JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

ARTICLE

pubs.acs.org/JAFC

Food Matrix Effects on in Vitro Digestion of Microencapsulated Tuna Oil Powder

Zhiping Shen,*[†,‡] Christina Apriani,[§] Rangika Weerakkody,[†,‡] Luz Sanguansri,[†,‡] and Mary Ann Augustin[†,‡]

[†]CSIRO Preventive Health National Research Flagship

[‡]Division of Food and Nutritional Sciences, 671 Sneydes Road, Werribee, VIC 3030, Australia

[§]School of Agriculture and Food Systems, Land and Food Resources, University of Melbourne, VIC 3010, Australia

ABSTRACT: Tuna oil, containing 53 mg of eicosapentaenoic acid (EPA) and 241 mg of docosahexaenoic acid (DHA) per gram of oil, delivered as a neat microencapsulated tuna oil powder (25% oil loading) or in food matrices (orange juice, yogurt, or cereal bar) fortified with microencapsulated tuna oil powder was digested in simulated gastric fluid or sequentially in simulated gastric fluid and simulated intestinal fluid. The level of fortification was equivalent to 1 g of tuna oil per recommended serving size (i.e., per 200 g of orange juice or yogurt or 60 g of cereal bar). The changes in particle size of oil droplets during digestion were influenced by the method of delivery of the microencapsulated tuna oil powder. Lipolysis in simulated gastric fluid was low, with only 4.4−6.1% EPA and ≤1.5% DHA released after digestion (as a % of total fatty acids present). After sequential exposure to simulated gastric and intestinal fluids, much higher extents of lipolysis of both glycerol-bound EPA and DHA were obtained (73.2−78.6% for the neat powder, fortified orange juice, and yogurt; 60.3−64.0% for the fortified cereal bar). This research demonstrates that the choice of food matrix may influence the lipolysis of microencapsulated tuna oil.

KEYWORDS: Microencapsulation, tuna oil, n-3 fatty acids, lipolysis, food matrix

INTRODUCTION

Long chain n-3 polyunsaturated fatty acids (LC n-3 PUFAs) play an important role in human health.[1] The intake of these fatty acids has been associated with improving adult cardiovascular health,[2] alleviating neural disorders,[3] assisting in infant brain and vision development,[4] and anticarcinogenic activity.[5−7] However, LC n-3 PUFAs are highly susceptible to oxidation and degradation. Lipid oxidation leads to impaired flavor and nutritional value, shortening the shelf life of LC n-3 PUFA-containing products.[8] Microencapsulation is effective for stabilizing LC n-3 PUFAs and for masking the inherent fishy taste and odor of fish oils. Microencapsulated LC n-3 PUFA oils have been used to fortify a range of food products.[4,9,10]

The microcapsule formulation has the potential to alter the rate at which the microencapsulated oil is lipolysed and absorbed in the human body and the extent to which the digested fat reaches the systemic circulation.[11−14] The ingestion of emulsified fish oil has been compared with that of a fish oil gelatin capsule in humans.[11] The study showed that there was greater absorption of LC n-3 PUFAs, as evidenced by EPA and DHA levels in plasma phospholipids, on ingestion of the emulsified form.[11] However, a human clinical study comparing the bioavailability of fish oil gelatin capsules and microencapsulated fish oil in complex coacervate powders added to milk shakes showed that there was equivalent bioavailability of LC n-3 PUFAs.[12] There is potential to alter the site of the release of oil after digestion by the appropriate design of encapsulant matrices.[15]

The rate of lipid digestion within the stomach and small intestine depends on many factors, including the activity of lipases in various parts of the gastrointestinal tract, the size of the emulsion droplets, and the nature of the interface of the emulsified droplets. Pancreatic lipase has been shown to have greater access to fats emulsified with proteins (e.g., caseinate and whey protein isolate) than those stabilized by phospholipids and nonionic surfactants.[16−21] It has been found that gastric lipolysis was suppressed when interfacial active substances were incorporated into a food matrix.[22]

The components of a food and its microstructure affect the bioavailability of nutrients.[23] Fat-containing foods may be developed to manipulate lipid bioavailability. The rate and extent of lipid digestion in the presence of a food matrix are affected by the dimension of the exposed surface area of the matrix and the type and concentration of the food components. Moreover, it is also affected by the permeability of the food matrix to low molecular weight biological molecules, such as enzymes and acids.[20] A recent review[24] suggested that postprandial lipemia is increased when a fat meal is taken with digestible starches and fructose, whereas the inclusion of some fibers had the converse effect. In vivo lipid digestion studies showed that some soluble viscous fibers (e.g., guar gum, pectin, and gum arabic) inhibit lipolysis of triacylglycerols.[25] The addition of chitosan and pectin into beef patties decreased in vitro lipid digestion, whereas the addition of cellulose had no significant effect.[26] Other components, such as multivalent cations (Ca^{2+} and Mg^{2+}), can also alter lipid digestion as they form insoluble soaps with free fatty acids or bile salts in the small intestine.[20] When fish oil is added to a food system, the bioavailability of its LC n-3 PUFAs is influenced by the matrix and composition of the food product.[27]

Received: April 13, 2011
Accepted: July 3, 2011
Revised: June 28, 2011
Published: July 03, 2011

ACS Publications © 2011 American Chemical Society

Most in vivo or in vitro studies have attempted to show bioequivalence between a gelatin capsule and a formulated microencapsulated LC n-3 PUFA oil in a food product or examined differences in bioavailability among emulsions stabilized by various surfactants.[11−13,21,22] An understanding of the digestion of LC n-3 PUFAs in different microencapsulated oil formulations and food matrices during in vitro digestion is lacking.

In this work, tuna oil was delivered as a neat microencapsulated tuna oil powder (25% oil loading) or in food matrices (cereal bar, yogurt, and orange juice) fortified with microencapsulated tuna oil powder. The encapsulants used in the production of the microencapsulated tuna oil powder were a heated mixture of glucose, processed resistant starch, and sodium caseinate. The cereal bar, orange juice, and yogurt were fortified with the microencapsulated tuna oil powder at a level equivalent to 1 g of tuna oil per recommended serving size (i.e., per 200 g of orange juice or yogurt or 60 g of cereal bar). The samples were digested in simulated gastric fluid (SGF) or sequentially in SGF and simulated intestinal fluid (SGF + SIF). The samples were visualized using light microscopy, and the levels of free EPA and DHA released were measured after digestion.

