Low-Molecular Weight Organic Compositions of Acid Waters from Vegetable Oil Soapstocks

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ABSTRACT: Alkaline extracts (soapstocks) from canola, corn, cottonseed, peanut, soybean, and sunflower oil refining were acidified, and identities and concentrations of the low-molecular weight organic components of the resulting acid waters were determined by gas chromatography, followed by mass spectroscopy, and by high-performance liquid chromatography. The main components of each acid water sample, in order of decreasing concentration and after omitting the fermentation product lactic acid, were phosphoric acid, α-glycerophosphate, and glycerol from canola; *myo*-inositol, phosphoric acid, α-glycerophosphate, and *myo*-inositol-1-phosphate from corn; glycerol, α-glycerophosphate, *myo*-inositol-1-phosphate, and β-glycerophosphate from cottonseed; phosphoric acid, glycerol, and *myo*-inositol from peanut; α-glycerophospho-1-myo-inositol, *myo*-inositol-1-phosphate, α-glycerophosphate, and glycerol from soybean; and α-glycerophosphate, glycerol, *myo*-inositol-1-phosphate, and β-glycerophosphate from sunflower.
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KEY WORDS: Acid water, canola, composition, cottonseed, glycerophosphate, maize, *myo*-inositol phosphate, peanut, soapstock, soybean, sunflower, vegetable oil.

Vegetable oil refining sometimes involves water extraction of phosphatides and gums and usually involves alkaline extraction of other hydrophilic material. The latter extract, commonly called soapstock or foots and comprising 2% or more of the initial oil volume, is a complex mixture of water, fatty acids, phosphatides, proteins, carbohydrates, and entrained vegetable oil. When not immediately added to animal feed, it is acidified. The resulting dewatered lipid fraction and sometimes the emulsion fraction, when it appears, are included in animal feed, while the aqueous fraction (acid water) is discarded at high cost to the processor. The acid water should contain the bulk of the remaining water-soluble material in soapstock after fatty acid protonation, including products resulting from the exposure of lipids and carbohydrates to heat and highly basic and acidic solutions, as well as products of any fermentation that occurred during processing or storage.

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This article describes the identification and quantitation of the low-molecular weight organic components of the acid waters from soapstocks of soybean, canola, sunflower, peanut, cottonseed, and corn oils, the first, third, fourth, fifth, sixth, and ninth most commonly produced vegetable oils, respectively (1). Analysis was by gas chromatography (GC) of trimethylsilyl (TMS) derivatives, followed by electron-ionization mass spectroscopy (EIMS) and chemical-ionization mass spectroscopy (CIMS), and by high-performance liquid chromatography (HPLC) with strong-acid ion exchange resin. To our knowledge, there has been no such investigation of acid waters, although the rough composition of acid water from soybean oil soapstock is available (2), and some proposals have been advanced for soapstock and acid water utilization (3–8). This work on acid waters follows a similar project on ethanol stillages from cane molasses, citrus waste, cornstarch, and sweet whey (9,10). Both projects were designed to find components of underutilized by-product streams that could be economically recovered and to learn more about the composition of the materials from which they were derived. We did not intend in either project to find the average composition of each stream by analyzing many samples of each; instead, we emphasized discovering the general composition and main components of streams from many sources.

The following article (11) describes the identification and quantitation of the low-molecular weight organic components of the acid water from coconut soapstock. It appears separately because coconut acid water is almost totally different in composition from the six acid waters presented here, which are qualitatively quite similar to each other.

EXPERIMENTAL PROCEDURES

Soapstock sources and separation. Soapstocks were obtained from vegetable oil and feed processors such as Archer Daniels Midland (Decatur, IL) and CanAmera Foods (Toronto, Canada), as well as from the Southern Regional Research Center of the U.S. Department of Agriculture (New Orleans, LA). They were separated, after gentle heating of the thicker samples, by acidification to pH 1.3–1.5 with 2.8N H_2SO_4, which added 20–40% to the resulting acid water vol-

umes. The mixtures were centrifuged at 8000 × *g* for 15 min to separate acid water, emulsion, and organic fractions. In some cases, cooling in an icewater bath after centrifugation was necessary for cleaner separation. A second 20-min centrifugation of the acid waters at 8000 × *g* removed small amounts of emulsion and organic fraction from them. All three fractions from each soapstock were collected, and their volumes were measured. The acid waters were neutralized with 2–5N NaOH to near pH 7, adding a further 12–30% to their volumes, and were passed successively through 0.45- and 0.22-μm filters, yielding clear-colored solutions that were stored at 4°C.

The neutralized acid waters were evaporated to dryness by spreading 1–3 mL onto glass plates held at 50°C for 1–2 h. Residues were scraped off with a razor blade. This technique yielded more consistent samples than the rotary vacuum evaporation used in our stillage research (9,10).

