AGRICULTURAL AND FOOD CHEMISTRY

Physicochemical Stability of Maize Germ Oil Body Emulsions As Influenced by Oil Body Surface–Xanthan Gum Interactions

CONSTANTINOS V. NIKIFORIDIS AND VASSILIOS KIOSSEOGLOU*

Laboratory of Food Chemistry and Technology, Department of Chemistry, Aristotle University of Thessaloniki, GR-54124 Thessaloniki, Greece

Two types of oil body cream, differing in protein content and composition, were prepared from maize germ. The cream rich in extraneous germ proteins (OB-A) constituted of oil bodies with a significantly lower size compared to the cream (OB-W) which was practically free from the extraneous germ proteins. In addition, the stability of the former cream against oil body coalescence was much higher upon long-term aging. Dilution of both creams to a 5% oil body level produced emulsions that were very unstable against creaming. The creaming stability was greatly improved following addition of 0.1% xanthan, the result being more spectacular in the case of the cream rich in extraneous maize germ proteins. The enhancement of the physical stability of oil droplets upon long-term storage is attributed to electrostatic xanthan gum–oil body surface protein interactions, verified by ζ -potential measurements, that lead to the diminution of depletion flocculation effects and to enhancement of steric stabilization due to the adsorption of the polysaccharide molecules to the oil body surface.

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

INTRODUCTION

Maize germ is a material very rich in lipids, with the crude fat content reaching values as high as 40% (1). As a result, the byproduct of wet- or dry-milling maize kernel processing is exploited industrially for the extraction of corn oil using organic solvents, usually hexane. In spite of the obvious advantages of this approach for corn oil recovery, that is, high extraction efficiency and low cost, the use of an organic solvent may result in environmental pollution, while safety problems, due to organic solvent inflammability, could also be a serious problem. To avoid the use of organic solvents, it has been recently proposed by a number of investigators that oil recovery from oil-rich plant seeds, including maize, could be effected by using aqueous extraction media to recover the oil in the form of a natural oilin-water emulsion (2-4).

This idea is based on the fact that in maize germ, as well as in other oil-rich materials (5-8), the lipids are found in the form of oil bodies that carry at their surface a stabilizing membrane. This membrane is made up of a layer of phospholipids and proteins, called oleosins, which constitute approximately 20% of the surface material. The hydrophobic middle section of the oleosin molecules at the surface is partly embedded in the phospholipids 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 protruding away from the surface (9).

When the oil bodies are extracted using aqueous media, the resulting oil-in-water emulsion may exhibit high physical stability, which is believed to be 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 (9). According to the results of two very recently published studies, the stability against aggregation and creaming of oil bodies, extracted from either soybean (2) or sunflower seed (10), depends heavily on the pH and the 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 with the properties of the mixed phosholipid—oleosin layer at the surface that does not yield when two neighboring oil bodies approach each other and start to interact.

Nikiforidis and Kiosseoglou (11) reported that 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 very high stability of the oil bodies in the recovered cream was attributed to the presence of an adsorbed mixed surface membrane around the triglyceride core, which was believed to be made up of a primary mixed layer of phospholipids and oleosins and a secondary layer of extraneous germ proteins. It was argued by the authors that the presence of the extraneous germ proteins play a key role in the long-term stabilization of oil bodies against coalescence and oil separation.

The oil bodies extracted from maize germ as well as from other sources tend to aggregate strongly, and as a result, the natural emulsions prepared by diluting the oil body cream to a relatively low oil content exhibit very low storage stability against creaming (2, 10, 11). One recently published paper (4) demonstrated

^{*}To whom correspondence should be addressed. Tel: +30-231-0-997834. Fax: +30-231-0-997779. E-mail: kiosse@chem.auth.gr.

that pectin addition to a diluted oil body cream, extracted from soybean, improves dramatically the physical stability of the emulsion. The authors attributed the improvement in stability to oil body surface—polysaccharide interaction that enhanced the repulsive steric forces operating between neighboring droplets and prevented their close approach and flocculation.

