Hydrophobic Interactions in the Purification of Soybean Lecithin

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ABSTRACT: Phospholipids have a nonpolar fatty acyl chain and a polar head group, which give them unique physicochemical behavior. They form micellar structures with glycerides, as in the hydration of lecithin, where mixed bilayers of phospholipids and glycerides are formed. In hydrated lecithin, the 70:30 phospholipid/glyceride composition shows the maximum interaction energy and corresponds to the most stable mixed layer. During purification of commercial soybean lecithin (CSL) with acetone, it was observed that the triglyceride extraction curves corresponded to an exponential function shared by the acetone and phospholipid phases. During CSL purification, a fraction of the triglycerides was dissolved, and the rest remained attached to the phospholipids, thus making total separation by simple contact with the solvent impossible. The behavior of these glyceride–phospholipid systems is controlled by such factors as Van der Waals forces, configurational entropy, and alterations in the structure of the adjacent water, similar to the hydrophobic interactions that exist between proteins and lipid chains in biological membranes. In this study, equilibrium plots for the phospholipid/triglyceride/acetone system were obtained and provided evidence of hydrophobic interaction.
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KEY WORDS: Acetone, acetone insolubles, extraction, hydrophobic interaction, lecithin, phospholipid/triglyceride mixtures.

Bimolecular layers of phospholipids and other lipids are presumed to be adsorbed through their polar groups to layers of proteins, thus forming the framework for biological interfaces in biomembranes. The lipid composition and the specific structure of the polar and nonpolar fractions of the phospholipid group play an essential role in how phospholipids function in membranes. At the same time, the asymmetrical distribution of phospholipid fatty acid chains has a structural significance because phospholipids adsorb to other lipids and proteins, forming complicated complexes in biomembranes (1).

Similar interactions are likely to occur in commercial soybean lecithin (CSL), used in food technology as a dispersant for water-insoluble compounds, which is composed of 60–65% phospholipids and 35–40% triglycerides. The interaction between mixtures of natural triglycerides and phospholipids has been examined in monolayers on water (2) and in bulk systems (3). However, despite its obvious importance, the interaction between soybean phospholipids and triglycerides has not been widely studied.

Triglycerides are slightly polar lipids and have extremely low solubility in water, but when spread on water surfaces, they form stable monolayers. Surface balance studies of mixed monolayers with well-characterized triglycerides and egg lecithin showed large differences between the surface properties of different triglycerides in mixed films. In infrared (IR) spectrometry, a 1:3 tristearin/egg lecithin mixture had a carbonyl band that was lowered by 50 cm^{-1}, indicating hydrogen bonding. However, no pronounced molecular interaction between tristearin and lecithins was recorded with differential thermal analysis or X-ray diffraction (4).

In this study, the bulk-phase soybean triglyceride/phospholipid interactions were investigated in a nonaqueous environment.

MATERIALS AND METHODS

Powdered phospholipids were obtained from CSL with detailed composition [acetone-insolubles (phospholipids), 60–65%; acetone-solubles (oil + free fatty acids), 30–35%; moisture and volatile matter (1%); and hexane-insolubles (extraneous matter), 0.2%] by treatment with cold acetone and intense shaking. The mixture was left to settle before decanting. The process was repeated until a light-yellow powder was achieved (97% phospholipids), which was freed from solvent in a vacuum oven at 50°C (see A-type and B-type extractions, next paragraph). Distilled acetone and dried distilled acetone were used as solvents. A cylindrical stainless-steel vessel, provided with a variable-speed mechanical stirrer, was used as extraction instrument (5).

Extraction methodology. Triglyceride extractions were performed according to the technique developed by Maroto *et al.* (6). For A-type extractions, a 1:1 CSL/acetone ratio was used. For B-type extractions, a 1:0.75 CSL/acetone ratio was used for the first stage and a 1:0.5 ratio for the following ones. A- and B-type extractions were performed twice, in five 15-min stages and five 30-min stages (Figs. 1 and 2).

