BGJ4480017 ดร.ไพรัตน์ โสภโณดร

รายงานวิจัยฉบับสมบูรณ์

โครงการ "การผลิตน้ำมันปลาที่เพิ่มสัดส่วนกรดไขมันไม่อิ่มตัวชนิดโอเมก้า-3 ในรูปผงแบบไมโครเอนแคบซูเลท"

โดย

รศ. ดร. ไพรัตน์ โสภโณดร อาจารย์ที่ปรึกษา

นางสาวอุทัย กลิ่นเกษร นักศึกษาผู้รับทุน

เมษายน 2548

สัญญาเลขที่ BGJ/17/2544

รายงานวิจัยฉบับสมบูรณ์

โครงการ "การผลิตน้ำมันปลาที่เพิ่มสัดส่วนกรดไขมันไม่อิ่มตัวชนิดโอเมก้า-3 ในรูปผงแบบไมโครเอนแคบซูเลท"

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มหาวิทยาลัยสงขลานครินทร์

สนับสนุนโดยสำนักงานกองทุนสนับสนุนการวิจัย

กิตติกรรมประกาศ

ขอขอบพระคุณรองศาสตราจารย์ ดร. ไพรัตน์ โสภโณดร อาจารย์ที่ปรึกษา ดร. ภาวินี ชินะโชติ อาจารย์ที่ปรึกษาร่วม Assoc Prof. Dr. D.J., McClements และ Prof. Dr. E.A., Decker อาจารย์ที่ปรึกษาพิเศษ ที่กรุณาให้คำปรึกษาแนะนำในการค้นคว้าวิจัยและการเขียนรายงานฉบับนี้

ขอบขอบคุณโครงการปริญญาเอกกาญจนาภิเษก และโครงการทุนวิจัยองค์ความรู้ใหม่ในการสร้างนักวิจัยระดับปริญญาเอก สำนักงานกองทุนสนับสนุนการวิจัย ที่ให้ความอนุเคราะห์สนับสนุนเงินทุนในการวิจัยและการใช้เครื่องมืออุปกรณ์ที่จำเป็นในงานวิจัยครั้งนี้

อุทัย กลิ่นเกษร

บทคัดย่อ

รหัสโครงการ: BGJ/17/2544

ชื่อโครงการ: การผลิตน้ำมันปลาที่เพิ่มสัดส่วนกรดไขมันไม่อิ่มตัวชนิดโอเมก้า-3 ในรูปผงแบบไมโครเอนแคบซูเลท

ชื่อนักวิจัย: รศ. ดร. ไพรัตน์ โสภโณดร อาจารย์ที่ปรึกษา
นางสาวอุทัย กลิ่นเกษร นักศึกษาผู้รับทุน
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การศึกษาผลของความเข้มข้นของตัวเร่งปฏิกิริยา อุณหภูมิ และอัตราส่วนโดยโมลของสารตั้งต้นต่อการเกิดปฏิกิริยาทรานส์เอสเทอริฟิเคชั่นระหว่างน้ำมันปลาทูน่าและเมทธิลเอสเทอร์ของกรดไขมันโอเมก้า-3 (กรดไขมันอีพีเอและดีเอชเอ) พบว่ากรดไขมันอีพีเอและดีเอชเอในน้ำมันปลาทูน่าเพิ่มขึ้นมากที่สุดที่ระดับความเข้มข้นของตัวเร่งปฏิกิริยาเท่ากับ 1.5 เปอร์เซนต์ การเพิ่มของกรดไขมันดังกล่าวในน้ำมันปลาทูน่าจะมากขึ้นเมื่ออุณหภูมิเพิ่มขึ้นจนให้ค่าสูงสุดที่อุณหภูมิ 80 องศาเซลเซียส เมื่อเพิ่มอัตราส่วนโดยโมลของสารตั้งต้น (เมทธิลเอสเทอร์ของกรดไขมันโอเมก้า-3 ต่อน้ำมันปลาทูน่า) กรดไขมันอีพีเอและดีเอชเอในน้ำมันปลาทูน่าเพิ่มมากขึ้นและเพิ่มมากที่สุดเมื่ออัตราส่วนดังกล่าวเท่ากับ 4:1 จึงกล่าวได้ว่าปฏิกิริยาทรานส์เอสเทอริฟิเคชั่นโดยมีสารเคมีเป็นตัวเร่งปฏิกิริยาสามารถใช้ในการเพิ่มสัดส่วนกรดไขมันโอเมก้า-3 (กรดไขมันอีพีเอและดีเอชเอ) ในน้ำมันปลาทูน่าได้ อย่างไรก็ตามการสูญเสียปริมาณ ไตรกลีเซอไรด์ระหว่างการเกิดปฏิกิริยาทำให้ได้ปริมาณผลผลิตน้อย การเพิ่มปริมาณผลผลิตในปฏิกิริยาจึงเป็นส่วนที่น่าสนใจในการศึกษาต่อไป การศึกษาผลของน้ำผึ้งข้าวโพดชนิดแข็งต่อความคงตัวของอิมัลชั่นแบบน้ำมันในน้ำของน้ำมันปลาทูน่าที่ทำให้อยู่ตัวด้วยเลซิทีน (อิมัลชั่นปฐมภูมิ) และเลซิทีน-ไคโตแซน (อิมัลชั่นทุติยภูมิ) รวมทั้งความคงตัวต่อการเกิดออกซิเดชั่นของน้ำมันปลาทูน่าผงในรูปไมโคร เอนแคบซูเลท พบว่าอิมัลชั่นทุติยภูมิมีความคงตัวต่อการเกิดการรวมตัวกันของเม็ดไขมันดีกว่าอิมัลชั่นปฐมภูมิหลังผ่านการให้ความร้อน (อุณหภูมิ 30-90 องศาเซลเซียส นาน 30 นาที) และในสภาวะที่มีความเข้มข้นของเกลือสูง (200 มิลลิโมลาร์ของโซเดียมคลอไรด์) การเติมน้ำผึ้งข้าวโพดชนิดแข็งเป็นการลดความคงตัวของอิมัลชั่นปฐมภูมิ แต่เพิ่มความคงตัวของอิมัลชั่นทุติยภูมิ ไมโครแคบซูลของน้ำมันปลาทูน่าสามารถผลิตได้โดยการทำแห้งอิมัลชั่นทุติยภูมิแบบสเปรย์ดราย ไมโครแคบซูลหรืออิมัลชั่นผงที่ได้จะมีความชื้นน้อยกว่า 3 เปอร์เซ็นต์ มีปริมาณน้ำมันปลาทูน่ามากกว่า 85 เปอร์เซ็นต์ และสามารถละลายน้ำได้เร็วภายใน 1 นาที นอกจากนี้พบว่าอุณหภูมิในการทำแห้ง (165-190 องศาเซลเซียส) ไม่มีผลต่อโครงสร้างของแคบซูลผงที่ได้ ผลการศึกษาการเกิดออกซิเดชั่นพบว่าน้ำมันปลาทูน่าในรูปผงแบบ

ไมโครแคบซูลมีความคงตัวต่อการเกิดออกซิเดชั่นดีกว่าน้ำมันปลาทั่วไป อัตราการเกิดออกซิเดชั่นของไมโครแคบซูลที่เก็บรักษาที่ระดับความชื้นสัมพัทธ์ 11 และ 33 เปอร์เซ็นต์ เกิดขึ้นเร็วกว่าที่ระดับความชื้นสัมพัทธ์ 52 เปอร์เซ็นต์ การผลิตน้ำมันปลาทูน่าในรูปผงแบบไมโครเอนแคบซูเลท โดยการทำแห้งอิมัลชั่นที่มีผิวสัมผัสแบบหลายชั้น พบว่ามีคุณสมบัติทางเคมีกายภาพและความคงตัวต่อการเกิดออกซิเดชั่นที่ดี เป็นการเพิ่มแนวทางในการใช้ประโยชน์น้ำมันปลาทูน่าในอุตสาหกรรมอาหาร

คำหลัก: น้ำมันปลาทูน่า กรดไขมันโอเมก้า-3 ทรานส์เอสเทอริฟิเคชั่น ผิวสัมผัสหลายชั้น อิมัลชั่นแบบน้ำมันในน้ำ ไมโครเอนแคบซูเลท

Abstract

Project Code : BGJ/17/2544

Project Title : Production of Microencapsulated Fish Oil Enriched with Omega-3 Fatty Acids

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Project Period : 42 months (Sep 2001 – March 2005)

The effect of reaction conditions (catalyst concentration, temperature, substrate mole ratio) on transesterification of tuna oil with ω-3 fatty acid methyl ester (ω-3 FAME) using sodium methoxide as catalyst was studied. The catalyst concentration of 1.5% w/w of reactants showed the highest incorporation of EPA and DHA. Incorporation of EPA and DHA were increased with increasing reaction temperature ($P<0.05$) and the highest incorporation was observed at 80°C. The highest EPA and DHA incorporation occurred at the mole ratio of ω-3 FAME and tuna oil of 4:1. It was found that chemical transesterification could enrich ω-3 PUFA, especially EPA and DHA, in tuna oil. However, loss of TG between transesterification lead to lower TG yields, which is an interesting area for further study. The effect of corn syrup solids (0-25%) on the stability of tuna oil-in-water emulsions stabilized by lecithin alone (primary emulsions) or by lecithin-chitosan membranes (secondary emulsions) was examined. The effects of relative humidity (11, 33 and 52%RH) and storage temperature (20 and 37°C) on oxidative stability of spray-dried microencapsulated tuna oil was investigated. The results from physicochemical properties evaluation indicated that the secondary emulsions had better stability against droplet aggregation than primary emulsions after being exposed to thermal processing (30 to 90°C for 30 minutes) and high sodium chloride contents (200 mM NaCl). The addition of corn syrup solids decreased the stability of primary emulsions, but increased that of secondary emulsions. Microcapsules of emulsified tuna oil could be produced by spray drying of secondary emulsions containing 20% corn syrup solids. The powders had relatively low moisture contents (< 3%), high oil retention levels (> 85%) and rapid water dispersibility (< 1 minute). The structure of the microcapsules was unaffected by drying temperature from 165 to 195°C. The oxidative stability of the powders was studied. The results showed that spray-dried tuna oil-in-water emulsions stabilized by lecithin-chitosan membranes were more oxidatively stable than bulk oils. Lipid oxidation was more rapid during storage at lower relative humidity (11 and 33% compared to 52% RH). We have demonstrated that a novel interfacial engineering technology, based on production of multilayer membranes around oil droplets, is effective for producing spray-dried encapsulated tuna oil. The powdered tuna oil produced by this method has good physicochemical properties and oxidative stability, which may lead to its more widespread utilization as a food additive.

Keywords: tuna oil, ω-3 fatty acids, interesterification, multilayer membranes, O/W emulsion, microencapsulation

Introduction

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

Generally, fish oils have complex mixtures of fatty acids with varying chain lengths and degree of unsaturation. Overconsumption of fish oils to obtain ω-3 PUFAs may increase the intake of cholesterol and other saturated fatty acids by consumers (*5;6*). Therefore, concentration or enrichment of fish oil in ω-3 PUFAs could help avoid such a concern. Interesterification is a reaction that exchanges carbonyl groups of fatty acids within and between TG molecules and it is used to modify the structure and composition of oils to improve the physical and nutritional properties of TG (*7*). Presently, there are two types of interesterification in use, i.e. enzymatic and chemical interesterification. There has been numerous research of fish oil enriched with ω-3 PUFAs using enzymatic interesterification (*8;9;10;11*). Enrichment by chemical interesterification has not been reported. Chemical interesterification has some advantages over enzymatic modification, which include lower catalyst cost, use of existing and more available industrial procedures and equipment, and shorter reaction times (*12*).