The aim of the present study was to use xanthan gum in order to improve the stability of a maize oil body suspension against flocculation, creaming, and possibly coalescence. This anionic polysaccharide tends to form in solution structures, exhibiting high low-shear and weak gel properties that lend stability to colloidal suspensions. On the other hand, as xanthan is a nonadsorbing biopolymer, its presence in emulsion systems may result in strong depletion flocculation phenomena and phase separation (12). There are, however, reports indicating that the molecules of xanthan may interact with those of whey protein and produce hybrids with improved functionality (13-15). Since the strength and extent of polysaccharide-oil body surface interactions may be affected by the composition of the interfacial membrane, two different types of maize oil body cream were prepared from an initial aqueous oil body extract by applying different methods of recovery: one cream consisting of oil bodies with surfaces enriched in maize germ extraneous proteins by recovering the oil bodies from an initial aqueous maize oil body extract through pH manipulation (11) and a second cream with oil body surfaces practically free from extraneous germ proteins by repeatedly washing the oil bodies of the initial extract with a sucrose solution.

MATERIALS AND METHODS

Materials. A byproduct enriched in maize germ, provided by an industrial mill situated in northern Greece (Karanikas Mills, S.A.), was used as a raw material for the aqueous extraction of oil bodies. The maize was a mixture of different common varieties grown in northern Greece. Intact germs from the sample were first collected by hand and then subjected to comminution, using a Brown mill fitted with knives, to pass through a 0.8 mm mesh sieve. Food grade xanthan gum was obtained from Fluka AG (Buchs, Switzerland).

Preparation of Oil Body Cream. Two oil body cream samples, one rich in exogenous germ proteins and a second practically free from their presence, were prepared. The process is divided into two steps. The first step was common for both creams and involved extensive alkaline extraction that resulted in aqueous oil body dispersion. The extract was then divided into two parts, and the oil body cream samples were recovered either by acid coagulation or by repeatedly washing with an alkaline sucrose solution to remove extraneous proteins. Alkaline extraction of oil bodies was effected by a method based on the modification of the process applied by previous investigators (2, 5, 10) and described in detail in a recently published paper (11). The comminuted germ material (250 g) was initially soaked in deionized water, in a ratio of 1/5 (w/v); the pH was adjusted and kept constant at 9 using 0.1 M NaOH solution with constant agitation for 24 h with the use of a mechanical stirrer (Kika Labortechnik, Malaysia), at 1200 rpm. 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 germ residue was then again extracted with deionized water at pH 9, the two extracted oil body dispersions were combined into one, and the resulting pooled dispersion was subjected two times to centrifugation (Firlabo SV11, France) at 3800g for 30 min to remove insoluble solids. The supernatant containing the oil bodies was divided into two parts. In the first, the recovery of oil bodies in the form of a cream was effected by adjusting the pH to 5 and centrifuging at 3800g 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. Following stirring for 1 h to break the aggregates, the pH value was again lowered to 5, the dispersion was centrifuged at 3800g, and the final resulting cream (OB-A) was collected. The second part of the suspension was mixed with an equal volume of a 0.5 M sucrose solution at pH 9 and centrifuged at 3800g. The cream at the top was then recovered and treated one more time with the alkaline sucrose solution. The final resulting washed cream (OB-W) was collected. The two cream samples obtained by applying two different isolation methods were stored at 5 °C, following the addition of 0.01% NaN₃ (Riedel de Haen), and analyzed for moisture, fat, and protein.

Determination of Protein, Fat, and Moisture. The determination of protein, fat, and moisture was conducted according to standard methods of the AOAC (*16*). 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 it was placed in the oven, the sample 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 (C. Gerhardt, U.K.) method.

Protein Electrophoresis. The adsorbed to the oil body surface maize germ protein was analyzed by SDS-PAGE (17) using 4.5 and 12.5% (v/w) acrylamide solutions for the stacking and separating gels, respectively. To solubilize the proteins, the oil body cream samples were mixed (1:5) with the extraction buffer containing 50 mM Tris-HCl, 5 M urea, 1% w/v SDS, and 4% β -mercaptoethanol at pH 8.0. After 1 h incubation at room temperature, an electrophoresis sample buffer of 125 mM Tris-HCl, 5 M urea, 1% SDS, 20% glycerol, and 4% β -mercaptoethanol was added (1:1). Following boiling for 2 min and the application of two freeze-thaw cycles, the supernatant 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 (Sony, DSC-W90). Determination of the protein constituent molar mass was performed with the aid of the Gel-Pro Analyzer (Media Cybernetics, Bethesda, MD).

