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ARTICLE

In Vitro Assessment of the Bioaccessibility of Tocopherol and Fatty Acids from Sunflower Seed Oil Bodies

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The in vitro digestibility (proteolytic and lipolytic) and bioaccessibility of nutritionally important compounds (α-tocopherol and fatty acids) have been studied for natural sunflower (*Helianthus annuus*) oil body suspensions in comparison to artificial emulsions emulsified with polyoxyethylene-20-sorbitan-monolaurate (Tween 20) or whey protein isolate. Proteolytic digestion of emulsions with pepsin (pH 2) promoted significant increases in mean particle size of the whey protein isolate stabilized emulsion (1.8−2.9 μm) and oil bodies (2.3−22.5 μm) but not the Tween 20 stabilized emulsions. SDS-PAGE of proteolytic digestion products suggested degradation of the stabilizing oleosin protein (ca. 18−21 kDa) in oil bodies. The rate of oil body hydrolysis with lipase was significantly slower than the lipase-catalyzed hydrolysis of the artificial emulsions and exhibited a prolonged lag phase. Results from simulated human digestion in vitro suggested that the mean bioaccessibility of α-tocopherol and total fatty acids from oil bodies (0.6 and 8.4%, respectively) was significantly lower than that from the Tween 20 stabilized emulsion (35 and 52%, respectively) and the whey protein isolate stabilized emulsion (17 and 33%, respectively). These in vitro results suggest that oil bodies could provide a natural emulsion in food that is digested at a relatively slow rate, the physiological consequence of which may be increased satiety.

KEYWORDS: α-Tocopherol; fatty acid; *Helianthus annuus*; oil body; emulsion; digestion bioaccessibility

INTRODUCTION

Plant seeds store cellular triacylglycerol (TAG) in spherical droplets called oil bodies that are utilized as a source of energy during seed germination. These oil bodies (typically 0.5−2.5 μm in diameter) are mainly composed of a TAG core (94−98% w/w) surrounded by a monolayer of phospholipids (0.5−2.0%) embedded with small alkaline proteins specific to oil bodies called oleosin (15−26 kDa molecular mass; 0.5−3.5%) and some minor proteins called caleosins and steroleosins (*1*−*5*). These proteins provide a thickened surface layer that prevents coalescence of oil bodies in the cytosol of oilseed cells (*1*, *6*) and, together with a net negative charge at neutral pH, prevent coalescence ex vivo when oil bodies are dispersed to form a suspension.

Oil bodies isolated from plant seeds into aqueous media are therefore a natural emulsion that may represent a vehicle to deliver natural, minimally processed, pre-emulsified oil into appropriate food systems. Sunflower oil is an accepted dietary source of lipid for human consumption, having a favorable composition of fatty acids from a nutritional perspective, and is also a rich source of α-tocopherol. This oil-soluble vitamin has been widely reported as an important powerful antioxidant in the human diet, which may exert beneficial effects in humans including protection against cardiovascular disease and cancer (*7*−*9*). Oil bodies isolated from sunflower seeds, and indeed other plant species, are enriched in tocochromanols (*10*, *11*) . However, what is not known is the rate of oil body digestion, and associated nutrient release, in the gastrointestinal tract.

The bioavailability of micronutrients from food sources is a critical concept in understanding their functionality in human health. In vitro techniques, designed to mimic the human digestive system (namely, conditions in the stomach and small intestine), have been used to evaluate the digestibility and bioaccessibility of a range of micronutrients, including vitamin E, from fruits and vegetables (*12*, *13*). These techniques are becoming increasingly popular due to the labor intensive, time-consuming, and costly nature of human studies. Moreover, good correlations have been found with carotenoid bioaccessibility data derived from investigations in vitro and in vivo with humans (*12*); in vitro methods are useful as preliminary assessment of bioavailability, but should be followed by in vivo studies.

To date, only simple lipolytic assays have been used to assess the action of lipase on natural oil bodies (*14*−*16*). Often this has been examined from the perspective of lipase activity in the seed itself, although work by Beisson et al. (*16*) has examined the effect of human pancreatic lipase on the lipolysis of almond oil bodies. Although of undoubted interest, these assays do not take into account the digestive processes that take place in the stomach prior to lipase hydrolysis in the small intestine, for example, the action of proteolytic enzymes at acidic pH. This enzymic action

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would be vital in determining the digestive fate of these protein-covered oil bodies. Furthermore, no studies have demonstrated the relative digestive bioaccessibility of micronutrients, for example, vitamin E, from oil bodies.

This study was undertaken to examine, in vitro, the effect of simulated gastric and duodenal digestion on sunflower oil body integrity in comparison to processed sunflower oil-in-water emulsions prepared with different emulsifying agents (Tween 20 and whey protein). In addition, these simulated digestive processes have been combined in sequence to compare the bioaccessibility of important nutritional components of oil bodies, namely, fatty acids and α-tocopherol (vitamin E), with the same components in formulated emulsions.

