JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Extraction and Characterization of Oil Bodies from Soy Beans: A Natural Source of Pre-Emulsified Soybean Oil

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Soybeans contain oil bodies that are coated by a layer of oleosin proteins. In nature, this protein coating protects the oil bodies from environmental stresses and may be utilized by food manufacturers for the same purpose. In this study, oil bodies were extracted from soybean using an aqueous extraction method that involved blending, dispersion (pH 8.6), filtration, and centrifugation steps. The influence of NaCl (0–250 mM), thermal processing (30–90 °C, 20 min) and pH (2–8) on the properties and stability of the oil bodies was analyzed using ζ-potential, particle size, and creaming stability measurements. The extracted oil bodies were relatively small ($d_{32} \approx 250$ nm), and their ζ-potential went from around +12 mV to −20 mV as the pH was increased from 2 to 8, with an isoelectric point around pH 4. The oil bodies were stable to aggregation and creaming at low (pH = 2) and high (pH $\geq$ 6) pH values but were unstable at intermediate values (3 $\leq$ pH $\leq$ 5), which was attributed to their relatively low ζ-potential. The oil bodies were stable to aggregation and creaming at relatively low salt concentrations (NaCl $\leq$ 25 mM, pH 7) but were unstable at higher values as a result of electrostatic screening effects. The oil bodies were stable to thermal processing from 30 to 90 °C (0 mM NaCl, pH 7), but there appeared to be a change in their interfacial properties (decrease in ζ-potential) at temperatures exceeding 60 °C. These results suggest that oil bodies extracted from soybeans have similar or improved stability compared to soybean oil emulsions produced from bulk ingredients and may provide a new way of creating functional soy products for the food industry.

KEYWORDS: Soybean; oil; emulsion; oil bodies; oleosin; extraction

INTRODUCTION

Soybeans are one of the most important bean sources in the world, providing vegetable protein and oil for millions of people and supplying important functional ingredients to the food, health care, pharmaceutical, and chemical industries (*1*). Soybeans have been widely consumed as a traditional food in Asia, but foods such as soymilk and tofu are also increasingly consumed in other parts of the world. Research has shown that soybean foods may provide protection against heart disease, cancer, cardiovascular disease, and osteoporosis (*2, 3*).

Industrially, edible oils are usually extracted from oilseeds using organic solvents, such as hexane or isopropanol (*1, 4, 5*). Solvent extraction has persisted in the oilseed industry as the principal method of extraction of edible lipids because of high extraction efficiencies (high yields) and relatively low cost. On the other hand, volatile organic solvents contribute to industrial emissions, and some of them (such as hexane) can easily ignite, requiring expensive safety measures and extensive training to protect workers' health. Moreover, the relatively harsh processing conditions associated with solvent extraction processes can lead to severe degradation of protein functionality (*6–8*). Consequently, there has been a tendency to develop alternative extraction processes based on nontoxic, nonvolatile solvents that can simultaneously extract oil and protein, without adversely affecting their functionality, including supercritical carbon dioxide and aqueous-based extraction (*4, 7, 8*). A number of studies have reported that aqueous based extraction processes that utilize enzymes are particularly efficient for simultaneous extraction of both oil and proteins (*9–17*).

Traditionally, oil extracted from soybeans is refined, purified, and sold to the food industry as a bulk liquid where it is utilized in a variety of products (*1*). Nevertheless, for certain applications there may be advantages in developing extraction processes that preserve the natural state of the soybean oil in the seed, that is, in the form of oil bodies that are coated by a layer of phospholipids and oleoresin proteins (*18–23*). In nature, the

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10.1021/jf071008w CCC: $37.00
Published on Web 09/20/2007

oleosin proteins provide the oil bodies with physical and chemical protection against environmental stresses, such as moisture and temperature fluctuations and the presence of oxidative reagents. In principle, food manufacturers could benefit from the preexisting natural protection of the soybean oil bodies to obtain a product that has improved stability during food processing, storage, transport, and utilization. The oil bodies extracted from soybeans could be used in food products in place of emulsified soybean oil, for example, in dressings, sauces, dips, beverages, and desserts. Additional advantages of using natural soybean oil bodies in foods, rather than emulsified bulk soybean oil, are that neither emulsifiers nor homogenization procedures are required.

The successful utilization of soybean oil bodies in food products requires a thorough understanding of their functional performance under different environmental conditions. The objective of this work was therefore to determine the influence of pH, ionic strength, and thermal processing on the properties and stability of oil bodies extracted from soybeans using an aqueous-extraction method.