Preparation of Dilute Oil Body Emulsion. Following crude fat determination the oil body cream samples, OB-A and OB-W, were diluted to an oil content of 10% (v/v) with deionized water, containing 0.01% (w/v) NaN₃ to prevent microbial growth. The resulting diluted creams were then divided into portions of 10 mL and mixed with equal volumes of a 0.2% (w/v) xanthan gum solution, prepared by mixing for more than 5 h at room temperature, to obtain o/w emulsion samples 5% in oil and 0.1% in xanthan. Control samples not containing xanthan were also prepared by diluting with deionized water. The pH values of the diluted emulsions (coded OB-A and OB-W emulsions) were then adjusted using a 0.1 M HCl solution to pH 7, 6, 5, 4, and 3. Four series of each one of the 5% in oil OB-A and OB-W emulsions were prepared to study the effect of pH on emulsion properties.

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 sample dilution with deionized water to an approximate oil content of 0.005% combined with mechanical stirring for 5 min. Droplet size distribution measurements were conducted both on freshly extracted and on aged oil body cream samples.

Measurement of Oil Body ζ **-Potential.** Oil body cream samples were diluted with deionized water to an approximate oil concentration of 0.01%. The pH of the diluted sample was adjusted using either 0.1 M HCl or NaOH. The diluted oil body suspension was subjected to electrophoresis with a Lazer Zee Meter 501 (PenKem, Bedford Hills, NY) at 100 V in the instrument's cell while the droplets were visualized on a monitor.

Evaluation of Emulsion Stability. To assess the stability of diluted oil body emulsions against creaming, 10 mL quantities of each sample were 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.

Measurement of Viscosity. OB-emulsion or xanthan gum solution rheological properties were determined at 25 °C with the aid of a Brookfield DV II LV viscometer (Brookfield Engineering Laboratories, Stoughton, MA) combined with an SC4 small sample adapter.

Statistical Analysis. Each type of oil body cream was prepared in triplicate by conducting three independent aqueous extractions. Each stock cream was diluted to prepare one set of emulsion samples. The determination of analytical characteristics of the creams and the measurement of the emulsion physicochemical properties were conducted at least 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 Cream Composition and Physicochemical Characteristics. The moisture, crude fat, and total protein ($N\% \times 5.7$) of OB-A were 63.5, 22.0, and 7.5%, respectively. On the other hand, the composition of the cream recovered by washing with the sucrose solution (OB-W) was as follows: moisture, 46.5%; crude fat, 43.8%; total protein, 1.6%. The much higher fat content in the latter should be connected with its relatively lower protein content, which apparently did not prevent the cream layer from becoming more compressed and concentrated during the centrifugation of the system. In the case of the OB-A cream, the presence of a thicker protein layer at the oil body surface prevented their close approach during cream recovery and led to the retention of relatively higher amounts of water.

As was reported in our previous paper (11), a very high proportion of the protein present in the OB-A cream was made up of extraneous germ proteins. On the other hand, the oleosins, determined by densitometry, constituted about 37% of the total proteins of the cream (11). It was not clear whether the extraneous protein molecules were present at the oil body surface from the beginning of their extraction or whether they were coagulated and adsorbed to the surface when the pH of the system was adjusted to 5. As shown in Figure 1, almost all the proteins in the cream recovered by washing with the sucrose solution belonged to those of the oleosin fraction. These proteins with a molecular weight of 15 and 16 kDa constitute an integral part of the oil body surface membrane and contribute to their physical stabilization by forming a mixed layer with the phospholipids (6, 7). The almost complete absence of extraneous maize germ proteins from OB-W cream indicates that the oil body surface was initially covered almost entirely by oleosins, while the coextracted extraneous germ proteins were mainly present in the continuous phase of the oil body dispersion and were removed during the washing process that followed the recovery of the crude cream.