MATERIALS AND METHODS

Materials. Sunflower seeds were purchased from Lembas Ltd. (Sheffield, U.K.). Deionized, polished water (14−18 mega ohms (MØ) cm; total organic content < 30 ppb; pH 6.8) was used to recover oil bodies and dilute where appropriate. Whey protein isolate (BiPRO) was a gift from Davisco Foods International, Inc., Eden Prairie, MN). Tween 20, pepsin (2800 units/mg of protein), pancreatin (porcine, 8xUSP specifications), lipase (from porcine pancreas type II, 100−400 units/mg of protein), and sodium deoxycholate were all products of Sigma Chemical Co. (Dorset, U. K.). Unless otherwise stated, all reagents used were of analytical grade.

Recovery and Purification of Oil Bodies. Sunflower seeds were homogenized (Kenwood BL315 blender, Havant, U.K.) in 10 mmol L^{-1} sodium phosphate buffer, pH 7.5 (1:5 w/v, 0.02% w/v sodium azide) for 2 min. The slurry was then filtered through three layers of cheesecloth and the filtrate centrifuged at 10000*g* (Beckman J2-21 centrifuge; fixed rotor JA-10) for 30 min (10 °C). The crude oil bodies, which collected as a creamy pad at the top of the mixture, were then carefully picked from the media and resuspended in 9 M urea (1:5 w/v), vigorously shaken, and then left on a rotary roller mixer (SRT 2 roller, Stuart Scientific, Staffordshire, U.K.) for 10 min. The mixture was then centrifuged as above and the creamy pad isolated. The pad was washed with urea twice further under identical conditions. This pad was then washed with buffer (see above) another three times to remove urea from the preparation.

Proximate Composition of Purified Oil Body Preparations. The moisture content of the oil body cream was determined gravimetrically following vacuum drying at 50 °C for 24 h. The lipid content of the dried oil body preparation (ca. 0.5−1 g) was determined gravimetrically using repeated extraction with diethyl ether (*17*). The protein content of the defatted dried oil bodies was determined using the bichinconinic acid (BCA) assay (*18*) following solubilization of proteins in 2% sodium dodecyl sulfate (SDS) solution at 90 °C. Bovine serum albumin was used as a protein standard. All determinations were conducted in triplicate.

Preparation of Oil/Water (o/w) Emulsions. *Sunflower Seed Oil Body Suspension*. Once the lipid contents of the purified oil body preparations were determined (see above), suspensions were formulated by dispersing oil bodies in 0.09% NaCl to provide a final suspension of 5% oil w/v. These suspensions were prepared in triplicate and stored at 5 °C for no longer than 12 h before use.

Processed Sunflower Oil Emulsion. Sunflower oil was extracted in bulk from sunflower seeds using diethyl ether before rotary evaporation of solvent. This oil was used to formulate emulsions (5% oil; 0.09% NaCl solution) using whey protein isolate or Tween 20 as emulsifier (1% w/v). Oil was added to the saline solution containing emulsifier and then pre-emulsified with an ultraturax homogenizer (IKA Werke, Staufer, Germany) at 10000 rpm. To produce emulsion droplets with a monomodal distribution and within the size range reported for purified oil bodies (0.5−2.5 μm) it was necessary to then pass the preparations once through a high-pressure homogenizer (Emulsiflex C-5, Glen Creston, Stanmore, U.K.) at 5000 psi. Emulsions were prepared in triplicate and stored at 5 °C for no longer than 12 h before use.

Particle Size Analysis. Emulsion (5% oil w/v) size (diameter) distributions in 0.09% NaCl solution were determined before and after digestion with pepsin (see below) by laser diffraction using the Malvern Mastersizer S (Malvern Instruments, Worcs., U.K.) fitted with a 320 mm lens and a small sample dispersion unit. Sample obscuration was maintained at 17−20%, and the presentation code was 3 NAD. The fundamental size distribution derived from this technique is volume based; that is, reported percentage distribution within a given size category implies the percentage of the total volume of particles in the entire distribution. The particle size measurements are hereby reported as the volume mean diameter: $d_{43} = \sum n_i d_i^4 / \sum n_i d_i^3$, where n_i is the number of droplets of diameter d_I. Each individual particle size measurement was determined from the average of two readings made per sample.

Pepsin Digestion of Oil Bodies and Emulsions. Pepsin solution (36 mg mL^{-1}, 0.1 M HCl) was added (1 mL) to the emulsion (10 mL; 5% oil w/v, 0.09% NaCl), and the sample (measured pH 2) was placed on a rotary roller mixer in an incubator at 37 °C for 30 min. To stop the reaction 0.9 M sodium bicarbonate solution (1.5 mL) was added to raise the pH of the media (>6.5). The emulsions were assessed for particle size (see above) and protein composition. Prior to protein analyses the before and after digestion whey protein stabilized emulsions and oil bodies (8 mL) were defatted with the addition of chloroform/methanol (2:1; 15 mL), and following centrifugation the interfacial material containing proteins was isolated with a Pasteur pipet, dried under a stream of nitrogen, and then resuspended in 1 mL of 2% SDS solution for protein analyses (see below).

