Effect of Degumming Reagents on the Recovery and Nature of Lecithins From Crude Canola, Soybean and Sunflower Oils

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Six reagents (water, citric acid, phosphoric acid, oxalic acid, acetic anhydride and maleic anhydride) were evaluated for their effectiveness in degumming three crude vegetable oils (canola, soybean and sunflower). All chemical reagents tested were found to be significantly more effective than water in removing lecithin material from all three oils except for acetic anhydride degumming of canola. Citric and phosphoric acids were found to be very effective in reducing phosphorus levels in canola oil (91 and 93% removal, respectively). For soybean oil, all reagents except water showed excellent degumming ability by removing 98% phosphorus, while in the case of sunflower oil, maleic anhydride and oxalic acid produced the highest level of phosphorus removal (95 and 90%, respectively). Both citric acid and acetic anhydride were effective in removing Fe from all three oils during degumming (84 to 94%), while phosphoric acid showed slightly lower values (73 to 87%).

No significant changes in the phospholipid composition or fatty acid profiles of the phospholipid classes were observed as a result of degumming with the various chemical reagents. In general, canola phospholipids were lowest in palmitic, stearic and linoleic acid and contained the highest levels of oleic acid when compared to soybean and sunflower phospholipids. Both citric and acetic anhydride were found to influence the removal of an unknown glycolipid significantly. Canola lecithin was shown to contain a greater amount of glycolipids than sunflower and soybean lecithins.

Commercial lecithin is a naturally occurring mixture of phospholipids (PL) obtained during the refining of crude vegetable oils. For food grade lecithin extraction, 2% hot water or steam is generally used, as this preserves the integrity of the PL and reduces the occurrence of chemical changes (1). The PL hydrate and swell by imbibing water and become insoluble in oil. Approximately 5 to 20% of the PL are not removed from the oil by water degumming (2). To improve removal, a phosphoric acid pretreatment is sometimes used prior to alkali refining. This treatment can be harsh and often produces a lecithin material which is unsuitable for most food applications.

A number of investigators (3-9) have reported on the degumming properties of various chemical reagents. Citric acid is of particular interest as it is currently used by many Canadian processors. A recent study by Diosady et al. (9) has shown that citric acid is a very effective degumming reagent for canola oil. There is, however, very little information concerning the effects of these chemical reagents on the composition and quality of the recovered lecithins.

The objectives of this study were to compare the degumming effectiveness of water, citric acid, phosphoric acid, acetic anhydride, maleic anhyhdride and oxalic acid on crude canola, soybean and sunflower oils and to determine the compositional changes in the lecithin material brought about by these degumming agents.

MATERIALS AND METHODS

Crude undegummed canola oil (mixed cultivars of *Brassica napus* and *Brassica campestris)* and sunflower oil *(Helianthus annus)* were supplied by C.S.P. 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), digalactosyl diglyceride (DGDG)].

Degumming. Samples of crude oil (50 g) were placed in 250-ml centrifuge bottles and heated to 60 C in a constant temperature water bath. The levels (Table 1) and procedures for adding the degumming agents were given by Diosady et al. (9). Following degumming, the oil was cooled to 40 C and centrifuged at 575 × g for 15 min. The degummed oil was separated from the gummy lecithin residue by decantation.

Acetone fractionation. The crude lecithins were concentrated and freed of water and neutral oil by washing in acetone (10). The acetone insolubles (AI) were placed in vials, purged with nitrogen, tightly sealed and stored in a desiccator at < 0 C.

Phosphorus and iron analysis. Phosphorus and iron in the crude and degummed oils were determined using

TABLE 1

Level of Chemical Degumming Reagents

Reagent	Reagent added (g/Kg oil)	Water added (oil basis)
Citric acid (50% w/v)	2.5	2%
Oxalic acid[a] (10% w/v)	2.0	—
Phosphoric acid (85%)	1.7	2%
Acetic anhydride (97.3%)	2.5	2%
Maleic anhydride (powder)	2.5	2%

[a]Added as a 10% aqueous solution.

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the AOCS Official Method (10) and atomic absorption spectrophotometry (11), respectively.

