Effect of Degumming Reagents on the Composition and Emulsifying Properties of Canola, Soybean and Sunflower Acetone Insolubles

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Acetone insolubles (AI) extracted from crude canola, soybean and sunflower oils using six degumming reagents (water, citric, phosphoric and oxalic acids plus acetic and maleic anhydride) were separated into phospholipid (PL) components by HPTLC. The separated PL were quantified by phosphorus determination. Statistical analysis of the PL composition data indicated that the chemical degumming reagents did not dramatically alter the PL profiles although some significant differences were observed.

Acetone insolubles recovered by water degumming produced the most stable oil-in-water emulsions. Those AI isolated by citric acid, acetic anhydride and maleic anhydride treatments produced slightly less stable emulsions but showed good potential as emulsifying agents. Phosphoric and oxalic acid treatments produced AI with very poor emulsifying properties.

Commercial lecithin is a complex mixture of phospholipids (PL), glycolipids (GL), neutral lipids (NL) and other non-PL compounds. Vegetable lecithins are characterized by a relatively high percentage of free and bound carbohydrates. Erdahl (1) has characterized oil-free commercial soybean lecithin as containing 82.5% PL, 15% GL and 2.5% NL.

The major phospholipids contain structural groups that impart both hydrophobic and hydrophilic properties to the molecule, and the balance between these groups determines their emulsification characteristics. PC promotes O/W emulsions; PE and PI promote W/O emulsions while natural lecithin, with a blend of phospholipids, promotes weak W/O and O/W emulsions (2).

The PL composition of vegetable lecithins has been investigated: soybean lecithin (3,4), crude and degummed soybean oil (5), total lipid extract of sunflowerseed (6,7), total lipid extract of rapeseed (8,9) and canola gums (10). Very few papers, however, have been published on the PL composition of canola lecithins and none on the quantitative changes in composition as a function of degumming reagents.

Lecithin is used in foods primarily as an emulsifier. However, other important applications include: wetting agent, releasing agent, viscosity modifier, crystal inhibitor and as an anti-splattering agent in margarine (2,11,12). Because the surface active properties of lecithin depend on composition as well as physical structure, the degumming efficiency of various reagents could modify the PL composition to a point where emulsification properties are affected. One such modification could involve the levels of hydratable and nonhydratable PL. A previous paper has shown qualitative changes in composition produced by various degumming agents (13).

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The objectives of this study were to quantitatively determine the changes in the PL composition of canola, soybean and sunflower acetone insolubles (AI) prepared with six degumming reagents, to compare the emulsification potential of all AI preparations, and to correlate compositional data (PL and fatty acid) with emulsion stability.

MATERIALS AND METHODS

Materials. Crude undegummed canola oil (mixed cultivars of *Brassica napus* and *Brassica campestris*) and sunflower oil *(Helianthus annus)* were supplied by CSP Foods Ltd. (Saskatoon, Saskatchewan). Soybean oil *(Glycine max)* was obtained from Canadian Vegetable Oil Processors, Hamilton, Ontario. Phospholipid standards [phosphatidylcholine (PC), lysophosphatidylcholine (lyso PC), phosphatidylethanolamine (PE), phosphatidylglycerol (PG), phosphatidic acid (PA) and phosphatidylserine (PS)] were obtained from Sigma Chemical Co., St. Louis, Missouri. Phosphatidylinositol (PI) was purchased from Supelco, Inc., Bellefonte, Pennsylvania, as were the glycolipids: (esterified sterylglucoside (ESG), monogalactosyl diglyceride (MGDG), and digalactosyl diglyceride (DGDG)).

Degumming, acetone insolubles and fatty acid profile. The degumming procedure, preparation of the AI and determination of the fatty acid profiles were described in a previous paper (13).

