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Aqueous Extraction of Oil Bodies from Maize Germ (*Zea mays*) and Characterization of the Resulting Natural Oil-in-Water Emulsion

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Oil bodies in the form of an adequately dispersed suspension were extracted from maize germ by applying aqueous extraction. The effect on the yield of oil body extraction of parameters, such as pH of aqueous medium, state of germ comminution, and number of successive extraction steps applied, was evaluated, indicating that an extraction yield as high as 95% could be reached when a finely comminuted germ material is extracted 3 times under alkaline conditions. The extracted oil body suspension was coagulated by pH manipulation, and the resulting cream, consisting of intact oil bodies, was studied with respect to protein composition, particle size distribution, and oil body ζ potential. Changes in particle mean diameter resulting during oil body cream storage as well as the creaming behavior of emulsions prepared by cream dilution with water were also investigated. The findings are discussed in terms of the presence at the oil body surface of an adsorbed mixed layer made up of phospholipids, oleosins, and extraneous germ proteins that determine the physical stability of oil droplets upon long-term storage.

KEYWORDS: Maize; germ; oil bodies; emulsion; stability

INTRODUCTION

Plants store lipids, mainly triglycerides, in the form of distinct spherical organelles called oil bodies or oleosomes that serve as energy stores to support active metabolism periods, such as seedling growth during germination (*1*). The oil bodies originating from the seeds of a number of plant species exhibit many structural and compositional similarities with regard to their triglyceride, phospholipid, and protein constituents (*2*, *3*). The remarkable stability of oil bodies against external physical and chemical stresses is attributed to a protective mixed surface layer surrounding the neutral lipid core. This layer is made up of phospholipids and a number of proteins with a relatively low molecular weight (15−26 kDa), called oleosins, as well as some other minor proteins, such as caleosin (*4*, *5*).

Many edible oils from oil-rich seeds are prepared by applying organic solvent (usually hexane) extraction, a process that is followed by refining of the extracted crude oil. Although this process is characterized by high extraction efficiency and low cost, the use of volatile organic solvents very often results in environmental pollution, while safety problems, because of organic solvent inflammability, are also a serious matter of concern. Recently, a number of researchers (*6*−*8*) have put forward the idea of extracting the oil from oil-rich plant seeds in the form of a natural emulsion by using aqueous extraction media. This approach has the obvious advantages connected with omitting a toxic and inflammable organic solvent, while the product of the aqueous extraction would be a physically stable dispersion system and could be exploited in the preparation of food products appearing in the form of oil-in-water emulsions.

Maize germ is a very rich source of lipids (*9*), and because of that, the byproduct resulting from maize processing (starch production or maize kernel milling) is exploited industrially for the extraction of corn oil. Because the lipids in the germ are in the form of oil bodies, their extraction with aqueous media is expected to result in a highly stable dispersion. The stability of maize oil bodies as well as those originating from other sources (*2*, *10*) is the consequence of the presence at the droplet surface of a layer of phospholipids in direct contact with the triglyceride molecules of the core and oleosins, which constitute 20% of the surface material. The hydrophobic middle section of the oleosin molecules at the surface is partly embedded in the phospholipid layer and partly in direct contact with the triglyceride molecules of the core, while the two amphipathic N and C terminals of the protein molecules are exposed to the aqueous phase. The high stability of oil bodies at neutral environments was associated with the negative charge of the droplet surface in combination with steric repulsive forces originating from the protruding hydrophilic parts of the oleosin molecule. According to the results of two very recently published studies, the stability against aggregation and creaming of oil bodies, extracted either from soybean (*6*) or sunflower seed (*8*), heavily depended upon the pH and ionic salt content of the dispersion aqueous phase, indicating that the surface charge is a crucial parameter in determining the oil body stability against aggregation and creaming. On the other hand, their remarkable stability against coalescence could be partly connected to the properties of the mixed phosholipid−oleosin

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layer at the surface that does not yield following the very close approach of two oil bodies.