Utilization of oils high in omega-3 fatty acids (ω-3 FA) in food is limited due to their high susceptibility to oxidation. Lipid oxidation can be reduced by addition of antioxidants and/or by microencapsulation of the oil (*13;14;15;16*). Microencapsulation is a process whereby particles of sensitive or bioactive materials are covered with a thin film of a coating material (*17*). The encapsulated substance (*e.g.*, fats, oils, aromas, flavors) is usually referred to as the "core" material, whereas the film surrounding the core is usually called the "wall" material (*17;18;19*). The wall material is usually composed of relatively low molecular weight carbohydrates, such as corn syrup solids and/or maltodextrin (*13;14;20;21;22*). Nevertheless, corn syrup solids and maltodextrin cannot be used in isolation because they are not effective at forming and stabilizing emulsified oil. Instead, hydrophobic core material is usually homogenized in the presence of an aqueous solution containing an emulsifier (*e.g.*, surfactant, phosopholipid or biopolymer) that forms a protective coating around the oil droplets, and then corn syrup solids and/or maltodextrin are mixed with the resulting emulsion (*16;18;23;24*). The emulsion is then dried to remove the water (*e.g.*, by spray or freeze drying), which leads to the formation of oil droplets surrounded by emulsifier molecules that are entrapped within a carbohydrate matrix. Many researchers have used mixtures of maltodextrin or corn syrup solids with proteins, such as whey protein and sodium caseinate for microencapsulation (*20;21;22*). On the contrary, there is much less information about the mixing of maltodextrins or corn syrup solids with other biopolymers such as chitosan. Because a stable emulsion containing small droplets is critical for microencapsulation, it is important to select an appropriate emulsifying system, as well as the conditions required to obtain a stable emulsion before the drying process. The production of emulsions containing droplets surrounded by multiple-layered interfacial membranes may prove to be an effective means of improving the stability of microencapsulated oils.

In this study, the fatty acid composition of fish oil (tuna oil) was chemically modified to enrich ω-3 PUFAs, primarily EPA and DHA, by using sodium methoxide ($NaOCH_3$) as

catalyst. The effects of reaction time and temperature, catalyst concentration, and reactants mole ratio were studied. Lipid and fatty acid composition of tuna oil were analyzed before and after transesterification. The effect of corn syrup solids on the stability of tuna oil-in-water emulsions stabilized by lecithin alone or by lecithin-chitosan membranes was examined. The effect of environmental stresses, such as pH, ionic strengh and thermal treatment were studied. The impact of spray drying on the properties and dispersibility of encapsulated tuna oil powders and the effects of relative humidity (RH) on oxidative stability of spray-dried microencapsulated tuna oil were investigated.

Objectives

1. To determine the optimal condition for the enrichment of fish oil with ω-3 PUFAs by chemical transesterification reaction.
2. To assess the processing conditions for the microencapsulation of fish oil.

Materials and Methods

Materials; Crude tuna oil used for transesterification was obtained from Chotiwat Manufacturing Co., Ltd. (Songkhla, Thailand). The degumming, refining, bleaching, winterization and deodorization of the oil were carried out according to Rungsilp (1998). Degummed, bleached and deodorized tuna oil used for emulsion preparation was obtained from Maruha Co. (Utsunomiya, Japan; 16 wt% EPA; 14.1 wt% DHA; lipid hydroperoxides, 0.35±0.01 mmol/kg oil; thiobarbituric acid reactive substances, 0.12±0.01 mmol/kg oil; no detectable tocopherols). Fatty acid methyl ester standards were purchased from Aldrich Chemical Co, Inc. (Milwaukee, WI). Sodium methoxide was obtained from Fluka Chemie (Buchs, Switzerland). Powdered chitosan ("medium molecular weight", ≈ 250 kDa) was purchased from the Sigma-Aldrich Chemical Co. (St. Louis, MO). As stated by the manufacturer the properties of this chitosan were: viscosity of 1 wt% solution in 1 wt% acetic acid = 200 – 800 Cps; degree of deacetylation = 75% - 85%; maximum moisture content = 10 wt%; maximum ash content = 0.5 wt%. Powdered lecithin (Ultralec P; acetone insolubles, 97%; moisture. 1 wt%) was donated by ADM-Lecithin (Decatur, JL). Corn syrup solids (DRI SWEET®36, Code 335249; dextrose equivalent, 36; molecular weight ≈ 0.5 kDa; total solids, 97.2 wt%; moisture, 2.8 wt%; ash, 0.2 wt%) was obtained from Roquette America. Inc. (Keokuk, IA) All other chemicals were reagent grade or better. Distilled and deionized water was used for the preparation of all solutions..

Methods

1. Transesterification of tuna oil

1.1 Preparation of free fatty acids from tuna oil

Preparation of free fatty acids from tuna oil was carried out according to Senanayake and Shahidi (*25*) and Wanasundara and Shahidi (*26*) with slight modification. Tuna oil (25 g, treated with 200 ppm butylhydroquinone, BTHQ) was saponified using a mixture of KOH (5.75g), water (11 ml) and 95% aqueous ethanol (66 ml) for 1 h at 62°C under nitrogen condition. After saponification, distilled water (50 ml) was added to the saponified mixture and the unsaponifiable matter was extracted with 2 x 100 ml of hexane and discarded. The saponifiable matter in the aqueous layer was acidified to pH 1.0 with 6 N HCl and the free fatty acids were extracted into 50 ml of hexane. The hexane layer containing free fatty acids was then dried over anhydrous sodium sulfate and the solvent was removed using a vacuum rotary evaporator at 40°C. The free fatty acids were stored at -20°C under nitrogen until use.

1.2 Preparation of ω-3 fatty acids concentrates from tuna oil by urea complexation

The separation of ω-3 fatty acids from the hydrolyzed fatty acids mixture of tuna oil was carried out using urea-fatty acid adduct formation according to Senanayake and Shahidi (*25*) and Wanasundara and Shahidi (*26*). Free fatty acids (10 g) were added under constant stirring to a hot (60°C) solution of 30 g urea in 150 ml of 95% aqueous ethanol. The solution was heated and stirred until clear, then it was allowed to crystallize at room temperature for 5 h and then kept at 4°C for 24 h for further crystallization. The formed crystals were separated from the liquid by vacuum filtration. The filtrate was diluted with an equal volume of water and acidified to pH 4-5 with 6 N HCl and equal volume of hexane was subsequently added and the mixture was stirred for 1 h, then transferred to a separatory funnel. The hexane layer containing free fatty acids was separated from the aqueous layer and washed with distilled water to remove any remaining urea and then dried over anhydrous sodium sulfate. The solvent was subsequently removed at 40°C using a vacuum rotary evaporator.

1.3 Preparation of ω-3 fatty acids methyl ester

Preparation of n-3 fatty acids methyl ester was carried out according to Jham, Teles and Campos (*27*) with modification for scale up. The n-3 fatty acids (25 g) were hydrolyzed with 500 ml of KOH in methanol (0.5 M) at 100°C for 5 min in tightly capped glass bottles. After hydrolysis, the mixture was esterified with 200 ml of HCl in methanol (4:1 vol/vol) and then heated in an oil bath for 15 min at 100°C. The mixture was cooled and then 1000 ml of distilled water was added. The fatty acid methyl ester was extracted with 2 x 500 ml of hexane. The hexane layer was dried quickly over anhydrous sodium sulfate and the solvent was removed using a vacuum rotary evaporator at 40°C. The n-3 fatty acids methyl ester was stored at -20°C in nitrogen until use. The fatty acid composition of this sample was determined using gas chromatography (GC).

1.4 Enrichment of ω-3 fatty acids (Chemical transesterification)

Sodium methoxide-catalyzed transesterification was carried out in screw capped tube and gently stirred on a magnetic-stirrer hot plate. The reactants, tuna oil and ω-3 fatty acids methyl ester were dried in test tube at 60°C under vacuum of 30 cmHg for 15 min. The mole ratio of tuna oil to n-3 fatty acids methyl ester was changed using different amount of n-3 fatty acids methyl ester. After the temperature of reactants had reached the reaction temperature (60, 70 and 80°C), sodium methoxide powder (0.5, 1.0 and 1.5% w/w of reactants) was added. After 5 h, the reaction was stopped with the addition of 2 ml of dilute citric acid (4% w/v) then washed with 3 volume of 2 ml of distilled water. The oil phase (reaction product) was dried using anhydrous sodium sulfate. The lipid class of oil was analyzed by thin-layer chromatography / flame ionization detector analyzer (TLC/FID) and the fatty acid composition was analyzed by gas chromatography (GC).

1.5 Gas chromatography (GC) analysis

The reaction products were analyzed by thin-layer chromatography (TLC) on silica gel 60 plates (*28*) using a two-step development system. In the first step, the plates were developed for 8 cm with n-hexane:diethyl ether:formic acid (50:20:0.3, vol/vol/vol). The second development (10 cm) was made with n-hexane:benzene (50:50, vol/vol). The bands were visualized in iodine tank and the bands that corresponded to triglyceride (TAG) were scraped from the TLC plates and methylated to fatty acid methyl ester using the procedure according to Jham et al. (*27*). The gas chromotography was carried out using a Perkin Elmer Autosystem XL (The Perkin-Elmer Corporation, Norwalk, CT) equipped with a PERMABOND-FFAP DF-0.25 fused-silica capillary column 25 m x 0.25 mm id

(MACHEREY-NAGEL, Germany) and FID detector, and operated in a split mode with split ratio 100:1. The injector and detector temperatures were 245 and 250°C, respectively. The column temperature was held at 150°C for 0.5 min, then programmed to 170°C at 4°C/min, further programmed to 195°C at 5°C/min, the final temperature was 215°C at 10°C/min from 195°C and held for 7.5 min. The carrier gas used was helium set at a flow rate of 0.5 ml/min (15 psi). The content of fatty acid methyl ester as mg/g oil was calculated with 19:0 as internal standard according to Joseph, and Ackman (*29*).

1.6 TLC-FID analysis

The lipid composition of the oils was determined on silica-gel-coated quartz rods (Chromarod-SIII, Iatron Laboratories, Inc., Japan) using a two-step development system. The first development (8 cm) was made with n-hexane:diethyl ether:formic acid (50:20:0.3, vol/vol/vol). After development, the solvent was removed by flushing the rods with a stream of nitrogen and drying in air for ca. 5 min. The rods were then placed in the second solvent system, and developed for 10 cm with n-hexane:benzene (50:50, vol/vol). The lipid composition was determined on an Iatroscan Model TH-10 analyzer equipped with a flame ionization detector (Iatron Laboratories, Inc., Japan).

2. Production of microencapsulated tuna oil

2.1 Preparation of tuna oil-in-water emulsion

A concentrated tuna oil-in-water emulsion (15 wt% oil, 3 wt% lecithin) was made by blending 15 wt% tuna oil with 85 wt% aqueous emulsifier solution (3.53 wt% lecithin) using a high-speed blender (M133/1281-0, Biospec Products, Inc., ESGC, Switzerland), followed by three passes at 5,000 psi through a single-stage valve homogenizer (APV-Gaulin, Model Mini-Lab 8.30H, Wilmington, MA). This primary emulsion was diluted with aqueous chitosan solution to form a secondary emulsion (10 wt% tuna oil, 2 wt% lecithin and 0.4 wt% chitosan). Any flocs formed in the secondary emulsion were disrupted by passing it once through a high-pressure valve homogenizer at a pressure of 4,000 psi (*30*). The electrical charge on the droplets changed from negative (~ -52 mV) for the primary emulsion to positive (~ + 57 mV) for the secondary emulsion when the chitosan was present, which indicates that the cationic chitosan molecules adsorbed to the surface of the anionic lecithin-coated emulsion droplets (*30;31*). Primary and secondary emulsions containing varying concentrations of corn syrup solids (0 – 25 wt%) were prepared by mixing the initial primary and secondary emulsions with corn syrup solids solutions. The pH of the final emulsions was adjusted back to 3.0 using HCl or NaOH.

2.2 Evaluation of environmental stresses for tuna oil emulsions

The influence of environmental stresses on the properties of primary and secondary emulsions in the absence and presence of corn syrup solids with the same oil concentration (5 wt%) was studied:

pH. All emulsion samples were initilally prepared at pH 3.0 and then the pH of the emulsions was adjusted to the desired pH (3-8) by adding NaOH solution. The emulsions (10 mL) were transferred into glass test tubes (internal diameter = 15 mm, height = 125 mm), which were then stored at room temperature prior to analysis.

NaCl. Emulsions containing NaCl (200 mM) were prepared by adding NaCl solution to primary and secondary emulsions. Emulsions sample (10 mL) were then transferred into glass test tubes (internal diameter = 15 mm, height = 125 mm), and stored at room temperature prior to analysis.