High performance liquid chromatography (HPLC). The major PL in the AI mixtures were separated, identified and collected using a Spectra Physics Model 8000B HPLC, equipped with a μ Porasil analytical column (Waters Associates, Framingham, Massachusetts) and guard column packed with 37–50 μm silica. The column was thermostated at 35 C, and the injection volume was 10 μl. A model 450 variable wavelength UV detector (Waters Associates) set at 210 nm and 0.10 AUFS was used to monitor the effluent. HPLC-grade n-hexane, 2-propanol, chloroform and methanol obtained from Fisher Chemical Co. were filtered through 0.45 μm membrane filter (Millipore Corp., Bedford, Massachusetts). Double distilled deionized water was filtered through a Norganic cartridge (Millipore Corp.) and a 0.45-μm membrane filter.

The mobile phase consisted of three solvents: hexane, 2-propanol and a mixture of water and 2-propanol (1:1, v/v). Helium purging was used during each run to remove dissolved gases. The flow rate was two ml/min. The solvents were programmed for stepwise gradient elution (Fig. 1) using the procedure of Hax and Guerts van Kessel (12). Analysis and regeneration time were 30 and 20 min, respectively. The column was routinely cleaned with 200 ml methanol and 200 ml chloroform.

The PL in the AI mixtures were identified by comparing retention times with standard PL. The standards were run separately and in spiked samples. The areas were electronically integrated. The major PL fractions (PC, PE and PI) were collected directly from the HPLC column, stabilized with BHT (0.01%) and stored at < 0 C for fatty acid analysis.

Gas liquid chromatography (GLC). A Hewlett Packard model 5830 A gas chromatograph equipped with a six-ft glass column packed with 20% DEGS on Chromasorb WAW (80 × 100 mesh) was used for fatty acid analysis. The individual PL fractions separated by HPLC analysis were collected in four-ml reactivials, evaporated to dryness and redissolved in 0.4 ml of heptane. The fatty acids were rapidly methylated by alkali catalyzed transesterification (1.0 ml of 2 N methanolic KOH). After methylation, 0.3 ml of the heptane phase was transferred to a one-ml reactivial and the solvent evaporated to dryness under nitrogen. Two drops of heptane were added to dissolve the methyl esters and one to two μl injected into the GC.

Open column chromatography. A modification of the procedure of Rouser (13) was used to separate and quantitate the neutral lipid and glycolipid fractions in the AI samples. Silica gel (70–230 mesh, Kieselgel 60, Merck) was equilibrated to 10% water and washed with chloroform. A short column (2.5 cm × 5 cm) was packed and a Whatman #1 filter paper disk placed on top. Ten ml of AI (60 mg in $CHCl_3$) was applied to the column and eluted with diethyl ether (100 ml) at a flow rate of two ml/min. The neutral fraction was collected into a pre-weighed flask, rotary evaporated to dryness and weighed. Acetone was then used to elute the glycolipids in a similar manner. The percent neutral lipids and glycolipids were determined gravimetrically, and the percent PL was calculated by difference.

The experimental design consisted of a 6 × 3 factorial arrangement conducted according to a split-plot design with two replications. The main plot factor involved six degumming reagents, while the split-plot factor consisted of three varieties of vegetable oils. Statistical analysis was performed using the GLM (General Linear Model) procedure developed by statistical Analysis System (14). Probability levels of $p<0.05$ were considered significant.

RESULTS AND DISCUSSION

Degumming effectiveness. The efficiency of degumming was evaluated directly by comparing residual phosphorus (P) and iron (Fe) levels in the degummed oils and indirectly by the recovery of AI. Results in Table 2 show that all chemical reagents used in this study were significantly more effective than water in removing P from the three crude vegetable oils with one exception: acetic anhydride degumming of canola. Citric, phosphoric and oxalic acids and maleic anhydride did not significantly differ in their ability to degum all three oils.

In terms of reducing the P levels in canola oil, both citric and phosphoric acids were very effective [91% and 93% reductions, respectively (Table 3)]. Results from a study by Diosady et al. (9) agree closely with the present findings and substantiate the superior degumming properties of citric acid on canola oil. For soybean oil, all reagents except water (86% P removal) showed excellent degumming properties by removing P at the 98% level. Maleic anhydride and oxalic acid proved to be the best reagents for sunflowr oil degumming with 95% and 90% P removal, respectively.