Although the study of purified from various sources oil bodies is useful from the theoretical point of view, it is essential for practical reasons to extract the bodies at a high yield using aqueous media containing chemicals that are permitted for food processing. In this way, the resulting natural emulsions may contain extraneous seed proteins, which, when present at the oil droplet surface, may significantly affect their physicochemical properties. Thus, the physical stability of oil bodies may to some extent depend upon the presence of adsorbed proteins present at the outer droplet surface along with the oleosins. Because the nature and concentration of these proteins at the surface may differ depending upon the oil body source and, possibly, the extraction conditions, natural emulsions based on oil bodies are expected to exhibit appreciable differences in their physicochemical properties, depending upon oil body origin.

The aim of this study was to assess the possibility of exploiting maize germ to prepare physically stable natural oil-in-water emulsions. An initial target of the work was to optimize the conditions of aqueous oil body extraction and obtain a concentrated oil body cream at the highest possible yield. The investigation was completed by studying the key physicochemical properties of oil-in-water emulsions based on the extracted maize oil body cream.

MATERIALS AND METHODS

Materials. Mature and physically dried in the air maize seeds were harvested from maize plants (*Zea mays* L., Limagrain LG 2244) cultivated in a local farm situated in northern Greece. The seeds were first ground to a size lower than 8 mm with the aid of a domestic mill fitted with knives, operating for 3 min at 2800 rpm. A fraction enriched in germ material was then obtained using a sieve with a mesh size of 4 mm, and the intact germs (moisture content of 7.5%) were collected by hand. Part of the germ raw material was then subjected to milling, using a Brown mill fitted with knives, and the resulting flour was separated into two fractions by employing a sieve with a mesh size of 0.8 mm.

Isolation of Oil Bodies. The process of oil body isolation is divided into two steps: an alkaline extraction step resulting in an aqueous oil body dispersion and a coagulation step that leads to a concentrated oil body cream. Extraction of oil bodies was effected by a method based on the modification of the process applied by previous investigators (*1*, *6*, *8*). The intact germ material or the two comminuted germ fractions (100 g) was initially soaked in deionized water, in a ratio of 1:5 (w/v), for 24 h at room temperature. During this time period, the mixture was constantly agitated with the use of a mechanical stirrer (Kika Labortechnik, Malaysia), at 1200 rpm, while the pH was adjusted and kept constant at 3, 6, or 9 using 0.1 M HCl or NaOH solutions. The mixture was then subjected to intensive agitation (speed set at position 2) for 40 s by employing a Braun Blender (type 4249, Germany), and the resulting dispersion was filtered through a filter made up of three layers of cheesecloth. The extracted germ residue was then mixed again with deionized water (1:5), and the pH of the mixture was adjusted to 3, 6, or 9 using 0.1 M HCl or NaOH solutions and kept constant while continuously stirring for 2 h. At the end of this period, the mixture was again subjected to intensive agitation in the blender for 40 s and filtered through the cheesecloth filter. The oil body extraction procedure was repeated 1 more time. The yield of oil extraction was determined indirectly from the fat content of the extracted germ residue at the end of each one of the extraction steps and subtracted from the initial fat content of the germ.

To recover the oil bodies from the extract, the three extracted oil body dispersions were combined together, the resulting pooled dispersion was subjected 2 times to centrifugation (Firlabo SV11, France) at 3800*g* for 30 min, and the precipitated solid material at the end of each centrifugation step was discarded. On the basis of results of preliminary tests conducted to determine the optimum pH value for droplet flocculation and creaming, the recovery of the oil bodies in the form of a cream (the "pad") from the aqueous extract was then effected by adjusting the pH to 5 and centrifuging at 3800*g* for 30 min. The creamed layer at the top of the centrifuge tube was carefully removed and dispersed in deionized water (1:5), and the pH was adjusted to 8.5. After stirring for 1 h to break the aggregates, the pH value was again lowered to 5 and the dispersion was centrifuged at 3800*g*. The cream was then washed 1 more time with water, and the recovered final cream was analyzed for moisture, fat, and protein. The fat content of the cream and that of the initial germ material was then used to calculate the final yield of the oil body extraction and recovery from the extract processes, expressed as a percentage of the germ total crude oil content.

