Oil-in-Water Emulsions Formulated with Sunflower Lecithins: Vesicle Formation and Stability

L.G. Pan, M.C. Tomás*, and M.C. Añón

Centro de Investigación y Desarrollo en Criotecnología de Alimentos (CIDCA)–Facultad de Ciencias Exactas, Universidad Nacional de La Plata–Consejo Nacional de Investigaciones Científicas y Técnicas (UNLP–CONICET), (1900) La Plata, Provincia de Buenos Aires, Argentina

ABSTRACT: Oil-in-water emulsions (30:70, vol/vol) were formulated with sunflower lecithin to characterize the destabilization processes and the vesicles formed. Dispersions containing levels of 0.1% lecithin were more stable against coalescence than the control system. When the lecithin concentration was increased to 0.5%, the presence of spherical structures, such as vesicles, was recorded that occluded the emulsion inside. Vesicles underwent a creaming process, and a narrow coalescence zone was detected in the upper layers of the samples. As the lecithin concentration was increased, more vesicles were formed, representing as much as 80% of the system volume. A reduction in the average size of vesicles was observed at high lecithin concentrations (2.5 and 5.0%). The vesicle size distribution changed as a function of lecithin concentration, decreasing the ratio of large to small particles in the same way. Coalescence took place in zones where large-volume vesicles were in contact in the upper portion of the tube sample. The results obtained suggest that sunflower lecithins present interesting emulsifying properties that may prove useful in food technology.

Paper no. J10697 in *JAOCS 81*, 241–244 (March 2004).

KEY WORDS: Emulsion stability, oil-in-water emulsions, phospholipids, sunflower lecithins, vertical scan analyzer, vesicles.

In food industry, natural or modified soybean lecithin is used in a wide variety of applications, including as emulsifiers–dispersing agents, viscosity regulators, fat replacers, and wetting or antispattering agents (1,2). The major components of vegetable lecithins include PC, PE, PI, and a small amount of PA (3,4). Oil-in-water emulsions (O/W) are present in many food products, e.g., mayonnaise, salad dressings, and margarines and spreads (5,6). In these systems, the presence of phospholipids as emulsifiers and stabilizing agents yields products with acceptable attributes. Thus, model systems have been designed to study the behavior of these complex systems for their application in food technology (7).

Crude sunflower oil containing approximately 0.3% phosphatides represents another important source of lecithin (8). However, few studies have been carried out on emulsifier applications. Most have been concerned with their composition as well as their physical and chemical properties (9). The large volume of sunflower oil produced in Argentina represents a potential source of lecithin. Thus, basic information on the utilization of this commodity is required. This study reports the stability of O/W emulsions: The aims of the present work were to study O/W emulsions formulated with sunflower lecithin and to characterize the formation of vesicles and their destabilization.

EXPERIMENTAL PROCEDURES

Sunflower lecithins. Sunflower gums (obtained by water degumming of crude sunflower seed oils) were treated with acetone (acetone/gum ratio 3:1, vol/vol) to eliminate residual oil, and were subsequently freeze-dried.

O/W emulsions. Refined sunflower seed oil was provided by a local oil industry and was used in the formulation of emulsions. O/W emulsions (30:70, vol/vol) were prepared at room temperature in an Ultra-Turrax T25 homogenizer using an S 25 N–10 G dispersing tool (7.5 mm rotor diameter) at a rate of 10,000 rpm for 1 min.

Sunflower lecithin had previously been dissolved in the oil phase at 30°C for 30 min with moderate agitation. The emulsifier concentration in the oil ranged from 0.1 to 5.0% (wt/vol of the continuous phase).

Microscopy. Emulsions were observed at initial times (time = 0 min) with a Leica DMLB optical microscope, operated with transmitted light and phase contrast. Micrographs were taken with a Leica DC 100 camera (Bensheim, Germany). Emulsions were analyzed with a Mastersizer Micro Particle Analyzer (Malvern Instruments Ltd., Malvern, United Kingdom), which is a laser diffraction-based particle size analyzer. Particle size distribution by volume and number were determined. Mean diameters $D[4,3]$ and $D[1,0]$ were calculated as follows:

$$D[4,3] = \Sigma V_i d_i^{\,4} / \Sigma V_i\, d_i^{\,3} \qquad [1]$$

$$D[1,0] = \Sigma n_i d_i / \Sigma n_i \qquad [2]$$

where V_i is the relative volume in class i and d_i is the mean class diameter.