Thermal Treatment. Emulsion samples (10 mL) were transferred into glass test tubes (internal diameter = 15 mm, height = 125 mm), which were then stored in a water bath for 30

min at a fixed temperature (30, 60 and 90°C). The emulsions were then placed immediately into a cold water bath for 10 min and stored at room temperature prior to analysis.

2.3 Characterization of physicochemical properties of tuna oil emulsions

After the emulsions had been subjected to environmental stress their physicochemical properties were characterized:

Creaming Index. The creaming stability of emulsion samples (10 ml or 67 mm) was monitored at room temperature by visual observation of the height of the serum layer formed at the bottom of glass tubes (H_S), expressed as a percentage of the total height of the emulsions in the tubes (H_E): Creaming Index = $100 \times (H_S/H_E)$.

Mean droplet diameter. The particle size distribution of the emulsions was measured using a static light scattering instrument (Malvern Mastersizer Model 3.01, Malvern Instruments, Worcs., UK). To prevent multiple scattering effects the emulsions were diluted with pH-adjusted acetate buffer prior to analysis so the droplet concentration was less than 0.02 wt%.

The electrical charge (ξ-potential). The electrical charge of oil droplets in the emulsions was determined using a particle electrophoresis instrument (ZEM5003, Zetamaster, Malvern Instruments, Worcs., UK). The emulsions were diluted to a droplet concentration of approximately 0.008 wt% with pH-adjusted double-distilled water prior to analysis to avoid multiple scattering effects.

2.4 Preparation of microencapsulated tuna oil emulsions.

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

2.5 *Characterization of the properties of microencapsulated tuna oil emulsion*

Aw. Water activity of spray-dried samples was measured by AquaLab Water Activity Meter (Series 3, Decagon Devices, Inc., Pullman WA) at 25 °C

Moisture content. Duplicate samples of approximately 2 g of powder were placed in aluminum pans and dried for 24 h at 70 °C and 29 in. Hg in vacuum oven (Fisher Scientific, Fairlawn, NJ). Moisture content was calculated from the weight difference (*32*).

Scanning electron microscopy. Internal and surface morphology of the powders were evaluated by Scanning Electron Microscopy (SEM) using the method of Hardas *et al.* (*22*). The images were viewed by scanning electron microscope at 3.0-5.0 kV (JEOL 5400., JEOL, Japan).

Extraction of free oil. Fifteen-mL hexane was added to 2.5 g powder. The mixture was mixed with a vortex mixer (Fisher Vertex Genie 2, Scientific Industries, Inc, Bohemia) for 2 min and then centrifuged (Sorvall RC-5B Refrigerated Superspeed Centrifuge, Du Pont Company, Wilminngton, Delaware) at 8,000 rpm for 20 min. The supernatant was filtered, the filter paper (Whatman, Maidstone, Kent, U.K.) washed twice with hexane (*22;32*) and hexane was evaporated in a rotary evaporator (RE 111 Rotavapor, Type KRvr TD 65/45, BUCHI, Switzerland) at 70 °C, and the solvent-free extract was dried at 105 °C. The amount of encapsulated oil was determined gravimetrically.

Extraction of encapsulated oil. Two-mL of acetate buffer (pH 3.0) was added to 0.5 g powder free of surface oil and vertexed for 1 min. The resulting solution was then extracted with 25 mL hexane/isopropanol (3:1 v/v). The tubes were then shaken for 15 min at 160 rpm using an automatic shaker (Innova 4080 Incubator Shaker, New Brunswick Scientific Co. Inc., NJ), and centrifuged for another 15 min. The clear organic phase was collected and the aqueous phase reextracted with the solvent mixture (*22;32*). After filtration through anhydrous Na_2SO_4 the solvent was evaporated in a rotary evaporator (RE 111 Rotavapor, Type KRvr TD 65/45, BUCHI, Switzerland) at 70 °C, and the solvent-free extract was dried at 105 °C. The amount of encapsulated oil was determined gravimetrically.

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

From the quantitative determinations above detailed, encapsulation efficiency (EE) was calculated as follows:

EE = Encapsulated oil (g/100 g powder) × 100 / total oil (g/100 g powder)

2.6 Oxidative stabilitystudied of microencapsulated tuna oil

Spray-dried tuna oil powders (3 g each) were placed in open glass beakers (20 mL, 3.5 cm diameter) and the beakers were placed in desiccators with relative humidity (RH) of 11, 33 or 52 %, prepared using saturated LiCl, $MgCl_2$ and $Mg(NO_3)_2$ solutions (*33*). All desiccators were then incubated at 37 °C.

Lipid hydroperoxides were measured by a modifiled method of (*34*) after an extraction step in which 0.3 mL of reconstituted emulsion (100 mg of emulsion powder in 0.3 mL of acetate buffer) was added to 1.5 mL of isooctane-2-propanal (3:1 v:v) followed by vortexing three times for 10 s each and centrifuging for 2 min at 3400 g (Centrific™ Centrifuge, Fisher Scientific, Fairlawn, NJ). Next, organic phase (0.2 mL total volume containing 0.015 to 0.2 mL of lipid extract depending on the oxidation state of the lipid) was added to 2.8 mL of methanol-butanol (2:1 v:v), followed by 15 μL of thiocyanate solution (3.94 M) and 15 μL of ferrous iron solution (prepared by mixing 0.132 M $BaCl_2$ and 0.144 M $FeSO_4$ in acidic solution). The solution was vortexed, and the absorbance at 510 nm was measured after 20 min. Lipid hydroperoxide concentrations were determined using a cumene hydroperoxide standard curve.

Thiobarbituric acid reactive substances (TBARS) were measured according to Mei *et al* (*35*). A thiobarbituric acid (TBA) solution was prepared by mixing 15 g trichoroacetic acid, 0.375 g TBA, 1.76 mL of 12 N HCl, and 82.9 mL H_2O. One hundred milliliters of TBA solution was mixed with 3 mL of 2 % butylated hydroxytoluene in ethanol and 2 mL of this solution was mixed with 1 mL of reconstituted emulsion (5 mg of emulsions powder in 1.0 mL of acetate buffer). The mixture was vortexed and heated in a boiling water bath for 15 min, cooled to room temperature, and centrifuged at 3400 g for 25 min. Absorbance was measured at 532 nm. Concentrations of TBARS were determined from standard curves prepared using 1,1,3,3-tetraethoxypropane.

3. Statistics.

All data represent the mean of two different trials and results are reported as the mean and standard derivation of these measurements. The data were subjected to the analysis of variance (ANOVA) univariate method ($P \geq 0.05$). Comparison of means after the ANOVA test was performed using the Duncan's multiple range test.

Results and Discussion

1. Chemical transresterification of tuna oil

1.1 Effect of reaction time on recovery of TG and incorporation of n-3 PUFA into tuna oil.

The lipid composition of refined tuna oil and n-3 FAME are shown in Table 1. The main component was TG for refined tuna oil and FAME for ω-3 FAME with 99.2 and 98.8 %, respectively. It was clearly shown that the purification and methylation methods used in this study were lighly efficient. The major fatty acids present in tuna oil were C16:0, C18:1, C20:5 and C22:6 (Table 2). As a result of urea complexation, C16:0 and 18:1 were eliminated almost completely while C20:5 and C22:6 were enriched from 41.1 to 74.8 and 266.1 to 565.9 mg/g oil, respectively. Haagsma *et al.* (*5*) and Wanasundara and Shahidi (*26*) have reported similar results for urea complexation experiments carried out for cod liver and seal blubber oils, respectively.

Table 1. Lipid composition (% peak area) of refined tuna oil and n-3 fatty acids methyl ester (n-3 FAME)[a].

Lipid composition[b]	Refined tuna oil	n-3 FAME
FAME	ND[c]	98.80±0.20
TG	99.19±0.05	ND
FFA	0.22±0.16	0.46±0.45
DG	0.14±0.12	0.13±0.16
MG	0.45±0.22	0.62±0.46

[a] Mean ± SD from duplicate determination

[b] Lipid composition, FAME = fatty acid methyl ester, TG = triglyceride, FFA = free fatty acid, DG = diglyceride, MG = monoglyceride

[c] ND = not detected

Table 2. Fatty acid composition (mg/g oil) in refined tuna oil, n-3 fatty acids methyl ester (n-3 FAME) and transesterified tuna oil[a].

Fatty Acids	Refined tuna oil	n-3 FAME	Transesterified tuna oil[c]
C14:0	23.35±0.34	0.81±0.05	9.81±0.88
C16:0	161.74±0.68	ND[b]	70.11±1.03
C16:1	49.17±0.01	29.57±0.26	26.44±0.96
C18:0	37.05±1.44	ND	19.48±1.27
C18:1	131.72±0.04	0.75±0.30	61.28±1.65
C18:2	22.50±0.13	8.73±1.84	8.14±0.35
C20:4	15.97±0.11	32.32±0.40	14.70±0.43
C20:5	41.09±0.30	74.75±1.07	60.39±0.70
C22:1	15.92±1.59	ND	12.28±0.17
C22:4	23.91±0.09	56.01±0.70	25.29±0.72
C22:6	266.12±0.91	565.94±0.11	388.14±1.57
Others	211.46±0.66	231.12±0.40	303.94±0.88

[a] Mean ± SD from duplicate determination

[b] ND = not detected

[c] Tranesterification was carried out at 80°C for 5 h, the mole ratio of refined tuna oil per n-3 FAME was 1:4 and $NaOCH_3$ amount was 1.0% by weight of reactants.

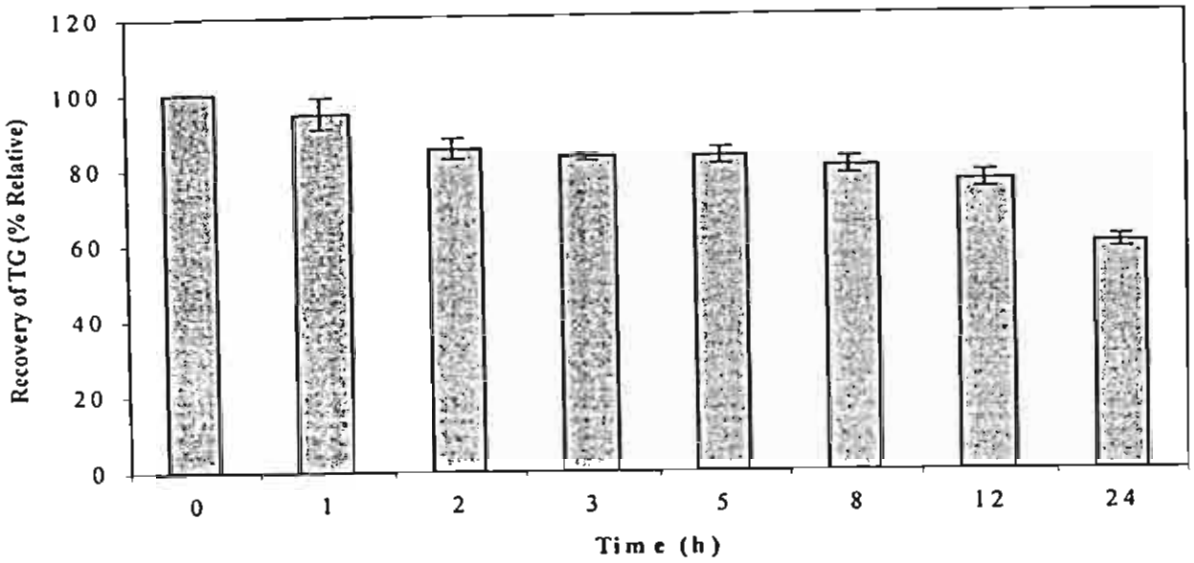

Figure 1. Effect of reaction time on recovery of TG. Transesterification was carried out at 80°C for 24 h, the mole ratio of tuna oil to n-3 FAME was 1:4 and NaOCH, amount was 1.0% by weight of reactants.