MATERIALS AND METHODS

Materials. High fat soybeans (code: 5601T) were obtained from the Crops laboratory at the University of Tennessee (Knoxville, TN). Analytical grade hydrochloric acid (HCl) and sodium hydroxide (NaOH) were purchased from the Sigma Chemical Company (St. Louis, MO). All other chemicals were obtained from Fisher Scientific (Pittsburgh, PA). Distilled and deionized water from a Nanopure water system (Nanopure Infinity, Barnstead International, IA) was used for the preparation of all solutions.

Isolation of Soybean Oil Bodies. Oil bodies were physically isolated from a total homogenate of mature soybean seeds using an aqueous-based flotation–centrifugation method (*24*). Briefly, soybeans (45 g) were soaked in a buffer solution (10 mM Tris-HCl buffer, pH 8.6) overnight at 4–6 °C. Soaked beans (100 g) were homogenized in 200 mL of buffer solution (3 mM $MgCl_2$ and 100 mM Tris-HCl, pH 8.6) using a commercial food processor (DLC-1941TX, Cuisinart, Shelton, CT) for 20 s at the low speed setting and then for 40 s at the high speed setting. The soybeans were further homogenized with a high speed blender (Bio Homogenizer M133/1281-0, Bio Spec Products Inc., Bartlesville, OK) for 1 min at the high speed setting. The slurry was filtered through three layers of cheesecloth, and then the remaining filter cake was pressed. Typically, about 40–45 g of filter cake remained in the filter. The filtrate was collected and then centrifuged at 30 000 rpm for 20 min in 4 × 70 mL centrifuge tubes (Sorvall A-641 rotor, SORVALL ULTRA 80, Thermo-scientific, Waltham, MA). The resulting cream layer was collected and suspended in a chilled buffer solution (10 mM Tris-HCl buffer, pH 8.6) using a Wheaton Potter Elvehjem tissue grinder (Safe-Grind 55 mL, Fisher Scientific) with an 8 in. drill press (5-speed model 8050, Tradesman). The homogenized sample was then returned to a clean centrifuge tube and the centrifugation repeated. Cream layer collection, suspension, and centrifugation constituted one complete wash cycle, and for all samples, a total of three wash cycles were carried out. The final cream layer (sometimes referred to as the "pad") was stored at 4 °C for a maximum of 2 days prior to use. Typical yields were approximately 8–9 g (wet weight) of purified oil bodies from 45 g of seeds.

Characterization of Oil Body Composition. The proximate analysis of the soybean variety (5601T) used in this study has previously been determined (*25*): moisture = 14.7%, fat = 20.9%, protein = 40.4%, and ash = 5.4 ± 0.2% (the ash content was determined in our laboratory using a muffle furnace, at 500 °C for 5 h). The composition of the cream layer extracted from the soybeans was determined by proximate analysis (*26*): moisture = 46.7 ± 0.3%, ash = 0.328 ± 0.002%, fat = 40.1 ± 1.4%, protein = 8.8 ± 0.6%, and extraneous matter = 4.1 ± 1.6%. The moisture content was determined by oven drying (105 °C for 5 h) the ash content using a muffle furnace (500 °C for 5 h), the fat content by using the Soxhlet method (hexane extraction for 13 h), and the protein content by using the Kjeldahl method. The extraneous matter content was determined by difference: 100 – %fat – %protein – %moisture – %ash. The extraneous matter was likely to have been carbohydrates and/or phospholipids. The mass ratio of oil/protein in the initial soybeans was 0.52:1, whereas that in the cream layer was 4.6:1, indicating that most of the protein was removed by the extraction process. The total yield of oil from the original soybeans was 36%, which indicated that the majority of oil was not extracted using this method and remained in the filter cake. The purpose of this study was to isolate and characterize the properties of oil bodies, rather than to optimize the efficiency of the aqueous extraction process. Nevertheless, optimization of the extraction procedure to obtain a greater fraction of the oil bodies from the soybeans would be a valuable subject for future work, particularly if this procedure was going to be used industrially.

