulsions were less than 1.8 $\mu$ m, with chitosan-starch emulsions exhibiting the smallest median oil droplet size and chitosan-pullulan the largest; chitosan-starch-pullulan emulsions had the widest oil droplet size distribution. Encapsulation efficiency of all prepared microcapsules was approximately 63%. Median particle size ranged from 4 $\mu$ m for chitosan-starch-pullulan microcapsules to 11 $\mu$ m for chitosan-pullulan microcapsules. All formulations exhibited acceptable water activity and moisture contents for use as food ingredients. There were no significant differences in oxidation induction time (OIT) of the three formulations suggesting that they may provide similar levels of protection in terms of oxidative stability.

### **KEYWORDS:**

fish oil, spray drying, microencapsulation, emulsion, chitosan, high-amylose starch, pullulan

## INTRODUCTION

Well established health benefits are associated with consumption of polyunsaturated fatty acids (PUFAs), and in particular omega-3 fatty acids such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), yet dietary consumption of these healthful lipids remains low in the United States (Kris-Etherton, Taylor et al. 2000). In order to help consumers achieve recommended daily intakes, there is growing interest in incorporating omega-3 containing lipids, such as fish oil, into a variety of food products. Incorporation of such oils is technically challenging due to high susceptibility of polyunsaturated lipids to oxidation, off odors and flavors associated with common lipid sources, and food matrix incompatibilities.

These challenges can be alleviated by microencapsulation. Microencapsulation transforms the liquid oil into a solid powder. The powder form is generally easier to work with and has greater stability because the oil is afforded protection by the encapsulating shell material. PUFA-rich oils have been encapsulated in a variety of wall materials including modified starches, glucose, trehalose, maltodextrins, hydroxypropyl methyl cellulose, lecithin, chitosan, corn syrup solids, gum arabic, pullulan, whey protein, sodium caseinate, gelatin, alginate, and glycosylated proteins (Minemoto, Adachi et al. 1999; Minemoto, Hakamata et al. 2002; Jimenez, Garcia et al. 2004; Drusch, Serfert et al. 2006; Velasco, Marmesat et al. 2006; Drusch and Mannino 2009). The most commonly used processing technique for generating encapsulated PUFAs is spray drying, but other techniques such as extrusion, fluidized bed coating and polymer gelation, coacervation and electrostatic self-assembly also have been used (Forsell, Myllarinen et al. 2007; Shaw, McClements et al. 2007; Drusch and Mannino 2009).

In general, a material functions effectively as an encapsulant for lipid based core materials if it has the following properties; the ability to form films, water solubility, bland flavor, low viscosity, emulsifying capability, stability in the glassy state and low cost (Rishch and Reineccius 1995; Madene, Jacquot et al. 2006; Jin, Perrie et al. 2008). The most effective materials are those that will form a complete wall or shell around the oil and maintain the integrity of this shell during storage, processing, and potentially in the food as well (Jimenez, Garcia et al. 2006). Chitosan exhibits many of these properties but is limited by its high viscosities at low concentrations. Additionally, the metal-chelating ability of chitosan may afford protection against oxidation during processing. Serfert, Drusch and Schwarz (2008)

indicate that metal chelation plays an important role in preventing oxidation in the feed emulsion.

The purpose of this work was to evaluate the performance of chitosan blended with high-amylose starch, chitosan blended with pullulan, and chitosan blended with both high-amylose starch and pullulan as wall components for microencapsulation of fish oil by spray drying. High-amylose starch, also known as resistant starch (type RS2) is a natural form of amorphous starch that has been linked to health benefits associated with resistance to hydrolysis in the small intestine (Koksel, Basman et al. 2008). High-amylose starch was selected as a wall material based on previous work in our laboratory indicating favorable oxygen barrier properties of chitosan-high-amylose starch films, as well as its bland taste and low water-holding capacity. Pullulan, a natural polysaccharide comprised of  $\alpha(1\rightarrow6)$  linked maltotriose units, was selected based on its high water solubility, bland taste, favorable oxygen barrier properties and excellent film forming abilities (Leathers 2005).

## **MATERIALS AND METHODS**

### **Materials**

Chitosan with DD=95% and low molecular weight (15 mPA·s as a 1% solution as reported by supplier) was donated by Primex, Inc. (Siglufjordur, Iceland). High-amylose starch (Hi-maize 260) was donated by National Starch (Bridgewater, NJ). Pullulan was donated by Hayashibara (Okayama, Japan). Fish oil (from anchovies and sardines) with total omega-3 content of 300mg/g (as reported by the supplier, triglyceride basis) was donated by Ocean Nutrition (Nova Scotia, Canada). All reagents were purchased from Sigma-Aldrich (St. Louis, MO) and were ACS reagent grade or higher.

### **Microcapsule Preparation.**

For all formulations, chitosan was dissolved in 1% (v/v) aqueous acetic acid at a level of 4% (w,w). High-amylose starch and/or pullulan were added to achieve the following ratios of chitosan (C), starch (S), and pullulan (P): 60% C:40% S, 60% C:40% P, and 60% C:20% S:20% P. High-amylose starch was suspended in 1% (v/v) aqueous acetic acid in a Parr pressure reactor (Parr Instrument Company, Moline, IL) prior to combining with chitosan and pullulan. The starch suspension was heated to 160°C under 0.4 MPa of nitrogen gas and held for 30 minutes to

achieve a stable suspension. Fish oil was added immediately prior to homogenizing at a level of 50% (w/w) based on wall material and the mixture was rapidly stirred with a non-aerating overhead stirrer with propeller style blade (Kraft Apparatus, New York, NY) to prepare a course feed emulsion. Course emulsions then were passed twice through a two-stage pressure homogenizer (Model 15MR, APV, Everett, MA) at 20/5 MPa (200/50 Bar). Feed emulsions were flushed with nitrogen and were spray dried within 24 hours of preparation on a Buchi 190 mini spray drier (Flawil, Switzerland) equipped with a 0.5 mm two fluid nozzle. Air pressure was set at 0.55 MPa. Inlet and outlet temperatures were maintained at 160°C and 90°C respectively. Microcapsules for general characterization were stored in glass jars at room temperature, while those for characterization of oxidative stability were packaged in oxygen barrier bags, nitrogen flushed, and stored at -80°C.

### **Feed Emulsion Characterization**

**Viscosity.** Viscosity of the feed emulsion was measured in duplicate on a rotational viscometer with LV2 spindle (Brookfield, Middleboro, MA). Emulsions were kept in a 25°C water bath prior to measuring viscosity.

**Oil droplet size.** Oil droplet diameter (median, 90th percentile and percent under 2µm) was measured in triplicate using a laser diffraction particle size analyzer (Horiba LA-700, Horiba Instruments Inc, Irvine, CA) with water as a dispersant. Droplet diameter was measured on the same day of emulsion preparation and also was monitored over a 45 day storage period. Feed emulsions were stored at room temperature for the 45 day period.

### **Microcapsule Characterization**

**Water Activity.** Water activity was measured at 25°C on an Aqualab Series 3TE water activity meter (Decagon Devices, Pullman, WA). Reported values are the mean of three measurements.

**Moisture Content.** Moisture content was measured by drying overnight at 100°C in a vacuum oven with admission of dried air. Measurements were made in duplicate.

**Surface Lipid, Inner Free Lipid and Encapsulation Efficiency.** Surface lipid and inner free lipid content were measured by solvent extraction. Microcapsule samples (~1g) were first rinsed three times with 10ml of hexanes through a fine sintered glass filter. Lipid removed in

this step was considered ‘surface lipid.’ The solvent was dried to constant weight and percent surface lipid was determined gravimetrically based on theoretical total lipid content. Samples then were transferred to a glass jar and 40ml of hexane were added. Microcapsules were shaken in hexane for 48 hours at 250rpm. Samples then were filtered and solvent was evaporated. Percent ‘inner-free lipid’ was determined gravimetrically based on theoretical total lipid content. Measurements were carried out in duplicate. Encapsulation efficiency was calculated based on theoretical total lipid content and measured surface lipid.

**Oxidation Induction Time.** A TA Instruments (New Castle, Delaware) differential scanning calorimeter (DSC) equipped with a pressure cell was used to evaluate relative oxidative stability by measuring oxidative induction time (OIT) according to ASTM D6186-08 (ASTM 2008). A sample of microcapsules (8-12mg) was placed in an open pan in the pressure cell and heated rapidly to 120°C. After equilibrating for 2 minutes at this temperature, oxygen (3.45MPa) was introduced to the cell. The sample was held isothermally at 120°C under 3.45MPa of oxygen until an oxidation exotherm was observed. OIT was reported as the time in minutes from the time oxygen was introduced to the cell to the time the peak onset was observed. Measurements were made in duplicate.

**Morphology.** Microcapsule morphology was characterized by scanning electron microscopy (SEM) on a Leo 1550 field emission SEM (Zeiss, Peabody, MA). Microcapsules were adhered to the stub with sticky tape and sputter coated with gold (~5nm).

**Particle Size.** Median particle diameter was measured in triplicate using a laser diffraction particle size analyzer (Horiba LA-700, Horiba Instruments Inc, Irvine, CA) with ethanol as a dispersant.

### **Statistical Analysis**

The effects of wall material composition on feed emulsions and microcapsule properties were evaluated using one-way ANOVAs with  $\alpha=0.05$ . Means comparisons were carried out using the Tukey-Kramer HSD test. Changes in oil droplet size during storage were analyzed by linear regression. Statistical analyses were performed with JMP Statistical Software, version 7.0 (Cary, NC).