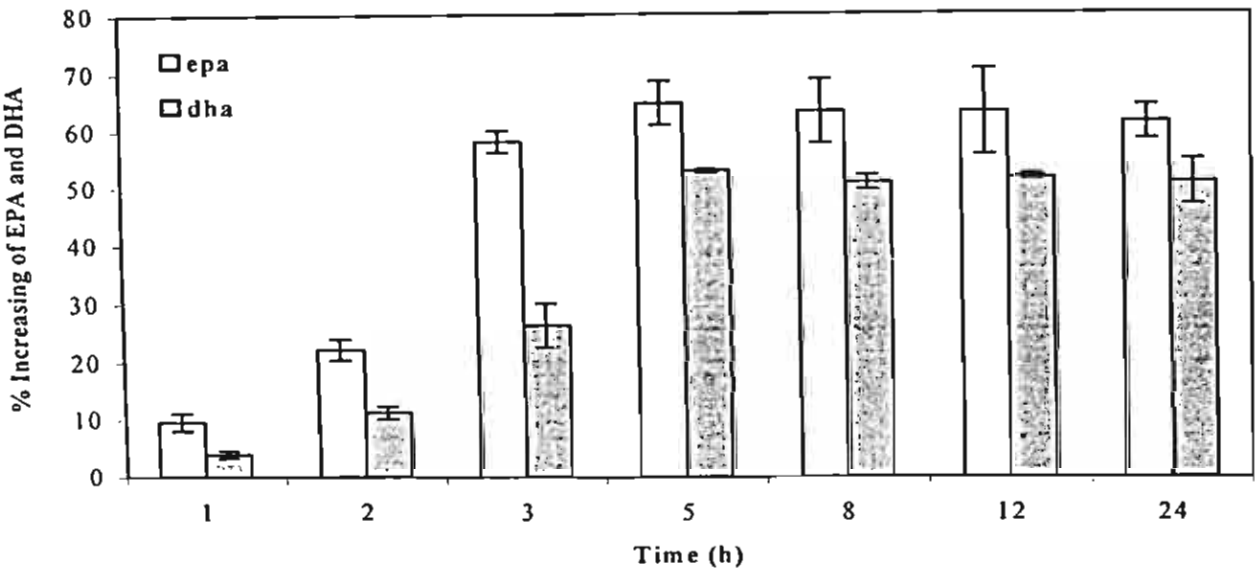

Figure 2. Effect of reaction time on incorporation of EPA and DHA in to tuna oil with transesterification. Transesterification was carried out at 80°C for 24 h, the mole ratio of tuna oil to n-3 FAME was 1:4 and NaOCH, amount was 1.0% by weight of reactants.

The dependence of reaction rate of transesterification on time was studied at 80°C for 24 h in the presence of 1.0 % w/w of sodium methoxide ($NaOCH_3$). The yield of TG, in term of relative amount comparing to the initial amount , recovered from transesterified products is shown in Figure 1. When the reaction time increased, the TG yield was decreased ($P<0.05$), showing that after 24 h the TG was only 59% of the initial value. However, the TG yield did not significantly decrease from 3 to 8 h of reaction time ($P\geq0.05$). The decrease of TG was

due to the formation of di- and monoglycerides (*12*) and saponification producing soaps and methyl esters(*36;37*).

The fatty acid composition and EPA and DHA concentration in TG of transesterified tuna oil is given in Table 2 and Figure 2, respectively. The fatty acid composition of tuna oil before transesterification was different from fatty acid composition after transesterification. After transesterification for 5 h, EPA and DHA were increased from 41.1 to 60.4 and 266.1 to 388.1 mg/g oil, respectively. The further the ester interchange progressed, the more EPA and DHA methyl esters were consumed and the more EPA and DHA were incorporated into TG. Thus, the EPA and DHA concentrations in TG were increased with increasing reaction time until 5 h ($P<0.05$, Figure 2). After 5 h, EPA and DHA concentration were not significantly increased upto 24 h ($P\geq0.05$), indicating that the reaction was probably complete. Konishi *et al.* (*12*) found that the ester interchange between TG and methyl stearate took place rapidly at 60°C , i.e. *ca.* 2 h. It has been reported that if the reaction mixture has a sufficient concentration of catalyst, the actual interesterification reaction is extremely fast (*36*). Previous research has shown that the time of reaction varies widely (5 min – 6 h) depending on reaction conditions, such as the catalyst level, temperature and solubility of the catalyst in the reactants (*36;37*). Konishi *et al.* (*12*) revealed that in certain cases, the intersterification can take as long as 24 h, even with catalyst pre-activation. Moreover, it is impossible to predict the onset of the reaction and it was very difficult to obtain only a partial interesterification, so most reactions were conducted until equilibrium was reached (*36*). Therefore, time of the transesterification for 5 h was required under our reaction conditions.

1.2 Effect of reaction conditions on recovery of TG and incorporation of EPA and DHA

The effect of reaction conditions including catalyst concentration, reaction temperature and mole ratio of reactants on the recovery of TG was given in Figure 3. All factors showed the same responses in TG recovery, the TG level were significantly decreased ($P<0.05$) when the reaction conditions increased. From Table 3, the catalyst concentration and reaction temperature better correlated with the recovery of TG than the mole ratio of reactants.

The incorporation of EPA and DHA into TG of tuna oil increases their net concentration compared with those in the original tuna oil. Incorporation of EPA and DHA increased with increasing concentration of $NaOCH_3$ ($P<0.05$) indepently of reaction temperature (Figure 4 and 5). Increased incorporation of stearic acid into soybean oil with increasing amount of $NaOCH_3$ has been reported to result in improved ester interchange (Konishi et al., 1993). The oil quality and the efficiency of the drying system used play critical roles in determining the dosage of $NaOCH_3$ (*38*). In general, $NaOCH_3$ can be used in low levels, (0.2-0.5% w/w), if the starting oil is well refined and dry (*28;39;40*). In this study, more $NaOCH_3$ was used due to the presence of moisture, free fatty acids and peroxide substances, which might impair catalyst performance (*36;37;38*).

Temperature affects the transesterification kinetics. The dependence of incorporated EPA and DHA on temperature was studied at 60, 70 and 80°C (Figure 4 and 5). The incorporation of EPA and DHA increased with increasing temperature ($P<0.05$), due to higher ester interchange. Konishi *et al.* (*12*) reported similar results in the case of transesterification of soy bean oil with methyl stearate. Interesterification can be carried out at 0-240°C depending on the type of catalyst used (*41*). In general, sodium methoxide ($NaOCH_3$) is active at temperature around 50-90°C (*36*) but interestrification can be initiated at 70-120°C by 0.05-0.5% $NaOCH_3$ (*38*). Addition of antioxidants or an inert atmosphere treatment should also be considered for the case of high reaction temperature in order to prevent oxidative deterioration of oil especially fish oil due to the high level of polyunsaturated fatty acids.

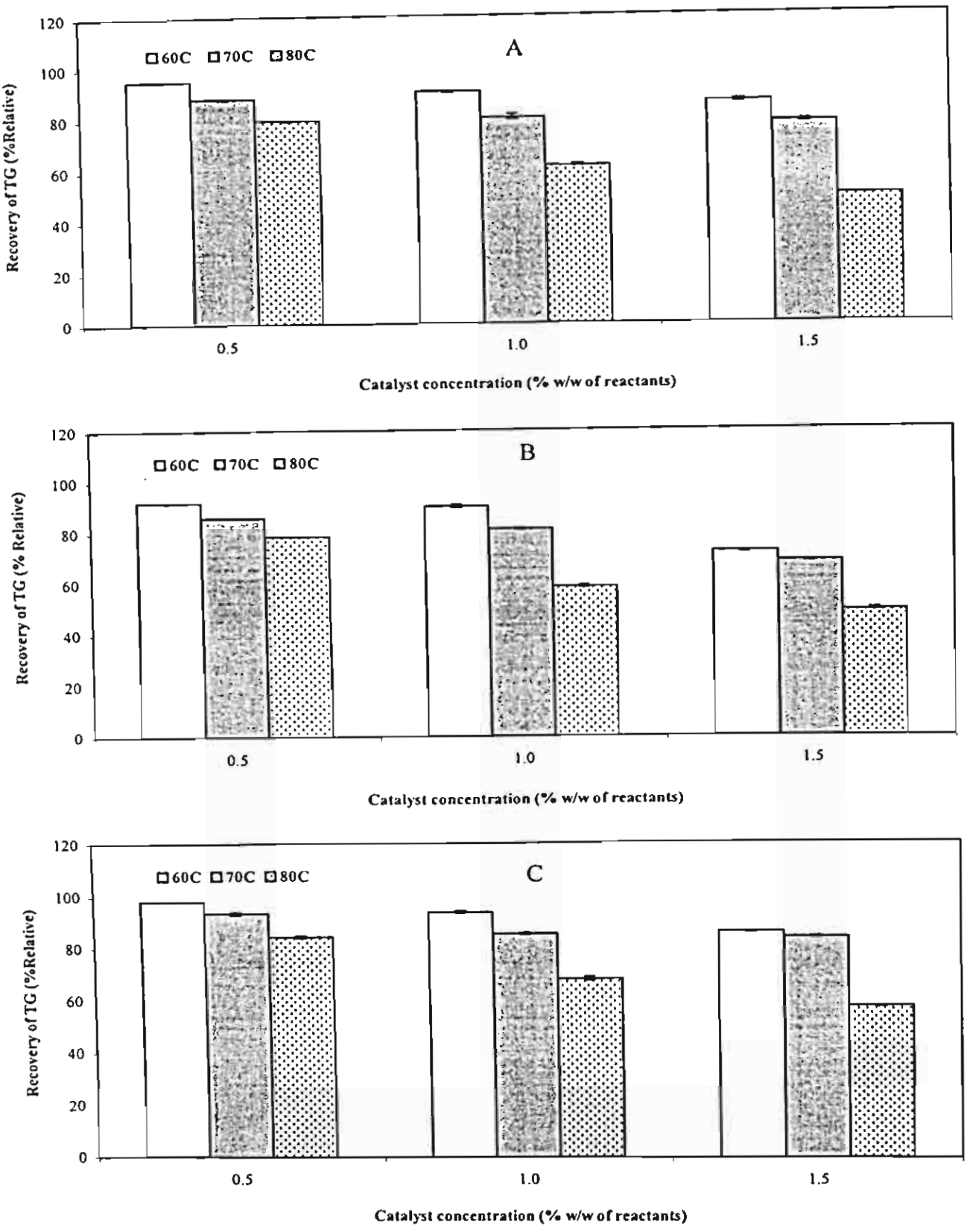

Figure 3. Effect of reaction conditions on TG recovery of tuna oil after transesterification with the mole ratios of tuna oil to n-3 FAME were 1:2 (A), 1:3 (B) and 1:4(C) at 60, 70 and 80°C. The amount of catalyst ($NaoCH_3$) were 0.5, 1.0 and 1.5 % weight of reactants.

Table 3. Linear regression coefficient of full factorial design-effects for response variables (TG recovery, EPA and DHA) on tranesterification of tuna oil.

Reaction conditions	TG recovery (%)	EPA Increasing (%)	DHA increasing (%)
Catalyst concentration	-0.718**[a]	0.796**	0.817**
Reaction temperature	-0.545**	0.315*	0.369**
Mole ratio of reactants	-0.256*	0.381**	0.206

[a] * $P< 0.05$, ** $P<0.01$

Figures 4 and 5 show the incorporation of EPA and DHA into tuna oil at various ω-3 FAME to tuna oil molar ratios. With ω-3 FAME as the acyl donor, EPA and DHA incorporation into TG of tuna oil increased as the molar ratio increased ($P<0.05$) and highest at 1:4 molar ratio of tuna oil to ω-3 FAME. Akoh *et al.* (*42*) studied the total incorporation of EPA and DHA into trilinolein at various n-3 FAME to trilinolein molar ratio. They found that, the incorporation of EPA did not increase beyond a molar ratio of EPA ethyl ester/trilinolein of 4, the incorporation of DHA increased as the molar ratio increased up to 5. Schuchardt *et al.* (*43*) revealed that alcoholysis reaction requires 1 mol of a TG and 3 mol of alcohol, which the similar reaction pattern as in this study (tranesterification of tuna oil requires 1 mol of oil and 3 mol of FAME). However, some excess level of the FAME should be used to increase the incorporation of fatty acids. This is limited by the economic implication since it increases the cost. For this study a mole ratio of tuna oil to ω-3 FAME of 1:4 was found necessary.