According to Wiedermann (15), an effective degumming agent should reduce the level of P in crude vegetable oils to below 50 ppm. In the case of soybean and sunflower oils, all reagents except water effectively reduced P below 50 ppm. However, only citric and phosphoric acids succeeded in producing acceptable P levels in crude canola oil.

Based on the total P content of the crude undegummed oils and the subsequent reduction of P with water degumming, canola and sunflower oils were shown to contain 40% "nonhydratable" PL (NHPL), while soybean oil contained only 14% NHPL.

Degumming effectiveness was also estimated by measuring residual Fe in the degummed oils (Table 2). Results show that citric acid and acetic anhydride reduced the levels of Fe by 90% and 84%, respectively, in canola oil, 86% and 90% in soybean oil, and 94% and 93% in sunflower oil (Table 3).

Citric acid was found to be the most effective reagent overall in terms of its ability to remove P and Fe from all three crude oils. Citric acid was superior to phosphoric acid in terms of its excellent ability to chelate pro-oxidant Fe. Although acetic anhydride proved to be an effective reagent in removing both P and Fe from sunflower and soybean oils, its ability to remove P from canola was limited.

Acetone insolubles. The average percent AI in sunflower, canola and soybean oils was 0.58%, 1.69% and 2.79%, respectively (Table 2). In all cases except for acetic anhydride degumming of canola oil, the use of

TABLE 2

Percent Acetone Insolubles (undegummed oil), ppm Phosphorus and ppm Iron in Degummed Oils[a]

Degumming reagent	Oils								
	Canola			Soybean			Sunflower		
	% AI	ppm P	ppm Fe	% AI	ppm P	ppm Fe	% AI	ppm P	ppm Fe
Crude	—	617	4.67	—	716	6.63	—	239	7.39
Water	1.53	247	2.50	2.22	100	2.17	0.47	95	1.91
Citric acid	2.31	57	0.49	2.47	14	0.91	0.65	36	0.43
Phosphoric acid	1.73	45	1.21	2.94	15	1.79	0.68	35	0.94
Oxalic acid	1.89	78	1.31	2.80	16	2.83	0.55	24	2.48
Acetic anhydride	0.87	251	0.72	3.07	12	0.69	0.61	35	0.51
Maleic anhydride	1.82	94	4.15	3.24	18	1.51	0.54	13	5.09
	1.69[b]			2.79[a]			0.58[b]		

[a]Mean of 2 determinations.

[b]Mean % AI summed and averaged over all degumming agents.

chemical reagents increased the yield of AI over that of water.

HPLC analysis of the acetone insolubles. A quantitative analysis of the PL composition of the crude AI samples by HPLC was not possible. Although linear standard curves were prepared, the calculated PL concentrations in actual AI samples were unreasonable. The problem was very likely due to variations between the fatty acid composition of the PL standards and PL samples. According to Jungalwala et al. (16), detection of PL in the near UV region is dependent primarily on the degree of unsaturation of the fatty acids rather than on the polar head groups of the PL.

A qualitative assessment of changes in PL composition brought about by the extracting abilities of the various degumming reagents was obtained by comparing peak areas within a single AI sample. Only the three major PL (PC, PE and PI) were investigated.

TABLE 3

Percent Removal of Phosphorus and Iron from Crude Oils

Degumming reagents	Oils					
	Canola		Soybean		Sunflower	
	% P	% Fe	% P	% Fe	% P	% Fe
Water	60	47	86	67	60	74
Citric acid	91	90	98	86	85	94
Phosphoric acid	93	74	98	73	85	87
Oxalic acid	87	72	98	57	90	66
Acetic anhydride	59	84	98	90	85	93
Maleic anhydride	85	12	98	70	95	31

Figure 1 represents the profile of water-degummed canola AI. Peaks labeled A were tentatively identified as neutral lipids and glycolipids. Standard glycolipids, mono- and digalactosyl diglyceride (MGDG and DGDG) and esterified sterol glycoside (ESG) eluted immedi-