Determination of Protein, Fat, and Moisture. The determination of protein, fat, and moisture was conducted according to standard methods of the Association of Official Analytical Chemists (AOAC) (*11*). The moisture content of maize germ before and after oil body extraction as well as that of the final oil body cream was determined gravimetrically, following vacuum drying in an oven at 60 °C, for 24 h. Before the sample was placed in the oven, it was mixed with dry sand (previously heated at 105 °C until constant weight) to prevent sample agglomeration and moisture entrapment. The fat of the resulting dry sample was then extracted for 12 h with petroleum ether in a Soxhlet apparatus and quantified gravimetrically. The protein content of the samples was determined according to the Kjeldahl method.

Protein Electrophoresis. The adsorbed oil body surface maize germ protein was analyzed by sodium dodecyl sulfate−polyacrylamide gel electrophoresis (SDS−PAGE) (*12*) using 4.5 and 12% (v/w) acrylamide solutions for the stacking and separating gels, respectively. To solubilize the proteins, the oil body samples were treated with a 0.0625 M Tris buffer containing 2% (w/v) SDS, 10% (w/v) glycerol, 0.1% (w/v) bromophenol blue, and 5% β-mercaptoethanol. After boiling for 2 min and the application of two freeze−thaw cycles, the subnatant containing the protein (3−4 mg/mL) was recovered by centrifugation and applied on the electrophoresis gel. Protein fractions were fixated by immersing into a 12.5% (w/v) trichloroacetic acid solution. The gels were stained with Coomasie Brilliant Blue G-250 and photographed with the aid of a digital camera (Kodak DC3400). Determination of the protein constituent molar mass was performed with the aid of the Gel-Pro Analyzer (Media Cybernetics, Bethesda, MD).

Measurement of Oil Body ζ Potential. Oil body cream samples were diluted with deionized water to an approximate oil concentration of 0.01%, and the droplet aggregates were dispersed into single oil bodies by sonication for 15 min using an UP100H ultrasonic apparatus (Hielscher, Germany). The pH of the diluted sample was then adjusted using either 0.1 M HCl or NaOH; the dispersion was again sonicated; and the pH value was rechecked. The diluted oil body suspension was subjected to electrophoresis with a Lazer Zee Meter 501 (PenKem, Bedford Hills, NY) at 100 V in the cell of the instrument, while the droplets were visualized on a monitor.

Measurement of Oil Body Size Distribution. Oil body size distribution was determined with the aid of a laser light-scattering instrument (Malvern Mastersizer 2000, U.K.). The samples were analyzed in triplicate. Measurements were performed at room temperature following cream sample dilution with deionized water to an approximate oil content of 0.005% combined with either mechanical stirring for 5 min to determine the size of aggregates or intensive sonication for at least 15 min and frequency of 24 kHz, employing the UP100H ultrasonic processor to disperse the droplet aggregates completely and determine the average size corresponding to single oil bodies. Droplet size distribution measurements were conducted on both freshly extracted as well as aged oil body cream samples.

Evaluation of Emulsion Stability against Creaming and Aggregation. To assess the stability of oil bodies against creaming, the oil body cream was diluted with deionized water to obtain oil body suspensions with a 5 or 10% oil content and the pH was adjusted to 6.5 or 3.8. The suspension was then extensively sonicated for 20 min, and the pH of the sonicated samples was measured and readjusted to 6.5 or 3.8, when needed. A quantity of 10 mL of the dispersed single-droplet suspensions were then placed in sealed cylindrical vessels and left at room temperature while visually monitoring the serum volume separated with time. The height of the serum separated at the bottom of the vessel, expressed as a percentage of the total sample height, was plotted against storage time, indicating the development of the creaming process.

Statistical Analysis. At least three extraction series from the same batch of seeds were performed to assess the yield of oil body extraction (CV of 6.36%), while the total yield of the combined processes of oil body extraction and recovery from the extract was also measured at least in triplicate (CV of 7.17%). The physicochemical properties of the stock oil body cream and the oil-in-water emulsions based on cream dilution were evaluated in triplicate. Data were subjected to analysis of variance (one-way ANOVA) with the aid of SPSS 16 software. Variations among samples were evaluated by least significance difference (LSD) at the 5% level of probability ($p = 0.05$).