Vertical scan analyzer (QuickSCAN). All dispersions were evaluated by optical characterization using a vertical scan analyzer (QuickSCAN) as described previously (6). The basic features of this equipment are the following: The sample to

*To whom correspondence should be addressed at CIDCA, 47 y 116, (1900) La Plata, Provincia de Buenos Aires, Argentina.
E-mail: mabtom@hotmail.com

be analyzed is contained in a cylindrical glass measurement cell. Near the cell is a mobile reading head composed of a pulsed near-IR light source (λ = 850 nm) and two synchronous detectors. The transmission detector receives the light, which passes through the sample (0°), while the back-scattering detector receives the light back-scattered by the sample (135°). The whole reading head moves along the test tube, scanning the entire length of the sample (about 65 mm) and acquiring, at a given time, transmission (T) and back-scattering (BS) data every 40 µm. Thus, it is possible to obtain curves giving the percentage of transmitted and back-scattered light flux, relative to external standards, as a function of the sample height in millimeters. In all cases, the results are expressed in comparison to a reference profile (time = 0 min), yielding corresponding ΔT and ΔBS profiles. In this way, it is possible to discriminate between particle migration (sedimentation, creaming) and particle size variation (flocculation, coalescence) processes. Creaming/clarification kinetics were followed by measuring peak thickness variations as a function of time of the BS profiles.

RESULTS AND DISCUSSION

Micrographs corresponding to O/W emulsions formulated with the addition of 0 to 5.0% sunflower lecithin can be observed in Figures 1A–E, respectively. When the lecithin concentration was 0.1%, it was possible to observe—as in the control system—the oil droplets emulsified in the aqueous continuous phase. At a concentration of 0.5%, sunflower lecithins yielded spherical structures, such as vesicles. This phenomenon was also recorded at high levels of the emulsifier assayed. It is noteworthy that these vesicles occluded the emulsified oil droplets inside (Figs. 1C–E). In addition, although being in contact with each other, they did not coalesce instantly. Moreover, by using phase-contrast microscopy it was possible to observe that the vesicles presented light-dispersing edges. These results are in agreement with the formation of different types of vesicles in model systems (10).

Emulsions are unstable systems in a physicochemical sense, evolving from an homogeneous system at the beginning to complete phase separation. By monitoring the optical properties of an emulsion by means of QuickSCAN, we could distinguish between particle size variation and particle migration processes. When only particle size variation exists (e.g., coalescence), the ΔBS profiles will always be negative because the particle size increases as the particle density diminishes. When the destabilization process involves only particle migration, the plot of ΔBS will detect a positive region corresponding to the creamed phase and a negative lower region caused by clarification.

In Figure 2A the differential scanning profiles corresponding to an O/W emulsion without the presence of lecithins (control system) can be observed. A rapid separation of the creamed phase followed by a destabilization by coalescence took place and was recorded at the top of the tube. The positive ΔT profiles are in accordance with the creaming process, denoting a clarification zone at the tube bottom.

The differential BS profiles corresponding to emulsions with 0.5 and 1.0% sunflower lecithin are shown in Figures 2B and 2C, respectively. In the early stages, both systems underwent a creaming process, registered by a negative ΔBS in the tube bottom and a positive ΔBS—for short times—in the upper portion of the sample, producing a creamed phase. Furthermore, this phase was destabilized by coalescence. When the lecithin concentration was increased from 0.5 to 1.0%, a wide upper zone associated with the creamed phase was observed,