The presence of extraneous proteins at the oil body surface influences the droplet surface ζ -potential, as shown in **Figure 2**. When they constitute only a minor fraction of the total proteins of oil body surface, as in the case of OB-W cream, the ζ -potential value at any pH appears to be marginally but significantly less negative than that corresponding to oil bodies of the OB-A cream. As a consequence, the point of zero charge for the oil bodies in the OB-W cream is higher. This value is still much lower compared to that reported by Tzen (5, 6) for extensively purified maize germ oil bodies, something that should be rather attributed to the incomplete removal of all the extraneous proteins in our study.

The two creams also differed with respect to their oil body size, with that of the OB-A cream being significantly lower (p < 0.05; $d_{3,2} = 0.45$ and 0.79 μ m for OB-A and OB-W, respectively) (**Figure 3**). Since the mean volume diameter of the initial oil body alkaline extract was 0.31 μ m, it is assumed that the process of recovery of the oil bodies from their extract led to some oil droplet coalescence, probably as a result of repeated centrifugation steps



Figure 1. SDS-PAGE profiles of molecular marker (lane 1), maize germ (lane 2), OB-A cream (lane 3), and OB-W cream (lane 4) proteins.



Figure 2. Influence of pH and xanthan on the ζ -potential of OB-W (a) and OB-A (b) creams: (**D**) OB-W; (**D**) OB-W, 0.1% xanthan; (**•**) OB-A; (\diamond) OB-A, 0.1% xanthan.

applied for the preparation of the cream. The very limited droplet coalescence in the case of the OB-A cream should be attributed to the presence of extraneous germ proteins in the initially alkaline oil body extract. Following pH adjustment, these proteins become adsorbed to the oil body surface and form a thick layer that constitutes a physical barrier that does not allow the close approach of oil bodies during centrifugation, thus preventing the rupture of the thin continuous phase film that forms between the oil bodies, when they are compressed against each other. Additionally, the presence of the extraneous proteins at the oil body surfaces is bound to enhance emulsion stability as a result of a much thicker and more viscoelastic adsorbed surface layer compared with that of the OB-W sample. The presence of a much thinner membrane at the surface of the OB-W cream oil droplets leads apparently to a higher degree of droplet coalescence during cream recovery. It must be stressed, however, that the presence of the mixed surface oleosin-phospholipid layer propably offers a degree of protection to the oil bodies that prevents their extensive coalescence.

The higher degree of protection offered by the thicker surface layer to the oil bodies of the OB-A cream during recovery from



Figure 3. Photographs and d_{3,2} data of oil bodies of a diluted extract and of OB-W and OB-A creams at two aging times.



Figure 4. Oil body size distribution curves of (\bigcirc) fresh oil body extract and of (\Box) OB-A and (\triangle) OB-W emulsions aged for 15 days.

their alkaline extract is also reflected in the remarkable stability of this cream against droplet coalescence during storage. As shown in Figure 3, the mean droplet size $(d_{3,2})$ of both creams increased following storage at 5 °C for 15 days. This increase, however, in the case of the OB-A cream should be mainly connected with oil body flocculation phenomena, since the size of the individual oil bodies, as may be seen in the respective photograph, does not appear to have suffered any spectacular change with aging. This becomes more apparent from the data of Figure 4, which presents the oil body size distribution both for the initial oil body extract and for the OB-A and OB-W aged emulsions. Comparing the data presented in Figure 4 with the photographs of the aged OB-A emulsion leads to the conclusion that the shift to the right of the distribution curve resulting from aging would not have been possible if the emulsions were not extensively flocculated. In the case of OB-W cream both large droplets and droplet aggregates may be spotted in the photograph of the cream aged for 15 days, an indication that this cream was not as stable as the former one with respect to both oil body flocculation and coalescence. Taking into account that the oil and the protein content of the OB-W cream are 43.8 and 1.6%, respectively, and the mean volume diameter $(d_{3,2})$ of the nonaged cream is 0.79 μ m, one may calculate the protein surface load per unit surface, which is approximately 4.8 mg/m². This protein load is considered very satisfactory for the stabilization of oil droplets against coalescence as it should correspond to a layer thicker than a protein monomolecular surface. Considering, however, that the oleosins, which constitute the main proteins of the surface of OB-W cream droplets, have a very low molecular weight and, in addition, they lay flat at the interface (5), it may be hypothesized that the