Protein Analyses. Concentrations of protein in the 2% SDS solution (see above) were determined using the BCA assay. All solutions were then diluted to provide an overall concentration of 10 mg mL^{-1} before resolving the different protein molecular weights with SDS-PAGE as reported previously (*11*). A control sample containing just the pepsin preparation was also run to aid possible identification of proteolytic bands.

Lipase Hydrolysis of Oil Bodies and Emulsions. A simple turbidimetric assay was used to compare the relative rates of lipolytic hydrolysis of oil body suspensions and processed emulsions (whey protein isolate and Tween 20) by pancreatic lipase in the presence of excess bile salts (25 mmol L^{-1} sodium deoxycholate (*19*)). In principle, as emulsion droplets are hydrolyzed, fatty acid products are incorporated into micelles that are optically clear, resulting in a reduction in measured absorbance or turbidity. The absorbance (365 nm) of 0.05% o/w emulsion or oil body dispersion (oil weight basis; total volume = 1 mL) was monitored over a 5 min period following the addition (50 μL) of lipase solution (100 mg mL^{-1}). Results were expressed as the percent absorbance of the original solution. Appropriate controls were run to confirm no loss of turbidity of emulsions in the absence of lipase. All experiments were conducted in triplicate at 20 °C.

Bioaccessibility of Fatty Acids and Vitamin E. The digestion procedure was a slight modification of that described by Garrett et al. (*20*). Pepsin solution (36 mg mL^{-1}; 0.1 M HCl) was added (1 mL) to 10 mL of o/w emulsion or oil body dispersion (5% w/v oil, 0.09% NaCl) and then incubated at 37 °C for 30 min on a roller mixer. The pH of the media was then raised to 7.0 with the addition of ca. 1.5 mL of 0.9 M sodium bicarbonate before the addition of 1.2 mL of a pancreatin/bile salt mixture (100 mmol L^{-1} sodium deoxycholate; 5 mg mL^{-1} pancreatin; 100 mmol L^{-1} sodium bicarbonate solution) and additional lipase solution (300 μL; 100 mg mL^{-1}). The preparation was then incubated for a further 30 min at 37 °C. At the end of the incubation, 500 μL of 2.5 mmol L^{-1} Orlistat [1-(3-hexyl-4-oxo-oxetan-2-yl)tridecan-2-yl 2-formylamino-4-methylpentanoate] in methanol was added to inhibit further lipase activity. Final concentrations of constituents in the preparation were 2.5 mg mL^{-1} pepsin, 2.1 mg mL^{-1} lipase, 8.3 mmol L^{-1} sodium deoxycholate, and 0.4 mg mL^{-1} pancreatin. Micelles were separated from emulsified oil droplets by ultracentrifugation. Aliquots of each digestate (6 mL) were transferred to Quick-Seal Polyallomer tubes (Beckman Coulter, Buckinghamshire, U.K.) and then placed in a 50.3 Ti rotor and centrifuged at 165000*g* at 4 °C for 95 min (Beckman L8-M ultracentrifuge). The aqueous fraction was collected from the centrifuge tube using an 18 gauge needle fitted to a 5 mL syringe and then filtered (0.22 μm) before storage at −80 °C. For an explanation of modifications see the Discussion.

Analysis of Fatty Acids and Vitamin E. Fatty acids were extracted from the micellar fractions (1 mL) using chloroform/methanol as per Bligh and Dyer (*21*) to achieve a relative concentration of chloroform/methanol/water of 1:2:0.8 during monophasic extraction and 1:1:0.9 during biphasic separation. Extracted free fatty acids were then derivatized using methanolic sulfuric acid (1%) at 50 °C (*22*). The fatty acyl groups of the oil, and of the oil bodies, extracted from sunflower seeds were also derivatized to allow fatty acid compositional analysis. Identification and

quantification of fatty acid methyl esters (FAMEs) was carried out using GC-MS (Fisons 800 GC and Fisons MD800 quadrapole mass spectrometer; 70 eV ionization energy and 450 μA emission current) equipped with a Chromapak CP-Wax 52 CH column (25 m × 0.25 × 1.2 μm). FAMEs were identified by comparison of retention times and mass spectral data against authentic standards and the NIST/LIBTX library. Vitamin E (α-tocopherol) in emulsions and micellar fractions was recovered as described previously for carotenoids (*20*) and then quantified/identity confirmed by molecular mass determination using HPLC with fluorescence detection as described previously (*11*) coupled to a mass spectrometer (Micromass, Waters, U.K.) with an APCI interface and selected ion monitoring of the protonated α-tocopherol molecule, $[M + H]^+ = 431$ m/z (*23*, *24*). Settings on the mass spectrometer were cone voltage, 18 V; 4.09 kV capillary voltage; source temperature, 150 °C; and 350 °C desolvation temperature. Gas flow was set at 570 L h^{-1}.