Proximate analyses. Proximate analyses of soapstocks and neutralized acid waters were conducted by Woodson-Tenent Laboratories (Des Moines, IA). For soapstocks, moisture was determined by distillation with toluene [AOCS Official Method Ca 2a-45 (12)], total nitrogen was measured after combustion in a Leco apparatus [AOCS Recommended Practice Ba 4e-93 (12)], total fatty acids were found after saponification [AOCS Official Method G 3-53 (12)], and phosphatides were calculated as thirty times the total phosphorus measured by the spectrophotometric molybdovanadophosphate method [AOAC Official Method 958.01 (13)]. For neutralized acid waters, moisture was determined by evaporation in a 135°C forced-draft oven [AOAC Official Method 930.15 (13)], total nitrogen was measured as above, fat was found gravimetrically after HCl hydrolysis [AOAC Official Method 954.02 (13)], ash was determined by combustion at 600°C [AOAC Official Method 942.05 (13)], and carbohydrate was that matter that was not moisture, fat, ash, or crude protein, the last being 6.25 times total nitrogen.

Derivatization reactions. Powders obtained from 0.5 mL of neutralized acid waters (3.0 and 1.5 mL of canola and peanut solutions, respectively) were added to 500 μL pyridine, 450 μL hexamethyldisilazane, and 50 μL trifluoroacetic acid, all from Pierce (Rockford, IL), to form volatile TMS derivatives (14). The samples were heated at 70°C for 1 h with periodic shaking until a single liquid phase was present. Small amounts of derivatized proteins and other components settled to the bottom of the reaction vial during this time.

Preparation of standards. Samples of 0.1–0.8 mg of pure chemical standards purchased mainly from Sigma (St. Louis, MO) were derivatized as before. As noted previously (10), amino acid standards required up to 2 h at 70°C to obtain satisfactory results. In addition, when high *myo*-inositol concentrations were present, penta-TMS derivatives were noticed along with the expected hexa-TMS derivative (10).

GC. Derivatized samples were separated in a Hewlett-Packard (Avondale, PA) 5890A gas chromatograph with a 30 m × 0.25 mm i.d. × 0.1 μm film thickness DB-5 fused-silica capillary column (J&W Scientific, Folsom, CA) and a flame-ionization detector. Injector and detector temperatures were 270°C, the split ratio was 1:100, and the column He flow rate was 0.95 mL/min. Injection volumes were 3 μL. Two temperature programs were used (9): (i) 50°C for 10 min, followed by a 2.5°C/min rise to 150°C, which was held for 10 min, followed by a 20°C/min increase to 280°C; and (ii) 150°C for 10 min, followed by a 2.5°C/min rise to 250°C, held for 20 min, followed by a 5°C/min rise to 280°C. In both cases, the final temperature was held for 20 min. Peaks were integrated with a Hewlett-Packard 3392A integrator.

Response factors. GC response factor equations were developed for known components over concentration ranges that bracketed experimental values. They were linear with concentration, with all regression coefficients >0.99.

Mass spectroscopy (MS). EIMS and CIMS was performed with a Finnigan (San Jose, CA) Magnum ion-trap mass spectrometer with the two GC temperature programs and a 30 m × 0.25 mm i.d. × 0.25 μm film thickness DB-5 fused-silica capillary column with a split ratio of 1:50. CIMS was carried out with ammonia gas. Injection volumes were 1 to 2 μL of derivatized acid water.

Peak identification. Components were identified by a three-step procedure: (i) EIMS fragmentation patterns of GC peaks were compared to an on-line library of standards; (ii) CIMS spectra of the GC peaks gave molecular weights of the components they represented; and (iii) GC retention times of derivatized standards, along with the large number obtained in related projects (9–11), were compared to the retention times of the peaks in question.

HPLC. HPLC was conducted on underivatized but neutralized samples of the acid waters with an ISCO (Lincoln, NE) Model 2350 pump, a Bio-Rad (Richmond, CA) Cation H Micro-Guard precolumn and HPX-87H column, a Knauer (Berlin, Germany) Model 786 differential refractometer, and a Beckman (Berkeley, CA) Model 165 variable wavelength detector set to 210 nm. The eluent was 0.01N H_2SO_4 at 0.75 mL/min. Injection volumes were 20 μL, and the column temperature was 35°C.

RESULTS AND DISCUSSION

Soapstock and acid water characterization. Properties such as soapstock pH, relative volumes of different fractions after acidification, and dry weights of the acid waters after neutralization varied greatly (Table 1). Cottonseed soapstock yielded a large organic fraction volume, while all but cottonseed and peanut soapstocks gave more than 40% acid water, even excluding the dilution caused by H_2SO_4 addition. Large emulsion volumes occurred with all but the canola and cottonseed soapstocks. The former yielded almost no organic and emulsion fractions after neutralization. Canola and peanut neutralized acid waters had low dry weights, while those of cottonseed and sunflower acid waters were high. These differences are expected, not only because the oils come from different plant species but also because processing techniques vary among refiners. For instance, a second canola

TABLE 1
Characteristics of Soapstocks and Separated Fractions

Sources	Soapstock pH	Volumes of fractions (%)[a] Acid water	Emulsion	Organic	Dissolved solids concentration in acid water (g/L)
Canola	11.2	97	1	2	24.8
Corn	7.2	42	26	32	89.4
Cottonseed	10.2	21	0	79	148
Peanut	9.1	28	56	16	52.7
Soybean	7.0	47	27	26	86.6
Sunflower	5.6	42	32	26	105

[a]Correcting for dilution by H_2SO_4 solution.

sample from a different processor, not subjected to further analysis, was much higher in both organic and emulsion fractions and dissolved solid concentration in the neutralized acid water than the one analyzed here. It is also quite possible that different samples of the same soapstock from the same processor might vary greatly.