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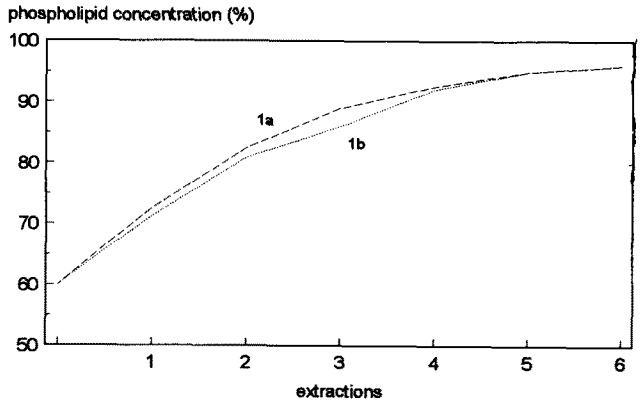

FIG. 1. Phospholipid concentration (%) at each extraction stage. A-type extraction, (. . . .), 15-min stages; B-type extraction (- - - -), 30-min stages.

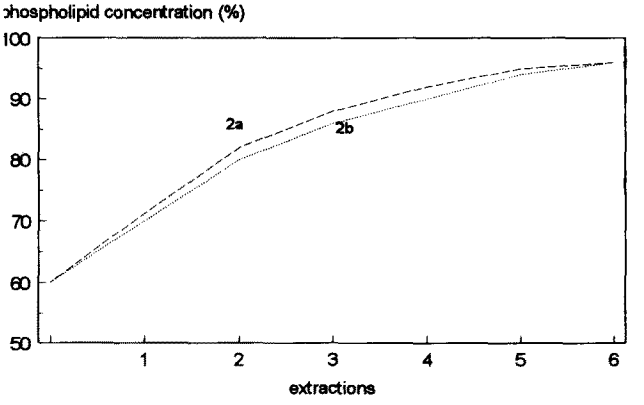

FIG. 2. Phospholipid concentration (%) at each extraction stage. A-type extraction, (. . . .), 15-min stages; B-type extraction (- - - -), 30-min stages.

The experimental conditions summarized in curve 2a (Fig. 2), which corresponds to minimum solvent consumption and the shortest extraction time, were chosen as the most favorable for the extraction process and were used in this study to determine the equilibrium values for the soybean triglyceride/phospholipid/acetone system. The compositions of the extract (clear solution) and the sludge (refined material) were analyzed at each stage of the extraction process (Fig. 3).

Interpretation of figures. In Figure 3, the ordinate represents the concentration of insoluble solid *B* (phospholipid), expressed as:

$$N = B/(A + C) \text{ [mass of } B\text{/mass of } A \text{ (acetone)} + C \text{ (triglyceride)]} \quad [1]$$

in extract (curve K′G′) and sludge (curve KG), regardless of whether the solid was soaked with the solution or not. In the abscissa, the *C* concentration in the extract (*X*) and in the sludge (*Y*) was expressed as a $C/(A + C)$ ratio while disregarding the concentration of *B*. In the sludge, total triglyceride concentration included both free triglycerides and triglycerides attached to phospholipids:

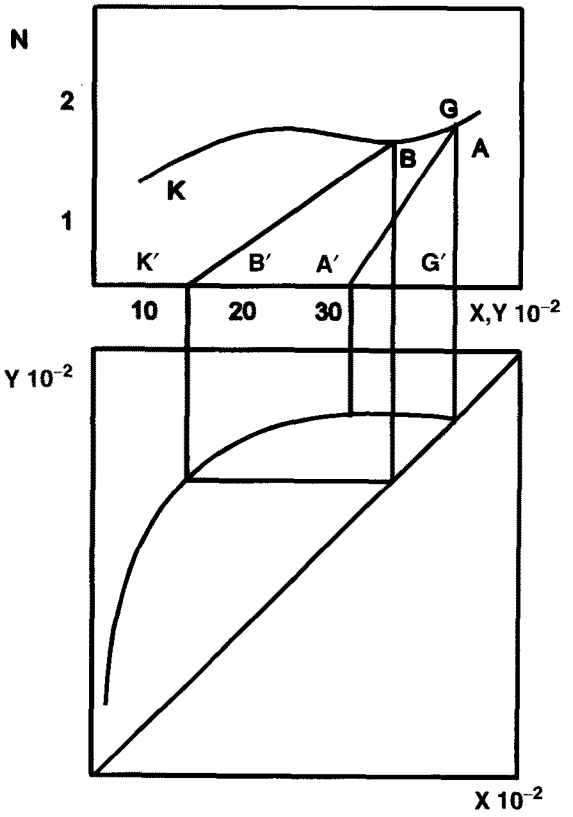

FIG. 3. Equilibrium composition for the soybean triglyceride/phospholipid/acetone system.

$$X = C_{extract}/(A + C) \quad [2]$$
$$Y = C_{sludge}/(A + C) \quad [3]$$

For instance, when CSL was still acetone-free, as before lixiviation, $A = 0$ and $Y = 1$. For pure solvent *A*, *B* and $C = 0$, thus $N = 0$ and $X = 0$.