MATERIALS AND METHODS

Materials. The ingredients used in the formulation of the microencapsulated tuna oil powder were Hi-DHA tuna oil (Nu-Mega Ingredients Pty Ltd., Australia), sodium caseinate (Myopure, Petersham, NSW, Australia), glucose monohydrate (Penford, Australia), and a high amylose corn starch (Hylon VII) (National Starch Food Innovation, Australia).

The food matrices, orange juice (Just Juice, National Foods, Australia) and Nestle diet yogurt (Peach Mango, Nestle, Australia), were purchased from a local supermarket. The cereal bar was made at CSIRO, as described below. The ingredients used to make the cereal bars were baking cocoa powder (Nestle, Australia), butter (Fonterra, Australia), fat-free marshmallows (Pascall Confectionery, Victoria, Australia), and rice pops breakfast cereal (Home brand, Woolworths, Australia). These were all obtained from a local supermarket.

The enzymes used in the preparation of SGF and SIF were pepsin, from porcine stomach mucosa (800−2500 units/mg of protein), pancreatin, from porcine pancreas (8 × USP of amylase), and porcine bile extract, and all were obtaiined from Sigma-Aldrich (United States).

The chemicals used in the extraction and analysis of fats were methyl tricosanoate (≥99.0%), 2-propanol [high-performance liquid chromatography (HPLC) grade], tetrahydrofuran (THF, HPLC grade), isooctane, hexane, and butylated hydroxytoluene (BHT, ≥99.0% FCC, Kosher) and were all obtained from Sigma-Aldrich (Sydney, Australia). BHT 0.025% (w/v) was added into isooctane, THF, and hexane to protect the LC n-3 PUFAs from oxidation during extraction and methylation.

Preparation of Microencapsulated Tuna Oil Powder. The formulation of the microencapsulated tuna oil powder was 25% tuna oil, 25% sodium caseinate, 25% glucose, and 25% processed Hylon VII (dry basis). The processed Hylon VII was prepared separately by heating 20% total solid (TS) Hylon VII dispersion in a retort (200 °C, 20 min), mashing the retorted material, and diluting to 10% TS prior to homogenization at 35 MPa using an APV Rannie homogenizer (APV Australia Pty Ltd., North Clayton, Victoria, Australia).

For preparation of the microencapsulated tuna oil powder, a mixture of sodium caseinate and glucose (26% TS) was combined with the processed Hylon VII (10% TS). The final protein and carbohydrate mixture (17% TS) was heated in a retort (100 °C, 40 min). The mixture was then cooled down to 60 °C before the addition of tuna oil, which had been preheated to 60 °C, to obtain a pre-emulsion (21.4% TS). This pre-emulsion was homogenized at 35/10 MPa using an APV Rannie homogenizer. The homogenized emulsion was spray dried into powder using a GEA Niro Production Minor Spray dryer (GEA Process Engineering Pty Ltd., Blackburn, Australia) using inlet and outlet temperatures of 180 and 80 °C, respectively. The powders were packed into aluminum foil sachets and kept at 4 °C until use.

Table 1. Fat Content of Microencapsulated Tuna Oil Powder and Fortified Food Formulations Used for in Vitro Digestion Studies[a]

sample weight	fat from added tuna oil powder (g)	fat from food matrix (g)
powder (0.8 g)	0.20	n/a
orange juice (40 g)	0.20	<0.2
yogurt (40 g)	0.20	<0.04
cereal bar (12 g)	0.20	0.83

[a] Fortified food samples used for in vitro digestion were 40 g of orange juice, 40 g of yogurt, or 12 g of cereal bar containing 0.8 g of tuna oil powder. These amounts represent one-fifth of the recommended serving size for these food products. The level of fortification was equivalent to 1 g of tuna oil per serving size (i.e., per 200 g of orange juice or yogurt or 60 g of cereal bar).

Preparation of Food Matrices with Microencapsulated Tuna Oil Powder. The formulations of the food matrices fortified with microencapsulated tuna oil powder are listed in Table 1. The level of fortification was equivalent to 1 g of tuna oil per recommended serving size (i.e., per 200 g of orange juice or yogurt or 60 g of cereal bar).

Preparation of Cereal Bar. Marshmallows (5.0 g) coated with melted butter (0.9 g) were heated in the microwave oven for 30 s and then manually beaten into a smooth creamy paste. Microencapsulated tuna oil powder (0.8 g) and cocoa powder (0.8 g) were added, and the mixture was manually beaten to homogeneity. Rice pops (4.5 g) and a drop of orange essence were then mixed in. The final mixture was pressed into a tray lined with grease proof baking paper, covered, and refrigerated for 15 min. The prepared bars were wrapped in grease proof baking paper, placed inside a foil bag, and sealed by heating. The bars were stored at −20 °C until used for in vitro digestion studies.

Fortification of Orange Juice and Yogurt with Microencapsulated Tuna Oil Powders. Microencapsulated tuna oil powder (0.8 g) and the food sample (38.2 g) (orange juice or yogurt) were shaken to homogeneity in a Schott bottle. These samples were freshly prepared on the day of use.

Preparation of Reagent Solutions for in Vitro Digestion. In vitro digestion fluids were prepared as described,[28] except that these solutions were purged with argon.

SGF. The SGF solution was prepared on the day when in vitro digestion was carried out. Sodium chloride (20 ± 0.01 g) was dissolved in 800 mL of Milli-Q water and adjusted to pH 1.2, using 37% HCl in water (w/v). Pepsin (3.2 ± 0.01 g) was added, and the solution was mixed for 30 min. The solution was finally made up to 1000 mL, with Milli-Q water, purged with argon, and stored at 4 °C until required.