## RESULTS AND DISCUSSION

### Feed Emulsion Properties

Oil droplet diameters and viscosities of fish oil emulsions are shown in Table 4.1. Median oil droplet diameters for all formulations were significantly different ( $p < 0.001$ ), but were all unimodal and smaller than  $1.8\mu\text{m}$ . Of the formulations evaluated, the chitosan-starch fish oil emulsion produced the smallest oil droplets with the narrowest size distribution, with a median oil droplet diameter less than  $1.1\mu\text{m}$  and ninety percent of oil droplets smaller than  $1.5\mu\text{m}$ . This emulsion, however, was also less stable as evidenced by an increase in oil droplet size during storage (Figure 4.1). The median oil droplet diameter of the chitosan-starch emulsion increased significantly during storage from  $1.06\mu\text{m}$  at day 0 to  $1.38\mu\text{m}$  at day 45 ( $p = 0.002$ ); 90<sup>th</sup> percentile values increased from  $1.45\mu\text{m}$  to  $2.63\mu\text{m}$  ( $p < 0.001$ ), and percent under  $2\mu\text{m}$  decreased from 99.7% to 78.8% ( $p < 0.001$ ). Oil droplet diameters did not change significantly from day 0 to day 45 for chitosan-pullulan emulsions ( $p = 0.85$ ,  $p = 0.79$ , and  $p = 0.79$  median, 90<sup>th</sup> percentile, and % under  $2\mu\text{m}$  respectively) and chitosan-starch-pullulan emulsions ( $p = 0.098$ ,  $p = 0.32$ , and  $p = 0.16$  for median, 90<sup>th</sup> percentile, and % under  $2\mu\text{m}$  respectively). For spray drying applications, emulsions are generally used within a few days of preparation, therefore it is not necessary for feed emulsions to possess long term stability. However, there was an interest in monitoring stability of the emulsions over storage to gain insight into the effectiveness of chitosan as an emulsifier. Chitosan has known abilities to stabilize emulsions through electrostatic, steric, and viscosity modifying effects (Agullo, Rodriguez et al. 2003). The level of chitosan in all three formulations was maintained at 60% of the wall matrix composition for the purpose of forming stable emulsions with small oil droplet size. Neither high-amylose starch nor pullulan were expected to afford satisfactory emulsion stability on their own. Koksel et al (2008) found that resistant starch did not improve the emulsifying properties of soy protein, but also did not have a detrimental effect on soy protein emulsions. The lower stability of the chitosan-starch emulsion is most likely due to the lower viscosity of this formulation compared to the others. Higher viscosities prevent droplet flocculation and coalescence by slowing oil droplet movement (Tsaliki, Pegiadou et al. 2004). Pullulan has been shown to reduce oil droplet size and improve emulsion stability, effects that were attributed to a viscosity increase and polymer-polymer interactions (Tsaliki, Pegiadou et al. 2004; Kshirsagar, Yenge et al. 2009). In our work pullulan, did not appear to reduce initial oil droplet size and the chitosan-pullulan feed emulsion had the

widest oil droplet size distribution; however, pullulan did seem to have a stabilizing effect on the emulsions during storage.

## **Microcapsules Properties**

### **Microcapsule Shape and Surface Morphology**

Microcapsules were generally spherical with wrinkled surfaces as indicated by SEM images (Figure 4.2). Pullulan incorporation resulted in fiber-like structures that wrapped around and between the microcapsules. These fibers were more prevalent in chitosan-pullulan microcapsules as compared to chitosan-starch-pullulan microcapsules. This is explained by the higher pullulan concentration in the former (40% w/w of total wall components versus 20%). Fiber like structures also were observed by Kshirasagar et al (2009) when pullulan was incorporated at levels higher than 1% (w/w) in gum arabic turmeric oleoresin microcapsules. The fibrous nature of the chitosan-pullulan microcapsules was visually apparent without the aid of a light microscope. Fibers in the chitosan-starch-pullulan microcapsules were only apparent under magnification and these microcapsules appeared to behave as a powder. Optical images of the three formulations are shown in Figure 4.3.

### **Particle Size**

Median particle diameter of the prepared microcapsules ranged from 3.6 $\mu\text{m}$  to 10.7 $\mu\text{m}$ . The 90<sup>th</sup> percentile diameter ranged from 12.3 $\mu\text{m}$  to 51.0 $\mu\text{m}$ . The median diameter of these microcapsules is markedly smaller than many other spray dried fish oil microcapsules. For example fish oil microcapsules prepared with n-octenylsuccinate-derivitized starch, gum arabic, sugar beet pectin, highly branched cyclic dextrin, caseinate, maltodextrin and whey protein isolate have been reported to have median particle diameters in the range of 20-40 $\mu\text{m}$  (Kagami, Sugimura et al. 2003; Kolanowski, Ziolkowski et al. 2006; Drusch, Serfert et al. 2007; Jafari, Assaidpoor et al. 2008). Chitosan has been shown to produce relatively small spray dried microcapsules. Kosaraju, D'ath, and Lawrence (2006) report median particle size of 9.9 $\mu\text{m}$  for spray dried olive leaf extract microcapsules prepared with chitosan and several authors report median particle sizes generally ranging from 3-10 $\mu\text{m}$  for pharmaceutical microcapsules prepared with chitosan under similar conditions (Giunchedi, Genta et al. 1998; Desai and Park 2005; Oliveira, Santana et al. 2005; Liu, Desai et al. 2006). Chitosan-pullulan microcapsules had

significantly higher median particle sizes than both of the other formulations ( $p < 0.001$ ); however, chitosan-pullulan and chitosan-starch 90<sup>th</sup> percentile particle sizes were not significantly different, and 90<sup>th</sup> percentile particle size of chitosan-pullulan-starch microcapsules was significantly smaller than the other formulations ( $p < 0.001$ ). The larger median particle size of chitosan-pullulan microcapsules is mainly attributed to the higher viscosity of the feed emulsion which would be expected to result in larger droplet sizes during atomization (Drusch, Serfert et al. 2007). The fibrous nature of pullulan containing formulations also impacts the particle size results.

### **Encapsulation Efficiency and Lipid Distribution**

All formulations had encapsulation efficiencies of approximately 63%. There were no significant differences in encapsulation efficiency or surface oil content between the formulations ( $p = 0.9347$ ). The levels of surface oil (approximately 27% for all formulations) in these microcapsules is quite high, but the small particle size of these microcapsules relative to other fish oil microcapsules as mentioned above should be taken into account. Research in our lab has shown that when chitosan-starch microcapsules were prepared with a larger nozzle (0.7 mm rather than 0.5mm used for all formulations in this study) that surface lipid dropped to approximately 11%. Particle size of chitosan-starch microcapsules prepared with the larger nozzle was still quite small (median=10.2 $\mu$ m, 90<sup>th</sup> %ile= 37.1 $\mu$ m). It is likely that larger droplets produced from the larger nozzle facilitated more efficient coverage of the oil droplets with a polymer film.

It is well established that the oil fraction in spray dried lipid microcapsules resides in different locations within the microcapsule, and that different extraction procedures are more or less efficient in recovering the lipid (Kim, Chen et al. 2005; Drusch and Berg 2008). Buma (1971) identified four distinct types of extractable fat in spray dried dairy products; “the surface fat, the outer layer fat in the surface layer of the particle, fat, that can be extracted by the solvent through capillary forces and fat, that can be reached by solvent through holes left by already extracted fat.” Kim, Chen, and Pearce (2005) identify three types of fat in spray dried dairy powders — surface free fat, inner free fat and encapsulated fat. Free-fat can be considered as a combination of fat residing on the surface and fat that is within the microcapsules matrix but accessible to organic solvents (Buma 1971; Kim, Chen et al. 2005; Drusch and Berg 2008). In

this work two fractions of free fat were quantified. First, surface fat was collected by rinsing with hexane. Samples with surface fat removed then were exposed to hexane for 48 hours with shaking to collect fat referred to as inner-free fat. While there were no significant differences in surface fat content of the different formulations, microcapsules prepared with chitosan and starch only had significantly higher levels of inner free fat than those prepared with chitosan and pullulan and those prepared with chitosan, starch, and pullulan ( $p=0.0040$ ). Drusch and Berg (2008) investigated effects of drying temperature and oil load level on extractable fat (free fat) levels and reported that higher inlet and outlet temperatures and higher oil loads resulted in higher levels of extractable fat (both surface and inner free fat fractions). Since all formulations in our work were dried at the same temperatures and had the same oil load level this does not explain the differences observed in our formulations. It also has been shown that smaller oil droplet sizes in feed emulsions produced lower levels of extractable fat (Drusch & Berg, 2008). It seems reasonable to expect smaller oil droplets to result in less inner free fat; however, in our study chitosan-starch microcapsules had the smallest oil droplet sizes in the feed emulsions of the three formulations and the highest inner free fat levels. Drusch and Berg (2008) also saw a relationship between particle size and extractable oil with smaller particle sizes resulting in higher levels of extractable oil (surface and inner free fat) when oil load was fixed. This makes sense for surface fat as smaller particles have higher surface to volume ratios. For inner free fat it can be explained by a relationship between larger particles and greater air inclusion and/or cracks (Drusch and Berg 2008). Particle size does not completely explain the results in the present study. The chitosan-starch microcapsules were larger than the chitosan-pullulan-starch microcapsules but had higher inner free fat. The lowest level of inner free fat occurred for the chitosan-pullulan microcapsules and these were the largest particles. Our results seem to imply that pullulan may play a role in preventing extraction of fat in the microcapsule matrix.