Figure 5. Creaming behavior of OB-W (a) and OB-A (b) emulsions as influenced by pH in the absence (filled symbols) or presence (empty symbols) of xanthan: $(\blacklozenge, \diamondsuit)$ pH 3; (\blacksquare, \Box) pH 4; $(\blacktriangle, \bigtriangleup)$ pH 5; (\diamondsuit, \bigcirc) pH 6; (filled ×, open ×) pH 7.

repulsive steric stabilization forces operating between two interacting oil bodies in close proximity are expected to be relatively weak. On the other hand, the presence of exogenous maize germ proteins with a much higher molecular weight at the outer surface layer of the OB-A cream oil droplets will probably increase both the intensity of the steric forces as well as the strength of the surface membrane and hence enhance oil body stability against coalescence.

Stability of Diluted Oil Body Emulsions. The influence of pH and 0.1% xanthan gum on the stability against creaming of dilute oil-in-water emulsions based on OB-W and OB-A cream is shown in parts **a** and **b** of Figure 5, respectively. In general, the OB-W emulsions exhibited very low creaming stability, as serum separation at the bottom of the container became apparent only within a few minutes of aging, irrespective of the emulsion pH.



Figure 6. Viscosity—shear rate plots of OB-W (\blacklozenge) and OB-A (\diamondsuit) emulsions containing 0.1% xanthan and of 0.1% xanthan gum solution (×) at pH 6.



Figure 7. Influence of pH on the viscosity of OB-W (black bar) and OB-A (gray bar) emulsions containing 0.1% xanthan and of 0.1% xanthan gum solution (white bar) at 7.92 s⁻¹.

Additionally, the creaming process was completed within the first hours of storage. The OB-A emulsion creamed at a much lower rate, with the emulsion at pH 6 exhibiting the highest stability against creaming. One reason for the lower stability of the former emulsion might have been the larger mean droplet size compared to that of the oil drops in the latter. As a result, the oil bodies in the OB-W cream are expected to move faster to the top of the container. The influence of pH, however, on the rate of creaming of OB-A emulsion indicates that possible interactions between the oil bodies may lead to the formation of droplet networks exhibiting different degrees of resistance to buoyancy forces and, hence, different rates of creaming (19). In the OB-W emulsion the absence of exogenous proteins from the surface probably resulted in relatively weak oil body interactions, producing a network where droplet rearrangement took place at a relatively high rate, leading to emulsions very unstable with respect to creaming. In the case of OB-A emulsions, on the other hand, the coagulated exogenous germ protein molecules at the outer surface of neighboring oil bodies may interact, probably through hydrophobic bonding, leading to bridging flocculation. Combined with the van der Waals attractive oil body interactions, bridging flocculation is expected to lead to droplet networks that were more difficult to yield to buoyancy forces than the OB-W emulsion networks.