Thin layer chromatography (TLC). The AI were separated into individual PL fractions on LHP-K linear HPTLC plates (Whatman Inc., Clifton, New Jersey). Forty μl of solution containing ca. 30 mg/ml AI dissolved in chloroform were streaked across the bottom of the HPTLC plate and eluted for 30 min in a solvent mixture (chloroform:methanol:acetic acid:water, 75:45:3:1) developed by Allan and Cockcroft (15).

The PL and GL compounds were identified with reference standards run alongside the AI mixtures. The compounds were visualized by spraying with ninhydrin for PE, Dragendorf for PC and lyso PC, α-naphthol to distinguish the glycolipids and a general spray reagent described by Vaskovsky and Kostetsky (14) for the detection of all PL fractions.

The neutral lipids migrated with the solvent front, whereas the more polar PL generally remained near the bottom of the plate (except for PA) and the bulk of the glycolipids migrated to the top half (Fig. 1). Seven PL were visualized in each of the three oilseed samples. Six of the seven PL were identified: Lyso PC, PC, PI, PE, PA and PG (the latter compound present at relatively low levels). The remaining phosphorus-containing compound has been tentatively identified as "diphosphatidyl glycerol" (DPG). Excellent separation between PE, PG and DPG was achieved with this method.

Seven glycolipids bands were visualized with the α-naphthol spray reagent although only MGDG and DGDG

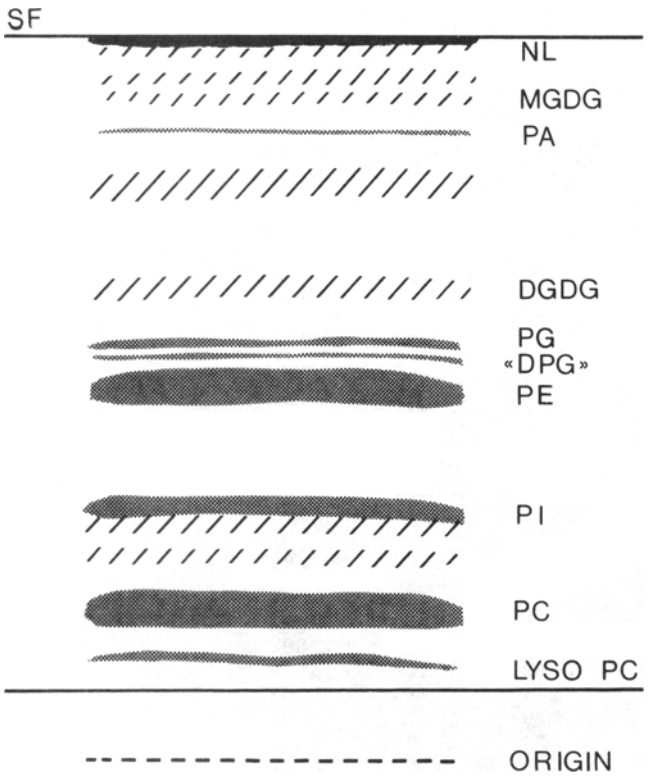

FIG. 1. Typical HPTLC analysis of acetone insolubles. Lysophosphatidylcholine (lyso PC); phosphatidylcholine (PC); phosphatidylinositol (PI); phosphatidylethanolamine (PE); diphosphatidylglycerol (DPG); phosphatidylglycerol (PG); digalactosyl diglyceride (DGDG); phosphatidic acid (PA); monogalactosyl diglyceride (MGDG); neutral lipid (NL). Solvent system: Chloroform/methanol/acetic acid/water (75:45:3:1, v/v/v/v). Development time: 30 min. Detection reagents: α-naphthol for glycolipids, molybdic acid reagent for phospholipids.

were identified with standards. Unidentified compound(s) remaining at the origin were purple when sprayed with α-naphthol, which strongly suggests the presence of glycolipids.