### **Moisture Content and Water Activity**

Chitosan-starch and chitosan-starch-pullulan microcapsules had very low moisture content and water activity (Table 4.2). Chitosan-pullulan microcapsules exhibited significantly higher moisture content and water activity ( $a_w$ ) than the formulations containing starch ( $p<0.0001$ ). While all values are low enough to prevent microbial growth, such low  $a_w$  values may not be ideal for long term storage. In general, oxidation of food products is believed to occur most

rapidly at water activities below 0.1 and in the range of 0.55 – 0.85, with  $a_w$  of 0.2-0.3 showing the slowest oxidation rates (Nawar 1996; Bell 2007). The stability observed in the 0.2-0.3 range is attributed to hydration of metal ions, free radical quenching by water, and prevention of oxygen contacting the product (Nawar 1996; Bell 2007). Water activity of microcapsules can be modified after preparation by exposing the microcapsules to varying moisture contents. It is possible that modifying the  $a_w$  could improve long term stability. Partanen and colleagues (2008) observed that oxidation was most rapid for whey protein isolate-flax oil microcapsules when stored at 0% RH ( $a_w < 0.1$ ) as compared to 11, 49, 75, and 91% RH. Grattard and colleagues (2002) observed slower oxidation rates for maltodextrin-flaxseed oil microcapsules when the  $a_w$  was in the range of 0.3-0.4 as compared to 0.15 (which showed slightly higher rates) and 0.6 and 0.8 (which showed much higher rates). The fact that carbohydrates are plasticized by water, decreasing their glass transition temperature ( $T_g$ ) and increasing molecular mobility and oxygen permeability must also be considered in selecting appropriate storage conditions and further research is required to elucidate the most appropriate conditions (Jin, Perrie et al. 2008).

### **Oxidative Stability**

Oxidative stability was evaluated by measuring oxidation induction time (OIT) using pressure differential scanning calorimetry (PDSC). In this method microcapsules are held isothermally under 3.45MPa of oxygen until an oxidation exotherm is observed. OIT is reported as the time from when oxygen is introduced to the cell to the time of peak onset of the oxidation exotherm. An example of the DSC curve obtained with this method is shown in Figure 4.4. The PDSC method is reproducible and closely correlated to measurement of oxidative stability index (OSI) for oils with low oxidative stability (Kodali 2005). Furthermore Kodali (2005) found an excellent correlation of OIT with temperature and developed an equation enabling conversion of OIT measured at one temperature to another temperature. There is no evidence in the literature of applying this method to lipid microcapsules and therefore comparison of the present formulations to other reported values is not possible. Rather, OIT was used for the sake of comparing the protective effect afforded by the different matrix formulations. The OITs of chitosan-starch-pullulan and chitosan-starch microcapsules were 19.7 and 19.4 minutes respectively and the OIT of chitosan-pullulan microcapsules was 17.6 minutes, although the difference was not significant ( $p=0.085$ ).

## CONCLUSIONS

Fish oil microcapsules were successfully generated using blends of chitosan, high-amylose starch and pullulan. Chitosan at the levels used in this study produced stable emulsions with favorable oil droplet sizes for spray drying and may be considered a useful component for generating microcapsules of lipophilic ingredients. Blending chitosan with other carbohydrates such as high-amylose starch and pullulan may provide more favorable microcapsule properties and easier-to-process emulsions than if chitosan is used alone. In the preceding chapter (Chapter 3) properties of microcapsules prepared from the same type of chitosan as a single component matrix were discussed. Chitosan combined with high-amylose starch exhibited lower oil droplet sizes in the feed emulsion than the same type of chitosan when used alone. Furthermore, all formulations evaluated in the present chapter exhibited higher oxidation induction times than microcapsules produced with the same type of chitosan when used alone. All formulations in this study, however, exhibited higher surface oil contents than microcapsules produced with the same type of chitosan when used alone. Blending chitosan with other materials enables a lower viscosity of the feed and therefore higher overall solids content and could improve the efficiency of the drying process.

Although favorable properties were observed for chitosan blends compared to chitosan alone (OIT, oil droplet size) some properties did not improve (encapsulation efficiency). Further work to more comprehensively evaluate the interactions between chitosan, high-amylose starch, and pullulan would be useful. Additionally, considering chitosan in combination with low molecular weight carbohydrates known to produce microcapsules with high encapsulation efficiency (eg. maltodextrins) would be worthwhile to investigate. Pullulan can produce fibrous materials and if it is used as a wall material it should be kept at a concentration lower than 20% of the total wall composition to avoid production of fibers, or used at levels higher than 20% if fibers are desirable. High-amylose starch suspensions exhibited a low viscosity and this type of starch may be useful as a bulking agent; however, more research is required to determine if the benefits of using this type of starch overcome the extra processing step required to produce a stable suspension.

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**TABLES**

**Table 4.1. Properties<sup>1</sup> of chitosan-starch (60:40 C:S), chitosan-pullulan (60:40 C:P), and chitosan-starch-pullulan (60:20:20 C:S:P) fish oil feed emulsions**

	60:40 C:S $\bar{x} \pm se$	60:40 C:P $\bar{x} \pm se$	60:20:20 C:S:P $\bar{x} \pm se$
viscosity (mPa s)	510 ± 40 <sup>a</sup>	1370 ± 150 <sup>ab</sup>	960 ± 140 <sup>b</sup>
oil droplet size, 50 <sup>th</sup> percentile (µm)	1.06 ± 0.04 <sup>a</sup>	1.80 ± 0.06 <sup>b</sup>	1.57 ± 0.13 <sup>c</sup>
oil droplet size, 90 <sup>th</sup> percentile (µm)	1.45 ± 0.01 <sup>a</sup>	3.02 ± 0.02 <sup>b</sup>	2.42 ± 0.18 <sup>c</sup>
% of oil droplets under 2 µm	99.7 ± 0.1 <sup>a</sup>	63.2 ± 2.0 <sup>b</sup>	79.5 ± 3.4 <sup>c</sup>

<sup>a,b</sup>Means within row followed by the same superscript are not significantly different at the  $\alpha=0.05$  level using the Tukey-Kramer HSD test

<sup>1</sup>Values are reported as mean ± standard error, n=4 for viscosity, n=6 for oil droplet properties  
Properties were measured on the day of preparation.

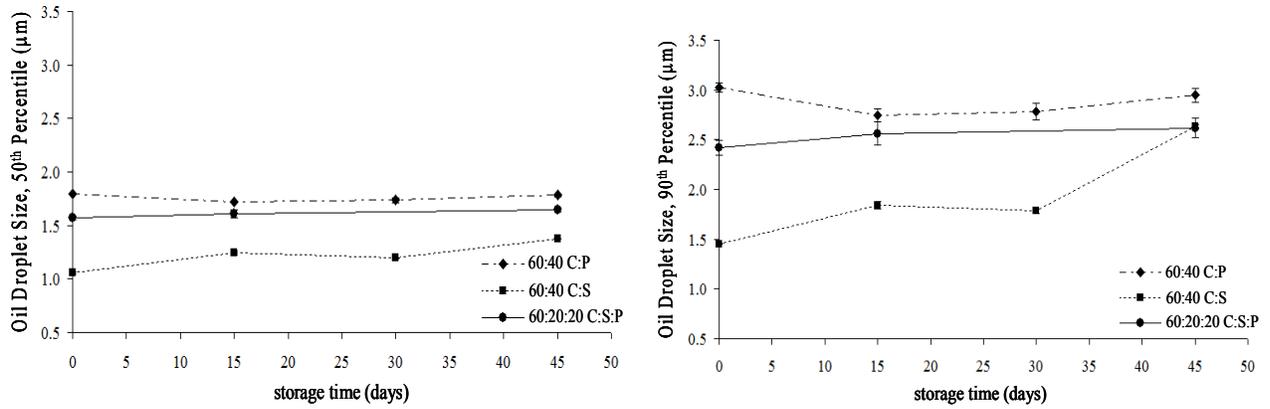
**Table 4.2. Properties<sup>1</sup> of chitosan-starch (60:40 C:S), chitosan-pullulan (60:40 C:P) and chitosan-starch-pullulan (60:20:20 C:S:P) fish oil microcapsules**

	60:40 C:S $\bar{x} \pm se$	60:40 C:P $\bar{x} \pm se$	60:20:20 C:S:P $\bar{x} \pm se$
encapsulation efficiency (%)	62.4 ± 0.5	62.9 ± 2.0	63.0 ± 0.3
surface fat (%)	37.6 ± 1.4	37.1 ± 1.9	37.0 ± 0.3
inner free fat (%)	9.1 ± 0.9 <sup>a</sup>	5.4 ± 0.2 <sup>b</sup>	6.7 ± 0.2 <sup>b</sup>
particle size, 50 <sup>th</sup> percentile (µm)	5.5 ± 0.2 <sup>a</sup>	10.7 ± 0.9 <sup>b</sup>	3.6 ± 0.1 <sup>a</sup>
particle size, 90 <sup>th</sup> percentile (µm)	39.5 ± 6.8 <sup>a</sup>	51.0 ± 4.9 <sup>a</sup>	12.3 ± 1.5 <sup>b</sup>
water activity (25°C)	0.065 ± 0.015 <sup>a</sup>	0.192 ± 0.021 <sup>b</sup>	0.065 ± 0.005 <sup>a</sup>
moisture content (%)	2.1 ± 0.4 <sup>a</sup>	6.7 ± 0.4 <sup>b</sup>	2.4 ± 0.6 <sup>a</sup>
oxidative induction time (min)	19.4 ± 0.3	17.6 ± 1.1	19.7 ± 0.2