RESULTS AND DISCUSSION

Oil Body Extraction and Recovery. Data on the yield of the oil body extraction process, calculated indirectly from the difference between the fat content of the maize germ material before and after the aqueous extraction process, are presented in **Table 1**. As was expected, all of the three parameters studied, that is the state of germ comminution, the pH of the extraction medium, and the number of the successive extraction steps applied, appeared to influence to an appreciable extent the yield of aqueous oil body extraction. The lowest extraction yield value (56.8%) was obtained in the case of intact germ material, extracted at pH 6 by applying only one extraction step, while the highest extraction yield value (95.3%) was obtained in the case of the extensively comminuted material that was extracted 3 times at pH 9.

The influence of the degree of germ comminution on the yield of extraction should be explained on the basis of the fact that the greater degree of milling results in a higher solid particle surface area in contact with the water and, therefore, in the increase of the possibility of oil body aqueous extraction. Thus, the extraction yield reaches its highest values at any pH and number of extraction steps applied, when the germ particles are of a size lower than 0.8 mm. One matter of concern when subjecting the germ to milling prior to extraction is the possibility of oil body coalescence into droplets of a larger size. However, preliminary oil droplet size measurements showed that the mean diameter of oil bodies extracted from finely milled germ as determined with the Malvern sizer was not significantly different from that of oil bodies obtained from the intact germ material.

The pH of extraction also appears to have a significant influence on the yield, with higher yield values obtained when oil body extraction was performed at pH 9 and lower yield values obtained when oil body extraction was performed at pH 3, at any combination of the other two parameter values. As was reported by Tzen et al. (*1*), the oleosomes obtained from maize possess an isoelectric point close to 6 and, as a result, the oil bodies would be expected to be less dispersible in water and, hence, extracted to a lesser extent at pH 6 than at pH 3. The higher than expected dispersibility and extractability of maize oil bodies at pH 6 should be attributed to the presence at the surface along with oleosins of storage proteins. The presence of these extraneous proteins at the surface layer was also reported for oil bodies extracted from other oil-rich materials (*6*, *8*) and should constitute storage proteins that were not removed under the extraction conditions prevailing or were first extracted and subsequently adsorbed to the droplet surface. The presence of these more acidic proteins compared to oleosins, which are expected to form a secondary layer around the more tightly bound alkaline oleosins, is expected to alter the surface charge of the oil bodies, and it is rather these proteins and not the oleosins that determine the oil body dispersibility dependence upon pH. It is therefore not surprising that high yield values are also observed at pH 6, where the oil bodies, as a result of the accompanying storage proteins, are highly dispersible in water.

The yield of oil body extraction also depends upon the number of successive extraction steps applied to the germ material. This effect, although sometimes significant, is less pronounced in the case of the finely comminuted germ when the extraction is performed at pH 6 or 9, apparently because these conditions allow for a more effective initial oil body extraction when applying even only one extraction step.

Table 1. Influence of Extraction Parameters (State of Comminution, pH, and Number of Extraction Steps) on the Yield of Aqueous Oil Body Extraction from Maize Germ

sample	pH	number of extractions	yield (%)[a]
whole germ	6	1	56.8 a
		2	67.6 c, d
		3	80.2 f, g
	9	1	64.7 b, c
		2	80.1 f, g
		3	84.2 g, h, i
	3	1	68.8 c, d, e
		2	77.6 f
		3	86.7 h, i, j
germ flour (>0.8 mm)	6	1	60.6 a, b
		2	70.9 d, e
		3	83.3 g, h
	9	1	69.4 d, e
		2	84.8 g, h, i, j
		3	87.4 h, i, j, k
	3	1	78.1 f
		2	86.5 h, i, j
		3	89.6 j, k, l
germ flour (<0.8 mm)	6	1	64.2 b, c
		2	73.3 e
		3	88.6 i, j, k, l
	9	1	80.1 f, g
		2	89.1 j, k, l
		3	91.6 k, l
	3	1	81.4 f, g
		2	92.6 l, m
		3	95.3 m

[a] Values with different letters indicate significant differences ($p = 0.05$).