Preparation of Oil Body Suspensions for Environmental Stress Tests. The influence of pH, ionic strength, and heating on the properties of the oil body suspension extracted from the soybeans was examined. An oil body suspension was prepared by mixing 10 g (wet weight) of cream layer with 30 g of buffer solution (10 mM sodium phosphate, pH 7) using the Wheaton Potter Elvehjem tissue grinder. Then 1 g of oil body suspension was diluted with 9 g of buffer solution of known pH and salt concentration (10 mM sodium phosphate, pH 2–8, 0–150 mM NaCl). The pH of the oil body suspension was adjusted back to the desired value using either 0.1 M HCl or NaOH. The oil body suspension was then stored at room temperature for 24 h prior to ζ-potential, light scattering, and creaming stability analysis.

For thermal processing studies, oil body suspensions (10 g) were transferred into glass tubes, stored in a water bath at a fixed temperature (30–90 °C for 20 min), cooled to room temperature, and then stored at room temperature prior to analysis.

ζ-Potential Measurements. Oil bodies were diluted to a concentration of approximately 0.005 wt % oil using a buffer solution to avoid multiple scattering effects. Diluted suspensions were injected directly into the measurement chamber of a particle electrophoresis instrument (ZEM 5003, Zetamaster, Malvern Instrument, Worcester, U.K.). The ζ-potential was then determined by measuring the direction and velocity of the oil body movement in an applied electrical field. The ζ-potential measurement was reported as the average and standard deviation calculated from measurement of at least two freshly prepared samples, with five readings taken per sample. ζ-potential measurements were made after the oil body suspensions were stored for 24 h at room temperature.

Particle Size Analysis. The oil bodies were diluted to an oil concentration of approximately 0.006% using buffer solution to avoid multiple scattering effects. The particle size distribution of the oil bodies was measured using a laser light scattering instrument (Mastersizer X, Malvern Instruments Ltd., Malvern, U.K.). This instrument measures the angular dependence of the intensity of laser light scattered by dilute oil bodies and then finds the particle size distribution that gives the best agreement between theoretical predictions and experimental measurements. A refractive index ratio of 1.08 was used by the instrument to calculate the particle size distributions. Measurements are reported as the surface-weighted ($d_{32} = \sum n_i d_i^3 / \sum n_i d_i^2$) mean particle diameter, where n_i is the number of droplets of diameter d_i. The particle size measurements are reported as the average and standard deviation of measurements calculated from a minimum of two freshly prepared samples, with two readings taken per sample. Particle size measurements were made after the oil body suspensions were stored for 24 h at room temperature.

Optical Microscopy. The microstructure of selected oil body suspensions was determined using optical microscopy (Nikon microscope Eclipse E400, Nikon Corporation, Japan). Oil body suspensions were gently agitated in a glass test tube before measurement to ensure that they were homogeneous. A drop of the oil body suspension was then placed on a glass slide and observed under the microscope at a magnification of 100× . Images of oil bodies were acquired using digital image processing software (Micro Video Instruments Inc., Avon, MA). Optical microscopy measurements were made after the oil body suspensions were stored for 24 h at room temperature.

Creaming Stability Measurement. Ten grams of oil body suspension was transferred into a glass test tube (internal diameter 15 mm,

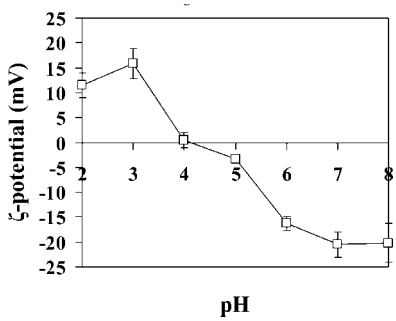

Figure 1. Dependence of the particle electrical charge (ζ-potential) of oil bodies extracted from soybean on pH.

height 125 mm), tightly sealed with a plastic cap, and then stored at room temperature for 7 days. After storage some of the suspensions separated into a number of layers: a "cream" layer at the top, a "suspension" layer in the middle, and a "serum" layer at the bottom. These layers could be distinguished by their visual appearance: the cream layer was optically opaque and whiter than the original oil body suspension, the suspension layer had the same appearance as the original oil body suspension, and the serum layer was either slightly turbid or transparent. The total height of the oil body suspension (H_T), the height of the serum layer (H_{SL}), and the height of the cream layer (H_{CL}) were measured. The extent of creaming was characterized by creaming indices, which were defined as $CI_{SL} = 100 \times H_{SL}/H_T$ for the serum layer and $CI_{CL} = 100 \times H_{CL}/H_T$ for the cream layer. The creaming indices provided indirect information about the extent of droplet aggregation in an oil body suspension, for example, the higher the creaming indices, the greater the particle aggregation.