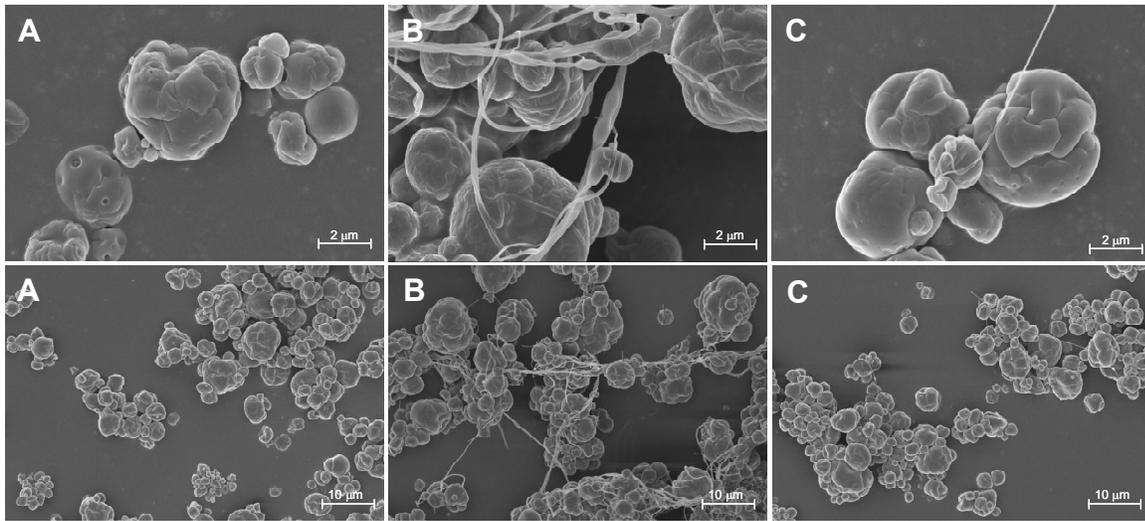
<sup>a,b</sup>Means within row followed by the same level are not significantly different at the  $\alpha=0.05$  level using the Tukey-Kramer HSD test

<sup>1</sup>Values are reported as mean ± standard error, n=4 for encapsulation efficiency, surface fat, inner free fat, moisture content, and oxidative induction time, n=6 for particle size and water activity. Properties were measured within one week of preparation, except for oxidation induction time which was measured within one month of preparation on microcapsules stored under nitrogen at -80°C.

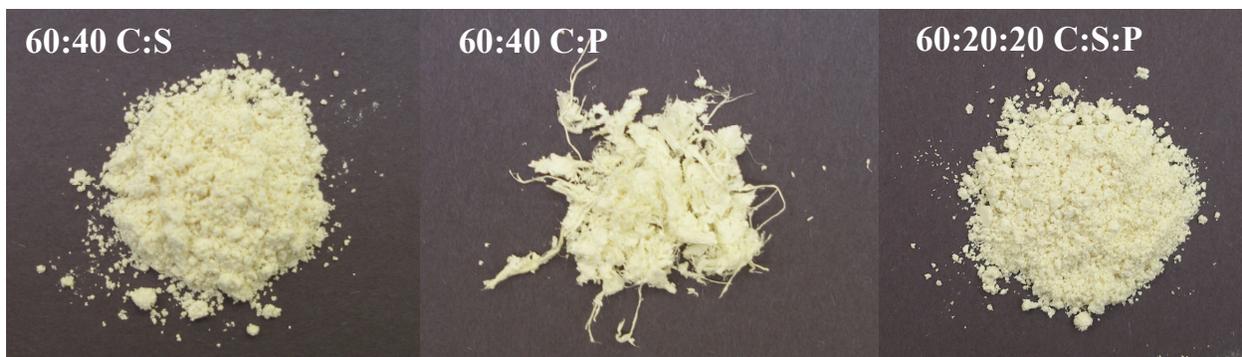
## FIGURES



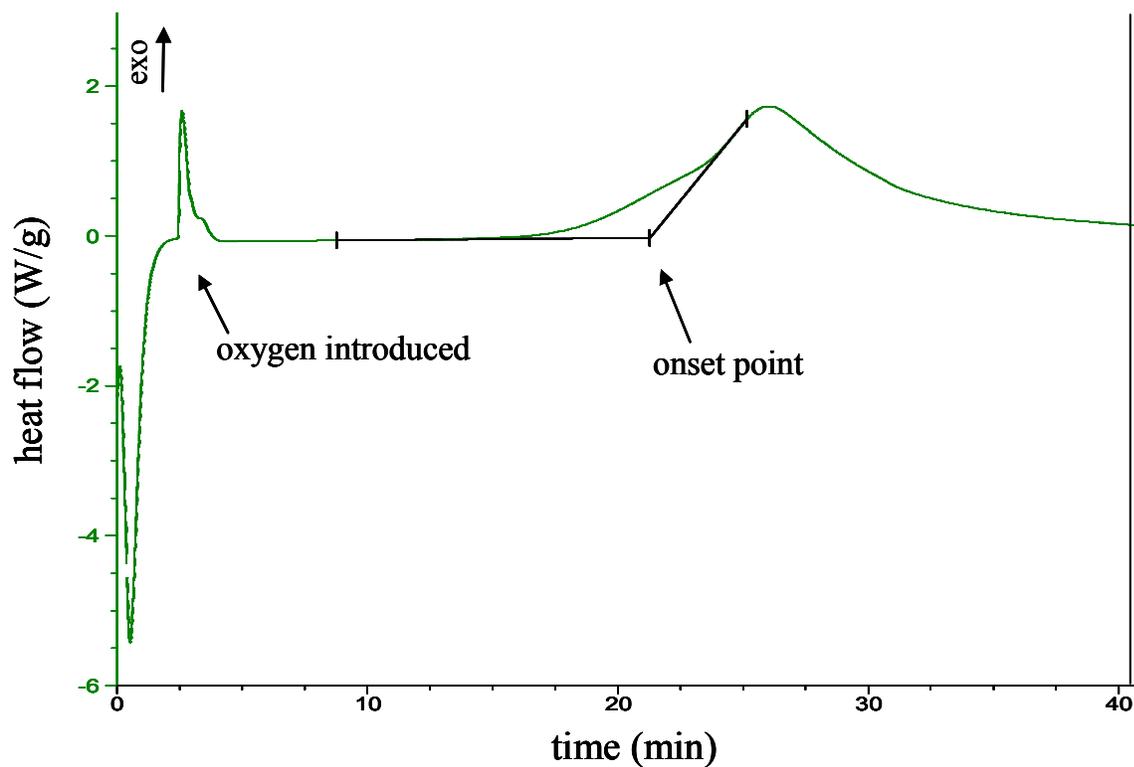
**Figure 4.1. Oil droplet sizes in chitosan-starch (60:40 C:S), chitosan-pullulan (60:40 C:P), and chitosan-starch-pullulan (60:20:20 C:S:P) fish oil feed emulsions during storage at ambient conditions. Median oil droplet sizes are shown on the left and 90<sup>th</sup> percentile sizes are shown on the right.**



**Figure 4.2 SEM images of fish oil microcapsules prepared with chitosan-starch (A), chitosan-pullulan (B), and chitosan-starch-pullulan (C). Top line images were recorded at 10,000 times magnification, bottom line recorded at 2,000 times magnification.**



**Figure 4.3. Optical images of chitosan-starch (60:40 C:S) chitosan-pullulan (60:40 C:P), and chitosan-starch-pullulan (60:20:20 C:S:P) fish oil microcapsules showing fibrous nature of chitosan-pullulan microcapsules.**



**Figure 4.4. Typical DSC thermogram obtained when measuring oxidative induction time of chitosan-fish oil microcapsules. Sample was heated to 120°C. Oxygen was introduced to the cell two minutes into the run and oxygen pressure is maintained at 500psi for the remainder of the run. Two minutes is subtracted from the onset point and this is reported as the oxidative induction time (OIT). As shown in this example sample would have an OIT of 21.4 min – 2 min = 19.4. Data shown is for chitosan-starch microcapsules.**

## **CHAPTER 5: OXIDATIVE STABILITY OF CHITOSAN-STARCH FISH OIL MICROCAPSULES DURING STORAGE AS MONITORED BY HEADSPACE SOLID-PHASE-MICROEXTRACTION GAS CHROMATOGRAPHY/MASS SPECTROMETRY (HS-SPME-GC/MS)**

### **ABSTRACT**

Oxidative stability of fish oil microcapsules prepared by spray drying with chitosan and high-amylose starch as matrix materials was evaluated over a 35 day storage period. Peroxide values (PVs) of surface and encapsulated oil were monitored separately. PVs of both fractions remained low throughout the storage period with no significant differences between the fractions through 21 days of storage. Between 21 and 35 days of storage, PVs of surface oil remained steady; however, PVs of encapsulated oil increased. Secondary oxidation products were monitored using HS-SPME-GC/MS. Twenty-nine volatile compounds were identified in the headspace of the microcapsules. These were attributed to both surface and encapsulated oil. Concentrations of propanal, 1-penten-3-ol, pentanal, hexanal, and 2,4-heptadienal, and total peak area increased during the storage period. Propanal, pentanal and 2,4-heptadienal contents increased the most during the first two weeks of storage while the other compounds remained fairly constant during that time. The most dramatic increases for all compounds were observed between 14 and 21 days of storage.

### **KEYWORDS:**

oxidative stability, HS-SPME, fish oil, omega-3, microcapsules, chitosan, high-amylose starch

## INTRODUCTION

Omega-3 polyunsaturated fatty acids ( $\omega$ 3 PUFAs), such as eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3), have been associated with numerous health benefits, including prevention of cardiovascular disorders, diabetes, arthritis, inflammatory bowel disease, autoimmune diseases, cancer, and depression (Young 2001; 2008). Furthermore, these fats play an important role in membrane fluidity, brain and eye development, and gene expression (Young 2001). Despite the well established health benefits, consumption of omega-3 containing lipids is generally below the recommended levels. In order to increase consumption there is growing interest in incorporating sources of omega-3 fatty acids, such as fish oil, into food products in which they do not naturally occur; such as bread and cereal products.