2. Production of microencapsulated tuna oil

2.1 Emulsification of tuna oil

2.1.1 *Influence of ionic strength and pH on emulsion stability.*

The influence of ionic strength (NaCl concentration) and pH on the stability of both lecithin-coated (primary emulsion) and lecithin-chitosan-coated (secondary emulsion) droplets in the absence and presence of corn syrup solids were examined. A fixed corn syrup solids concentration of 20 wt% was used to prepare the primary and secondary emulsions in these studies. A series of dilute emulsions (5 wt% oil) was prepared by dispersing the concentrated primary and secondary emulsions in a variety of aqueous solutions with different pH values (3 to 8) and ionic strengths (0 and 200 mM NaCl). The emulsions were stored at room temperature for 1 week and then the mean particle diameter, electrical charge (ζ-potential) and creaming stability were measured.

In all the emulsions, the magnitude of the ζ-potential decreased appreciably as the NaCl concentration was increased from 0 to 200 mM at pH 3 (Table 4), which can be attributed to electrostatic screening effects (*44*). In addition, all of the emulsions were stable to droplet aggregation at 0 and 200 mM NaCl at pH 3 (Table 4), which indicates that the combined strength of the attractive interactions between the droplets (*e.g.* van der Waals, depletion and hydrophobic) was insufficient to overcome the combined strength of the repulsive interactions (*e.g.*, electrostatic, steric and thermal fluctuation) even in the presence of salt (*44*).

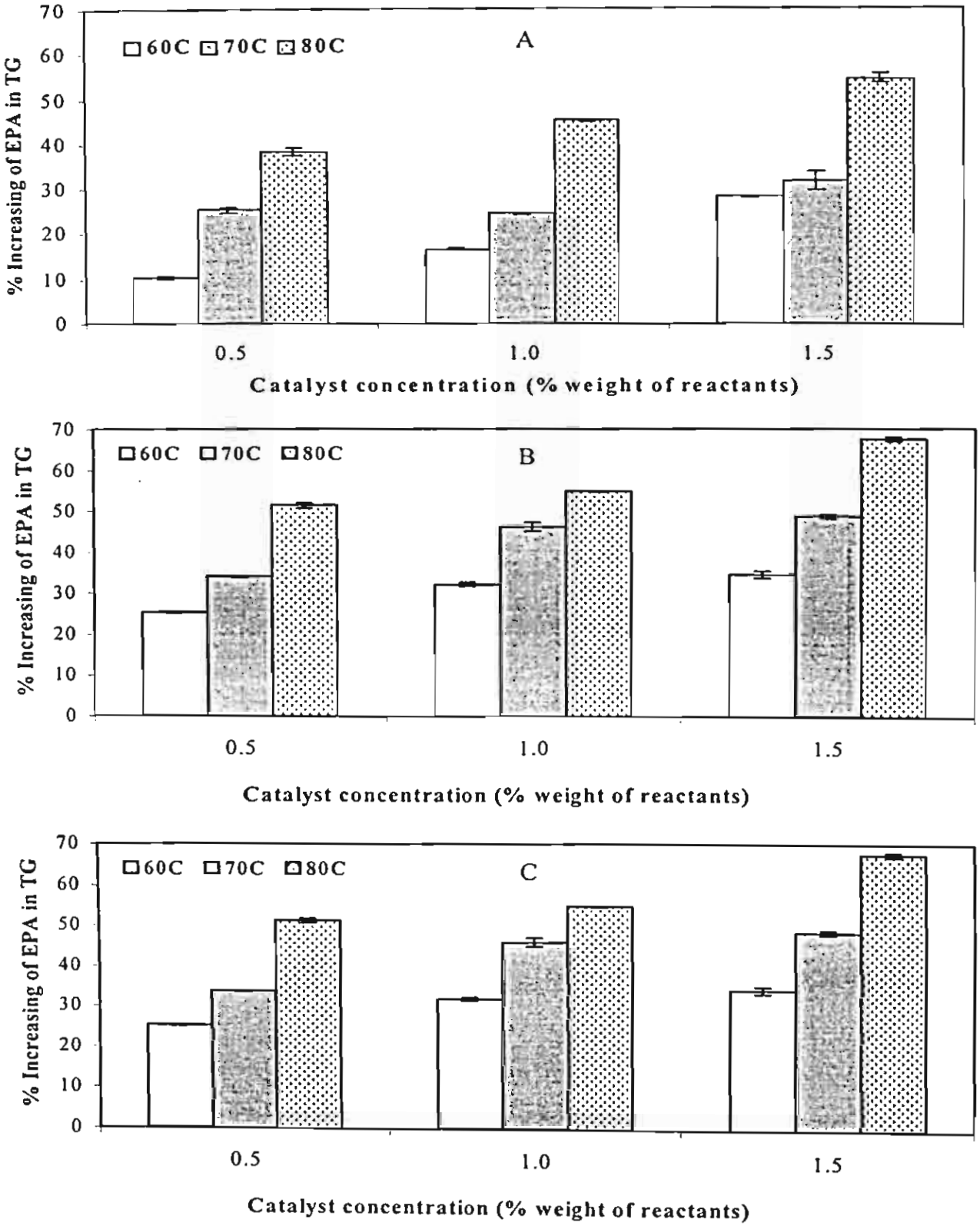

Figure 4. Effect of reaction condition on incorporation of EPA into TG of tuna oil after transesterification. The mole ratios of tuna oil to n-3 FAME were 1:2 (A), 1:3 (B) and 1:4(C) at 60, 70 and 80°C. The amount of catalyst ($NaOCH_3$) were 0.5, 1.0 and 1.5 % weight of reactants.

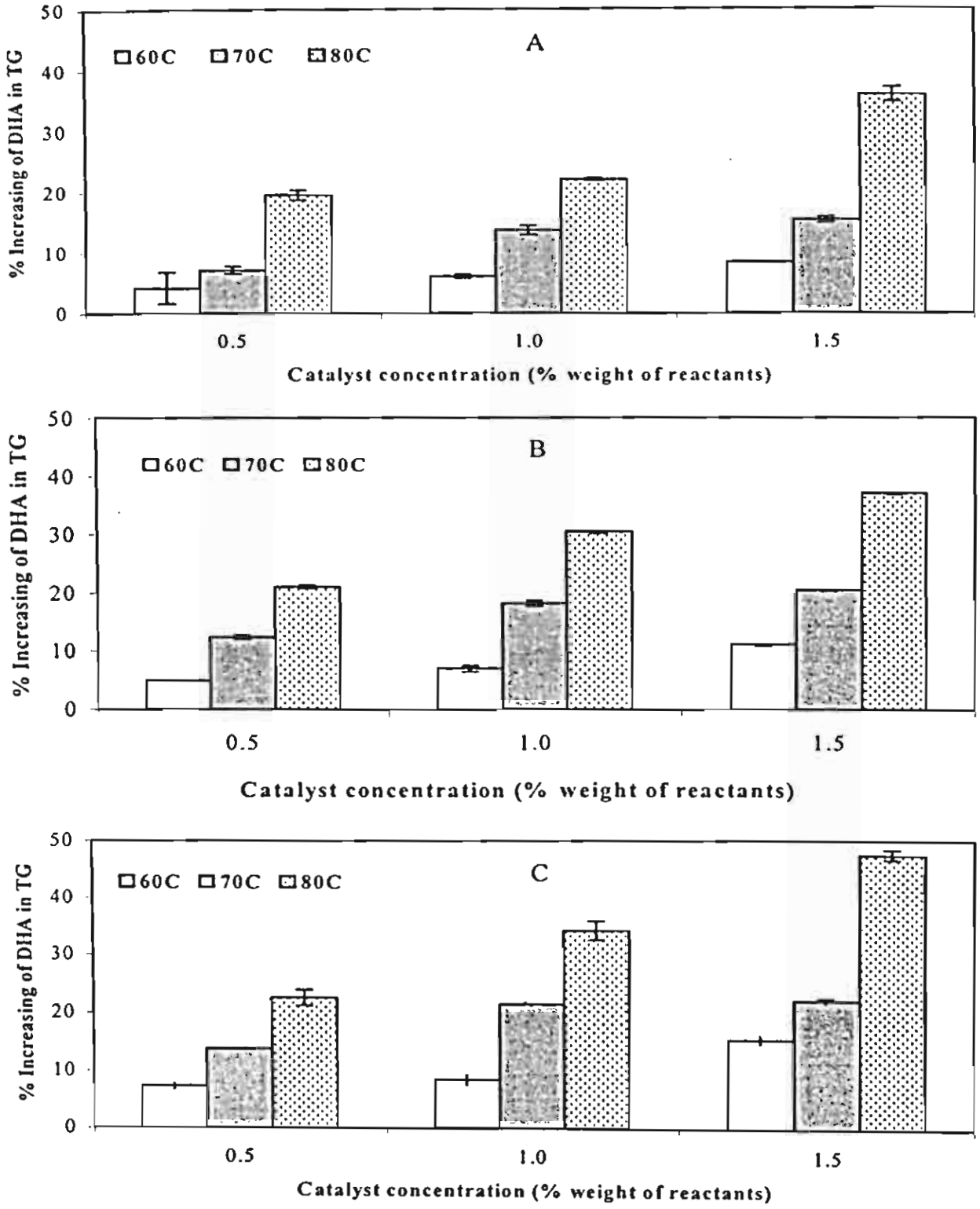

Figure 5. Effect of reaction condition on incorporation of DHA into TG of tuna oil after transesterification. The mole ratios of tuna oil to n-3 FAME were 1:2 (A), 1:3 (B) and 1:4(C) at 60, 70 and 80°C. The amount of catalyst ($NaOCH_3$) were 0.5, 1.0 and 1.5 % weight of reactants.

Table 4. Influence of NaCl (0 or 200 mM) and corn syrup solids, CSS (0 or 20 wt%) on mean particle diameter and electrical charge (ζ-potential) of emulsions consisting of 5 wt% tuna oil, 1 wt% lecithin, 100 mM acetate buffer (pH 3.0), and either 0 wt% chitosan (primary emulsion) or 0.2 wt% chitosan (secondary emulsion).

	NaCl concentration (mM)	
Measured Properties	0	200
Mean Particle Diameter (μm)		
Primary emulsion	0.39 ± 0.06	0.41 ± 0.06
Primary emulsion + CSS	0.38 ± 0.04	0.44 ± 0.08
Secondary emulsion	0.26 ± 0.01	0.26 ± 0.01
Secondary emulsion + CSS	0.27 ± 0.06	0.26 ± 0.04
Electrical Charge (ζ-potential)		
Primary emulsion	-52.8 ± 0.2	-27.1 ± 0.7
Primary emulsion + CSS	-51.8 ± 1.7	-26.5 ± 0.1
Secondary emulsion	55.5 ± 0.1	20.8 ± 1.3
Secondary emulsion + CSS	54.7 ± 1.8	19.3 ± 3.5

The ζ-potential of the lecithin-stabilized droplets in the primary emulsions was nagative at all pH values (Figure 6) due to the fact that the pK_a value of the anionic phosphate groups on lecithin are typically < 3 (*45*). The magnitude of the ζ-potential decreased slightly when the pH was raised from 3 to 8, which can be attributed to the electrostatic screening effect associated with the increase in ionic strength that occurs when the pH was increased. The ζ-potential of the lecithin-chitosan stabilized droplets in the secondary emulsions was positive at low pH values (< pH 7) but became negative at higher values (Figure 7). The cationic groups on chitosan typically have pK_a values around 6.3-7 (*46*), hence the chitosan begins to lose some of its charge around this pH. Consequently, there may have been a weakening in the electrostatic attraction between the chitosan and the lecithin-coated droplets, which may have led to the release of some of the adsorbed chitosan. Nevertheless, the fact that the ζ-potential of the droplets was less nagative in the secondary emulsions than in the primary emulsions at the same high pH values suggests that some of the chitosan remained adsorbed to the droplet surfaces.