Calculations and Statistics. The bioaccessibility of fatty acids and vitamin E was defined as a percentage release: [total substrate released into the micellar phase (μg)/total available substrate (μg)] × 100. All experiments were carried out on triplicate emulsion preparations; the data were statistically analyzed using StatGraphics Plus for Windows (V. 5.1). Values are expressed as means ± SD.

RESULTS

The sunflower seed oil body preparation (purified) contained 27.1 ± 3.4% moisture. On a dry weight basis the preparation typically contained 99.2 ± 2.3% lipid and 1.5 ± 0.2% protein. This emulsion was then diluted with saline solution to provide a 5% lipid content oil body dispersion (w/v) for further studies.

Particle Size Data. The particle size distribution of all emulsion droplets (oil bodies, Tween 20 and whey protein isolate stabilized emulsions), before and after incubation with pepsin can be seen in **Figure 1**. The volume mean diameters (d_{43}) of the Tween 20 and whey protein isolate stabilized emulsion droplets (1.5 ± 0.3 and 1.8 ± 0.5 μm, respectively) were smaller ($P = 0.03$) than those of oil bodies (2.3 ± 0.3 μm) prior to incubation with pepsin. All three emulsions had a monomodal distribution predigestion. Following pepsin incubation the diameter (d_{43}) of the whey protein isolate stabilized emulsion droplets (2.9 ± 0.4 μm) and oil bodies (22.5 ± 9.9 μm) increased significantly ($P < 0.05$), but no change was recorded for Tween 20 stabilized oil droplets (1.3 ± 0.1 μm). Moreover, a shift from a monomodal distribution to one with three apparent peak maxima was recorded for oil bodies when digested with pepsin.

Action of Pepsin on Proteins Present in the Emulsions. Qualitative examination of the protein molecular weights (SDS-PAGE) in whey protein isolate stabilized emulsions before and after incubation with pepsin (**Figure 2**) suggests the loss of bands at ca. 14 kDa and the increase in bands between 3.5 and 6.5 kDa. Moreover, in oil body emulsions there is a loss of protein bands between 14.3 and 20.1 kDa and an increase in bands between 6.5 and 14.4 kDa following incubation with pepsin. Comparison with proteins in the pepsin preparation itself suggests that these are genuine fragment products of pepsin catalysis.

Lipase-Induced Emulsion Destabilization. Examination of the lipase-catalyzed hydrolysis of different emulsions (**Figure 3**) as indicated by turbidity measurements (see Materials and Methods) revealed that the relative rate of oil body hydrolysis was significantly slower than those of the Tween 20 and whey protein isolate stabilized emulsions, which were similar. In comparison to the artificial emulsions, the oil bodies exhibited an apparent lag phase of between 30 and 60 s before appreciable levels of lipolysis were detected. At the end of the hydrolysis period (300 s) 54.5 ± 1.2% of the turbidity of the oil body emulsion remained, and only 5.5 ± 0.5 and 5.7 ± 0.2% of the turbidity of the Tween 20 and whey protein isolate stabilized emulsions remained, respectively ($n = 3$, ± SD).

Release of α-Tocopherol and Fatty Acids from Emulsions during Simulated Digestion. The α-tocopherol concentrations in the formulated 5% oil emulsions can be seen in **Table 1**. Following simulated gastric and then intestinal digestion and measurement of α-tocopherol in micellar fractions, the bioaccessibility (% transfer from emulsion to micellar fraction) for the different treatments was calculated and followed the order Tween 20 (35 ± 5.7%) > whey protein isolate (17 ± 1.3%) > oil bodies (0.6 ± 0.1%).

The four major fatty acids consistently present in the oil of the various emulsions (mainly as triacylglycerols >98%) were linoleic acid > oleic acid > palmitic acid > stearic acid in the relative proportions 70, 23, 5, and 2%, respectively. All further calculations were carried out on the basis of only these fatty acids. The total concentration of fatty acids was 450 mg per assay. Following recovery of the micellar fraction after simulated digestion, and analysis of free fatty acids present in micelles, the bioaccessibility of total and individual fatty acids was calculated (**Table 2**). The transfer of fatty acids from emulsion droplets to the micellar fraction was greatest in the Tween 20 (52.0 ± 9.1%) and the whey protein isolate stabilized emulsions (33.0 ± 1.7%), which were both greater ($P < 0.05$) than for oil bodies (8.4 ± 0.4%). Examination of the bioaccessibility of individual fatty acids suggests that palmitic acid and stearic acid were transferred to a greater extent than linoleic and oleic acid for all treatments, although this was significant for only the oil body treatment (see **Table 3**).