The highly unsaturated nature of omega-3 fatty acids is critical to their health benefits; however, this same property renders them highly susceptible to oxidative deterioration. Lipids react with oxygen to produce short-lived hydroperoxides which subsequently decompose to produce a variety of compounds, including aldehydes, ketones, and alcohols. The structure of a fatty acid greatly affects its susceptibility to oxidation. PUFAs are much more susceptible to oxidation than saturated lipids due to bis-allylic methylene groups which result in a great number of easily extractable hydrogen atoms (Kulas, Olsen et al. 2006). Oleic acid, with a single double bond, reacts approximately 10 times faster than its saturated counterpart, stearic acid, while linoleic acid (2 double bonds) reacts more than 100 times faster, and linolenic acid (3 double bonds) reacts almost 200 times faster (Pokorny, Yanishlieva et al. 2001). EPA (5 double bonds) and DHA (6 double bonds) are extremely susceptible to oxidation. The highly unsaturated nature of long chain omega-3 fatty acids such as EPA and DHA not only affects the rate at which oxidation occurs, but also results in a highly complicated outcome in terms of the products generated. Each double bond is a site for hydrogen abstraction, ultimately enabling formation of greater than 16 hydroperoxide isomers for EPA and 20 hydroperoxide isomers for DHA (Kulas, Olsen et al. 2006). This great number of isomers possible during the initiation stage results in a large quantity of possible secondary oxidation products. Many of these secondary oxidation products contribute unpleasant flavors and odors. Not only do these products make an omega-3 containing product unacceptable in terms of sensory attributes, but they indicate that the lipid has degraded and therefore the product no longer contains the desired level of omega-3 fatty acids,

and may even contain oxidation products that have negative health effects (Turner, McLean et al. 2006).

Microencapsulation is a commonly used technique for extending the shelf-life of PUFAs. In microencapsulation, the lipid is surrounded by or embedded in a matrix typically comprised of either carbohydrates, proteins, gums or a blend of such materials. Ideally, this ‘wall’ provides protection against damage caused by light, heat, and oxygen. Microencapsulation also can mask undesirable flavors or odors, facilitate processing, control the release rate and location of a compound, and impact bioavailability of the encapsulated material (Re 1998; Vilstrup 2001). Microcapsule properties depends on the processing methods and materials involved in preparation (Jin, Perrie et al. 2008). While it is generally expected that microencapsulation will improve the oxidative stability of a core material, microencapsulated products are still susceptible to oxidation. Certain microcapsule attributes may even render a product more unstable. For example the large surface area of spherical particles may result in exposure of a large amount of oil to air if oil remains on the surface of the microcapsules. Furthermore, it is not uncommon for air pockets to be incorporated into particles during processing; trapped air inside a microcapsule could eliminate the anticipated protective effect.

The purpose of this work was to evaluate the oxidative stability of fish oil microcapsules prepared with a blend of chitosan and high-amylose starch. A secondary goal of this work was to evaluate the value of headspace solid phase microextraction coupled to gas chromatography/mass spectrometry (HS-SPME-GC/MS) as a method to analyze intact microcapsules. The HS-SPME-GC/MS technique has been previously applied to analysis of fish oil supplemented milk (Jimenez-Alvarez, Giuffrida et al. 2008), fish oil-in-water emulsions (Iglesias, Lois et al. 2007), gelatin and caseinate-based fish oil microcapsules (Jonsdottir, Bragadottir et al. 2005), and fish oil microcapsules prepared by spray nebulization (Benedetti, Drusch et al. 2009).

## **MATERIALS AND METHODS**

### **Materials**

Chitosan with DD=95% and low molecular weight (15 mPA·s as a 1% solution as reported by supplier) was donated by Primex, Inc. (Siglufjordur, Iceland). High-amylose starch (Hi-maize 260) was donated by National Starch (Bridgewater, NJ). Fish oil (from anchovies and

sardines and including canola and sunflower oil) with total omega-3 content of 300mg/g (as reported by the supplier, triglyceride basis) was donated by Ocean Nutrition (Nova Scotia, Canada). All reagents were purchased from Sigma-Aldrich (St. Louis, MO) and were ACS reagent grade or higher.

### **Preparation of Microcapsules**

Starch was suspended in 1% aqueous acetic acid (v,v) by heating to 160°C under 0.4 MPa of nitrogen gas and holding for thirty minutes in a Parr Instruments pressure reactor (Parr Instrument Company, Moline, IL). Chitosan was dissolved in 1% aqueous acetic acid (v,v). Chitosan and starch suspensions were combined to produce a suspension with final polymer content of approximately 7% (w,v) and 60% chitosan, 40% starch on a weight basis. Fish oil was added immediately prior to homogenizing at a level of 50% (w,w) based on wall material and the mixture was rapidly stirred with a non-aerating overhead stirrer with propeller style blade (Kraft Apparatus, New York, NY) to prepare a coarse emulsion. Coarse emulsions then were passed twice through a two-stage pressure homogenizer (Model 15MR, APV, Everett, MA) at 20/5 MPa (200/50 Bar). Emulsions were flushed with nitrogen and were spray dried within 24 hours of preparation on a Buchi 190 mini spray drier (Buchi, Flawil Switzerland) equipped with a 0.5 mm two fluid nozzle. Air pressure was set at 0.55 MPa. Inlet and outlet temperatures were maintained at 160°C and 90°C respectively. Microcapsules for general characterization were stored in glass jars at room temperature, while those for characterization of oxidative stability were packaged in oxygen barrier bags, nitrogen flushed, and stored at -80°C.

### **Peroxide Value**

Microcapsules samples (~2g) were stored in uncapped glass jars at 35°C and 33% relative humidity. Saturated MgCl<sub>2</sub> solutions were used to achieve and maintain the target relative humidity. Peroxide value was measured on two fractions of fat extracted from the microcapsules. First surface fat was removed from a microcapsule sample by washing with hexane through a glass fritted filter. Hexane was evaporated under nitrogen and the peroxide value of the recovered lipid was measured. Microcapsules were transferred back to their original containers and sonicated for ten minutes in 10ml of a 7:3 (v,v) mixture of chloroform and methanol. Solvent was collected by filtering and evaporated with nitrogen. The peroxide value of lipid

recovered from this extraction was measured. Lipid extracted with hexane was considered surface lipid, while that extracted with chloroform/methanol was considered encapsulated lipid. Peroxide value was measured according to ISO 3976/IDF74:2006E (IDF, 2006). The extracted lipid was dissolved in chloroform/methanol (7:3, v,v), then 50 $\mu$ L of ammonium thiocyanate and 50 $\mu$ L of ferrous chloride were added. The solution was transferred to a glass cuvette and equilibrated for 10 minutes. The absorbance was then measured at 500nm. Peroxide value was calculated based on a standard curve generated from Fe<sup>3+</sup> according to the standard method.

### **HS-SPME-GC/MS**

For SPME analysis, 0.5g samples were stored in uncapped 10ml SPME vials. Samples were removed from the incubator at pre-determined time points and capped. Two milliliters of saturated sodium chloride were added by syringe. A CTC CombiPal autosampler (Zwingen, Switzerland) was used for exposure of the fiber to the headspace and for injection. Volatiles were adsorbed to the fiber during 15 minutes at 50°C with shaking. A 85 $\mu$ m Carboxen/PDMS fiber (Supelco, Bellefonte, PA) was used. A Hewlett Packard 5890A gas chromatograph (Valley Forge, PA) coupled to a Hewlett Packard 5972 mass selective mass spectrometer was used for separation and analysis of the volatiles. Volatiles were desorbed for 10 minutes at 250°C. Volatiles were separated on an HP-5ms column, 30m x 0.25 mm I.D. x 0.25 $\mu$ m film thickness. Injection temperature was 250°C. Initial oven temperature of 35°C was held for 3 minutes after which the temperature was increased to 70°C at a rate of 3°C/minute, then increased to 200°C at a rate of 10°C/minute and finally increased to 250°C at a rate of 20°C/minute. Detector temperature was set at 280°C GC-MS and the mass spectrometer was operated in scan mode from 40m/z to 550 m/z. Samples were analyzed in duplicate.

Initial compound identification was done using the MS library. Compound identities were subsequently confirmed for some compounds by matching retention times with standards and through the use of Kovats indices. For quantification of pentanal, hexanal, and 1-penten-3-ol standard curves were generated from the peak areas obtained from standard solutions. Standard solutions were prepared by dissolving in ethanol and then diluting in water to achieve the desired concentration.

## RESULTS AND DISCUSSION

General properties of the microcapsules used in this study are shown in Table 5.1. Details of these properties were discussed in Chapter 4.

### Peroxide Value

Peroxide values (PVs) were measured in surface oil and encapsulated oil fractions separately (Figure 3.1). There was no significant difference between the peroxide value of surface and encapsulated fractions during storage through day 21. Peroxide value of surface oil and encapsulated oil fractions were significantly different on day 28 ( $p=0.028$ ) and day 35 ( $p=0.0056$ ). Although statistically significant, these peroxide values are still quite low and do not indicate substantial oxidation of either fraction. It is generally expected that surface oil is more susceptible to oxidation than encapsulated oil as the encapsulated oil should be afforded protection by the wall components whereas surface oil is theoretically exposed to more air. In this work the peroxide value measurements indicated that the surface oil was oxidizing more slowly than the encapsulated oil. It is not uncommon for spray dried microcapsules to have air incorporated into the matrix, or to have large central voids within the particles (Forsell, Myllarinen et al. 2007). Air in the interior of the particle could facilitate oxidation of encapsulated oil as could other initiators such as metals.