Following addition of 0.1% xanthan, the stability of OB-W emulsions increased spectacularly, with the emulsion pH at 6 exhibiting the highest stability, although the systems were far from being characterized as stable emulsions (**Figure 5a**). On the other hand, the presence of xanthan in the OB-A emulsions enhanced their stability to such an extent that they did not show any sign of serum separation at any pH, following storage for long time periods (**Figure 5b**). Xanthan gum solutions are highly viscous systems exhibiting a pronounced shear-thinning behavior. As a result, the gum acts as an effective stabilizer of dispersion systems at relatively moderate or high concentrations (>0.1%), by entrapment of oil droplets or particles in the highly viscous micro regions of the solution enriched in xanthan (19, 20). At relatively low concentrations, however, the gum molecules may destabilize a dispersion system due to depletion flocculation (18). The destabilization of the system by a polysaccharide through depletion may be avoided only when the polysaccharide molecules interact with the emulsifier at the surface of the dispersed particles (e.g., with adsorbed protein molecules, mainly through ionic interaction). In fact, the stability of the system can be increased in that case through the enhancement of the repulsive steric stabilization interactions between approaching particles (21). As shown in Figure 6, both the OB-A and OB-W emulsions at pH 6, containing 0.1% xanthan, and the respective xanthan solution exhibit a pronounced shear-thinning behavior. Considering that in the absence of xanthan the rheological behavior of emulsions was Newtonian and their viscosity was too low to be measured with the Brookfield viscometer, it may be hypothesized that the rheology of both emulsions was dominated by the xanthan molecules that apparently were not prevented by the oil bodies from forming the well-known weak gel network structure. A similar behavior with respect to emulsion rheology was more or less observed at pH 7 (results are not shown). Figure 7 exhibits the influence of selected pH values on an emulsion containing 0.1% xanthan and the respective gum solution at a shear rate of 7.92 s⁻¹. At pH 6 as well as at pH 7, the viscosities of both emulsions were significantly different but were of the same order as the viscosity of the xanthan gum solution. At pH 5, however, and at any other lower pH (results are not shown), the viscosity of both emulsions dropped dramatically to a level well below that of the lower limit of the viscometer. On the other hand, as was expected, the viscosity of the 0.1% xanthan solution at the acidic pH range did not change significantly. It may, therefore, be concluded that at pH 5 or lower the gum network was severely disrupted as a result of the presence of the oil bodies in the system. This disruption, however, did not bring about any dramatic change in the creaming stability of the emulsions (Figure 5), indicating that the viscosity of the system, as influenced by the presence of the polysaccharide molecules, plays a secondary role in the stabilization of the oil bodies against creaming. As shown in Figure 2a, the ζ -potential value in the case of the OB-W cream became more negative above pH 5 while at pH 3 and 4 the sign of the ζ -potential value changed from positive to negative. Considering that the pK_a value of xanthan is close to 4 (22) and the polysaccharide chain at all the pH values studied carries an appreciable charge, it is assumed that extensive interaction between the oil body surface and the negatively charged polysaccharide molecules may have taken place. A similar but less spectacular shift in the ζ -potential values may also be noticed for oil bodies of the OB-A cream (Figure 2b). Apparently, the domination of the outer surface of the latter emulsion by exogenous germ proteins constitutes a barrier that did not allow extensive xanthan-oil body interactions. This limited interaction, however, apparently sufficed to prevent expulsion of the oil bodies from the system as a result of depletion flocculation. Additionally, the presence of polysaccharide molecules at the surface of the oil droplets is expected to increase the magnitude of the steric stabilization forces and protect the oil bodies from flocculation and creaming, an explanation also provided by Iwanaga et al. (4) for the stabilization of soybean oil bodies by pectin.

In conclusion, the presence of extraneous proteins at the surface of oil bodies enhances their physical stability again to coalescence, either during their isolation in the form of a concentrated cream or following cream storage for several days. Xanthan gum addition at a 0.1% level to dilute oil body

dispersions brought about a dramatic enhancement in their creaming stability, the result being more pronounced when the oil bodies carried at their surface appreciable amounts of extraneous maize germ proteins. Data on the ζ -potential indicated that xanthan—oil body surface interactions probably take place that influence the oil body dispersion stability against creaming.

LITERATURE CITED

- (1) Bajaj, S., Bhatia, N., & Bajaj, Y. P. S. Biotechnology in Nutritional Improvement of Maize. In *Biotechnology in Agriculture and Forestry*; Bajaj, Y. P. S., Ed.; Springer-Verlag: New York, 1994; Vol. 25, pp 485–512.
- (2) Iwanaga, D.; Gray, D. A.; Fisk, I. D.; Decker, E. A.; Weiss, J.; McClements, D. J. Extraction and characterization of oil bodies from soy beans: A natural source of pre-emulsified soybean oil. *J. Agric. Food Chem.* **2007**, *55*, 8711–8716.
- (3) Kapchie, V. N.; Wei, D.; Hauck, C.; Murphy, P. A. Enzyme-assisted aqueous extraction of oleosomes from Soybeans (Glycine max). J. Agric. Food Chem. 2008, 56 (5), 1766–1771.
- (4) Iwanaga, D.; Gray, D.; Decker, E. A.; Weiss, J.; McClements, D. J. Stabilization of soybean oil bodies using protective pectin coatings formed by electrostatic deposition. *J. Agric. Food Chem.* **2008**, *56* (6), 2240–2245.
- (5) Tzen, J. T. C.; Lie, G. C.; Huang, A. H. C. Characterization of the charged components and their topology on the surface of plant seed oil bodies. J. Biol. Chem. 1992, 267 (22), 15626–15634.
- (6) Tzen, J. T. C.; Cao, Y. Z.; Laurent, P.; Ratnayake, C.; Huang, A. H. C. Lipids, proteins, and structure of seed oil bodies from diverse species. *Plant Physiol.* **1993**, *101* (1), 267–276.
- (7) Tzen, J. T. C.; Peng, C. C.; Cheng, D. J.; Chen, E. C. F.; Chiu, J. M. H. A new method for seed oil body purification and examination of oil body integrity following germination. *J. Biochem.* **1997**, *121* (4), 762–768.
- (8) Frandsen, G. I.; Mundy, J.; Tzen, J. T. C. Oil bodies and their associated proteins, oleosin and caleosin. *Physiol. Plant.* 2001, *112* (3), 301–307.
- (9) Tzen, J. T. C.; Huang, A. H. C. Surface-structure and properties of plant seed oil bodies. J. Cell Biol. 1992, 117 (2), 327–335.
- (10) White, D. A.; Fisk, I. D.; Mitchell, J. R.; Wolf, B.; Hill, S. E.; Gray, D. A. Sunflower-seed oil body emulsions: Rheology and stability assessment of a natural emulsion. *Food Hydrocolloids* **2008**, *22* (7), 1224–1232.