The mean particle diameter of the primary emulsions remained low at all pH values (Figure7), suggesting that these emulsions were stable to droplet aggregation. The secondary emulsions were stable to droplet aggregation at pH < 5.0, but highly unstable at higher pH values, as deduced from the large increase in mean particle diameter. The instability of the secondary emulsions at higher pH values was probably because the ζ-potential was relatively low (Figure 6), which reduced the electrostatic repulsion between the droplets, thus leading to extensive droplet flocculation (*30*). In addition, partial desorption of chitosan molecules from the droplet surfaces may have led to some bridging flocculation.

The influence of corn syrup solids on the ζ-potential and mean particle diameter of the diluted primary and secondary emulsions at different pH (3-8) and ionic strength (0 or 200 mM NaCl) was also measured (Figures 6 and 7). At all pH and ionic strengths, there was no significant difference between the ζ-potential and mean particle diameter measurements of the primary emulsions in the absence or presence of corn syrup solids. In addition, there was no significant effect of corn syrup solids on the ζ-potential of the secondary emulsions at all pH and ionic strengths. Nevertheless, corn syrup solids did have a significant impact on the mean particle diameter of the secondary emulsions at pH 6 (Figure 7). The mean particle diameter was smaller in the secondary emulsion in the presence of corn syrup solids (0.76 ± 0.06 μm)

than in the absence of corn syrup solids (1.19 ± 0.17 μm). This effect might be due to the fact that corn syrup solids increase the viscosity of the aqueous phase, which retards the movement of oil droplets thereby inhibiting their aggregation (*47*). Nevertheless, the presence of the corn syrup solids could not prevent the extensive droplet aggregation observed in the secondary emulsions at higher pH values.

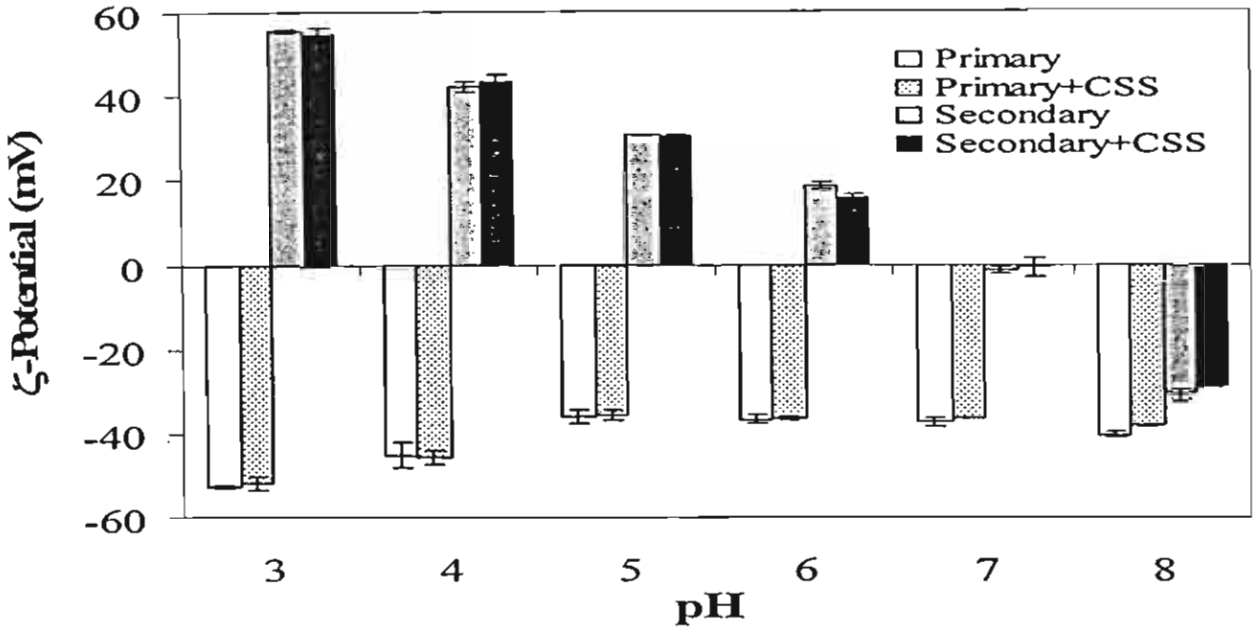

Figure 6. Influence of pH on electrical charge of emulsion droplets (ζ-potential) in primary (5 wt% tuna oil, 1 wt% lecithin, 100 mM acetate buffer, pH 3.0).and secondary emulsions (5 wt% tuna oil, 1 wt% lecithin, 0.2 wt% chitosan, 100 mM acetate, pH 3.0).in the absence and presence of 20 wt% corn syrup solids.

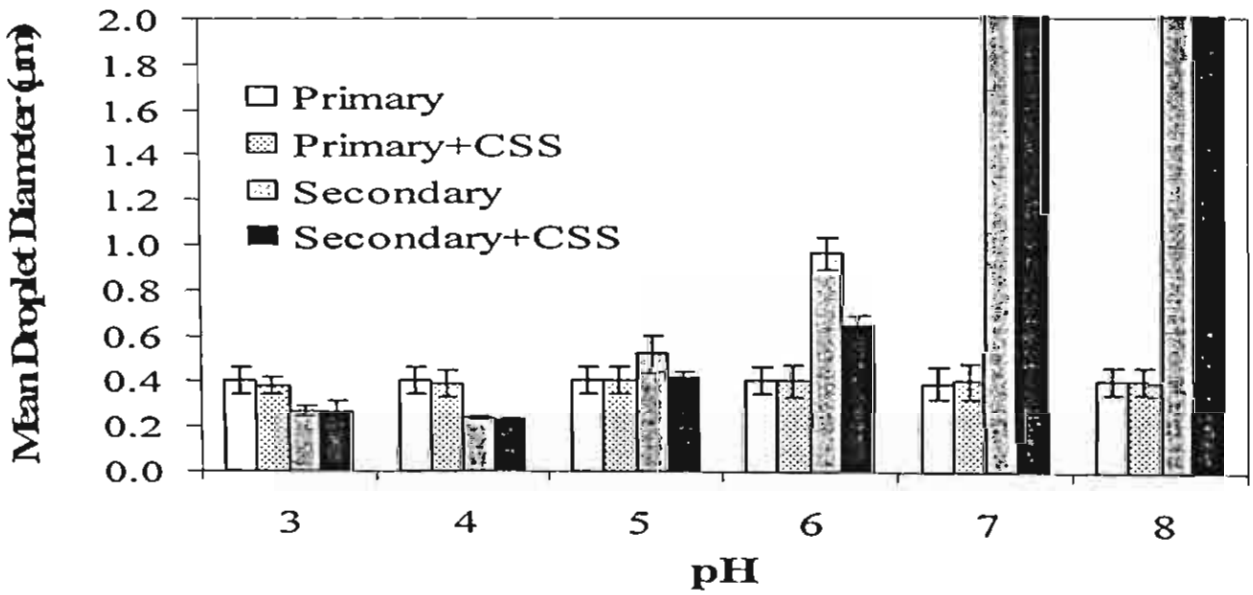

Figure 7. Influence of pH on mean diameter of emulsion droplets in primary (5 wt% tuna oil, 1 wt% lecithin, 100 mM acetate, pH 3.0) and secondary emulsions (5 wt% tuna oil, 1 wt% lecithin, 0.2 wt% chitosan, 100 mM acetate, pH 3.0) in the absence and presence of 20 wt% corn syrup solids.

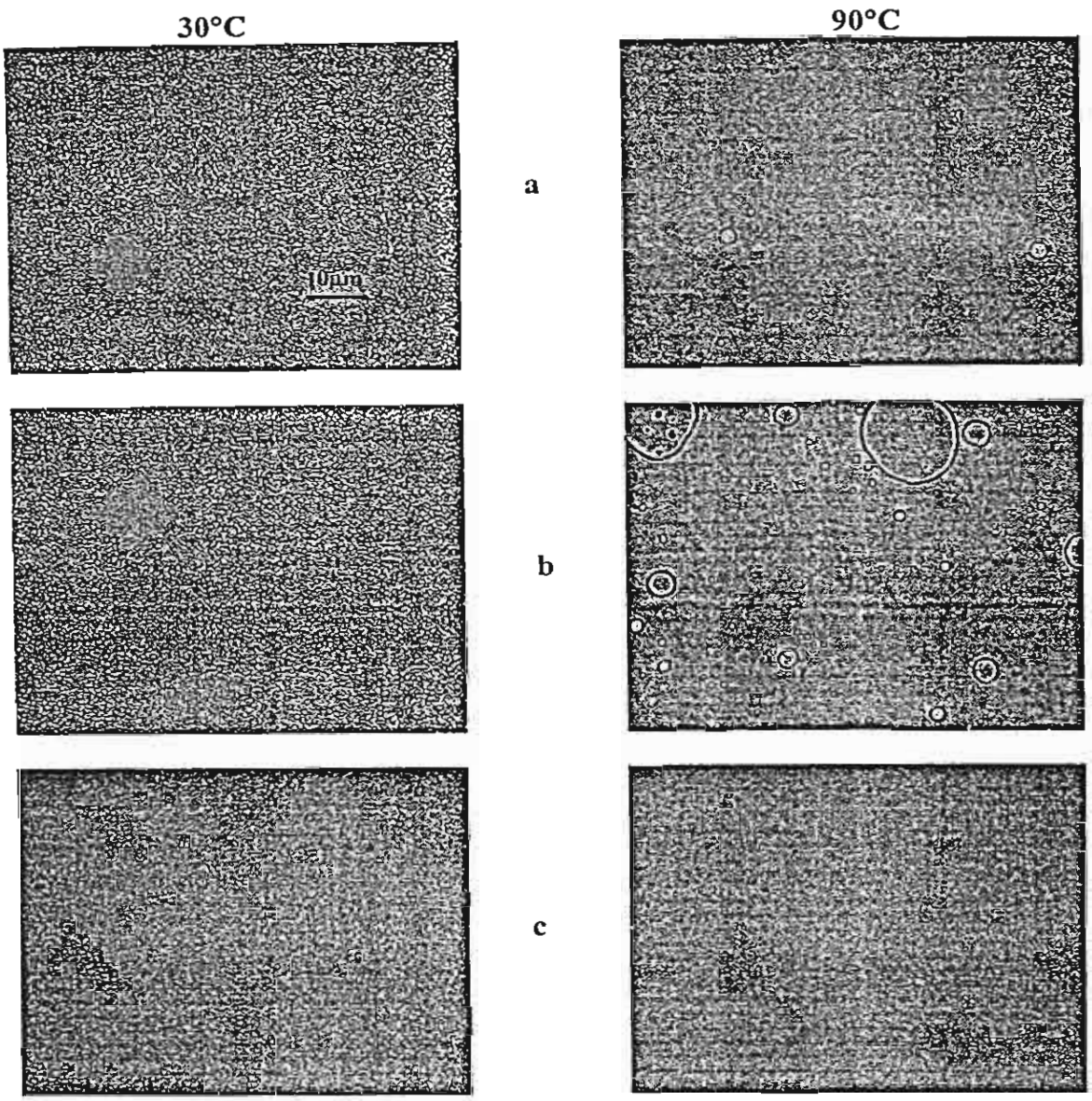

Figure 8. Dependence of microstructure on holding temperature (30 and 90 °C for 30 min) for a) primary emulsion in the absence of corn syrup solids, b) primary emulsion in the presence of 20 wt% corn syrup solids and c) secondary emulsions (similar results obtained in presence and absence of CSS).