Statistical Analysis. Experiments were performed at least twice using freshly prepared samples. Average and standard deviations were calculated from these measurements.

RESULTS AND DISCUSSION

Influence of pH on Oil Body Properties. The purpose of this series of experiments was to determine the influence of pH on the properties of oil bodies extracted from soybean. The electrical charge, particle size distribution, mean particle diameter, and creaming index of extracted oil bodies were measured (**Figures 1**–**3**). These measurements may provide important insights into the composition of the interfacial layer surrounding the oil bodies and also yield guidelines as to the type of food matrices (e.g., acid, neutral) in which oil bodies could be successfully utilized.

The ζ-potential of the oil bodies changed from around −20 mV at pH 8 to around +12 mV at pH 2, with the point of zero charge being around pH 4. This kind of behavior is consistent with protein-stabilized lipid droplets (*27*–*29*) and suggests that the oleosin protein coating remained around the oil bodies after the aqueous extraction procedure. Previous studies have found that the isoelectric point of oil bodies extracted from various plant sources was around pH 5–6 (*22*, *30*–*32*), which is about 1 to 2 units higher than the value determined in our study. The lipid body isolation procedure used in this study results in lipid bodies that contain olesin as well as other poorly characterized proteins. The lower isoelectric point of the lipid bodies in this study could be due to the formation of negatively charged surface active lipids. This could occur if some of the proteins associated with the lipid bodies were enzymes that could degrade lipids. For instance, soybeans contain phospholipases that can hydrolyze the functional groups of phospholipids (*33*). This could result in the conversion of phosphatidylcholine to phosphatidic acid resulting in an increase in negative charge. In addition, soybeans contain lipases that could hydrolyze lipids to produce free fatty acids that would also migrate to the interface of the oil bodies where they could alter the isoelectric point. Nevertheless, further work is required to establish the potential role of enzyme activity on the surface charge properties of oil bodies extracted from soybeans.

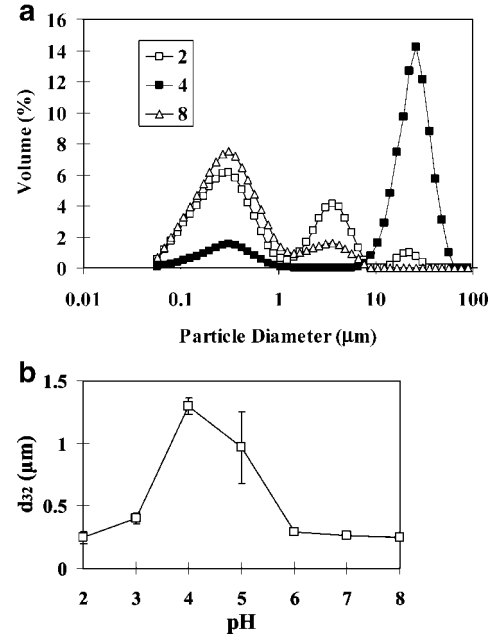

Figure 2. **a**. Particle size distribution of oil body suspensions at selected pH values. **b**. Dependence of the mean particle diameter (d_{32}) of oil bodies extracted from soybean on pH.

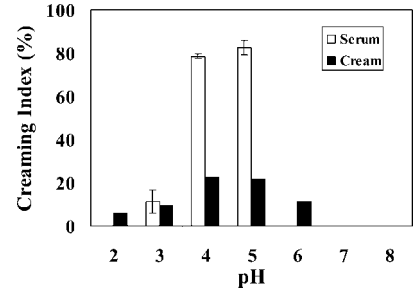

Figure 3. Dependence of the creaming stability of oil body suspensions from soybean on pH. The relative heights shown are those of the serum layer (clear or turbid) and the cream layer (white) observed in the oil body suspensions after 1 week of storage at room temperature.