Baik and colleagues (2004) found that PVs of surface oil in fish oil microcapsules prepared with sodium caseinate and corn syrup solids were ten times higher than those for encapsulated oil when stored at 30°C and 11% RH. PVs for these microcapsules were 17.4 mmol/kg post spray drying and therefore, oxidation as evidenced by PV had already proceeded beyond that of the microcapsules generated in this work. The bulk fish oil used in our study contained a mixture of tocopherols and citric acid whereas the bulk fish oil used by Baik et al (2004) did not contain antioxidants. It is likely that antioxidant composition of the bulk fish oil and drying temperatures which were lower (150°C) compared to 210°C for Baik et al (2004), assisted in controlling initial oxidation. Marquez-Ruiz et al (2003) found that surface and encapsulated oil oxidized at approximately the same rate for microencapsulated sunflower oil prepared without antioxidants, but that for sunflower oil containing tocopherol surface oil oxidized more rapidly than encapsulated oil. The same researchers have also reported that surface oil oxidized more rapidly for microencapsulated infant formula.

Drusch et al (2009) reported that peroxide values remained below 10 mmol/kg oil for greater than 15 days for fish oil microcapsules prepared with different blends of caseinate and glucose syrup. Benedetti and colleagues (2009) reported peroxide values in fish oil/palm oil blends encapsulated by spray congealing that gradually increased from 1.7 mmol/kg oil to 76.5 mmol/kg during storage at 20°C for 42 days. Shaw, McClements and Decker (2007) reported peroxide values ranging from less than 10 mmol/kg to greater than 160 mmol/kg for menhaden oil microcapsules prepared with electrostatically assembled layers of chitosan and lecithin and spray dried with varying amounts of corn syrup solids. Microcapsules dried with 5% and 20% corn syrup solids maintained peroxide values below 20 mmol/kg oil for 22 days of storage at 37°C and 33% RH, while those prepared with 1%, 2%, or 10% had reached peroxide values of 120 mmol/kg oil or higher by day 15. Secondary oxidation products (as monitored by measuring thiobarbituric acid reactive substances) remained low through 8 days of storage for all formulations and gradually increased after that for 5, 20, and 10% corn syrup solids microcapsules. Microcapsules from 1 and 2% corn syrup solids exhibited a sharp increase in secondary products between days 10 and 15 and declined afterward. It is difficult to directly compare the results presented herein to evaluate wall material performance with those in the literature due to differences in oil composition, antioxidant content of the oils, and storage conditions.

The peroxide value test measures the concentration of peroxides and hydroperoxides. These compounds are unstable intermediates in the oxidation process and readily decompose to yield secondary oxidation products such as aldehydes and ketones. It is therefore important to consider the possibility that low levels such as those observed herein may indicate that oxidation has progressed into the secondary phase (Kolanowski, Jaworska et al. 2007). The low levels of secondary oxidation products (Figure 5.3) however suggest that this is not the case. The high stability indicated by the peroxide value data is likely a combined result of effective functioning of the antioxidants in the oil and protection afforded by the chitosan-starch matrix.

### **Secondary Volatile Chemistry as Measured by HS-SPME-GC/MS**

Headspace-solid phase microextraction coupled with gas chromatography/mass spectrometry (HS-SPME-GC/MS) was used to monitor the formation of volatiles in the intact fish oil microcapsules during storage. Representative chromatograms are shown in Figure 5.2. The

compounds shown in Table 2 were detected in the headspace of microcapsules. The majority of these compounds have been previously detected in bulk fish oil, fish oil emulsions, or microencapsulated fish oil samples (Genot, Meynier et al. 2003; Jonsdottir, Bragadottir et al. 2005; Roh, Park et al. 2006; Jimenez-Alvarez, Giuffrida et al. 2008). The compounds 1-pentanol, 2-methyl-2-pentenal, 1-hexanol, 2-ethyl-2-pentenal, 1-heptanol, and 2-ethylhexanol have not been previously reported as detected by HS-SPME-GC/MS in fish oil samples, although 2-methyl-2-butenal and 2-ethyl-2-pentenal have been detected by supercritical carbon dioxide extraction (Roh, Park et al. 2006).

Jonsdottir and colleagues (2005) used HS-SPME-GC/MS to monitor oxidation in microencapsulated fish oil and suggested that hexanal, 2-nonenal and 2,4-decadienal were well suited for monitoring oxidation. The researchers used both a PDMS fiber and a PDMS/DBV fiber and reported that the PDMS/DBV fiber outperformed the PDMS fiber for detection of oxidatively derived volatiles. Microcapsules were prepared from cod liver oil with either caseinate and lactose (with Span80 or lecithin), caseinate and sucrose, caseinate and maltodextrin, or gelatin, gum acacia, and maltodextrin as wall materials. Hexanal, 2-nonenal, 2,4-decadienal, and total peak area were low for 4 weeks of storage at 4°C but generally increased after that initial period for caseinate/lactose/Span80, caseinate/sucrose, and caseinate/maltodextrin samples. Caseinate/lactose microcapsules with lecithin, caseinate/lactose microcapsules with  $\alpha$ -tocopherol, and gelatin/gum acacia/maltodextrin microcapsules showed lower overall peak area ratios during 20 weeks of storage at 4°C than the other caseinate blends. Differences were observed between microcapsules encapsulated with different wall materials. The compound 2,4-decadienal was not detected in the chitosan-starch microcapsules evaluated herein and 2-nonenal was not detected until day 35.

Benedetti and colleagues (2009) evaluated oxidation of spray-congealed fish oil/palm oil microcapsules using HS-SMPE-GC/MS with a Carboxen/PDMS fiber. The Carboxen/PDMS fiber has been reported to be more sensitive in detecting a wider range of volatiles produced during oxidation of fish oils (Iglesias, Lois et al. 2007). These researchers reported that propanal, 1-penten-3-one, hexanal, 1-penten-3-ol, 2,4-heptadienal, and E,Z-2,6 nonadienal were best suited for tracking as peak areas for these compounds increased during storage at 20°C for 6 weeks.

In our work the concentrations of hexanal, pentanal, and 1-penten-3-ol were quantified over the 35 day storage period. Areas for propanal and 2,4-heptadienal, and total peak area were also

monitored (Figure 5.3). All compounds were present at low levels at day 0 and increased during storage. Pentanal exhibited a gradual increase through the first four weeks of storage and then appeared to level off. Pentanal was well suited for monitoring due to the presence of a well defined peak. Jimenez-Alvarez and colleagues (2008) reported that pentanal was not detected in EPA or DHA triglycerides stored at 37°C for 6 hours, but was detected from ARA triglyceride. For monitoring degradation of EPA and DHA, pentanal may not be the best choice. However; considering that ARA has fewer unsaturation sites (4 versus 5 for EPA and 6 for DHA), presence of ARA degradation products would suggest that EPA and DHA are also degraded.

Hexanal and 1-penten-3-ol concentrations remained fairly constant through the first two weeks of storage, after which they increased rapidly, then appeared to level off and perhaps even gradually decline. Propanal and 2,4-heptadienal also exhibited rapid increases between days 14 and 28. Propanal content appeared to be declining after day 28. Good correlation between these compounds was observed and the correlations are shown in Table 5.3. The highest correlations were observed for propanal and 1-penten-3-ol and propanal and hexanal. Evolution of the individually considered compounds also correlated well to increases in total peak area during storage. Based on total peak area, the microcapsules appeared to be fairly stable for the first two weeks of storage.

Selecting compounds for monitoring oxidation in fish oil products is challenging. Often the most readily detected and quantified compounds do not necessarily correspond to the compounds that render a product unacceptable in terms of sensory acceptability. In relating sensory acceptability to hexanal levels, Iglesias et al reported that an “incipient rancid” odor corresponded to approximately 1.2 ppm of hexanal and a “rancid odor” corresponded to approximately 1.6 ppm in fish oil enriched milk (Iglesias, Lois et al. 2007). Hexanal content in the samples evaluated herein remained below 1 ppm through 21 days of storage and only reached a maximum of 1.5 ppm throughout the entire storage period. This suggests that the microcapsules had not oxidized to an extent that would render a product containing them unacceptable.

## **Conclusion**

The HS-SPME-GC/MS method used herein enabled detection of several volatile compounds related to off flavors in fish oil products. Evolution of hexanal correlated most closely to total peak area as compared to propanal, 1-penten-3-ol, pentanal, and 2,4-heptadienal. Headspace data showed a steady increase in volatile compounds after two weeks of storage at 35°C and 33% RH. The headspace and peroxide value data suggested that the microencapsulated fish oil was not highly oxidized even at the conclusion of the 35 day storage period.