SIF. Anhydrous potassium dihydrogen phosphate (17 ± 0.01 g) was dissolved in 750 mL of Milli-Q water followed by the addition of 192.5 mL of 0.2 M NaOH. The solution was adjusted to pH 6.8, using 1 M NaOH, and 3.15 ± 0.01 g of pancreatin was mixed in. The volume of the solution was made up to 1000 mL with Milli-Q water, and the mixture was stirred overnight at 4 °C. The solution was purged with argon and then stored at 4 °C overnight. The next day, the solution was removed from storage and equilibrated to room temperature before the addition of bile extract (6.25 ± 0.01 g).

Calcium Chloride Solution. Calcium chloride ($CaCl_2$) was prepared by dissolving 5.6 ± 0. 01 g of anhydrous $CaCl_2$ in 1000 mL of deionized water.

In Vitro Digestion of Neat Microencapsulated Tuna Oil Powders and Fortified Food Samples. Neat microencapsulated tuna oil powder (0.8 g) was dispersed in 38.2 g of deionized water. The fortified cereal bar (12 g) was manually broken into small pieces (∼2–3 mm) and dispersed in 28 g of deionized water to simulate comminution by mastication. The fortified orange juice and yogurt were used as is. The final weight of all test samples used for digestion was 40 g (Table 1). All SGF and SIF digestion processes were performed in duplicate.

SGF Digestion. SGF solution (50 mL) was added to the samples for digestion. A blanket of argon was applied to all solutions prior to tightly sealing the glassware. The mixture was incubated in a shaking water bath (Julabo, SW23, John Morris Scientific, NSW, Australia) at 37 °C/100 rpm for 2 h. After SGF digestion, each sample was divided equally into two different containers and stored at −20 °C until analysis.

Sequential SGF and SIF Digestion. A freshly SGF-digested sample was adjusted to pH 6.8 with 1 M NaOH. This sample was combined with 40 mL of SIF solution and further incubated for 20 min. $CaCl_2$ (10 mL) was subsequently added. A blanket of argon was applied to all solutions prior to tightly sealing the glassware. The sample was further incubated for 2 h and 40 min. At the end of the incubation period, the final weight of each digested sample was recorded. Each sample was then equally divided into two different containers and stored at −20 °C until analysis.

Measurement of pH and Microscopy. *pH Measurement.* The pH of dispersions of the initial food samples was measured. The pH of the food samples fortified with microencapsulated tuna oil powder was also measured before and after SGF digestion and after sequential SGF and SIF digestion.

Microscopy of Digested Sample. The digested sample was transferred onto a microscopy slide and then stained with "Oil Red O". The emulsion droplet was viewed using a microscope (Olympus BH-2, Olympus America Inc., United States) with magnification of 10 × 100. Images were taken using a ColorViewIIIu Soft imaging system camera (Brook Anco Corp., United States) with the aid of image processing software (AnalySIS getIT).

Extraction of Tuna Oil from in Vitro Digested Samples. A portion of the in vitro digested sample, estimated to contain 5 mg of microencapsulated tuna oil (i.e., 20 mg of microencapsulated tuna oil powder), was weighed accurately (±0.00001 g) into a 15 mL culture tube. Isopropanol (4 mL) was pipetted into the tube, and the liquid mixture was vigorously vortexed for 30 s. Hexane (4 mL) was then added into the sample, and the mixture was vortexed again. The mixture was blanketed with argon, tightly capped, and then centrifuged at 2000 rpm (J6-HC, Beckman) for 15 min, after which time the top solvent layer was withdrawn. A further 4 mL of hexane was then added to the residue remaining in the tube, and the mixture was again vortexed and centrifuged. This procedure was repeated twice, and the three solvent extracts were pooled. The solvent was evaporated at 60 °C under a continuous flow of nitrogen. After all of the solvent had been evaporated, 3 mL of hexane and 2 mL of internal standard (IS) (0.35 mg of methyl tricosanoate/mL of isooctane) were mixed with the extracted oil residue.

Methylation of the Oil Extract. The oil extract containing the IS was divided equally into two portions, one of which was subjected to acid methylation and the other to alkaline methylation. With acid methylation, all fatty acids (i.e., free fatty acids and glycerol-bound fatty acids) are esterified, whereas with alkaline methylation only glycerol-bound fatty acids are esterified.

Acid Methylation. Acid methylation was based on published methods.[29,30] Two milliliters of THF and 2 mL of sulfuric acid in methanol (4.5% v/v) were added to one portion of the oil extract. The tube was then blanketed under argon, tightly sealed, and vortexed. This mixture was then incubated in a water bath shaker (100 rpm) at 50 °C overnight. After incubation, 3 mL of NaCl in water (5%, w/v) and 0.5 mL of hexane were added, and the tubes were inverted for 30 s. The separated upper organic phase was withdrawn, washed with 4 mL of aqueous potassium bicarbonate (2%, w/v), and dried over anhydrous sodium sulfate. The fatty acid methyl ester (FAME) solution was then injected into the gas chromatograph (GC).

Alkaline Methylation. The alkaline methylation process was adapted from a published method.[31] The remaining portion of oil extract was combined with 200 μL of 2 M KOH in methanol, and the tube was then blanketed under argon and tightly sealed before being vortexed vigorously for 3 min. The solution was then neutralized with 200 μL of 2 M HCl containing trace methyl orange indicator. The solution was left to settle for 30 min before the top layer was withdrawn and injected into the GC.

Verification of the Oil Extraction Method. Direct methylation of the microencapsulated tuna oil powder was performed as described previously,[30] except that various methylation temperatures and times were examined, and the antioxidant BHT was added into all solvents used for extraction and methylation. Direct methylation of the oil extract from the powder was also carried out. This was done to confirm that the oil extraction method used in this study enabled complete extraction of the oil from the digested microencapsulated tuna oil powder. Acid methylation was performed in triplicate on both the powder and the oil extracted from the powder.

The purpose of testing temperature and time parameters was to enable more efficient utilization of the laboratory facilities, while still achieving a satisfactory analytical outcome. Both direct methylation and oil extraction followed by methylation were performed at 50 °C for 15 h (overnight) and 70 °C for 2.5, 3.0, and 3.5 h, using 0.15 ± 0.00001 g of microencapsulated tuna oil powder. In the previous study,[30] when methylation was carried out at 70 °C for longer than 2.5 h, there was loss of LC n-3 PUFAs when BHT was not added.