2.1.2 Influence of thermal treatment on emulsion stability.

The aim of these experiments was to examine the influence of thermal treatment on the stability of primary and secondary emulsions in the absence and presence of corn syrup solids (20 wt%). Emulsions with the same oil concentration (5 wt%) were held at temperatures ranging from 30 to 90 °C for 30 min, cooled to room temperature, and then stored for 24 h prior to measuring their mean particle diameter, ζ-potential and microstructure. There was no significant effect of heating or corn syrup solids on the ζ-potential of either the primary (-51 ± 2 mV) or secondary (57 ± 2 mV) emulsions. In addition, there was no significant effect of heating or corn syrup solids on the mean particle diameter of the droplets in the secondary emulsions (0.27 ± 0.03 μm). On the other hand, there was evidence of droplet coalescence in some of the primary emulsions after heating,

with d_{32} increasing from 0.40 ± 0.06 μm in the emulsions treated at 30°C (in the presence or absence of corn syrup solids) to 0.56 ± 0.02 μm (no CSS) and 1.54 ± 0.26 μm (+CSS) in the emulsions treated at 90°C. Thus, corn syrup solids appeared to promote droplet aggregation during heating. This finding was confirmed by optical microscopy, which showed that on average the individual droplets were larger in the primary emulsions containing corn syrup solids than in those without corn syrup solids (Figure 8). The increase in mean particle diameter in the primary emulsions observed at higher temperatures may have been due to a change in the optimum curvature of the lecithin molecules promoting coalescence, *e.g.*, due to dehydration of the head groups (*44;48*). The fact that droplet coalescence was greater in the primary emulsion containing corn syrup solids may have been because some of the lecithin was bound to the corn syrup solids (*49*) and was therefore unavailable to stabilize the droplets. Presumably, coalescence was not observed in the secondary emulsions because the droplets were coated with chitosan, which would have prevented the lecithin layers on different droplets from coming into close contact.

Table 5. Effect of inlet temperture on properties of spray dried tuna oil emulsion

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

* Reconstituted emulsion, for original emulsion $d_{3,2}$ = 0.26 ± 0.01 μm, Hydroperoxide = 0.86 ± 0.13 mmol / kg oil

3. Characterization of microencapsulated tuna oil.

3.1 Moisture and water activity.

The final effect of drying a product is a lower moisture content along with a lower water activity (*50*). The moisture content (1 – 3%) and water activity (0.1 – 0.25) of spray dried emulsion powders decreased with increasing air inlet temperature (Table 5). These results are consistent with the observations of (*51*) and (*52*), who found that the moisture content of spray dried products was highest when operated at lowest temperature. The maximum moisture specification of dried powders such as whole milk, cocoa and whole egg, is between 3% to 4%. In our study, this level of moisture content could be achieved even by

operating the spray-drier at the lowest air inlet temperature of 165 °C (feed rate = 2.2 L/h). (http://www.fao.org/documents/show_cdr.asp?url_file=/docrep/x5557e/x5557e0p.htm)

3.2 Free oil and encapsulated efficiency.

The amount of "free oil" in powdered emulsions is usually defined as that part of the oil that can be extracted with organic solvents (*53*). Nevertheless, it should be noted that the amount of free oil measured in an analytical test is highly dependent on the precise extraction conditions used. In a recent study, the "free oil" of powdered emulsions was considered to be equivalent to the hexane extractable oil (*54*). The amount of free oil in the powders (3.0-3.5 g/100 g powder) was found to be independent of air inlet temperature (Table 5). This result is similar to those of Danviriyakul and others (*54*) who found that the matrix system, but not the drying temperature, affected the amount of free oil. The encapsulation efficiency (EE) reflects the presence of free oil on the surface of the particles within the powder and the degree to which the wall matrix can prevent extraction of internal oil through a leaching process (*23*). In our study, the EE values (85 % to 87%) were unaffected by air inlet temperature (Table 1). Previous workers have reported EE values from 0% to 95% depending on the type and composition of wall material, the ratio of core material to wall material, the drying process used, and the stability and physicochemical properties of the emulsions (*13;14;15;22;23;24;32*). The EE value for the multilayer emulsion system used in our system was therefore towards the high end of previously reported EE values.

3.3 Powder morphology.

Many properties of microencapsulated systems result from their internal microstructure, such as the retention of core materials, flow properties and the protection of core materials from the environment (*55*). It is therefore important to characterize the internal structure of the powder. Drying temperature had no effect on the structure of the powder based on our scanning electron microscopy study (Figure 9). All powder samples contained approximately spherical particles with a diameter in the range 5-30 μm (Figure 9A). Some wrinkles or dimples on the surface were observed. These results are consistent with Hardas *et al.* and Danviriyakul *et al.* (*22;54*), who detected wrinkles or scars on the surface of the particles in spray-dried anhydrous milk fat powders consisting of sodium caseinate and maltodextrin. Wrinkles on powder particle surfaces have also been reported for other carbohydrate-based microcapsules (*16;20;56*) and were attributed to the results of mechanical stresses induced by uneven drying at different parts of the liquid droplets produced during the early stages of drying (*20;56*), to the movement of the moisture during the nonsaturated surface drying period (*57*), and to the effect of a surface tension-driven viscous flow (*20*). The powder particles appeared to be largely free of cracks but some the presence of pores was observed. These pores probably arise in the last phase of the drying process due to uneven shrinkage of the material (*58*). Porosity has been suggested to affect the extractability of fat from spray-dried milk powders through its effect on solvent penetration into the dry particles (*56*). The "free oil" measured using the solvent extraction procedure mentioned above may therefore have been due to the presence of these pores in the powder particles. A considerable part of the free oil consists of surface fat or of fat globules from the interior of the microcapsules (*58*). Previous studies suggest that it may be possible to reduce the level of pore formation and free oil by using amorphous lactose in the wall material to act as a barrier that limits the diffusion of the apolar solvent into the particles (*56*).

To study the inner structure of spray-dried microcapsules and how the core material is organized within the dry matrix, the capsules must be opened. The inner structure of the capsules (Figure 9B) indicated that in all cases the core material was in the form of small droplets embedded in the wall matrix. The mean diameter of the droplets was between 0.2

and 1.0 μm, which was very similar to the dispersed phased droplets in the liquid emulsions prior to drying. The inner structure of the capsules, including void, was similar to that reported for other spray-dried emulsions using carbohydrates-based wall materials (*16;22;54;56*). The formation of voids may be related to several mechanisms connected with atomization and spray-drying, *e.g.* desorption of dissolved gases from the emulsion during drying and subsequent expansion associated with the temperature increase that occurs in the particles during the latter stages of drying, and the formation of steam bubbles within the drying atomization (*55*).

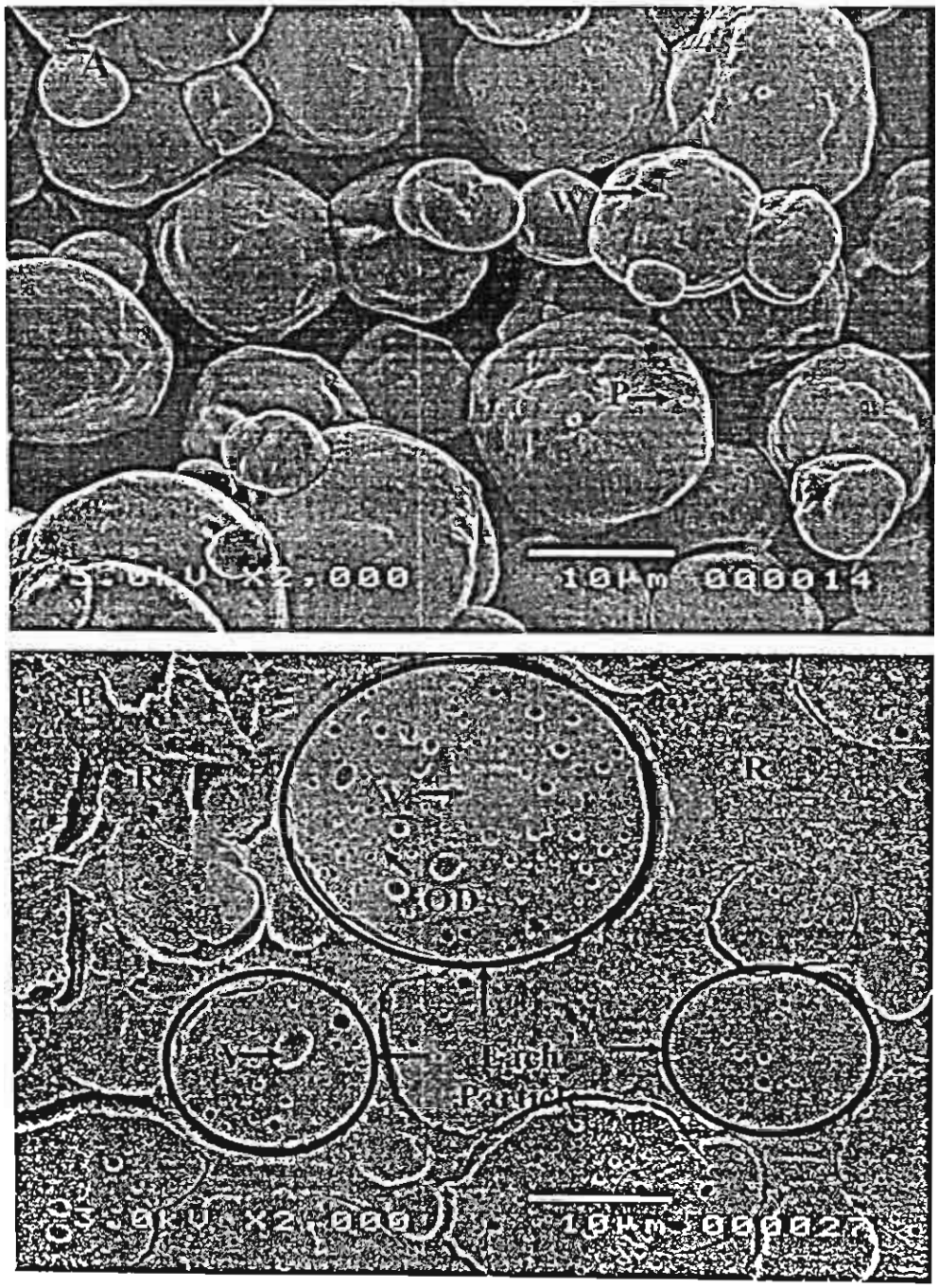

Figure 9. Representative micrographs showing the outer morphology (A) and inner structure (B) of tuna oil-containing capsules. W = wrinkle, P = pore, V = void, R = resin, OD = oil droplet or air cell.

4. Oxidative stability of microencapsulated tuna oil.

Previous work has shown that lipid oxidation in spray-dried powders does not correlate well with free or extractable fat (*59*). Therefore the oxidative stability of dried powder in this study was determined based on total lipid. The multilayer interfacial membrane on the dried emulsion droplets dramatically improved the oxidative stability of tuna oil when compared to bulk oil as indicated by both lipid hydroperoxide and TBARS formation (Figure 10a and b, respectively). These results are similar to those reported by Kagami and coworkers (*16*) who reported that microcapsules of the sodium caseinate-highly branched cyclic dextrin wall system improved oxidative stability of fish oil. Figure 11 shows lipid hydroperoxide and TBARS formation as a function of RH in the spray-dried fish oil powders. In the control samples, lipid hydroperoxide formation was in the order of 33 % RH > 11% RH > 52% RH. (Fig. 11a). A similar trend was observed in the development of TBARS during storage although TBARS in the samples at 11 and 33% RH became similar after 25 days of storage (Figures 11b)

Several investigators have suggested that lipid oxidation in foods is lowest at water activities close to the water monolayer, which falls between 0.2 and 0.3 for many foods, due to a decrease of the catalytic effect of transition metals, quenching of free radicals and singlet oxygen and/or retardation of hydroperoxide decomposition (*60;61*). However, other studies have suggested that this generalized view does not apply to a number of other foods. For instance, the encapsulated orange oil and methyl linoleate have been found to be more oxidatively stable in very dry (0 % RH) and very moist (> 75 % RH) states, whereas more rapid oxidation was found in the intermediate range (*62;63*). For this study, the oxidative stability of powders decreased when the RH increased from 11 to 33%. These results are similar to the datas from Minemoto *et al.* (*64*), they found that oxidation of encapsulated linoleic acid being higher as RH increased. However, the low oxidation in the powders at 52 % RH was observed in this study probably due to several chemical and physical changes in the powders during storage. First, lipid oxidation products may polymerize to produce brown-colored oxypolymers in the presence of other compounds such as amines, amino acids and antioxidants. These polymerization reactions can lead to inactivation of free radicals, a pathway that can influence oxidation rates and produce siginicant color formation in powders (*65*). Second, Maillard reaction products are well documented to reduce lipid oxidation (*66;67;68*). Wijewickreme *et al.* (*66*) reacted L-lysine with D-glucose, D-fructose, and D-ribose under different conditions and produced a Maillard reaction products with a potent antioxidant activity in the linoleic acid emulsions. For the present work, brown color formation (data not shown) was observed in the powder storage at 52 % RH. The formation of brown pigments could explain why lipid oxidation was slower in powders stored at high relative humidity. Third, when the powder was stored at a temperature higher than glass transition temperature (Tg) of corn syrup solids (Tg of corn syrup solids with DE = 36 at 52 %RH is ~ 6°C; *36*), the sugars can crystallize causing the powder particles to collapse (*69*). The collapse of powders is linked to a decrease in porosity, thus, lipid oxidation could be lower due to a decrease in the diffusivity of O_2 (*69*).