Particle size distribution measurements indicated that the majority of particles in the oil body suspensions were relatively small at pH values well away from the isoelectric point (**Figure 2a**). At pH 2 and 8, there was a large population of particles around 0.3 μm (>68 vol % at pH 2 and >85 vol % at pH 8) and another smaller population of particles around 3 μm. Optical microscopy observations indicated that these large particles were individual oil bodies (data not shown). At pH 4, the majority of the particles were extremely large, with >80 vol % having diameters above 10 μm (**Figure 2a**). The mean particle diameters (**Figure 2b**) and creaming indices (**Figure 3**) were relatively low for the oil body suspensions at low pH (pH 2) and high pH (pH 7 and 8), that is, $d_{32} < 0.30$ μm, $CI_{SL} = 0\%$, and $CI_{CL} < 10\%$. On the other hand, there was a large increase in mean particle diameter and rapid creaming in the oil body suspensions at intermediate pH values (pH 4 and 5). The oil body suspensions at pH 3 and 6 were only marginally stable, with a relatively low mean particle diameter but some evidence of serum or cream layer formation after 1 week of storage (**Figure 3**). These observations can be attributed to the pH

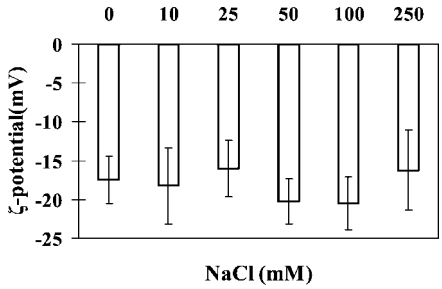

Figure 4. Salt dependence of the particle electrical charge (ζ-potential) on oil bodies extracted from soybean.

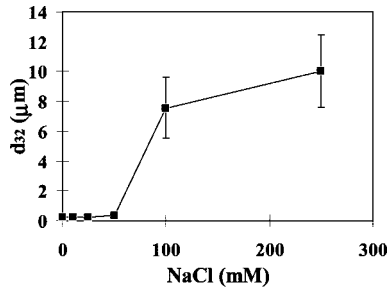

Figure 5. Salt dependence of the mean particle diameter (d_{32}) of oil bodies extracted from soybean.

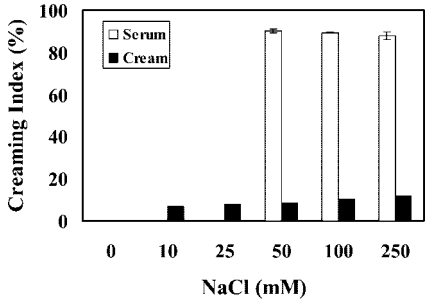

Figure 6. Salt dependence of the creaming stability of oil body suspensions from soybean. The relative heights shown are those of the serum layer (clear or turbid) and the cream layer (white) observed in the oil body suspensions after 1 week of storage at room temperature.

dependence of the electrostatic interactions between the oil bodies. At pH values far away from the isoelectric point of the oil bodies, there is a relatively strong electrostatic repulsion between the oil bodies that prevents them from aggregating (*34*). On the other hand, at pH values close to the isoelectric point, the oil bodies only have a weak electrostatic repulsion between them and so they tend to aggregate (*27, 35*). Optical microscopy observations indicated that these large particles were irregularly shaped flocs (data not shown).

Influence of NaCl on Oil Bodies. The purpose of these experiments was to examine the influence of salt on the stability of the oil body suspensions because this would provide guidelines on the type of food matrixes where oil bodies could be successfully utilized in the food industry. After preparation, oil bodies were stored at room temperature in the presence of salt (0–250 mM NaCl, pH 7.0), then their electrical charge and mean particle diameter were measured after 24 h of storage (**Figures 4** and **5**), and their creaming stability was measured after 1 week of storage (**Figure 6**).

The ζ-potential of the oil bodies was relatively insensitive to salt concentration (**Figure 4**); that is, it remained virtually unchanged at -18 ± 2 mV regardless of added NaCl concentration (0–250 mM). Usually the ζ-potential of colloidal suspensions decreases with increasing ionic strength

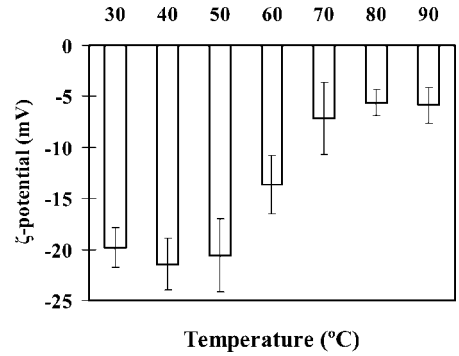

Figure 7. Dependence of the electrical charge (ζ-potential) on thermal treatment of oil bodies extracted from soybean (measured after cooling to room temperature).