FAME Analysis by GC. The GC method used was as previously described,[30] except that 1−3 μL aliquot of sample solution was injected with a split ratio of 1:50−1:10, depending on the FAME concentration. The fatty acid composition was in similar range to that previously published.[30] The amounts of EPA and DHA in the oil used for these experiments were 53 mg of EPA and 241 mg of DHA per gram of oil.

Calculation of EPA and DHA Concentrations. These were calculated as described in the AOCS official method.[32]

$$\text{EPA or DHA, mg/g sample} = \frac{A_x \times W_{IS} \times CF_x}{A_{IS} \times W_s \times 1.04}$$

where A_x = area counts for EPA or DHA, A_{IS} = area counts for IS, CF_x = theoretical correction factor for EPA (0.99) and DHA (0.97), W_{IS} = mass of IS added to the sample, in mg, and W_s = sample mass, in g.

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

RESULTS AND DISCUSSION

Validation of Methods for Assessment of LC n-3 PUFAs. This was carried out to establish the suitability of the conditions used in the method for analysis.

Conditions for Esterification of Extracted Oil and Direct Methylation of Microencapsulated Tuna Oil Powder. The oil contained within the microencapsulated tuna oil powder was analyzed following direct methylation of the powder as well as methylation of the oil extracted from the powder. In both methods, different combinations of time and temperature were examined. Comparable amounts of LC n-3 PUFAs were recovered after methylation at 50 (15 h) and 70 °C (3.5 h). In addition, these conditions gave the highest LC n-3 PUFAs recovery (EPA 43.69 mg/g oil and DHA 210.51 mg/g oil). The amounts of EPA and DHA obtained from the extracted oil were 97% of those obtained by direct methylation of the microencapsulated tuna oil

powder under corresponding methylation conditions (50 °C for 15 h and 70 °C for 2.5, 3.0, and 3.5 h). The small loss of 3% LC n-3 PUFAs is likely due to incomplete recovery of the solvent extracts after the multiple centrifugation, separation, and solvent evaporation steps, although it may also be due to incomplete oil extraction. The presence of the antioxidant, BHT, in all solvents used for extraction makes it unlikely that the loss is due to oxidation of the LC n-3 PUFAs.

The amounts of LC n-3 PUFAs recovered after methylation at 70 °C for 2.5 and 3.0 h were 93−94 and 96−98%, respectively, of that obtained from methylation for 3.5 h, irrespective of the method. In previous work,[30] methylation was performed at 70 °C for 2.5 h in the absence of BHT. In the current study, a longer reaction time (3.5 h) was used to improve the LC n-3 PUFA recovery, with the addition of BHT to prevent oxidation. To ensure efficient use of laboratory facilities, methylation was performed at 50 °C for 15 h (overnight) for all in vitro digested samples analyzed.

Physical Characteristics of Digests. The pH of the digests was measured, and the emulsion stability was assessed using light microscopy.

Table 2. pH Values of the Microencapsulated Tuna Oil Powder, Food Samples, and Digests before and after Exposure to SGF (2 h) and to Sequential SGF and SIF (5 h)[a]

sample	pH of sample (without fortification)	pH of sample (with fortification)	pH after SGF digestion	final pH after SGF and SIF digestion
tuna oil powder		7.4[b]	1.7	6.8
orange juice	3.7	3.8[c]	2.7	6.8
yogurt	4.2	4.3[c]	3.7	6.5
cereal bar	6.6[b]	6.5[c]	2.4	6.4

[a] The pH values were measured for duplicate digested samples. [b] pH values of 0.8 g of tuna oil powder or 12 g of cereal bar added to water, respectively (total weight of 40 g). [c] See the footnotes for Table 1 for information on food samples fortified with tuna oil powder.

pH of Food Matrix before and after Digestion. The pH value of each food matrix, with and without added microencapsulated tuna oil powder, both before SGF, after SGF, and after sequential SGF and SIF digestion, are shown in Table 2. The orange juice had the lowest pH (3.7), while microencapsulated tuna oil powder sample in deionized water had the highest pH (7.4) before digestion. After SGF digestion, the microencapsulated tuna oil powder sample had the lowest pH (1.7), while the three fortified food samples had a pH range 2.7−3.7, and this is attributed to the presence of buffering components in the food samples, such as proteins and citric acid. After SGF digestion, all samples were adjusted to pH 6.8 before subsequent exposure to SIF. All samples had a similar pH value after SIF digestion.

Microscopy of in Vitro Digested Samples. Following SGF and sequential SGF and SIF digestion, the samples (microencapsulated tuna oil powder and fortified orange juice, yogurt, and cereal bar) were visualized by bright field microscopy (Figure 1). Emulsion stability, as evidenced by changes in the particle size of oil droplets during digestion, is influenced by the composition of the surrounding medium. The presence of emulsifying agents in the digestion fluid and the food matrix is capable of displacing the surface-active components present at the original interface, facilitating the attachment of lipase and subsequent lipolysis. There were larger oil droplets in the SGF-digested samples with the neat microencapsulated tuna oil powder, fortified orange juice, and cereal bar than in the fortified yogurt (Figure 1).