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## TABLES

**Table 5.1. Properties of spray-dried fish oil microcapsules prepared with 60% chitosan and 40% starch at an oil load level of 50% based on carbohydrate weight.**

Property	Value <sup>1</sup> x ± se
encapsulation efficiency (%)	62.4 ± 0.5
surface fat (%)	37.6 ± 1.4
inner free fat (%)	9.1 ± 0.9
particle size, 50 <sup>th</sup> percentile (µm)	5.5 ± 0.2
particle size, 90 <sup>th</sup> percentile (µm)	39.5 ± 6.8
water activity	0.065 ± 0.015
moisture content (%)	2.1 ± 0.4

<sup>1</sup>Values are reported as mean ± standard error (se), n=4 for encapsulation efficiency, surface fat, inner free fat, and moisture content, n=6 for particle size and water activity

**Table 5.2. Volatile compounds identified in fish oil microcapsules stored for 35 days at 35°C and 33% RH. Fish oil was encapsulated in 60% chitosan, 40% starch at 50% oil load level.**

Compound	Retention Time (min)	Calculated Kovats Index	Flavornet Kovats Index
acetaldehyde*	1.4	-	-
propanal*	1.6	503	506
butanal <sup>ϕ</sup>	2.1	553	596
1-penten-3-ol*	3.1	667	NR
pentanal*	3.4	700	732
3-penten-2-one	4.3	732	NR
2-methyl-2-butenal	4.4	736	NR
pentenal <sup>ϕ</sup>	4.7	746	754
1-pentanol <sup>ϕ</sup>	5.2	764	766
cis-2-pentenol	5.3	768	NR
1-hexen-3-ol	5.5	775	NR
hexanal*	6.0	793	801
2-methyl-2-pentenal	7.4	828	NR
2-hexenal <sup>ϕ</sup>	8.3	849	844
1-hexanol <sup>ϕ</sup>	9.1	867	851
cis-4-heptenal <sup>ϕ</sup>	10.4	898	902
heptanal <sup>ϕ</sup>	10.5	900	957
2-ethyl-2-pentenal	11.8	925	NR
2-heptenal	13.2	953	951
1-heptanol <sup>ϕ</sup>	14.0	969	877
1-octen-3-ol	14.5	978	NR
octanal <sup>ϕ</sup>	15.5	998	1006
2,4-heptadienal*	15.8	1006	1011
2-ethyl-hexanol <sup>ϕ</sup>	16.8	1036	1015
octenal <sup>ϕ</sup>	17.5	1058	1060
3,5-octadiene-2-one <sup>ϕ</sup>	17.9	1070	1095
2-nonanone <sup>ϕ</sup>	18.5	1088	1093
nonanal <sup>ϕ</sup>	18.8	1097	1104
2-nonenal <sup>ϕ</sup>	20.1	1157	1147

\*Indicates compound was identified by EIMS library match plus Kovats Index, plus standard

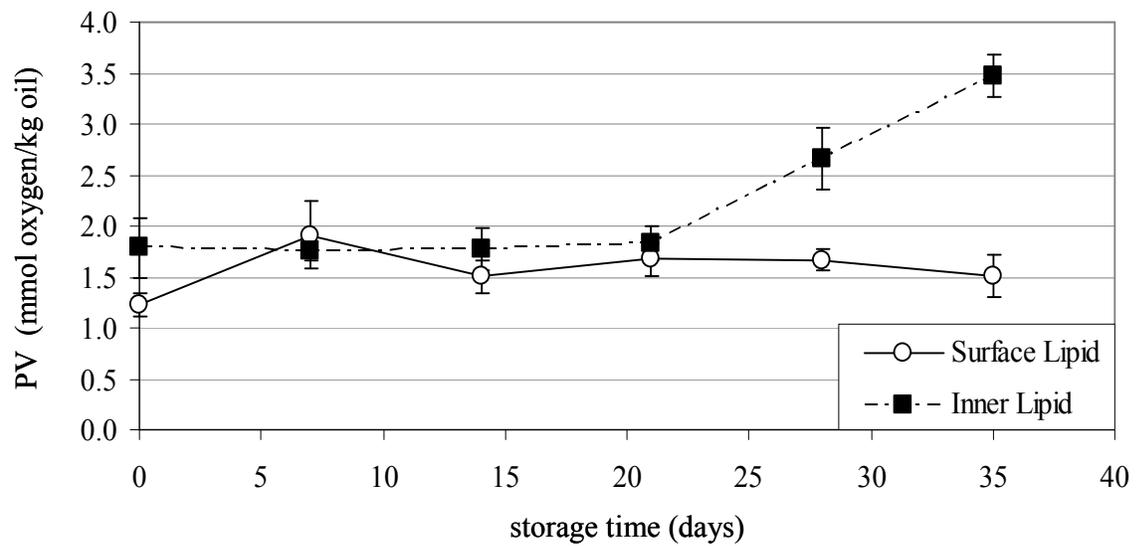
<sup>ϕ</sup>Indicates compound was identified by EIMS library match plus Kovats Index

All other compounds were identified by EIMS library match only

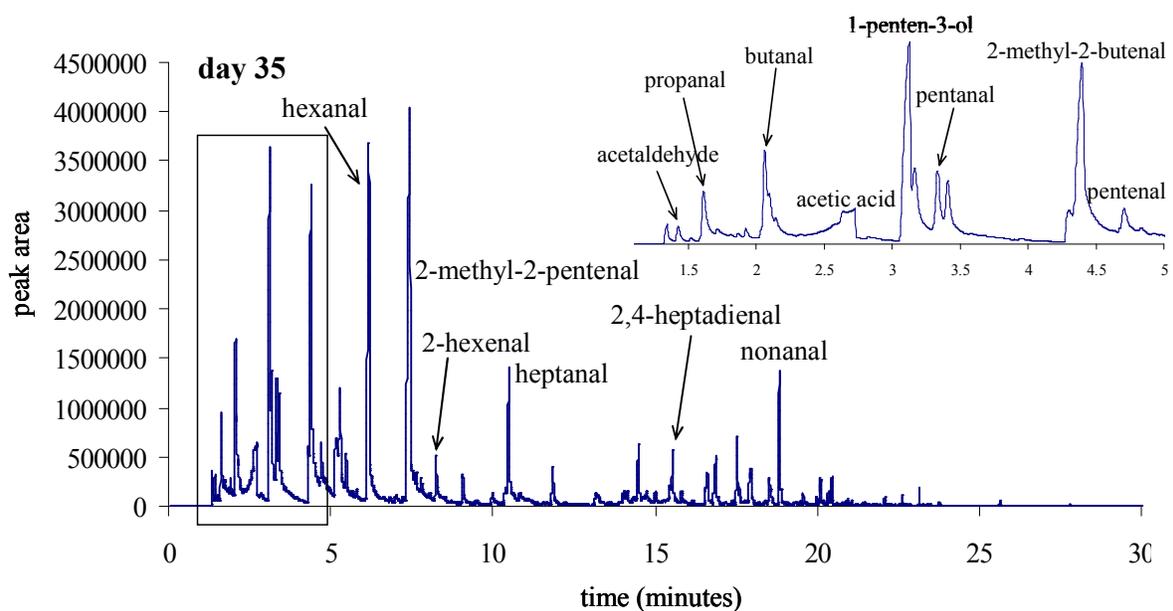
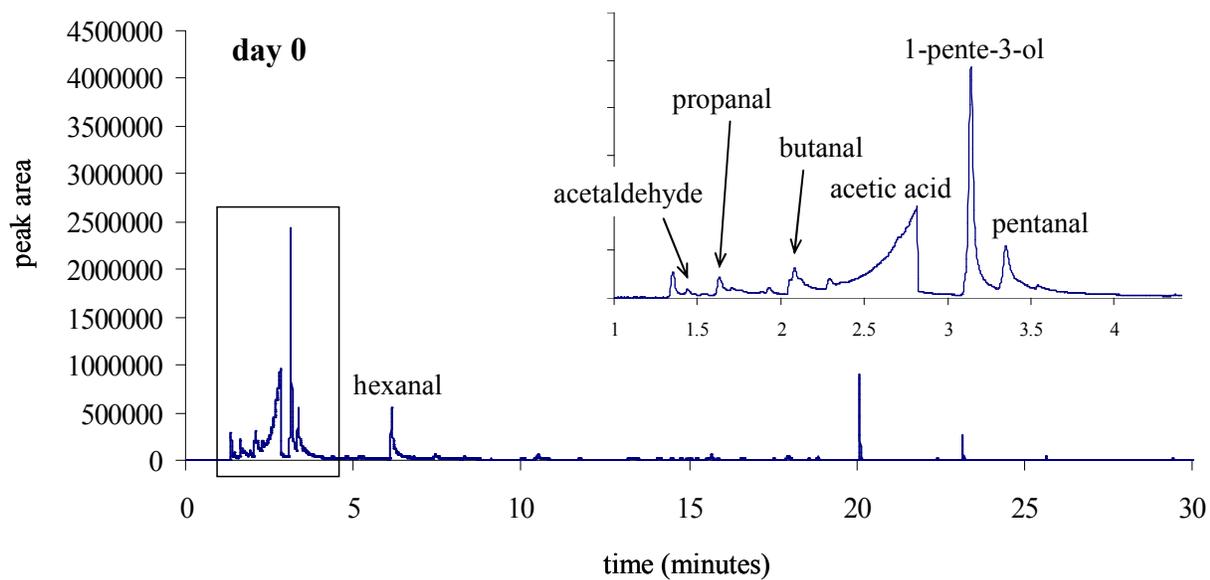
Flavornet Kovats Indices were obtained from the Flavornet aroma database at [www.flavornet.org](http://www.flavornet.org)

**Table 5.3 Correlation coefficients for evolution of select volatile compounds detected in fish oil microcapsules prepared from 60% chitosan and 40% starch at 50% oil load level over 35 days of storage at 35°C and 33%RH.**