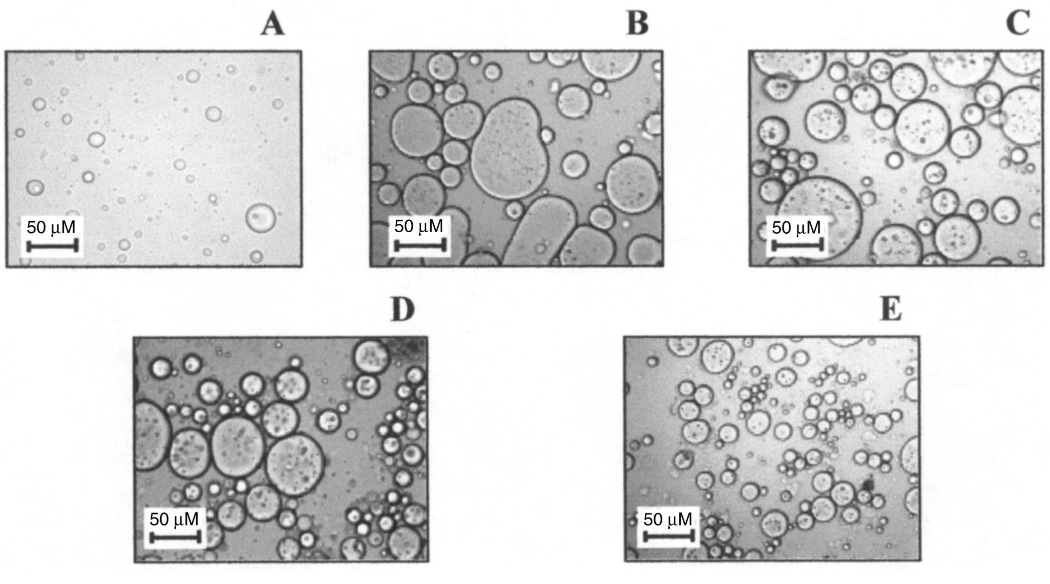

FIG. 1. Micrographs corresponding to oil-in-water (O/W) emulsions formulated with (A) a control system and (B) 0.5, (C) 1.0, (D) 2.5, or (E) 5.0% sunflower lecithin. Micrographs were taken at time = 0 min.