The size of the lipid droplets after in vivo gastric digestion normally ranges between 10 and 20 μm, irrespective of the initial droplet size.[20] Initial droplets larger than this size range tend to get broken down in the stomach, whereas when the initial droplets are much smaller, they tend to coalesce. Furthermore, the disintegration and coalescence of lipid droplets in the stomach continue until a steady state is reached, at which stage the rate of droplet disruption is balanced by the rate of droplet coalescence.[20]

An in vitro digestion method was used in the present study in which the physical process of digestion is simulated in a shaking water bath. This agitation does not accurately reflect the shearing,

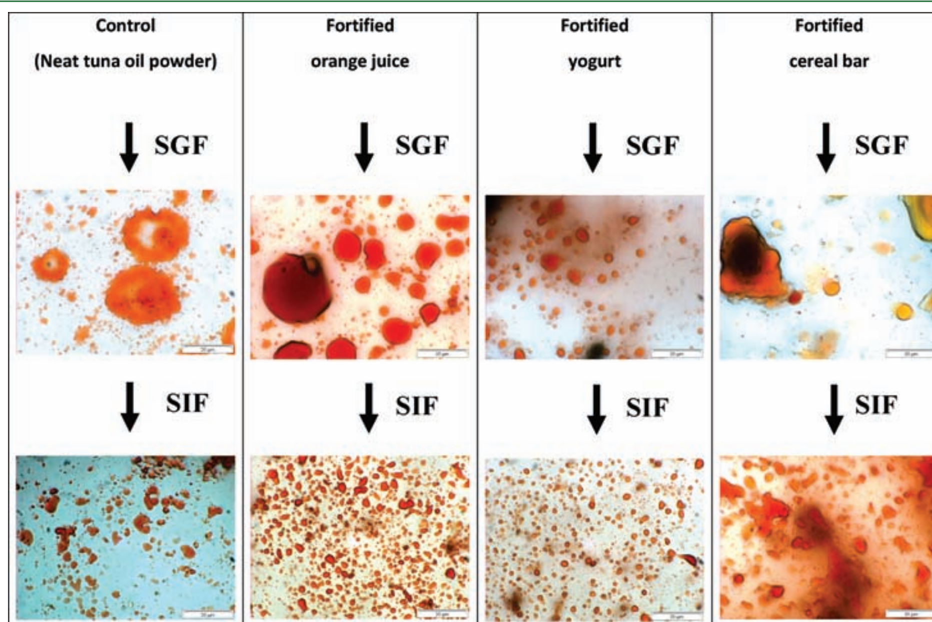

Figure 1. Bright field microscopy images of microencapsulated tuna oil powder and fortified food samples after exposure to SGF or sequential SGF and SIF.

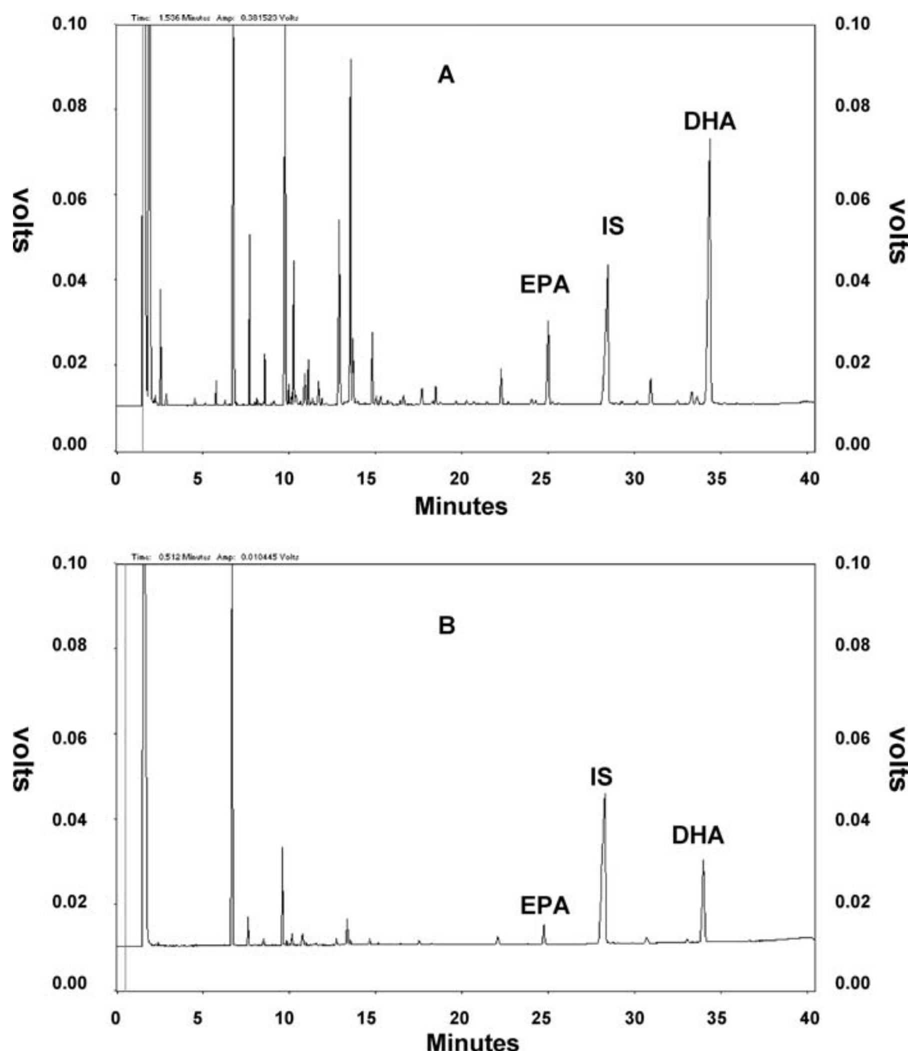

Figure 2. GC chromatograms of FAME analysis for oil extracted from neat microencapsulated tuna oil powder after sequential exposure to SGF and SIF (A) acid methylation (total fatty acids; i.e., glycerol-bound and free fatty acids) and (B) alkaline methylation (glycerol-bound fatty acid only); IS = internal standard (methyl tricosanoate).

mixing, and hydration that takes place during in vivo digestion.[23] Therefore, the steady state for droplet disintegration and coalescence may not be achievable by in vitro digestion.

For all samples, the oil droplet sizes were larger in the SGF digests than in the sequential SGF and SIF digests, and the latter were visibly more homogeneous (Figure 1). The digested microencapsulated tuna oil powder and fortified orange juice and yogurt samples had a similar and smaller lipid droplet size after exposure to SGF and SIF, as compared to the cereal bar sample. Because lipase acts at the interface, it is expected that lipolysis is related to the surface area available for lipase attack. Smaller lipid droplets have a larger surface area and are therefore more readily digested than bigger droplets, which have less surface area.[20] The oil droplets in the fortified cereal bar would therefore be expected to undergo a slower rate of lipolysis as compared to that of the other samples. However, as previously mentioned, oil droplet size is not the only factor governing the rate of lipolysis. Indeed, it should be noted that the cereal bar sample also contained the highest amount of total fat (Table 1).