Phosphorus analysis. The separated PL bands were charred by spraying the plates with 5% ammonium sulphate (16) and heating at 200 C for 10 min. The various PL and GL classes were seen as brown bands on a white background. The PL bands were marked by scoring and then ashed overnight at 500 C. The marked PL bands were scraped into centrifuge tubes and extracted with two ml of distilled water. The silica was vortexed and then centrifuged for five min at 1500 rpm. Clear 1.5-ml aliquots were placed in test tubes, tightly capped and boiled for one hr in the presence of three drops of 6N sulphuric acid prior to phosphorus analysis. After cooling in running water, 1.5 ml of Reagent C (17) were added and the samples incubated for one hr at 37 C. Absorbances were read at 750 nm with a Beckman model 35 spectrophotometer.

Emulsification. Oil and water emulsions were prepared by dispersing 0.05% AI in 60 ml paraffin oil with constant stirring and heating. Forty ml of water was added to this mixture and blended for two min in a Polytron homogenizer (Brinkmann Instruments, Rexdale, Canada). The final emulsion was prepared by passing the blended sample twice through a laboratory homogenizer (A-AN, Foss Electric, Hillerod, Denmark). Aliquots of the emulsion were transferred into 15-ml graduated tubes and centrifuged at 650 × *g* for 30 min. Phase separation was measured using the graduations on the tubes to obtain a relative assessment of the emulsion stability. Liquid paraffin was used for the oil phase as it produced a stable emulsion with a minimum amount of emulsifier (0.05% total volume basis). Emulsions prepared with a commercial vegetable oil were shown to be less stable than those using paraffin oil and therefore were not used in this study. Four determinations were performed on each AI mixture.

Statistical analysis. Statistical analysis was performed on all data using the GLM (General linear model) procedure developed by Statistical Analysis System (18). Probability levels of $p < 0.05$ were considered significant.

RESULTS AND DISCUSSION

Quantitative TLC analysis of acetone insolubles. The percentage of each PL class was calculated by dividing the phosphorus levels in each band by the total phosphorus content recovered from all bands. The results are shown in Table 1. The statistical analysis of the PL composition data indicated that the chemical degumming reagents did not dramatically alter the PL profiles. The following discussion will, therefore, briefly consider only the effects of water, citric acid and phosphoric acid.

Phosphoric acid is known for its ability to promote hydration of those PL considered to be nonhydratable (NHPL: lyso PC and PA), while the effects of citric acid degumming on NHPL removal have not been reported previously. Both citric and phosphoric acids were found to be significantly better than water in removing Lyso PC from each of the three oils. Citric acid was equivalent to water in extracting PA, while water was more effective than phosphoric acid in removing PA, which is considered to be a NHPL.

In the case of the hydratable PL, citric acid was shown to be less effective than water in PC removal. Water, citric and phosphoric acids were not significantly different from one another in terms of PE removal while citric acid was the most effective in removing PI from canola oil.

Racicot and Handel (5) reported that degumming removed each of the PL to some degree but that some phospholipids were removed more easily than others. They observed a significant decrease in the proportion of PC in degummed oils. Their results indicate that PL considered to be nonhydratable were being hydrated to some extent.

Our studies showed that although the PL were removed to a greater extent when using chemical degumming reagents than with water, the proportion of the individual PL did not change substantially. The PL composition of the three oilseeds' AI were compared using the results from water degumming. PC was present in similar levels in all three oilseed AI (33–35%). PE was present at the lowest levels in canola AI (21%) compared with 31% and 33% in sunflower and soybean, respectively. Sunflower was highest in PI (21%), while levels in canola and soybean AI were 15% and 17%, respectively. Lyso PC was found at roughly similar levels in the three oilseed AI (4–6%), as was PA (2–4%). However, PG plus DPG was