DISCUSSION

The composition and particle size of the purified sunflower oil bodies produced in this investigation were consistent with those reported previously, 0.5−2.5 μm (*25*). To avoid the surface area of droplets being a source of experimental variation monomodal processed emulsions were prepared using high-pressure homogenization to create a droplet distribution within the range for oil bodies previously reported. Although this was achieved, the volume mean diameter of the processed emulsions (mean = 1.4−1.5 μm) was smaller than that of the oil bodies (2.3 μm). Subsequently, it is not possible to completely rule out the impact of droplet size in the comparative digestion experiments; nonetheless, this study represents the first published attempt to compare the relative digestibility of natural oil bodies with artificial emulsions.

The exposure of protein-stabilized oil droplets (whey protein isolate and oil bodies) to pepsin under pH conditions equivalent to that of the stomach (pH 2) led to a significant increase in average droplet size, with a concomitant digestion of surface proteins. Examination of the emulsion by light microscopy (data not shown) revealed that coalescence of oil droplets had occurred in these cases. This explains the shift in the particle size data for the protein-stabilized emulsions. The two major proteins in the whey preparation used in this study were β-lactoglobulin (18.4 kDa) and α-lactalbumin (14.2 kDa). Examination of the protein molecular weights in the before and after pepsin incubated whey preparation suggests complete breakdown of α-lactalbumin but no extensive breakdown of the β-lactoglobulin protein under these conditions, implying specificity of the action of the pepsin enzyme. This is consistent with the findings of other authors who have shown that α-lactalbumin, but not β-lactoglobulin (unless heat treated), was hydrolyzed by pepsin in vitro (*26*, *27*). Examination of the protein molecular weights in the oil body preparation before and after pepsin incubation suggests the loss of oleosin (~18−21 kDa) during incubation and the appearance of protein fragments between 6.5 and 14 kDa, which may be breakdown products of oleosin. Oleosin has three structural regions: an amphipathic