Release of LC n-3 PUFAs after Digestion. The release of EPA and DHA after SGF digestion and after sequential exposure to SGF and SIF was determined. This involves estimating the efficiency of oil extraction from the digests after exposure to SGF or sequentially to SGF and SIF and measuring the released free fatty acids.

The difference between the total fatty acids (estimated after acid methylation) and the glycerol-bound fatty acids (estimated after alkaline methylation) was the free fatty acids released due to lipolysis. Figure 2 shows an example of the GC chromatograms obtained by FAME analysis, after acid methylation and alkaline methylation of a sample.

Food Matrix Effects on Efficiency of LC n-3 PUFA Extraction from Digests. Table 3 lists the measured and calculated amounts of EPA and DHA recovered from the various samples after exposure to SGF and to sequential SGF and SIF. The percentage recoveries of EPA and DHA are expressed as a % of the amount initially added to the food (formulated values).

The % recovery of LC n-3 PUFAs from the SGF digested samples was significantly ($P < 0.001$) influenced by the food matrix (EPA, 34.5−110.0%; and DHA, 33.5−102.9%) (Table 3). The lowest LC n-3 PUFA recovery was observed for the yogurt sample (EPA, 34.5%; DHA, 33.5%). This is due to coagulation of

Table 3. Measured and Calculated Values of EPA and DHA in Digests of Microencapsulated Tuna Oil Powder and Fortified Food Samples Exposed to SGF or Sequential SGF and SIF[a]

	after SGF digestion (measured)			after SGF digestion (calculated)		
sample	total fatty acid (mg)	% recovery[b]	glycerol bound (mg)	glycerol bound[c] (mg)	free fatty acid[d] (mg)	free fatty acid released[e] (%)
			EPA			
powder	8.95 ± 0.85 a,b	103.0	8.53 ± 0.45	8.28	0.46	5.2 ± 4.1 α
orange juice	7.07 ± 0.67 b	81.3	6.77 ± 0.71	8.36	0.38	4.4 ± 1.0 α
yogurt	3.00 ± 0.06 c	34.5	2.83 ± 0.11	8.20	0.54	6.1 ± 1.8 α
cereal bar	9.57 ± 0.27 a	110.0	9.15 ± 0.28	8.32	0.42	4.8 ± 0.2 α
			DHA			
powder	43.20 ± 4.08 d	102.9	42.67 ± 4.09	41.47	0.63	1.5 ± 0.1 β
orange juice	34.00 ± 3.15 d	81.0	33.96 ± 2.82	41.93	0.17	0.4 ± 1.0 β
yogurt	14.07 ± 0.19 e	33.5	14.04 ± 0.24	41.91	0.19	0.5 ± 0.4 β
cereal bar	41.91 ± 1.58 d	99.8	42.10 ± 1.92	42.18	−0.08	0.0 ± 0.8 β
	after SGF + SIF digestion (measured)			after SGF + SIF digestion (calculated)		
sample	total fatty acid (mg)	% recovery[b]	glycerol bound (mg)	glycerol bound[c] (mg)	free fatty acid[d] (mg)	free fatty acid released[e] (%)
			EPA			
powder	8.51 ± 0.32 f	97.9	1.86 ± 0.14	1.90	6.84	78.3 ± 1.7 γ
orange juice	8.16 ± 0.89 f	93.8	1.97 ± 0.04	2.10	6.64	76.0 ± 2.7 γ
yogurt	8.94 ± 0.39 f	102.8	1.92 ± 0.29	1.87	6.87	78.6 ± 2.9 γ
cereal bar	8.38 ± 0.85 f	96.4	3.03 ± 0.18	3.14	5.60	64.0 ± 3.9 δ
			DHA			
ME powder	40.31 ± 1.08 g	96.0	9.92 ± 0.95	10.33	31.77	75.5 ± 2.9 ε
orange juice	38.77 ± 3.66 g	92.4	10.43 ± 0.45	11.29	30.81	73.2 ± 2.4 ε
yogurt	41.98 ± 1.73 g	100.0	9.88 ± 1.36	9.88	32.22	76.5 ± 2.6 ε
cereal bar	37.00 ± 1.98 g	88.1	14.71 ± 0.64	16.72	25.38	60.3 ± 2.2 θ

[a] See the footnotes for Table 1 for information on food samples fortified with tuna oil powder. Theoretical amounts in digests for all samples (i.e., neat powder or fortified foods) were 8.74 mg of EPA and 42.10 mg of DHA (as glycerol-bound fatty acids); Measured data are the means ± SDs of duplicate digestions from two independent experiments. Values within a column for each fatty acid having the same Arabic or Greek alphabets are not significantly different ($P > 0.05$). [b] % recovery = [(recovered amount/theoretical amount in the sample) × 100]. [c] Calculated glycerol bound = [(measured value/recovery %) × 100]. [d] Calculated free fatty acid (mg) = (theoretical amount − calculated glycerol bound). [e] Calculated % free fatty released = [(calculated free fatty acid/theoretical amount) × 100].

the milk proteins in yogurt after SGF addition, which resulted in matrix solidification. This leads to entrapment of the LC n-3 PUFA and thereby impedes oil extraction efficiency.

After sequential exposure to SGF and SIF, there was 93.8−102.8% EPA and 88.1−100.0% DHA recovered from the oil extracts of the digested samples. There was no significant effect of the food matrix on the recoveries of EPA and DHA ($P = 0.437$ for EPA and $P = 0.055$ for DHA) (Table 3).

Food Matrix Effects on Free LC n-3 PUFAs Released by in Vitro Digestion. The percentages of free LC n-3 PUFAs released from the digests are listed in Table 3. These percentages indicate the portion of free EPA and DHA released from the triacylglycerols. An assumption made here was that the extracted oil had the same composition as that in the food samples where the oil extraction efficiency was not 100%.