TABLE 1

Relative Phospholipid Composition of Acetone Insoluble Mixtures

	Degumming reagents					
	Water	Citric acid	Phosphoric acid	Oxalic acid	Acetic anhydride	Maleic anhydride
	% Phospholipid[a]					
Canola AI						
PC	32.51	25.51	39.21	31.52	32.15	25.00
PE	21.09	22.04	17.89	18.29	22.32	13.52
PI	15.17	26.35	12.59	18.90	19.03	16.21
Lyso PC	4.59	8.65	11.79	7.51	6.20	8.42
PA	3.19	2.77	1.06	1.54	2.76	1.73
PG + DPG[b]	23.45	15.05	17.47	23.12	17.54	34.96
Soybean AI						
PC	32.54	27.90	29.09	28.31	31.45	31.08
PE	33.30	32.61	32.89	29.36	15.13	19.23
PI	17.29	16.18	16.39	26.15	22.01	16.18
Lyso PC	4.21	6.73	14.27	8.28	7.77	8.31
PA	3.50	4.37	1.80	2.22	8.96	2.94
PG + DPG[b]	9.17	12.22	5.56	5.77	8.82	21.88
Sunflower AI						
PC	34.97	24.86	35.60	20.54	36.77	24.92
PE	31.41	24.56	23.71	31.02	10.70	33.18
PI	21.38	15.93	18.65	20.15	16.68	12.23
Lyso PC	5.86	13.07	16.61	19.40	13.17	7.90
PA	2.39	2.87	1.17	2.30	7.40	1.67
PG + DPG[b]	3.98	18.01	4.25	6.56	15.28	20.11

[a]Percentage based on total phosphorus recovered on plate. Average of two determinations.

[b]PG and DPG were analyzed together as a single band.

See Fig. 1 legend for abbreviations.

TABLE 2

Percentage of Major Phospholipids in Water Degummed Acetone Insolubles[a]

Acetone insolubles	PC %	PE %	PI %	Reference
Canola	47.3	31.2	22.1	Present study[b]
	45.5	36.4	18.2	Ackman (19)
Soybean	39.1	39.9	20.8	Present study[b]
	39.7	34.9	25.4	Eichberg (20)
	42.9	34.1	22.4	Erdahl (1)
Sunflower	40.0	35.8	24.4	Present study[b]

[a]Phospholipid compositions were recalculated on the basis of the three phospholipid fractions totalling 100%.

[b]Average of 2 determinations.

See Fig. 1 legend for abbreviations.

found at considerably higher levels in canola (23%) than in soybean and sunflower (9% and 4%, respectively). Unknown phosphorus-containing compounds were found at levels between 6% and 12%.

A comparison of our results and those of other workers is presented in Table 2. No direct comparisons were possible for sunflower AI. However, based on literature values of the percent of PL in crude sunflower oil (6,21) the level of PC was higher (49–64%) and PE was considerably lower (21–23%).

Emulsification properties of AI. The results show that AI derived from simple water hydration produced the most stable emulsions (Fig. 2). Citric acid-, acetic- and maleic anhydride-extracted AI produced emulsions with equal stability, whereas emulsions prepared with AI from phosphoric and oxalic acid treatments were readily destabilized by centrifugation. Perhaps traces of phosphoric and oxalic acids were not completely removed by two acetone washings and contributed to poor emulsion stability.