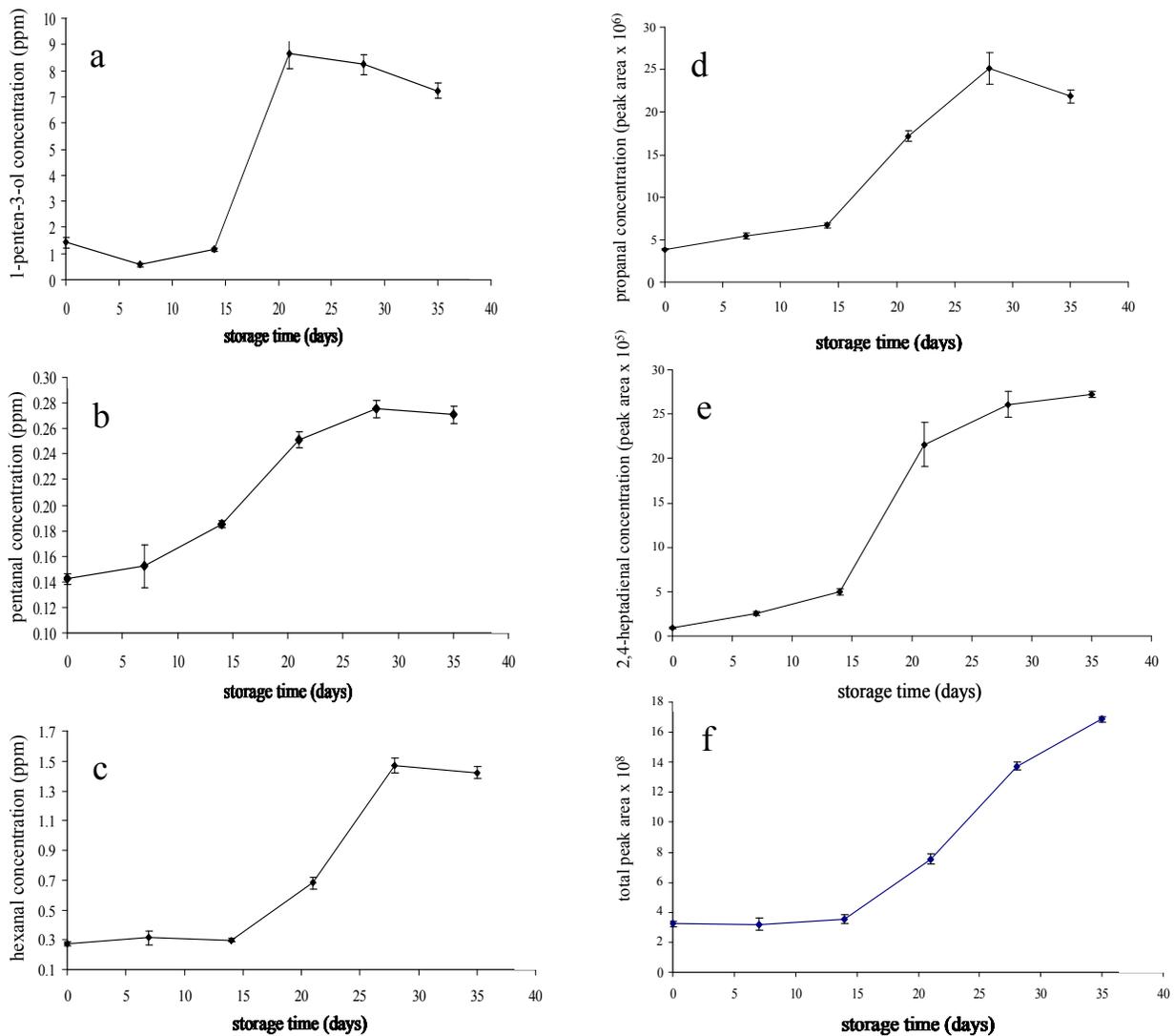
	propanal	1-penten-3-ol	pentanal	hexanal	2,4-heptadienal	total peak area
propanal		0.955	0.825	0.945	0.8637	0.904
1-penten-3-ol	0.955		0.823	0.925	0.900	0.881
pentanal	0.825	0.823		0.905	0.885	0.869
hexanal	0.945	0.925	0.905		0.901	0.962
2,4-heptadienal	0.8637	0.900	0.885	0.901		0.908
total peak area	0.904	0.881	0.869	0.962	0.908	



**Figure 5.1** Peroxide values of surface and encapsulated fractions of microencapsulated fish oil stored at 35°C and 33% RH for 35 days. Fish oil was encapsulated in 60% chitosan, 40% starch at an oil load level of 50%.



**Figure 5.2. Representative chromatograms for fish oil microcapsules prepared with 60% chitosan and 40% starch at 50% oil load level and stored for 35 days at 35°C and 33% RH. Inset sections represent the boxed portions of the graphs.**



**Figure 5.3. Evolution of select and total volatile compounds: (a) 1-penten-3-ol; (b) pentanal; (c) hexanal; (d) propanal; (e) 2,4, heptadienal; (f) total peak area in fish oil microcapsules during storage at 35°C and 33%RH over 35 days. Microcapsules were prepared from 60% chitosan and 40% starch at 50% oil load level.**

## **CHAPTER 6: CONCLUSIONS AND FUTURE WORK**

This work demonstrates that chitosan is a promising material for microencapsulation of lipid based ingredients by spray drying. Microcapsule properties are highly dependent on the properties of the feed emulsions. The fish oil emulsions prepared in this work had small oil droplet sizes and appeared to be stable during storage without the need for a separate emulsifying component. Chitosan of both degrees of deacetylation and molecular weights functioned well as an emulsifier and produced microcapsules with favorable properties. When blended with high-amylose starch and pullulan, stable emulsions and microcapsules were also formed. Several areas of future work related to this project that would be interesting to pursue are discussed below.

### **Investigate Chitosan as a Spray Drying Wall Material and Emulsifier in a More Systematic Manner**

In this work two types of chitosan differing in degree of deacetylation and molecular weight were investigated, however; the experimental design did not allow for direct evaluation of the effects of molecular weight or degree of deacetylation on performance as a wall material or emulsifier. It would be interesting to evaluate chitosan in these regards in a more systematic and comprehensive manner under fixed homogenization and drying conditions (including viscosity) to elucidate the effect of molecular weight and degree of deacetylation on feed emulsion and dried microcapsule properties. In conjunction with pursuit of such an endeavor, it would be useful to conduct an optimization of the drying process to determine if process efficiency and encapsulation efficiency can be improved by changing the drying temperatures, nozzle size, etc. For example, viscosity of the feed emulsion and drying temperatures may have a significant effect on surface oil contents due to differences in droplet atomization. Furthermore, drying temperatures near the glass transition temperature of the carbohydrate component can cause material to collect on the drier walls. Lower drying temperatures could improve recovery, but a balance between recovery and final moisture content and water activity is important.

The presence of free amine and hydroxyl groups along the chitosan backbone enable chemical modification including crosslinking. Crosslinking could have favorable effects on oil retention and barrier properties of the matrix and it would be worthwhile to consider the impact

of crosslinking on microcapsules properties. An investigation of chitosan as a material for fish oil microencapsulation could be further enhanced by evaluating taste masking properties and the effects of microencapsulation with chitosan on oil bioavailability.

### **Consider alternative materials to blend with chitosan for preparing fish oil microcapsules by spray drying**

While chitosan appears to function well in this application, it is limited in spray drying applications due to its high viscosity even at low concentrations. This limits the concentration of the feed emulsions and requires large amounts of solvent to be removed during drying reducing the efficiency of the process. In this work high-amylose starch and pullulan were selected for consideration as matrix materials in conjunction with chitosan. While incorporating these materials did allow a slight increase in the dissolved solids content of the feed emulsions, the increase was not dramatic and microcapsules produced from the blends had lower encapsulation efficiency than those prepared from the chitosan alone. It would be interesting to evaluate chitosan in combination with some low molecular weight materials that have been demonstrated effective as spray drying wall components such as maltodextrins and/or corn syrup solids. Lower molecular weight components could reduce free volume in the particles and therefore potentially improve oxygen barrier properties.

### **Further investigate interactions between chitosan, high-amylose starch, and pullulan.**

Despite the lack of improvement in encapsulation efficiency observed in the blends, combining chitosan with high-amylose starch and pullulan did seem to improve oxidative stability as evidenced by oxidative induction time. Evidence suggesting these materials produce films with low oxygen permeability was a primary driving force for selection of these materials and it would be interesting to further evaluate the oxygen and water vapor barrier properties of these materials in film form and in varying ratios.

### **Evaluate Chitosan-Fish Oil Microcapsules in Food Matrices with an Emphasis on Oxidative Stability During Storage**

In the present work oxidative stability of all microcapsule formulations was evaluated by measuring oxidative induction time (OIT) using pressure differential scanning calorimetry

(PDSC). This method allowed rapid comparison of the different microcapsules, but in that the method is an accelerated method care must be taken in relating the data to typical storage conditions. It would be worthwhile to monitor oxidation in chitosan-fish oil microcapsules under actual storage conditions using a wider range of tests including those that measure primary products (peroxide value, conjugated dienes and trienes) and secondary oxidation products (thiobarbituric acid reactive substances, anisidine value, and GC-MS monitoring of volatile secondary compounds in the headspace) as well as also possibly quantifying break down of DHA and EPA using GC-MS and monitoring oxygen consumption. Monitoring oxidation (and oxygen content) throughout processing (eg. before and after homogenization, after drying) would also be useful. It would also be beneficial to carry oxidative stability research out with fish oil that is free of antioxidants in order to get a better understanding of the protection afforded by the carbohydrate matrices. The fish oil used in this work contained a proprietary blend of antioxidants and therefore it was unclear in the long term storage study whether prevention of oxidation was due to the microcapsule structure of the antioxidants or a combination. It would also be nice to further evaluate the PDSC method for comparing oxidation in microcapsule systems by comparing results obtained with this method to those obtained through long term storage studies as well as other commonly used accelerated tests such as Oxipres or the Rancimat tests.

### **Further Evaluate Oxidative Stability of Fish Oil Microencapsulated in Chitosan Containing Matrices**

The microcapsules generated in this work were originally intended to be evaluated in a dairy matrix, however; preliminary research showed that chitosan interacted with dairy proteins to produce undesirable changes. Microcapsules prepared with chitosan in this work were also not readily soluble in water and microcapsules prepared with chitosan may therefore not be suitable for products requiring they be reconstitution in water or dispersed in a liquid matrix. They may however be well suited to bakery products, pasta products, and cereal products. Investigating microcapsules functioning in such products is suggested. Such investigation should include analysis of oxidative stability during storage as described above.