because of electrostatic screening effects (*34*). The relative insensitivity of the ζ-potential of oil bodies to the addition of NaCl may be due to the presence of some endogenous salt in the system or because of charge regulation effects. For example, the addition of monovalent cations (Na^+) may partially displace any divalent cations (Mg^{2+} or Ca^{2+}) associated with the anionic oil body surface at neutral pH, thereby counterbalancing the expected decrease in negative charge. The mean particle diameters of the oil bodies were relatively small ($d_{32} < 0.30$ μm) at NaCl concentrations ≤ 25 mM but increased appreciably when the salt concentration exceeded 50 mM (**Figure 5**). In addition, the oil body suspensions were relatively stable to creaming at NaCl concentrations ≤ 25 mM but formed a distinct serum and cream layer at higher salt concentrations (**Figure 6**). These results suggest that the oil bodies have relatively poor stability to aggregation in the presence of salt. The most likely explanation for this phenomenon is the ability of salt to screen the electrostatic repulsion between the oil bodies (*34*). The fact that the oil bodies only have a relatively low ζ-potential (<20 mV) may account for the fact that only a relatively small amount of salt (25–50 mM) is required to induce oil body aggregation and creaming instability.

Influence of Thermal Processing on Oil Bodies. Thermal treatment of foods is a commonly used processing operation in the food industry, for example, pasteurization, sterilization, baking, and so forth. Consequently, we examined the influence of thermal processing on the stability of the oil body suspensions. Oil body suspensions were held at temperatures ranging from 30 to 90 °C for 20 min, cooled to room temperature, and then stored at room temperature before analysis of ζ-potential, mean particle diameter, and creaming stability.

There was a pronounced decrease in the magnitude of the electrical charge on the oil bodies when they were heated above 70 °C, with the ζ-potential falling from around −20 mV at 30 °C to −7 mV at 90 °C (**Figure 7**). This decrease suggested that there was some alteration in the composition and/or structure of the interfacial layer surrounding the oil bodies. However, heating caused no significant change in the mean particle diameter of the oil bodies, with $d_{32} = 0.23 \pm 0.01$ μm from 30 to 90 °C. In addition, there was no evidence of serum or cream layer formation in any of the heated emulsions (data not shown). The good thermal stability of oil bodies has previously been demonstrated for both natural and artificial oil bodies from various plant sources (*36*). This good thermal stability suggests that heating does not cause an appreciable increase in the surface hydrophobicity of the oil bodies, as occurs in globular protein-stabilized lipid droplets (*37, 38*). The molecular origin of this effect may be

that oleosin proteins are proposed to have a "T"-shaped structure, with a hydrophilic head (the horizontal top part of the T) and a hydrophobic tail (the vertical stem part of the T). The hydrophilic head is exposed to water and believed to lie flat against the phospholipid–water interface that surrounds the oil bodies, whereas the hydrophobic tail penetrates through the phospholipid layer and into the triacylglycerol core of the oil bodies (*22*, *23*, *32*). Presumably, upon heating, this hydrophobic tail remains in its highly nonpolar environment and is not exposed to water.

Conclusions. This work has shown that stable oil bodies can be extracted from soybeans using an aqueous extraction method. The electrical properties and aggregation stability of the extracted oil bodies were determined as a function of pH, ionic strength, and thermal processing because these environmental stresses are commonly encountered in the food industry. The oil bodies proved to be stable to aggregation at pH values sufficiently far from their isoelectric point (pH 4) at relatively low salt concentrations (<50 mM), and they were stable to thermal processing (≤90 °C). Oil bodies extracted from soybeans and other plant sources may prove to be a useful new source of lipids for application in the food industry because they avoid solvent extraction processes and the necessity to homogenize bulk lipids to form emulsions. In addition, there is no need to utilize an emulsifier to prepare them, as there is for emulsified soybean oil. Finally, food manufacturers may be able to utilize the natural protection system inherent within oil bodies to stabilize the oil phase against chemical or physical degradation in foods.

ACKNOWLEDGMENT

We thank Dr. Vince Pantalone at the Department of Plant Sciences, University of Tennessee, for supplying the soybean samples.

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Received for review April 5, 2007. Revised manuscript received July 20, 2007. Accepted August 2, 2007. We thank the Tokyo University of Marine Science and Technology (Japan) for financial support of D.I. This material is based upon work supported by the Cooperative State Research, Extension, Education Service, United States Department of Agriculture, Massachusetts Agricultural Experiment Station (Project No. 831) and a United States Department of Agriculture, CREES, NRI Grant (Award No. 2005-01357).

JF071008W