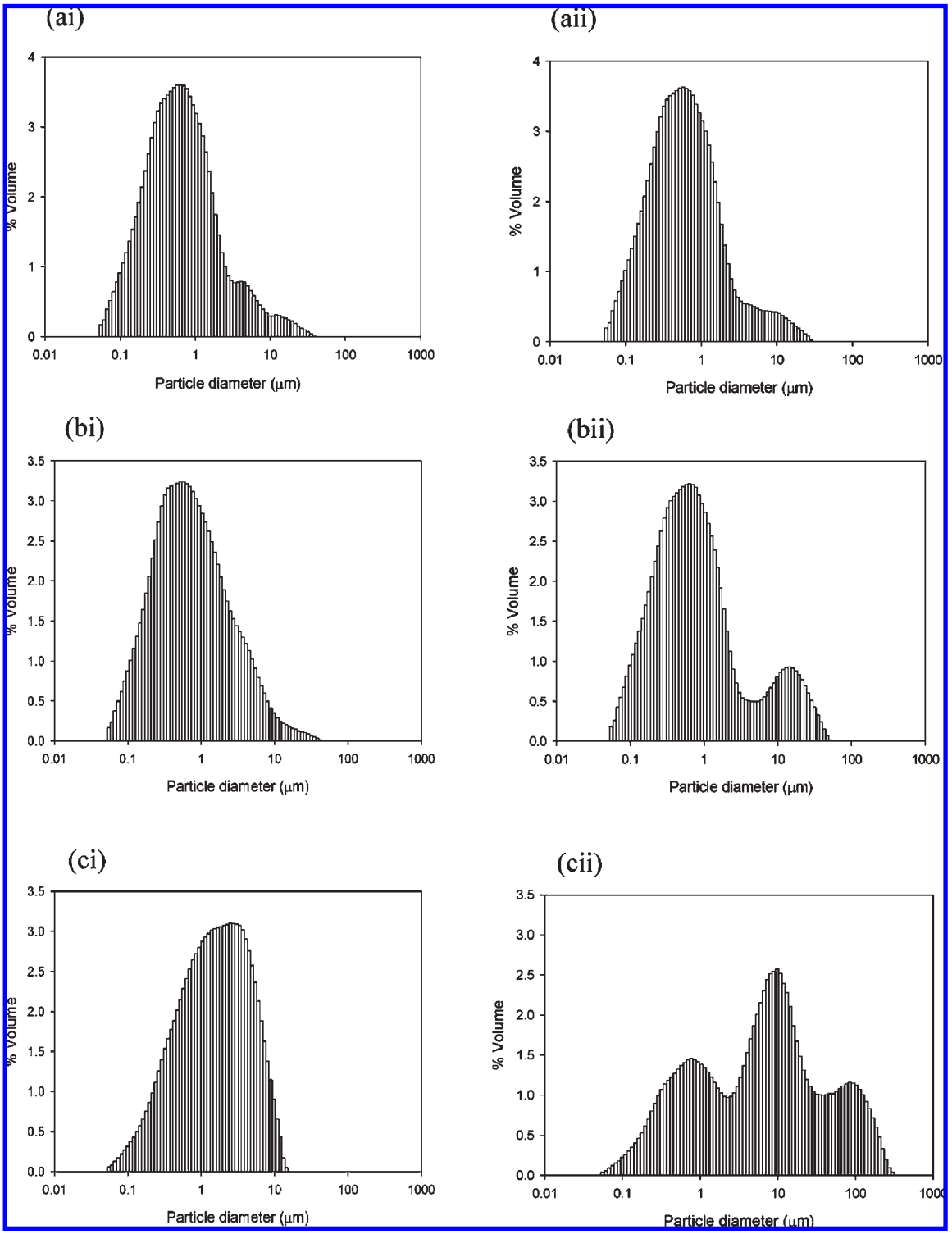

Figure 1. Particle size distributions (% volume) of sunflower oil emulsions emulsified with 1% w/v Tween 20 (**a**) or with 1% w/v whey protein (**b**) and oil bodies (**c**) before (**i**) and after (**ii**) digestion with pepsin solution (36 mg mL^{-1}, 0.1 M HCl) at 37 °C for 30 min.

N-terminal region, a central hydrophobic antiparallel β-strand domain, and an amphipathic C-terminal domain of variable length (*6*). It is likely that the protruding part of the oleosin molecule, which provides a strengthened layer on the surface, is susceptible to enzymatic cleavage and once digested leads to the weakening and consequential coalescence of oil bodies.

Pepsin hydrolyzes peptide bonds on the N-terminus side of aromatic residues (*28*). Given the amino acid sequence of oleosin protein in sunflower seed, there are 11 potential sites of pepsin action, and 4 of these peptide bonds are within the exposed domains of oleosin on the surface of oil bodies (*1*, *29*−*31*). Further work is required to elucidate the actual profile of oleosin degradation during digestion, but we have observed the generation of smaller peptides when sunflower seed oil bodies are exposed to stomach conditions in vitro (data not shown); these peptides are probably a result of the hydrolysis of peptide bonds on the exposed domains of oleosin.

However, a detailed study of the breakdown of oleosin and the fragment molecules, under conditions representing digestion in the human stomach, would be required to support this theory. In

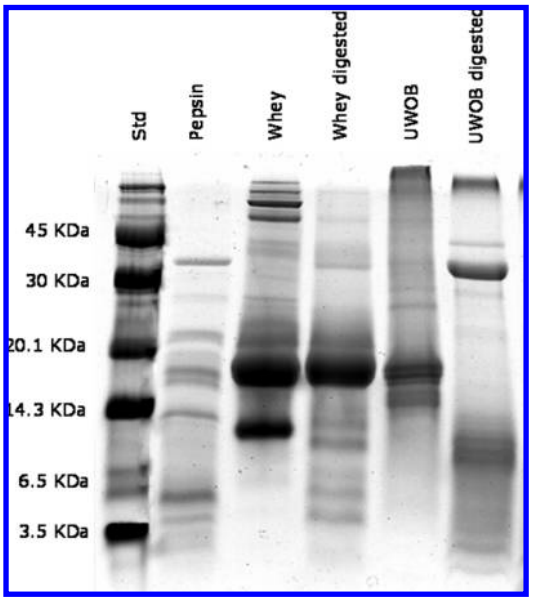

Figure 2. SDS-PAGE of proteins in whey protein isolate stabilized emulsion and purified oil bodies, UWOB (urea-washed oil bodies), before and after digestion with pepsin solution.

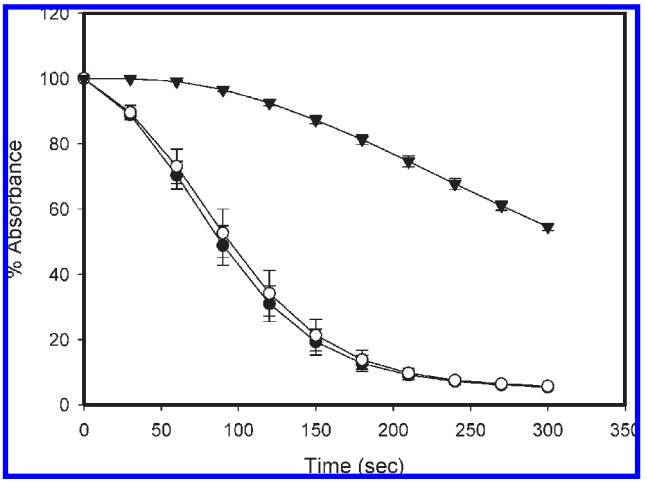

Figure 3. Comparative lipase hydrolysis of Tween 20 stabilized emulsion (●) whey protein isolate stabilized emulsion (○) and oil bodies (▼) all containing 0.05% w/v oil in the presence of bile salt (25 mmol L^{-1} sodium deoxycholate).