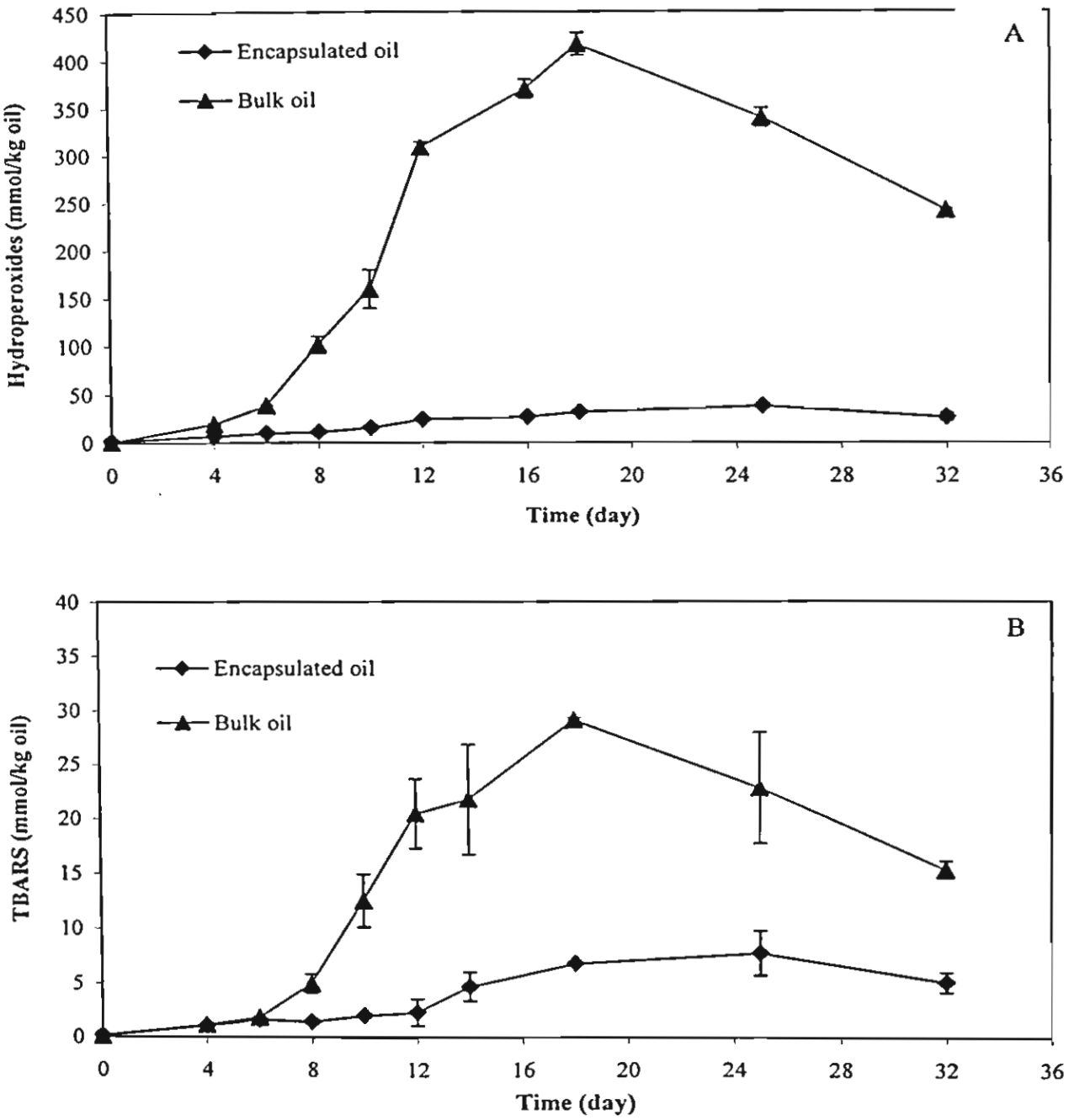

Figure 10. Formation of lipid hydroperoxides (A) and TBARS (B) in bulk oil and encapsulated oil (19.1% fish oil, 76.3 % corn syrup solid (DE 36), 3.8 % lecithin and 0.8% chitosan) during storage at 37 °C in 33 %RH.

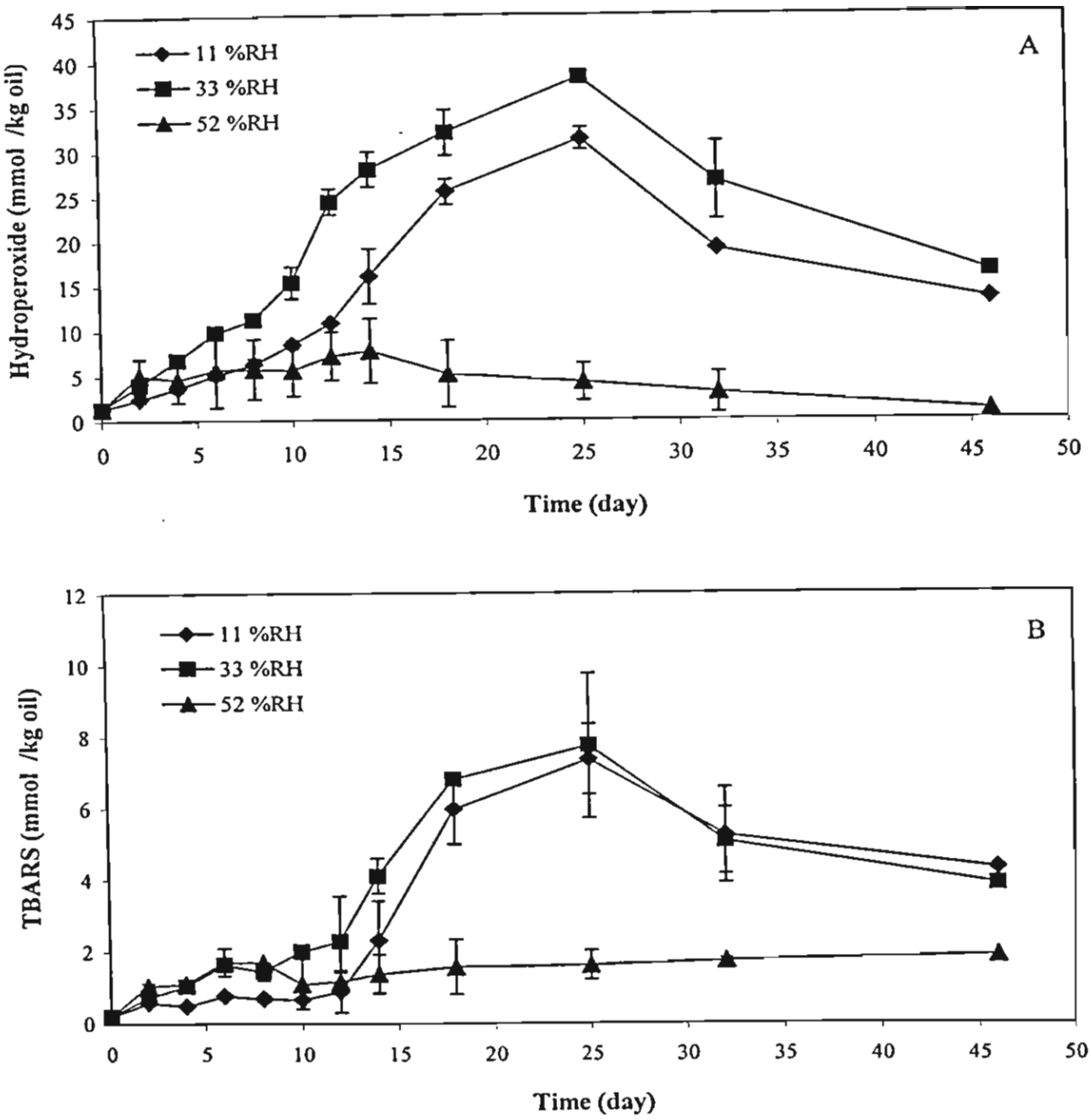

Figure 11 Formation of lipid hydroperoxides (A) and TBARS (B) in encapsulated oil powder during storage at 37 °C in various humidity.

Conclusion

This study has shown that chemical interesterification could enrich n-3 PUFA, especially EPA and DHA, in tuna oil. However, loss of TG between transesterification lead to lower TG yields, which would be an interesting area for further study. The stable tuna oil-in-water emulsions containing droplets stabilized by lecithin-chitosan membranes can be produced using an electrostatic deposition method, which involves adding a positively charged biopolymer to an emulsion containing negatively charged droplets. These emulsions remain stable to droplet flocculation and coalescence in the presence of quite high levels of corn

syrup solids (< 25 wt%), which is commonly used in the micro-encapsulation of oils. The multilayered emulsions have better stability to thermal processing and high ionic strengh. The high quality microencapsulated tuna oil can be produced by spray-drying oil-in-water emulsions containing corn syrup solids and oil droplets surrounded by multilayer interfacial membranes (lecithin:chitosan). The powdered tuna oil produced by this method has good physicochemical properties and oxidative stability when compared to bulk oil, which may lead to its more widespread utilization as a food additive.

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Output จากโครงการวิจัยที่ได้ทุนจาก สกว.

1. ผลงานดีพิมพ์ในวารสารวิชาการนานาชาติ

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2. การนำผลงานวิจัยไปใช้ประโยชน์

ผลงานวิจัยที่ได้ทำให้เกิดกระแสความสนใจในการศึกษาการเพิ่มสัดส่วนกรดไขมันโอเมก้า-3 โดยใช้สารเคมีเป็นตัวเร่งปฏิกิริยาเพิ่มขึ้น จะเห็นได้จากการที่มีผู้สนใจทั้งยุโรปและเอเชียจำนวนหนึ่งส่งอีเมลล์มาขอ reprint จากผู้วิจัย นอกจากนี้การผลิตอิมัลชั่นในรูปผงแบบไมโครเอนแคบซูลก็ได้รับความสนใจมากขึ้น โดยเฉพาะการผลิตอิมัลชั่นแบบผิวสัมผัสหลายชั้น (multilayer membranes O/W emulsion) ซึ่งเป็นระบบอิมัลชั่นที่สามารถป้องกันการเสื่อมเสียของสารประกอบที่ไวต่อปฏิกิริยาต่างๆ เช่น สารให้กลิ่นรส (aromaหรือ flavor) และกรดไขมันโอเมก้า-3 ได้ดี ทั้งนี้สารประกอบต่างๆ ที่ผลิตในรูปผงนั้น จะมีความสะดวกและง่ายต่อการจัดการทั้งด้านการบรรจุ ขนส่งและการใช้เป็นส่วนผสม (food ingredient) ในผลิตภัณฑ์อาหารต่างๆ

3. อื่นๆ

เข้าร่วมเสนอผลงานแบบโปสเตอร์หัวข้อ "Encapsulation of emulsifiled tuna oil in two layered interfacial membraned prepared using electrostatic layer-by-layer deposition" ในงานประชุมวิชาการ Nanotechnology Conference 2004, University of Massachusetts, Amherst, Massachusetts, USA (24 กันยายน 2547) และ งานประชุมวิชาการ 2005 IFT Annual Meeting, Ernest N. Morial Convention Center, New Orlean, Louisiana, USA (16-20 กรกฎาคม

ภาคผนวก

Characterization of Spray-Dried Tuna Oil Emulsified in Two-Layered Interfacial Membranes Prepared Using Electrostatic Layer-by-Layer Deposition

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