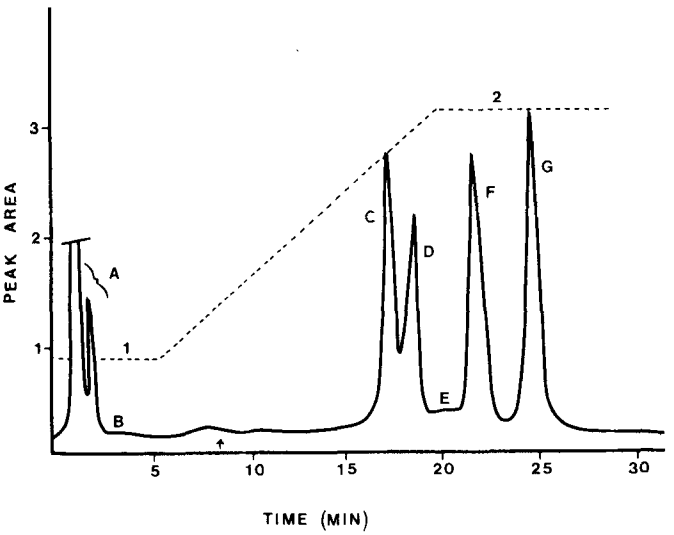

FIG. 1. High performance liquid chromatogram of water-degummed canola acetone insolubles 210 nm, AUFS 0.1, flow rate 2 ml/min. Chromatographic peaks: A, neutral lipids, mono and digalactosyldiglycerides and esterified sterol glycosides; B, phosphatidic acid; C, phosphatidylethanolamine; D, phosphatidylinositol; E, phosphatidylserine; F, unidentified glycolipid; G, phosphatidylcholine. Baseline adjusted to zero (arrow).

Chromatographic Gradient Solvent System

	Time	% Hexane	% 2-Propanol	% 2-Propanol + H_2O
1	0	43.6	53.4	3.0
2	22	43.6	38.4	18.0

TABLE 4

Percent Fatty Acid Composition of Individual PL Classes

Oil	PL[a]	Fatty Acid[b] 16:0	18:0	18:1	18:2	18:3
Canola	PC	7.12	1.12	56.74	31.54	4.18
	PE	11.43	2.12	42.10	42.23	4.17
	PI	19.44	2.16	31.21	38.98	7.21
Soybean	PC	14.80	3.75	12.29	62.31	6.91
	PE	19.12	3.19	8.64	63.82	5.34
	PI	27.73	6.33	10.62	49.15	6.17
Sunflower	PC	10.15	3.43	15.14	71.50	—
	PE	15.74	4.78	11.37	68.64	—
	PI	29.31	7.82	6.29	56.65	—

[a]PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol.

[b]The major fatty acids (listed) comprise >90% of the total fatty acid composition of each phospholipid class.

TABLE 5

Percent Neutral Lipids, Glycolipids and Phospholipids in Acetone Insoluble Mixtures Determined by Open Column Chromatography

Degumming agent	Lipid fraction	Acetone insolubles Canola	Soybean	Sunflower
Water	Neutral lipids	4.5	5.9	3.8
	Glycolipids	17.9	12.8	13.2
	Phospholipids	77.6	81.2	82.9
Citric acid	Neutral lipids	3.2	6.5	—
	Glycolipids	16.4	14.2	—
	Phospholipids	80.4	79.4	—
Phosphoric acid	Neutral lipids	3.4	8.3	—
	Glycolipids	14.1	13.3	—
	Phospholipids	82.5	78.6	—

ately after the solvent, indicating that these glycolipids were much less polar than the PL compounds. The large peak (F) between PC and PS was shown to be a glycolipid by TLC analysis; however, its identity was not determined. The method achieved complete separation between PC, PS and PA, with only partial resolution between PE and PI peaks.

AI samples prepared with acetic and maleic anhydride produced extra peaks. These peaks were shown to result from chemical reactions between the anhydrides and PE. Commercial PE produced two peaks when reacted with acetic anhydride and three peaks when reacted with maleic anhydride. Because one peak from maleic anhydride-treated PE eluted with a retention time similar to PI, all chromatographic runs with maleic anhydride-treated lecithins were not analyzed statistically.

Statistical analysis of the results indicated that the levels of PC, PE and PI in the degummed AI samples were not significantly altered by the type of degumming reagent used. However, an unidentified, highly polar glycolipid, eluting before PC and present in relatively high levels, was affected by the type of degumming agent. Citric acid treatment was shown to be significantly better at removing this glycolipid material from all three oils than water, phosphoric or oxalic acid treatments.