Table 1. Bioaccessibility of α-Tocopherol during Simulated Digestion of Emulsion and Oil Body Suspensions ($n = 3$, ± SD)

emulsion	available tocopherol[a] (μg)	released tocopherol[b] (μg)	bioaccessibility[c] (%)
Tween 20	299.0 ± 15.0 e	106.0 ± 12.0 e	35.0 ± 5.7 e
whey	307.0 ± 6.0 e	52.0 ± 2.9 f	17.0 ± 1.3 f
oil body	252.0 ± 11.0 f	1.5 ± 0.3 g	0.6 ± 0.1 g

[a] Total tocopherol per assay. Values within columns bearing different letters are significantly different ($P < 0.05$, ANOVA). [b] Total tocopherol per assay released into micellar phase. Values within columns bearing different letters are significantly different ($P < 0.05$, ANOVA). [c] Percent of total tocopherol per assay released into micellar phase. Values within columns bearing different letters are significantly different ($P < 0.05$, ANOVA).

terms of overall digestion, the potential implications of this would be a decrease in the available surface area for lipase activity, both in the stomach by gastric lipase and once the emulsion has been transferred from the stomach into the small intestine. This may result in a slower release of lipids and associated lipophilic compounds, for example, vitamin E from the emulsion into the micellar phase of the gut digestate.

The rate of pancreatic lipase induced emulsion destabilization was significantly slower for oil bodies than for the processed emulsions. This is consistent with the finding of Beisson et al. (*32*), who demonstrated that the specific activity of lipase on almond oil bodies, using the pH-stat technique, ranged from 18 to 38% of the specific activity on almond oil emulsified with gum arabic, depending on the lipase source. In that study the emulsion and oil bodies compared were both "around 2 μm", suggesting particle size/surface area were not the reasons for differences found. Furthermore, these authors suggested that the phospholipid/protein coat of oil bodies is responsible for reducing lipase activity. They also concluded that oil bodies behave like other proteolipid organelles, such as milk fat globules, when they are considered as a substrate for lipolytic activity. Indeed, other authors have shown that the interfacial composition of emulsions would have an effect on the rate of enzymatic hydrolysis (*33*). The physicochemical properties of the lipid/water interface will affect the absorption of the enzyme and the catalytic cleavage of fatty acids from the triglyceride molecule (*34*). To fully elucidate the effect of lipase hydrolysis on oil bodies, a refined study utilizing reconstituted oil bodies of different sizes would need to be tested in comparison to emulsions stabilized with known emulsifying agents.

Bioaccessibility represents a measure of the amount of food component that is released from the food matrix and is available for absorption. For lipophilic components, the efficiency of micellar incorporation is a prerequisite for absorption. The bioaccessibility model used in this investigation is a modification of a technique used to measure carotenoid bioavailability from baby food test meals (*20*). Further adaptations of this model and indeed other similar in vitro techniques have been utilized to measure bioaccessibility of carotenoids, phenols, and tocopherols from formulated foods, fruits, vegetables, and nuts (*12*, *35*−*40*). In this study, modifications to the original protocol included the addition of a lipase solution as well as pancreatin, because preliminary experiments had demonstrated that this was required to provide comparative estimates of fatty acid/vitamin E bioaccessibility for emulsions and oil bodies (data not shown). The original protocol does not take into account the lipolytic activity of gastric lipase, which is secreted in the human stomach under acidic conditions and can account for the hydrolysis of 10−30% of ingested fats (*41*−*44*). Thus, the traditional model may have underestimated the bioaccessibility of lipophilic micronutrients. Furthermore, in this study, a specific bile salt (sodium deoxycholate) was used at a defined physiologically relevant concentration for postprandial levels of bile salts (8.3 mmol L^{-1}) (*42*) in preference to a crude bile extract used in the original study because vitamin E was barely detectable in the micellar fraction when the bile extract was used. All in vitro digestion studies should only be considered as "approaching" physiological conditions encountered in vivo and thus, although often well correlated, are no replacement for a human study. Subsequently, discussion here is mostly limited to relative comparisons between treatments.