The oil body extract obtained from the more finely comminuted germ material (< 800 μm), by applying three successive extraction steps at pH 9, was selected, on the basis of the highest extraction yield, to recover the oil bodies in the form of a concentrated cream by lowering the pH value to 5 and extensively washing with deionized water at pH 8.5. The total yield of the combined processes of oil body extraction and recovery from the extract was 75.50%. This indicates that a significant part of the extracted oil bodies was lost during the recovery step possibly because not all of the oil bodies in the extract were aggregated at pH 5, and as a result, they did not cream to the top of the centrifuge tube. Nevertheless, this yield was much higher compared to the yield reported by Iwanaga et al. (*6*) for the extraction of oil bodies from soybeans, although the authors made it clear that optimization of the extraction process was not the main target of their work. In general, oil body extraction in the intact form from a number of seed sources, by applying the conventional extraction process, that is hydration of the material, mechanical grinding in a buffer, filtration, and finally, centrifugation, has the drawback of a relatively low oil body extraction yield. Kapchie et al. (*7*) reported that the final yield of soybean oleosome extraction and recovery can be significantly improved when the cell-wall tissue is disintegrated by mechanical or enzymatic treatment. In fact, treating the raw material with enzymes and applying three successive extraction steps led to a maximum yield of 84.65%, while the application of the conventional method resulted in oleosome yields lower than 45% of the total soybean fat. These workers, however, did not report whether enzyme treatment brought about an increase in oil body size, something

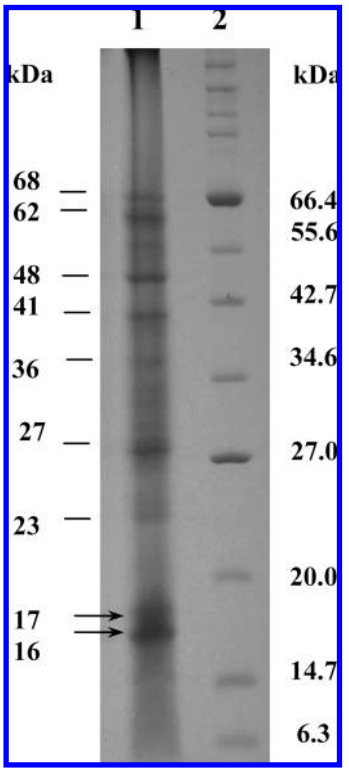

Figure 1. SDS−PAGE of proteins adsorbed at the surface of oil bodies obtained from maize germ (lane 1) and molecular-marker proteins (lane 2). Arrows point at zones with a molecular mass of 16 and 18 kDa possibly corresponding to oleosin proteins.

that may in turn result in the physical destabilization of the extracted natural emulsion upon storage or when subjected to processing treatments.

Oil Body Composition and Physicochemical Characteristics. The composition of the final oil body cream was moisture, 65.15%; crude fat, 26.24%; and total protein ($N \times 5.7$), 6.25%. On a dry weight basis, the protein constituted about 18% of the cream and the fat/protein mass ratio was about 5:1, a value very close to that reported by Iwanaga et al. (*6*) for soybean oleosomes obtained by the conventional method. The oleosins of the various seeds constitute, according to Tzen et al. (*2*), a much lower percentage of the total oil body and ranges between 1.5 and 3.5%, with the former corresponding to the oleosin of the maize oil bodies. White et al. (*8*) reported that the oleosin content of sunflower oil bodies is much higher and, on a dry weight basis, close 8%. It may, therefore, be hypothesized that an appreciable proportion of the protein of the oil bodies extracted from maize in this study is extraneous protein tightly adsorbed to the droplet surface that was not removed during the washing steps applied to the cream. **Figure 1** presents electrophoregrams for the protein constituents adsorbed to the oil body surface layer. The bands that correspond to protein constituents of molecular mass of 16 and 18 kDa could be attributed to proteins belonging to the oleosin fraction (*2*). These two proteins, according to the results of densitometric analysis of the electrophoresis zones, represent about 37% of the total proteins present in oil bodies. The rest of the bands corresponding to proteins of a higher molecular mass than the oleosins (between 23 and 67 kDa) should probably belong to the albumin and globulin fractions of maize germ (*12*), which are extracted under the alkaline conditions applied for oil body extraction. Proteins belonging to the the glutelin group have been, however, reported (*14*) to make about 25% of the total maize germ protein constituents, and because these proteins are