Fatty acid composition. The different degumming reagents had little effect on the fatty acid composition of the individual PL classes. Some minor variations in the fatty acid composition of the individual PL classes were noted, but in general, PC contained the highest levels of unsaturated fatty acid while those of PI were more saturated. As no significant differences were detected, the average fatty acid composition for each of the three major PL (PC, PE and PI) were determined and are presented in Table 4. These results are in close agreement with literature values (17–19). Among the saturated and unsaturated fatty acids of the individual PL classes, a trend was found to exist. When the PL were considered in the order PC, PE and PI, an increase in percent of saturated acids from PC to PI was noted, whereas among the unsaturated fatty acids the trend was towards greater unsaturation from PI to PC. This difference is believed to contribute to the difference in emulsifying properties of PC and PI. The former, possessing a higher degree of unsaturation, tends to promote o/w emulsions, while PI being more saturated shows a tendency to promote w/o emulsifying properties (20).

The fatty acid composition of canola PL differed from those of soybean and sunflower. Canola PL were lowest in palmitic, stearic and linoleic acids while containing the highest levels of oleic acid. The levels of linolenic acid in both canola and soybean PL were comparable.

Fractionation of acetone insolubles by column chromatography. The AI mixtures from water, citric and phosphoric acid-treated soybean and canola oils were fractionated and compared. In the case of sunflower oil, only water-degummed AI samples were separated because of insufficient material. The results are shown in Table 5. The NL ranged from 3.2 to 8.3%, the GL ranged from 12.8 to 17.9% and the PL ranged from 77.6 to 83%.

Ratios of GL to PL were used to assess any differences in AI products caused by the nature of the oils or the type of chemical degumming reagent used. The GL/PL ratio was highest in water-degummed canola AI (0.23) compared to the AI from water-degummed soybean (0.15) and sunflower (0.15).

A comparison of the AI from citric and phosphoric acid-degummed oils showed that the GL/PL ratios for citric acid AI were larger than those from phosphoric acid. Similar results were obtained in the HPLC analyses where the glycolipids were extracted to a greater degree by citric acid than by phosphoric acid from all three oils.

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REFERENCES

1. Carr, R.A., *J. Am. Oil Chem. Soc. 55*:765 (1978).
2. List, G.R., J.M. Avellaneda and T.L. Mounts, *Ibid. 58*:892 (1981).
3. Hayes, L.P., and H. Wolff, *Ibid. 33*:440 (1956).
4. Braae, B., B. Brimberg and M. Nyman, *Ibid. 34*:293 (1957).
5. Hvolby, A., *Ibid. 48*:503 (1971).
6. Ohlson, R., and C. Svensson, *Ibid. 53*:8 (1976).
7. Ayerbe, F.R., *Investigacion 28*:1 (1977).
8. List, G.R., C.D. Evans, K. Warner, R.E. Beal, W.F. Kwolek, L.T. Black and K.J. Moulton, *J. Am. Oil Chem. Soc. 54*:8 (1977).
9. Diosady, L., P. Sleggs and T. Kaji, *Ibid. 59*:313 (1982).
10. *Official and Tentative Methods of the American Oil Chemists' Society,* 3rd edn., edited by W.E. Link, AOCS, Champaign, IL, 1975.
11. *Official Methods of Analysis.* 12th edn., Association of Official Analytical Chemists, Washington DC, 1981.
12. Hax, W.M.A., and W.S.M. Guerts van Kessel, *J. Chromatogr. 142*:735 (1977).
13. Rouser, G., G. Kritchevsky, G. Simon and G.J. Nelson, *Lipids 2*:37 (1967).
14. *SAS user's guide,* SAS Institute Inc., Box 8000, Cary, North Carolina, 1979.
15. Wiedermann, L.H., *J. Am. Oil Chem. Soc. 58*:158 (1981).
16. Jungalwala, F.B., J.E. Evans and R.H. McCluer, *Biochem. J. 155*:55 (1976).
17. Sosulski, F., R. Zadernowski and K. Babuchowski, *J. Am. Oil Chem. Soc. 58*:561 (1981).
18. Weber, E.J., *Ibid. 58*:898 (1981).
19. Chapman, G.W., *Ibid. 57*:299 (1980).
20. Aneja, R., J.S. Chadha and R.W. Yoell, *Fette, Seifen, Anstrichm. 73*:643 (1971).

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