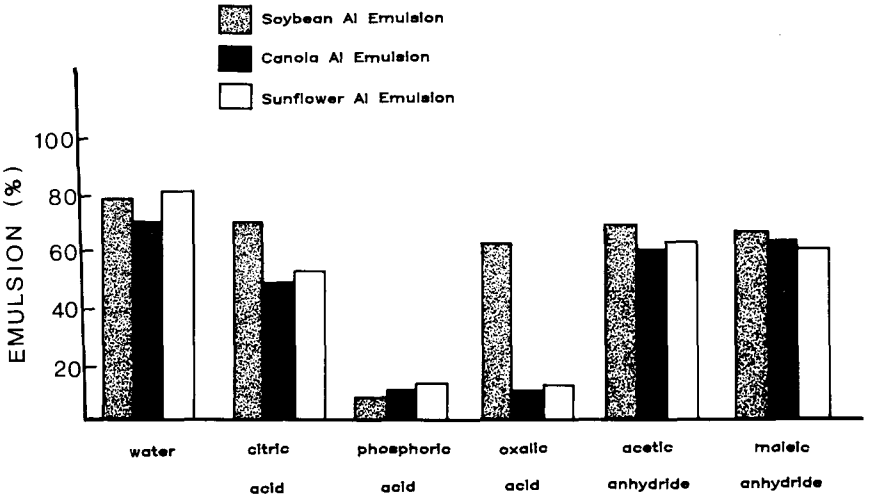

FIG. 2. Emulsion (60:40, oil/water) stabilizing ability of soybean, canola and sunflower acetone insolubles. Each bar represents the mean of 4 determinations.

TABLE 3

Fatty Acid Composition of Phospholipids from Canola, Soybean and Sunflower Acetone Insolubles

Acetone insolubles	Fatty acids[a]						
	16:0	18:0	18:1	18:2	18:3	% Sat.	% Unsat.
	% Composition[b]						
Canola	11.3	1.7	46.2	36.1	4.7	13.0	87.0
Soybean	19.2	4.1	10.5	60.1	6.1	23.3	76.7
Sunflower	16.8	5.0	11.2	67.0	—	21.8	78.2

[a]The major fatty acids listed comprise >90% of the total fatty acid composition.

[b]Average phospholipid fatty acid composition based on a weighted percent of PC, PE and PI in water-degummed AI from canola, soybean and sunflower (Table 2) and the individual fatty acid percentages for each phospholipid (13).

One objective of this study was to correlate the compositional changes in the PL and fatty acid profiles of the six AI with their respective emulsion stabilities. Weber (3) suggested that the physical properties of commercial lecithin are probably due to the proportion of PL in the lecithin and their respective fatty acid composition, as well as to the presence of other lipids.

Overall, soybean AI produce emulsions with slightly better stability than those from either sunflower or canola. The latter two AI show about the same effectiveness. Jakubowski (9) reported that the surface activity of rapeseed was less than that of soybean lecithin because of the lower PE content in rapeseed lecithins.

Our composition data does show water-degummed canola AI with lower levels of PE (21%) than water-degummed soybean (33%) or sunflower (31%) AI. Low levels of PE, however, did not correlate with poor emulsion stability.

When the average fatty acid profile of the total PL fraction from each oil was considered (Table 3), the PL of both soybean and sunflower AI were found to be equally unsaturated (77 and 78%, respectively) while canola was 87% unsaturated due mainly to high levels of oleic acid. Considering the similarity between soybean and sunflower profiles, fatty acid composition does not appear to account for the enhanced emulsifying properties of soybean AI.

The reagent that produced the greatest difference in emulsion stability was oxalic acid. It is not clear why oxalic acid-extracted soybean AI produced a stable emulsion while similarly treated canola AI did not. When the water-degummed AI of each oil are used as reference in a comparison of these two AI, the effect of oxalic acid on the change in composition of both AI was found to be very similar (Table 1). Both soybean and canola AI showed a reduction in PC, PE, PA and (PG + DPG) together with an increase in PI and Lyso PC.

The emulsifying properties of oxalic- and phosphoric acid-treated AI were generally poorer than those of water-degummed AI even though their PL compositions were similar. Rydhag (22) indicated that charged anionic PL (i.e., PI and PA) are responsible for improving the

emulsification properties of the lecithin mixture. This study showed that the levels of PI in phosphoric- and oxalic acid-extracted AI were not significantly different from those found in water-degummed AI. There was partial agreement, however, with PA, where the levels of PA in phosphoric acid-extracted AI were significantly lower.

The results of this preliminary emulsion study indicate that although the PL composition of the acetone insolubles did not vary dramatically, emulsion stability was affected by the various degumming treatments. Apart from the excellent emulsifying properties associated with water-degummed AI, only those AI recovered with citric acid and anhydride reagents showed potential as food grade emulsifying agents.

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