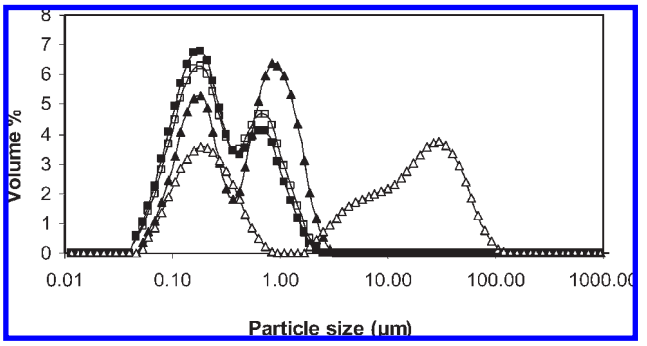

Figure 2. Particle size distribution of maize germ oil bodies in fresh and aged cream suspensions: (▲) cream stored for 1 day and mechanically dispersed in water, (■) cream stored for 1 day and dispersed in water by sonication, (△) cream aged for 25 days and mechanically dispersed in water, and (□) cream aged for 25 days and dispersed in water by sonication.

soluble in dilute alkali solutions, they should also be extracted with the oil bodies and probably adsorb to their surface. On the other hand, the percentage of proteins belonging to the prolamin fraction (zeins) in maize germ was found to be very low (*14*), and furthermore, these proteins are not expected to become extracted because they are only soluble in alcohol. As a result, the zeins are not expected to be found at the maize germ oil body surface. It may be concluded, therefore, that, although the proteins belonging to the albumin, globulin, and glutelin fractions do not constitute an integral part of the oil bodies in the germ structure, they are nevertheless tightly adsorbed to the surface of the extracted oleosomes and may affect the physicochemical properties of the oil body dispersion.

Extensive treatment of oil bodies with 9 M urea resulted in the partial removal of the extraneous proteins, as preliminary electrophoresis runs (data not shown) indicated. Washing with urea, however, resulted in the appearance of an oil layer at the top of the sample after only a few days of storage, suggesting that the extraneous proteins contributed along with the oleosins toward the stabilization of the maize oil bodies against coalescence. This observation, however, is in contrast to that made by White et al. (*8*), who reported that urea treatment did not affect the physical stability of oil bodies extracted from sunflower seed.

The surface area mean oil body size, $d_{3,2}$, was 0.30 μm, which is close to the size reported for other oil bodies extracted from either soybean (*6*) or sunflower seed (*8*). Particle size distribution data of fully dispersed oil body cream indicate that the largest part of the particles were of a very small size, while only a low fraction of particles were of a size around 1 μm (**Figure 2**).

The effect of pH on the ζ potential of maize oil bodies is shown in **Figure 3**. The ζ-potential value ranged from around + 35 mV at pH 3 to −40 mV at pH 7, while the point of zero was between pH 4.5 and 5. According to the results of previous studies (*1*, *2*), the zero charge point of the maize oil bodies is close to 6.2, a value 1.5 units higher than the one found in this study. Iwanaga et al. (*6*) also reported that the isoelectric points found for oleosomes extracted from soybeans were 1−2 pH units lower than those found by previous investigators. This was attributed by the authors to the possible formation of negatively charged surface-active lipids because of the degradation of lipids, such as phospholipids as a result of enzyme activity. Alternatively, the lower zero charge point of oil bodies could be connected to the presence at the surface of extraneous more acidic proteins compared to oleosins, which shield the oleosin surface layer and, as a result, bring about a reduction in the isoelectric pH value.

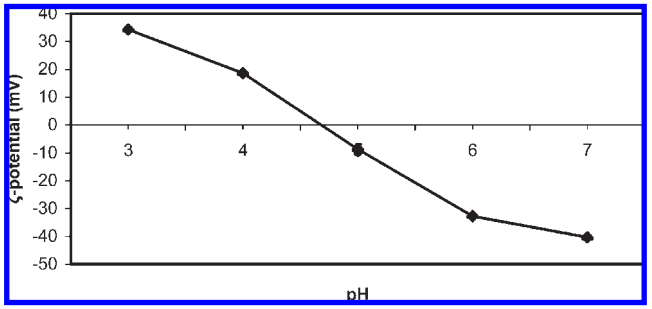

Figure 3. Dependence upon pH of the ζ potential of oil bodies extracted from maize germ.