There was only minimal release of free LC n-3 PUFAs after SGF digestion with only 4.4−6.1% EPA and ≤1.5% DHA released (as a % of total fatty acids present). Others have suggested that there is 10−30% lipolysis of the total triacylglycerols per meal consumed in vivo in the stomach.[20,22] This could be partly because the SGF used in this study does not contain gastric or lingual lipases.

After sequential exposure to SGF and SIF, the percentages of free LC n-3 PUFAs detected were markedly increased (Table 3). Pancreatic lipase plays a major role in digesting triacylglycerols to free fatty acids. Digestion was also assisted by the presence of potassium dihydrogen phosphate, bile salts, and $CaCl_2$ present in SIF. The extents of lipolysis of both glycerol-bound EPA and DHA obtained were 73.2−78.6% for the neat powder, fortified orange juice, and yogurt and 60.3−64.0% for the fortified cereal bar. There were no significant differences ($P > 0.05$) in lipolysis between the neat microencapsulated tuna oil powder and the fortified orange juice and yogurt samples. In comparison, the digested cereal bar sample had a significantly lower amount of LC n-3 PUFAs released ($P < 0.001$), showing that the type of food matrix affected the extent of LC n-3 PUFA lipolysis.

The lower amount of free LC n-3 PUFAs released after digestion of the cereal bar sample compared to the other samples may in part be due to the much larger lipid droplets in the digest of the cereal bar and, hence, the low total surface area available for lipase attack (Figure 1). Other studies have suggested that the presence of polysaccharides, for example, dietary fiber, can also alter lipid digestion.[20,24,25] As compared to the neat powder, orange juice, and yogurt samples, the cereal bar sample had the

highest content of dietary fiber. The cereal bar sample also had the highest fat content (Table 1), and some of this was solid fat due to the inclusion of butter in the formulation. The presence of solid fat promotes the coalescence of oil particles during digestion, which is a factor in slowing the rate of lipolysis.[18]

Pancreatic lipase has a high specificity for fatty acids esterified at the *sn*-1 and *sn*-3 positions of triacylglycerols.[33] The percentages of EPA and DHA esterified at the *sn*-2 position in tuna oil are about 37 and 52%, respectively; therefore, 63% EPA and 48% DHA would be at the *sn*-1 and *sn*-3 positions, respectively.[33] In this study, there were much higher percentages of free LC n-3 PUFAs released than glycerol-bound EPA and DHA at *sn*-1 and *sn*-3 positions. This can be due to the migration of *sn*-2 fatty acids to *sn*-1 and *sn*-3 positions after the fatty acids in *sn*-1 and *sn*-3 positions have been released.[19] Therefore, EPA and DHA anchored to the *sn*-2 position of the original triacylglycerol can potentially be digested, resulting in the higher amount of free LC n-3 PUFAs detected. However, the extent of EPA and DHA positional arrangement during lipid digestion requires further investigation.

This research has confirmed that lipolysis of microencapsulated tuna oil powder during in vitro digestion may be affected by the food matrix, its structure (e.g., solid versus liquid), but also by its composition. The lowest extent of lipolysis of the microencapsulated tuna oil during in vitro digestion was found when it was delivered in the cereal bar. This food vehicle contained the highest content of fat and polysaccharide (e.g., dietary fiber) and had the largest relative oil droplet size on exposure to SIF. The in vitro lipolysis of microencapsulated tuna oil powder delivered in orange juice or yogurt matrices was similar to that of the neat powder after sequential exposure to SGF and SIF. This study may have implications for the choice of food vehicle chosen for delivery of microencapsulated fish oil. Further studies are required to establish the bioequivalence of the microencapsulated fish oil delivered through various food products.

AUTHOR INFORMATION

Corresponding Author

*Tel: +61-3-97313260. Fax: +61-3-97313250. E-mail: zhiping.shen@csiro.au.

ACKNOWLEDGMENT

The technical assistance of Wayne Beattie in the production of microencapsulated tuna oil powders is gratefully acknowledged.

REFERENCES

(1) Ruxton, C. H. S.; Reed, S. C.; Simpson, M. J. A.; Milington, K. J. The health benefits of omega-3 polyunsaturated fatty acids: A review of evidence. *J. Hum. Nutr. Diet.* **2007**, *20*, 275–285.

(2) Roth, E. M.; Harris, W. S. Fish oil for primary and secondary prevention of coronary heart disease. *Curr. Atheroscler. Rep.* **2010**, *12*, 66–72.

(3) Dyall, S. C.; Michael, T. Neurological benefits of omega-3 fatty acids. *NeuroMol. Med.* **2008**, *10* (4), 219–235.

(4) Barrow, C. J.; Nolan, C.; Jin, Y. Stabilization of highly unsaturated fatty acids and delivery into foods. *Lipid Technol.* **2007**, *19*, 108–111.

(5) Larsson, S. C.; Kumlin, M.; Ingelman-Sundberg, M.; Wolk, A. Dietary long-chain n−3 fatty acids for the prevention of cancer: A review of potential mechanisms. *Am. J. Clin. Nutr.* **2004**, *79*, 935–945.

(6) Shahidi, F.; Miraliakbari, H. M. Omega-3 (*n*-3) fatty acids in health and disease: Part 1—Cardiovascular disease and cancer. *J. Med. Food* **2004**, *7*, 384–401.

(7) Robinson, L. E.; Clandinin, M. T.; Field, C. J. The role of dietary long-chain N-3 fatty acids in anti-cancer immune defence and R3230AC mammary tumor growth in rats: influence of diet fat composition. *Breast Cancer Res. Treat.* **2002**, *73*, 145–160.

(8) Wijesundera, C.; Ceccato, C.; Watkins, P.; Fagan, P.; Fraser, B.; Thienthong, N.; Perlmutter, P. Docosahexaenoic acid is more stable to oxidation when located at the *sn*-2 position of triacylglycerol compared to *sn*-1 (3). *J. Am. Oil Chem. Soc.* **2008**, *85*, 543–548.

(9) Augustin, M. A.; Sanguansri, L.; Bode, O. Maillard reaction products as encapsulants for fish oil powders. *J. Food Sci.* **2006**, *71*, E25–E32.