- (11) Nikiforidis, C. V.; Kiosseoglou, V. Aqueous Extraction of Oil Bodies from Maize Germ (*Zea Mays*) and Characterization of the Resulting Natural Oil-in-Water Emulsion. J. Agric. Food Chem. 2009, 57 (12), 5591–5596.
- (12) Ye, A.; Hemar, Y.; Singh, H. Enhancement of coalescence by xanthan addition to oil-in-water emulsions formed with extensively hydrolysed whey proteins. *Food Hydrocolloids* **2004**, *18* (5), 737–746.
- (13) Benichou, A.; Aserin, A.; Lutz, R.; Garti, N. Formation and characterization of amphiphilic conjugates of whey protein isolate (WPI)/xanthan to improve surface activity. *Food Hydrocolloids* 2007, 21 (3), 379–391.
- (14) Benichou, A.; Aserin, A.; Garti, N. Double emulsions stabilized by new molecular recognition hybrids of natural polymers. *Polym, Adv. Technol.* 2002, *13* (10–12), 1019–1031.
- (15) Laneuville, S. I.; Sanchez, C.; Turgeon, S. L.; Hardy, J.; Paquin, P. Small-angle static light- scattering study of associate phase separation kinetics in β-lactoglobulin + xanthan gum mixtures under shear. In *Food Colloids. Interactions, Microstructure and Processing*; Dickinson, E, Ed.; The Royal Society of Chemistry: Cambridge, 2005; pp 443–465.
- (16) AOAC. Official Methods of Analysis, 15th ed.; Association of Official Analytical Chemists: Washington, DC, 1992.
- (17) Laemmli, U. K. Cleavage of structural proteins during assembly of head of bacteriophage-t4. *Nature* **1970**, *227* (5259), 680–685.
- (18) Dickinson, E. Hydrocolloids as emulsifiers and emulsion stabilizers. Food Hydrocolloids 2009, 23 (6), 1473–1482.
- (19) Moschakis, T.; Murray, B. S.; Dickinson, E. Microstructural evolution of viscoelastic emulsions stabilised by sodium caseinate and xanthan gum. J. Colloid Interface Sci. 2005, 284, 714–728.
- (20) Drakos, A.; Kiosseoglou, V. Stability of Acidic Egg White Protein Emulsions Containing Xanthan Gum. J. Agric. Food Chem. 2006, 54 (26), 10164–10169.
- (21) McClements, D. J. Food Emulsions: Principles, Practice, and Techniques, 2nd ed.; CRC Press: Boca Raton, FL, 2005.
- (22) Young, S.-L.; Martino, M.; Kienzle-Sterzerl, C.; Torres, J. A. Potentiometric Titration Studies on Xanthan Solutions. J. Sci. Food Agric. 1994, 64, 121–127.

Received for review July 22, 2009. Revised manuscript received October 22, 2009. Accepted November 11, 2009.