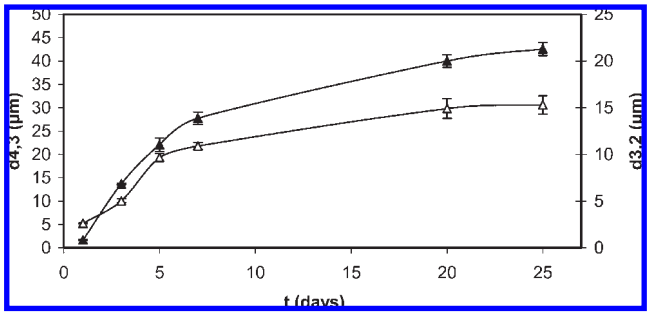

Figure 4. Development of the mean particle diameter, $d_{3,2}$ (△) and $d_{4,3}$ (▲), of a maize oil body cream with storage time.

Physical Stability of Oil Bodies and Oil-Body-Based Emulsions. After storage of oil body cream for a time period of up to 25 days, the droplets aggregated strongly and produced aggregates that resisted dispersion into single droplets, irrespective of surfactant (SDS) addition or the intensity and time of mechanical agitation applied (1400 rpm for at least 3 h). The mean particle diameters, $d_{3,2}$ and $d_{4,3}$, increased rapidly within the first 5 days of storage, while the rate of increase was much slower thereafter (**Figure 4**). This extensive oil body aggregation with storage was expected considering that the pH value of the cream was around 5, where the surface charge of the oil bodies is very low, leading to weak electrostatic repulsion and aggregation (*15*). The increase in particle size resulting from oil body coalescence might also have contributed to the increase of the mean particle size, taking into account the low oil body surface charge. As, however, shown in **Figure 2**, intensive sonication of the aged oil body suspension resulted in the complete dispersion of the aggregates into single droplets, as also verified with optical microscopy. As a result, in the case of the aged and sonicated emulsion, a mean droplet size, $d_{3,2}$, of a value very close to that corresponding to the fresh oil body cream, determined immediately after its recovery and easily dispersed by mechanical agitation only, was obtained (0.305 and 0.308 μm, respectively). These results indicate that the oil bodies in the relatively concentrated cream were very stable against coalescence, and because their surface charge was too low to protect the droplets, it is suggested that their high stability against coalescence was mainly connected to the mechanical properties of the protein membrane that surrounds the oil body triglyceride core. Considering that the oil and protein content of the cream is approximately 78 and 18%, respectively, on a dry weight basis and the mean surface volume diameter is about 0.3 μm, the surface protein load amounts to approximately 12 mg/m^2. It appears, therefore, that the protein surface layer is much thicker compared to the one corresponding to a protein monomolecular film, with a protein load of 1−2 mg/m^2. It is, apparently, this multiple surface layer that is made up of a primary mixed layer of phospholipids and partially embedded in the phospholipid layer

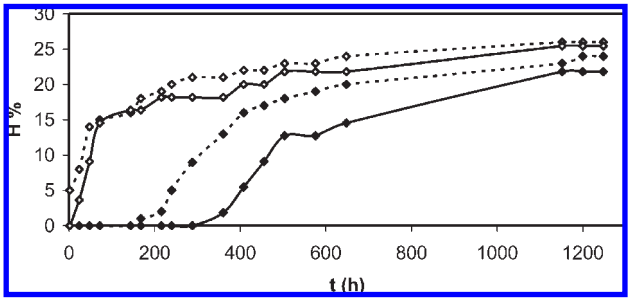

Figure 5. Creaming behavior of 5% (- - -) and 10% (—) oil body suspensions from maize germ at pH 6.5 (◇) and 3.8 (◆).

oleosin molecules and an external layer of extraneous germ seed proteins that protects the oil bodies from coalescing into ones with a larger size. In the case of the purified urea oil bodies, the surface layer is not thick enough to protect the oil bodies. As a result, oil separation at the top of the sample takes place during long-time storage.