In this study, the in vitro technique was used to measure the bioaccessibility of fatty acids and α-tocopherol from sunflower oil emulsions and oil bodies. The four fatty acids of interest here (linoleic, oleic, palmitic, and stearic) together account for >98% of the fatty acids in sunflower oil. The transfer of these fatty acids, in total or individually from emulsions to micelles, was significantly greater in the Tween 20 stabilized emulsion compared to the whey protein isolate stabilized emulsion and oil bodies. In fact, the transfer of fatty acids was significantly lower from oil

Table 2. Bioaccessibility of Fatty Acids during Simulated Digestion of Emulsion and Oil Body Suspensions ($n = 3$, ± SD)

	free fatty acid content in the micellar phase after digestion (mg)		
fatty acid	Tween	whey	oil body
palmitic (16:0)	19.0 ± 3.3	10.0 ± 0.4	4.2 ± 0.4
stearic (18:0)	7.3 ± 1.0	3.9 ± 0.2	2.0 ± 0.3
oleic (18:1)	54.0 ± 12.0	33.0 ± 1.6	9.4 ± 0.6
linoleic (18:2)	153.0 ± 26.0	102.0 ± 4.9	22.0 ± 1.5
total per assay (mg)	233.0 ± 42.0	149.0 ± 7.3	38.0 ± 1.7
bioaccesibility[a] (%)	52.0 ± 9.1	33.0 ± 1.7	8.4 ± 0.4

[a] Calculated on the basis of each emulsion containing 450 mg of total fatty acid per assay.

Table 3. Fatty Acid Composition of the Oil before Digestion and of the Free Fatty Acids in the Micellar Phase after Digestion

		free fatty acid composition in the micellar phase after digestion[a] (rel mol %)		
	fatty acid composition in parent oil (rel mol %)	Tween	whey	oil body
palmitic	5.3	8.2 ± 1.4	6.7 ± 0.3	11.1 ± 1.0
stearic	2.4	3.1 ± 0.5	2.6 ± 0.2	5.3 ± 0.8
oleic	23.0	23.2 ± 5.1	22.1 ± 1.1	24.7 ± 1.6
linoleic	69.8	65.7 ± 11.1	68.5 ± 3.2	57.9 ± 3.9

[a] Values for free fatty acid composition in the micellar phase after digestion are calculated from the results shown in **Table 2** from triplicate samples.

bodies compared to both artificial emulsions. The lower micellar incorporation of fatty acids from oil bodies witnessed in this study is likely a result of reduced action of lipase on the intact oil bodies (supported by the lipase catalysis experiment), which is a prerequisite for the transfer of free fatty acids to micelles. This may have been compounded by a reduction in surface area of oil bodies during the pepsin digestion stage (see **Figure 1**). In all three emulsion systems the relative bioaccessibility of palmitic and stearic fatty acids is greater than that of oleic and linoleic fatty acid (see **Table 3**). This is not surprising given that pancreatin contains a 1,3-specific lipase and that the unsaturated fatty acids are predominant at the sn-2 position of the triacylglycerol molecule. This profile of fatty acid release into the micellar phase is most acute in the oil body preparation. It is not clear at this stage why this should be, given that the oil bodies, and the oil used to make the processed emulsions, come from the same batch of sunflower seeds. Perhaps oil bodies have a nonrandom distribution of triacylglycerol molecules, with higher melting point molecules enriched just below the half-unit phospholipid bilayer, either to provide some structural benefit to the oil body or as a result of its the thermal history.

The oil bodies contained significant levels of α-tocopherol (ca. 81% of the amount in processed emulsions) and therefore can be justifiably compared to the processed emulsions in the bioaccessibility investigations. The percentage transfer of vitamin E from the emulsions to the micellar phase was highest ($P < 0.05$) for the Tween 20 stabilized emulsions, followed by the whey protein isolate stabilized emulsions, and was significantly lower for oil bodies. These results are in agreement with those of micellar incorporation of fatty acids and support the notion that oil bodies are not digested as rapidly as the artificial emulsions under the conditions used in this investigation. The bioaccessibility of vitamin E is highly dependent on the food matrix due to different locations within the matrix, different physiochemical properties, and the influence of associated compounds (*12*). In our study, however, a very simple food model has been compared, that is, an oil in water emulsion. Beyond the decrease in the surface area to volume ratio of oil bodies after pepsin digestion (which as a consequence could slow the rate of oil body digestion), it is unclear at this stage what other surface factors could also contribute to the relatively low percent bioaccessibility of tocopherol from oil bodies compared with the processed emulsion.

When all of the experimental results are considered together, and if they hold true in vivo, it would suggest that oil bodies are not as rapidly digested as similar processed emulsions with different surface properties. Potentially, this would lead to a reduced rate of absorption of lipids and associated lipophilic compounds that require micellar incorporation prior to intestinal absorption. However, Armand et al. (*42*) have shown that differences in the digestion of emulsions with different droplet sizes by gastric lipase and duodenal lipolysis do not necessarily result in overall differences in fat assimilation in healthy subjects because of efficient fat digestion by pancreatic lipase in the small intestine. This may be true, but what is also emerging from the literature is that the rate of digestion of lipids affects gastrointestinal tract physiology (*45*, *46*). Slower digestion of fats can result in increased satiety; therefore emulsion properties that promote slower digestion are of fundamental and applied interest and could be effectively employed to help reduce calorie intake.

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