(10) Kolanowski, W.; Jaworska, D.; Wei*β*brodt, J.; Kunz, B. Sensory assessment of microencapsulated fish oil powder. *J. Am. Oil Chem. Soc.* **2007**, *84*, 37–45.

(11) Raatz, S. K.; Redmon, J. B.; Wimmergren, N.; Donadio, J. V.; Bibus, D. M. Enhanced absorption of n-3 fatty acids from emulsified compared with encapsulated fish oil. *J. Am. Diet Assoc.* **2009**, *109*, 1076–1081.

(12) Barrow, C. J.; Nolan, C.; Holub, B. J. Bioequivalence of encapsulated and microencapsulated fish-oil supplementation. *J. Funct. Foods* **2009**, *1*, 38–43.

(13) Arterburn, L. M.; Oken, H. A.; Hoffman, J. P.; Bailey-Hall, E.; Chung, G.; Rom, D.; Hamersley, J.; McCarthy, D. Bioequivalence of docosahexaenoic acid from different algal oils in capsules and in a DHA-fortified food. *Lipids* **2007**, *42*, 1011–1024.

(14) Mu, H. Bioavailability of omega-3 long chain polyunsaturated fatty acids. *Supp AgroFood Industry Hi-Tech* **2008**, *19* (4), 24–26.

(15) Sanguansri, L.; Augustin, M. A. Microencapsulation and delivery of omega-3 fatty acids. In *Functional Food Ingredients and Nutraceuticals, Processing Technologies*, 1; Shi, J., Ed.; CRC Press, Taylor & Francis Group: United States, 2006; pp 297−327.

(16) Armand, M.; Borel, P.; Ythier, P.; Dutot, G.; Melin, C.; Senft, M.; Lafont, H.; Lairon, D. Effects of droplet size, triacylglycerol composition, and calcium on the hydrolysis of complex emulsions by pancreatic lipase: An in vitro study. *J. Nutr. Biochem.* **1992**, *3*, 333–341.

(17) Favé, G.; Coste, T. C.; Armand, M. Physicochemical properties of lipids: New strategies to manage fatty acid bioavailability. *Cell. Mol. Biol.* **2004**, *50*, 815–831.

(18) Golding, M.; Wooster, T. J. The influence of emulsion structure and stability on lipid digestion. *Curr. Opin. Colloid Interface Sci.* **2010**, *15*, 90–101.

(19) Mu, H.; Hoy, C. E. The digestion of dietary triacylglycerols. *Prog. Lipid Res.* **2004**, *43*, 105–133.

(20) McClements, D. J.; Decker, E. A.; Park, Y. Controlling lipid bioavailability through physicochemical and structural approaches. *Crit. Rev. Food Sci. Nutr.* **2009**, *49*, 48–67.

(21) Mun, S.; Decker, E. A.; McClements, D. J. Influence of emulsifier type on *in vitro* digestibility of lipid droplets by pancreatic lipase. *Food Res. Int.* **2007**, *40*, 770–781.

(22) Singh, H; Ye, A.; Horne, D. Structuring food emulsions in the gastrointestinal tract to modify lipid digestion. *Prog. Lipid Res.* **2009**, *48*, 92–100.

(23) Parada, J.; Aguilera, J. M. Food microstructure affects the bioavailability of several nutrients. *J. Food Sci.* **2007**, *72*, R21–R32.

(24) Lairon, D.; Play, B.; Jourdheuil-Rahmani, D. Digestible and indigestible carbohydrates: Interactions with postprandial lipid mechanism. *J. Nutr. Biochem.* **2007**, *18*, 217–227.

(25) Pasquier, B.; Armand, M.; Guillon, F.; Castelain, C.; Borel, P.; Barry, J. L.; Pleroni, G.; Lairon, D. Viscous soluble dietary fibers alter emulsification and lipolysis of triacylglycerols in duodenal medium *in vitro*. *J. Nutr. Biochem.* **1996**, *7*, 293–302.

(26) Hur, S. J.; Lim, B. O.; Park, G. B.; Joo, S. T. Effects of various fiber additions on lipid digestion during *in vitro* digestion of beef patties. *J. Food Sci.* **2009**, *74*, C653–C657.

(27) Schram, L. B.; Nielsen, C. J.; Porsgaard, T.; Nielsen, N. S.; Holm, R.; Mu, H. Food matrices affect the bioavailability of (n-3)

polyunsaturated fatty acids in a single meal study in humans. *Food Res. Int.* **2007**, *40*, 1062–1068.

(28) Kosaraju, S. L.; Weerakkody, R.; Augustin, M. A. *In-vitro* evaluation of hydrocolloid-based encapsulated fish oil. *Food Hydrocolloids* **2009**, *23*, 1413–1419.

(29) Christie, W. W. *Lipid Analysis. Preparation of Derivatives of Fatty Acids*, 3rd ed.; Christie, W. W., Ed.; The Oily Press, an imprint of PJ Barnes and Associates: Bridgwater, United Kingdom, 2003; pp 205−224.

(30) Shen, Z.; Augustin, M. A.; Sanguansri, L.; Cheng, L. J. Oxidative stability of microencapsulated fish oil powders stabilized by blends of chitosan, modified starch and glucose. *J. Agric. Food Chem.* **2010**, *58*, 4487–4493.

(31) Bannon, C. D.; Craske, J. D.; Hilliker, A. E. Analysis of fatty acid methyl esters with high accuracy and reliability. *J. Am. Oil Chem. Soc* **1985**, *62*, 1501–1507.

(32) American Oil Chemists' Society. (AOCS). Official Method Ce 1b-89. Fatty acid composition by GLC (marine oils). *AOCS Official Methods and Recommended Practices of American Oil Chemists' Society*: AOCCS Press: Champaign, IL, Reapproved 2009.

(33) Shen, Z.; Wijesundera, C. Evaluation of ethanolysis with immobilized *Candida Antarctica* lipase for regiospecific analysis of triacylglycerols containing highly unsaturated fatty acids. *J. Am. Oil Chem. Soc.* **2006**, *83*, 923–927.