An interesting observation regarding the stabilizing membrane around the oil bodies in the cream is that its structure presents many similarities to that of the adsorbed layer of the emulsions prepared with egg yolk. As in the case of oil bodies, the primary adsorbed film at the surface of egg-yolk-stabilized emulsion oil droplets is of a mixed nature made up of phospholipid as well as hydrophobic apolipoprotein molecules with a flexible structure. This layer is covered by a secondary much thicker layer made up of less surface-active protein molecules as well as protein aggregates that are intact or partially disrupted by micellar and granular supermolecular structures (*16*, *17*).

The oil body cream, despite its relatively low oil content (lower than 30%, on a wet basis), was very stable against creaming. This was probably due to the high protein content of the cream. The adsorbed protein molecules at the surface of two neighboring oil bodies may interact at a pH, where the surface charge is close to zero, possibly through hydrophobic forces, leading to the formation of a droplet network that does not yield under the influence of buoyancy forces, thus preventing serum expulsion and cream separation from the system upon storage. Similar results were reported by Papalamprou et al. (*18*), for a model salad dressing emulsion stabilized by lupin seed protein. These emulsions became very stable against creaming when the protein content was increased above a level, something that was attributed to the increase in the number of droplet−droplet interactions and the strengthening of the droplet network structure.

Dilution of the stock cream with deionized water to a final oil content of 5 and 10% and pH adjustment to 3.8 or 6.5 resulted in emulsions that were unstable against creaming (**Figure 5**). The pH and not the oil content appears to have a more pronounced influence on the rate of creaming, with the emulsions at pH 6.5 exhibiting serum separation at the bottom of the container within a few hours of storage. In the case of the samples at pH 3.8, the first signs of serum separation became apparent only after several days of storage.

According to White et al. (*8*) and Frandsen et al. (*4*), the pH range over which aggregation of purified urea sunflower oil bodies takes place is 4.0−6.6. Iwanaga et al. (*6*), reported that this range for nonpurified soybean oil bodies is between pH 3 and 6. These workers also observed that the emulsions in this pH range were unstable against creaming and suggested that this behavior was connected to the reduced net surface charge of the oil bodies in this pH range. In fact, when the oil body surface was coated by a pectin layer using electrostatic deposition, their creaming stability was improved because of the increase in the ζ potential and, hence, the enhancement of electrostatic repulsion

forces operating between oil bodies in close proximity (*19*). Considering that the maize oil bodies at pH 6.5 are more charged than at 3.8 (**Figure 3**), one would expect a lower rate of creaming at the former pH as a result of less extensive droplet interaction and aggregate formation than at the acidic pH. However, as was observed, the serum separated from the emulsion at pH 6.5 was much clearer than at pH 3.8. In the later sample, the serum remained turbid and became clear only in the final days of the storage period. This was an indication of a much more extensive aggregation at pH 6.5, involving all of the oil bodies, while at pH 3.8, it appears that there were many oil bodies that did not take part in aggregate formation, and it was these particles that creamed at a lower rate and produced the turbid appearance of the separated serum of the aged emulsions. Because the higher stability against aggregation and serum separation of oil bodies at pH 3.8 cannot be attributed to the magnitude of their surface charge, an explanation should be sought in the possible influence of the pH on the conformation of adsorbed at the outer layer extraneous protein molecules and the hydrophilic/hydrophobic balance between the exposed to water-phase protein groups.

In conclusion, oil bodies in the form of a natural oil-in-water emulsion cream can be obtained at a relatively high yield from maize germ by applying successive alkaline extraction steps to a finely comminuted germ material, combined with acidic aggregation and creaming of the extracted oil bodies. The extraction and aggregation conditions applied did not appear to influence the integrity of the recovered oil bodies. The oil bodies in the cream were very stable against coalescence during long-time aging, something that was attributed to the presence of an adsorbed mixed surface membrane around the triglyceride core. The surface membrane of oil bodies in the cream is believed to be made up of a primary mixed layer of phospholipids and oleosins and a secondary layer of extraneous germ proteins, with the later playing a key role in the long-term stabilization of oil bodies against coalescence and oil separation. At a neutral pH, the oil bodies aggregated strongly, and as a result, the natural emulsions, prepared by diluting the cream to relatively low oil content, creamed very rapidly. Despite the lower ζ potential and hence net surface charge at pH 3.8, less extensive creaming and serum separation was observed at this pH, indicating that other parameters may play a key role in oil body aggregation under these conditions.

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