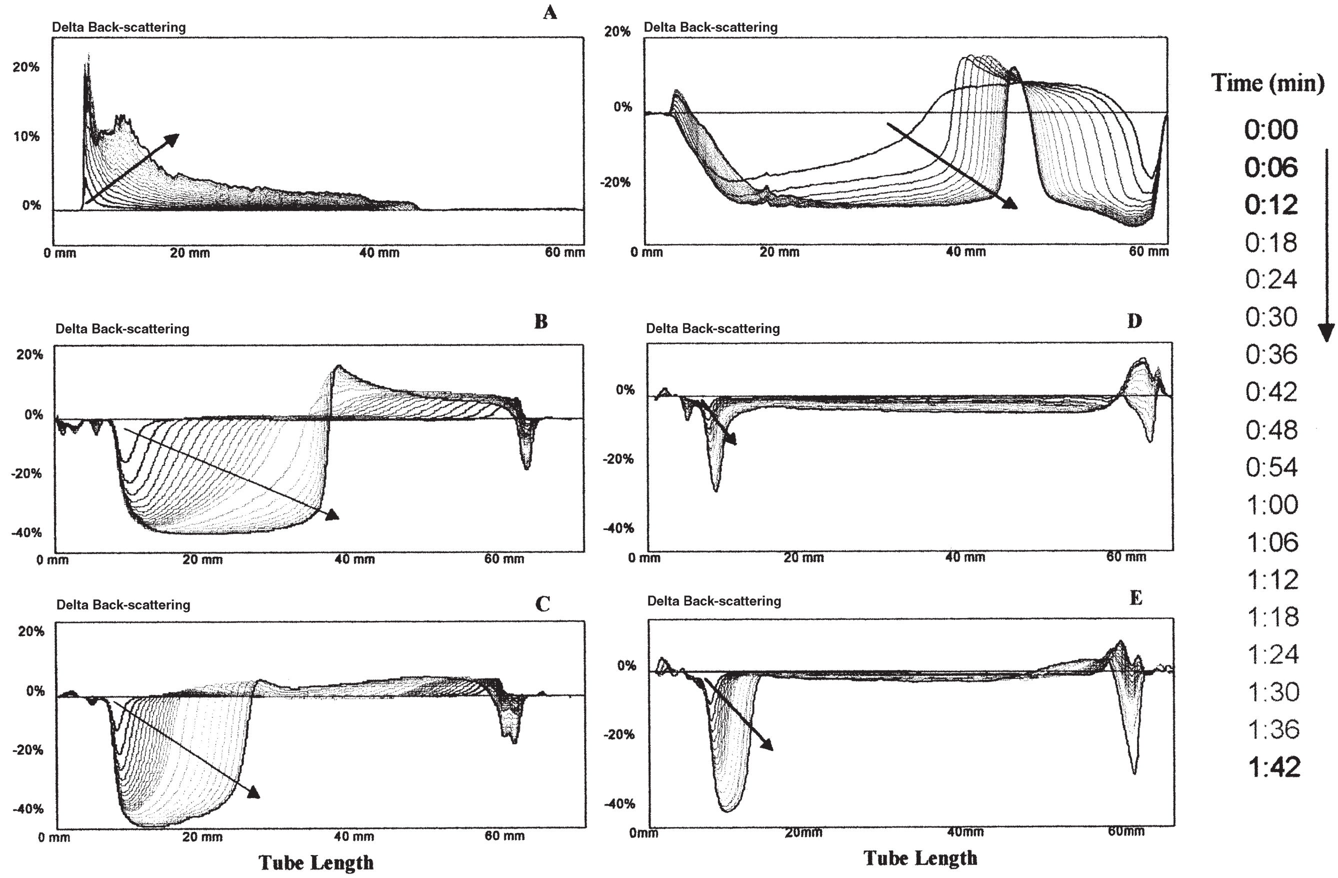

FIG. 2. Delta transmission (ΔT) and delta back-scattering (ΔBS) profiles corresponding to (A) the control system, and ΔBS profiles corresponding to O/W emulsions with (B) 0.5, (C) 1.0, (D) 5.0, and (E) 2.5% sunflower lecithin. Arrows denote time, as represented on the right scale. For other abbreviation see Figure 1.

involving the vesicles mentioned previously. However, the creaming kinetics at 1.0% were slower than those corresponding to 0.5%.

At high emulsifying agent ranges (5.0 and 2.5%), a different behavior was observed (Figs. 2D, E). For a concentration of 5.0%, the clarification zone at the tube bottom was significantly narrower than that for 2.5% (Fig. 2E) and lower levels of lecithins. In both cases, coalescence of the creamed phase was recorded. At these levels of lecithins, the ΔBS profiles indicated that vesicles occluded more than 80% of the system volume. A reduction in the average size of vesicles was detected (see Figs. 1D, E); nevertheless, coalescence of the large vesicles was observed in the upper portion.

The corresponding creaming/clarification kinetics of the O/W emulsions (shown in Fig. 3) were observed by recording the evolution of the peak thickness at the tube bottom. Dispersions prepared with different levels of lecithins were more stable against this destabilization process than the control system. Emulsions containing 0.5% lecithin presented a faster creaming process than systems containing 1.0%. At levels of 2.5 and 5.0%, clarification was hardly detectable, probably because vesicles occluded a great portion of the sample, preventing creaming.

The results obtained from the ΔBS profiles are in accordance with the corresponding microscopic analysis, indicating the presence of different size populations for emulsions with 1.0% of emulsifier. In the upper portion of the sample, large vesicles underwent a coalescence process, whereas a population of small vesicles was located in the bottom of the creamed phase.

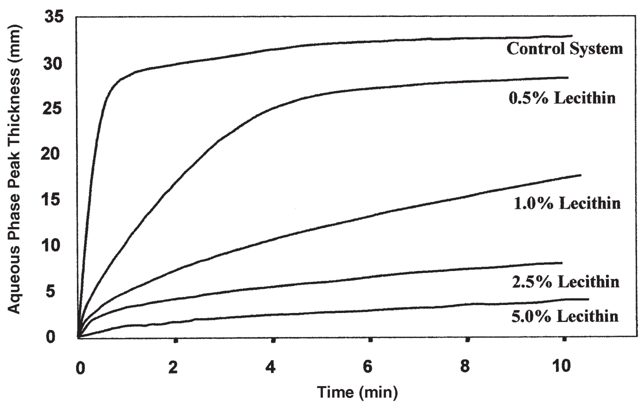

FIG. 3. Creaming/clarification kinetics for the different O/W formulations. For abbreviation see Figure 1.

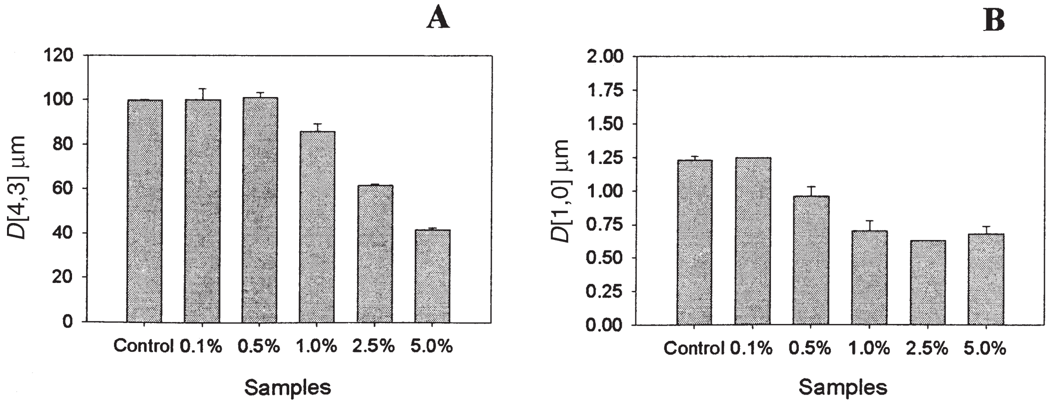

FIG. 4. Mean diameters (A) $D[4,3]$ and (B) $D[1,0]$ as a function of lecithin concentration. Error bars represent the SD.