RESULTS AND DISCUSSION

Soybean triglycerides and acetone are soluble in all proportions, and all possible combinations of the mixture were tested (curve KG, Fig. 3). According to this observation, simple contact between CSL and acetone should have been enough to separate triglycerides from phospholipids. However, in A-type, as well as in B-type extractions, intense shaking was necessary to bring CSL into contact with acetone, and several extraction stages were needed to get the desired 97% purity level in the phospholipids. Therefore, a chemical or physicochemical interaction between phospholipids and triglycerides may have occurred. The presence of nonpolar fractions in the phospholipid molecules may suggest that the interactions observed in this study were hydrophobic and occurred between the hydrophobic chains that constitute both phospholipids and triglycerides.

The origin and significance of these interactions have been

studied by several authors. Demel *et al.* (7) found that the behavior of mixed cholesterol/phospholipid films is governed by a number of factors, such as Van der Waals interactions, configurational entropy, and alterations in the water structure adjacent to the monolayers. Lecuyer and Dervichian (8) and Cornwell *et al.* (9) determined that the mean molecular area in the cholesterol/lecithin monolayer is smaller than the area expected from a simple additivity rule. This apparent contraction has been explained in terms of interaction and molecular association and has been defined as the "condensation effect." This condensation phenomenon is seen as the result of a balance of factors, principally chain-configurational terms, Van der Waals interactions, and water-structure changes (10). These effects depend on temperature, length, and slope of the chains, and on their resulting ability to interact with each other, with cholesterol, and with the substrate water (10). For instance, short-chain saturated phosphoglycerides undergo smaller Van der Waals interactions and, therefore, are less able to give "condensation effects" (10). Moreover, hydrophobic interactions between proteins and lipid chains have been observed (11), suggesting that similar interactions between phospholipids and triglycerides may also exist.

In Figure 3, the A′A and B′B lines represent the equilibrium data for each purification stage. These lines were not vertical, indicating that the triglyceride composition was higher in the sludge than in the extracts for all stages. This higher concentration was the result of the presence of free triglycerides and triglycerides attached to phospholipids, which increased the triglyceride concentration in the sludge. The solution in the sludge had the same composition as the solutions obtained from the extracts; therefore, the interaction between triglycerides and phospholipids seemed to be a good explanation for the higher triglyceride concentration in sludge. As a result, the equilibrium points for the whole extraction process were above the straight line $Y = X$ (Fig. 3).

The KG curve was not parallel to the x-axis, which showed that solids did not have the same degree of sedimentation or drainage for all solute concentrations (Fig. 3). This was also confirmed in A- and B-type extractions, where the separation of extracts and sludge was difficult at high triglyceride concentration. However, as the extraction process developed, separation became easier. The K′G′ line corresponded to extracts and coincided with the x-axis (Fig. 3), showing that the separation process had been appropriate because there were no solids in suspension. A further possibility for ordinate N not to be null would be that phospholipids are soluble in acetone.

Desnuelle *et al.* (2) worked with phospholipid/triglyceride mixed layers in water, which were put under variations of pressure until they were ejected to the surface. The ejection pressure depended on the phospholipid concentration. They observed that the least hydrophilic substance was not ejected to the surface when it reached its normal vaporization pressure. The substance was held on the surface by lateral cohesive forces exerted by hydrophilic substances. This delay is more important as the concentration of the hydrophilic substance increases. The phospholipid molecules seemed to be strongly anchored in water by their hydrophilic parts, while keeping the glyceride molecules on the surface attached to their hydrophobic fraction. The cohesion forces are probably exerted between the paraffinic chains of the triglycerides and the phospholipids (2).

In the present study, when the phospholipid concentration reached 80% in A- and B-type extractions, the viscosity of the system increased significantly from 33 to 15000 cp. These observations concurred with work by Desnuelle *et al.* (2) and Demel (10), who observed two clear changes in the ejection pressure at phospholipid concentrations ranging from 25–30% and 60–80% (2,10).

From the results obtained in this study, we concluded that there were hydrophobic interactions between fatty acids that constitute soybean triglyceride and phospholipid hydrophobic chains.

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