The evolution of $D[4,3]$ and $D[1,0]$ of the different systems assayed is shown in Figures 4A, B. One can observe that the value of $D[4,3]$ decreased when the lecithin concentration was higher than 0.5%. Levels of 2.5 and 5% lecithin presented a diminution in this parameter of approximately 40 and 60%, respectively. In considering $D[1,0]$, a decrease was detected when the emulsifier concentration was raised from 0.1 to 0.5%. Particle size distributions were equivalent for systems ranging from 1.0 to 5.0%.

The comparison of these parameters marked the presence of a small number of large particles occupying a representative volume of the emulsion. Furthermore, the incorporation of sunflower lecithin at higher levels than 1.0% had a greater effect on $D[4,3]$ than on $D[1,0]$. Thus, increasing the lecithin concentration reduced the size of large vesicles but had little effect on small emulsified droplets.

The results obtained by microscopic analysis, particle size distribution, and optical characterization showed that, from a concentration of 0.5% onward, sunflower lecithin yielded spherical structures (vesicles) that occluded the O/W emulsion. The vesicle size distribution changed as a function of lecithin concentration, decreasing the ratio of large to small particles in the same way. Also, an increase in lecithin levels slowed the creaming process. In all cases, coalescence took place in the upper portion of the samples. These data suggest that sunflower lecithins present interesting emulsifying properties for potential application in food technology.

ACKNOWLEDGMENTS

This work was supported by a grant obtained from Agencia Nacional de Promoción Científica y Tecnológica (ANPCyT)–República Argentina PICT-98-09-04286. Author L.G. Pan received a scholarship from CONICET and Molinos Río de La Plata S.A. The authors wish to thank Refinerías de Maíz S.A. (Unilever) for kindly providing the Mastersizer Micro Particle Analyzer.

REFERENCES

1. van Nieuwenhuyzen, W., Fractionation of Lecithins, *Eur. Food Drink Rev.*: *Process Technol.* 27–32 (1999).
2. van Nieuwenhuyzen, W., and B. Szuhaj, Effect of Lecithins and Proteins on the Stability of Emulsions, *Fett/Lipid 100*:282–291 (1998).
3. van Nieuwenhuyzen, W., The Industrial Uses of Special Lecithins, *J. Am. Oil Chem. Soc. 58*:886–888 (1981).
4. Larsson, K., Basic Concepts, in *Lipids: Molecular Organization, Physical Functions and Technical Applications,* The Oily Press, Dundee, United Kingdom, l994, pp. 2–6.
5. McClements, D.J., Emulsion Ingredients, in *Food Emulsions: Principles, Practice and Techniques*, CRC Press, New York, 1999, pp. 83–127.
6. Hassenhuettl, G.L., and R.W. Hartel, Margarines and Spreads, in *Food Emulsifiers and Their Applications*, edited by G.L. Hassenhuettl and R.W. Hartel, Chapman & Hall, New York, 1997, pp. 255–280
7. Pan, L.G., M.C. Tomás and M.C. Añón, Effect of Sunflower Lecithins on the Stability of Water-in-Oil (W/O) and Oil-in-Water (O/W) Emulsions, *J. Surfact. Deterg. 5*:135–143 (2002).
8. Carelli, A.A., M.I.V. Brevedan, and G.H. Crapiste, Quantitative Determination of Phospholipids in Sunflower Oil, *J. Am. Oil Chem. Soc. 74*:511–514 (1997).
9. Holló, J., J. Perédi, A. Ruzics, M. Jeránek, and A. Erdélyi, Sunflower Lecithin and Possibilities for Utilization, *Ibid. 70*:997–1001 (1993).
10. Szoka, F., and D. Papahadjopoulos, Liposomes: Preparation and Characterization, in *Liposomes: From Physical Structure to Therapeutic Applications*, edited by C.G. Knight, Elsevier Science, New York, 1982, pp. 51–82.

[Received July 14, 2003; accepted December 9, 2003]