Proximate analyses of the various soapstocks and neutralized acid waters also were quite different (Table 2). Canola and peanut soapstocks contained more water than the others, and, in general, high soapstock moisture led to high moisture in the corresponding acid water. The moisture in each soapstock sample could be roughly correlated with the amount of acid water and emulsion formed after acidification, while the fatty acid content was highly correlated with the amount of organic phase that resulted, with cottonseed soapstock in both cases being an outlier.

GC peak identification and quantitation. Gas chromatograms of the TMS-derivatized neutralized acid waters, obtained with 50°C→150°C and 150°C→250°C temperature profiles, are shown in Figure 1. Concentrations obtained by GC of each identified component are listed in Table 3.

Although there were obvious differences between the gas chromatograms of the different neutralized acid waters, the overall pattern of peaks was similar. In the low-temperature chromatograms, lactic acid, phosphoric acid, glycerol, glyceric acid, and β-glycerophosphate often yielded major peaks, along with a number of by-products of the trimethylsilylation reaction. Threitol and erythritol were often minor components, along with a number of unidentified peaks. In the high-temperature chromatograms, the same peaks were often seen: threitol, erythritol, and β-glycerophosphate again, and then α-glycerophosphate, several tautomeric forms of fructose, galactose, and glucose, gluconic acid, partially and fully TMS-derivatized forms of *myo*-inositol, *myo*-inositol-1-phosphate, *myo*-inositol-2-phosphate, sucrose, α-glycerophospho-1-*myo*-inositol, two forms of melibiose, and raffinose. Neutralized acid waters from corn, peanut, soybean, and sunflower soapstocks also had significant amounts of pinitol and *chiro*-inositol. Mannitol, sorbitol, and palatinose also occasionally appeared.

Although the largest peaks in both low- and high-temperature chromatograms were almost always identified, a sizable number of unknowns defied identification, even with the large number of derivatized standards submitted to GC here and elsewhere (9–11). Those standards tested here without success were 2-aminobutyric acid, γ-caprolactone, ε-caprolactone, chlorogenic acid, choline, ethanolamine, fucitol, *epi*-inonose, *scyllo*-inonose, isomaltose, 2-ketogluconic acid, 5-ketogluconic acid, nicotinic acid, nigerose, 2-phenylacetic acid, quebrachitol, ribonic acid, sequoyitol, tagatose, thiamine, α,α-trehalose, turanose, and xylonic acid. Some of the unknowns occurred in several samples, including those that produced peaks at retention times relative to glycerol (30.80 min) of 0.76, 0.79, 1.47, and 1.54 in the low-temperature chromatogram and at retention times relative to *myo*-inositol (27.74 min) of 0.54, 1.17, 1.33, 2.55, and 2.69 in the high-temperature chromatogram. Identification of unknowns was complicated by the fact that they in general did not yield usable mass spectra.

Nearly all the identified components can be found in plant tissue except lactic acid, which is almost certainly a product of anaerobic fermentation of unrefined vegetable oil or soapstock during processing or storage. In fact, lactic acid concentrations in the various soapstocks are strongly correlated with soapstock pH (Table 1). *Chiro*-inositol and pinitol have been reported in several agricultural products, such as soybeans (15–17), and *chiro*-inositol has been found recently in stillage derived from citrus waste at much higher levels than found here (10).

TABLE 2
Proximate Analyses of Soapstocks and Neutralized Acid Waters

	Canola	Corn	Cottonseed	Peanut	Soybean	Sunflower
Soapstocks (%)						
Moisture	96	48	36	78	55	58
Total nitrogen	0.066	0.048	0.251	0.016	0.085	0.203
Total fatty acids	1.7	36.6	40.4	18.4	33.7	25.5
Phosphatides from total phosphate	4.1	12.3	12.9	4.8	18.3	16.8
Neutralized acid waters (%)						
Moisture	97.4	93.0	86.7	95.7	93.7	88.6
Total nitrogen	0.091	0.064	0.061	0.032	0.067	0.178
Fat	<0.10	0.05	<0.10	0.02	0.03	0.37
Ash	2.35	5.83	1.39	4.14	4.69	7.68
Carbohydrate	0.00	0.75	11.5	0.03	1.21	2.23

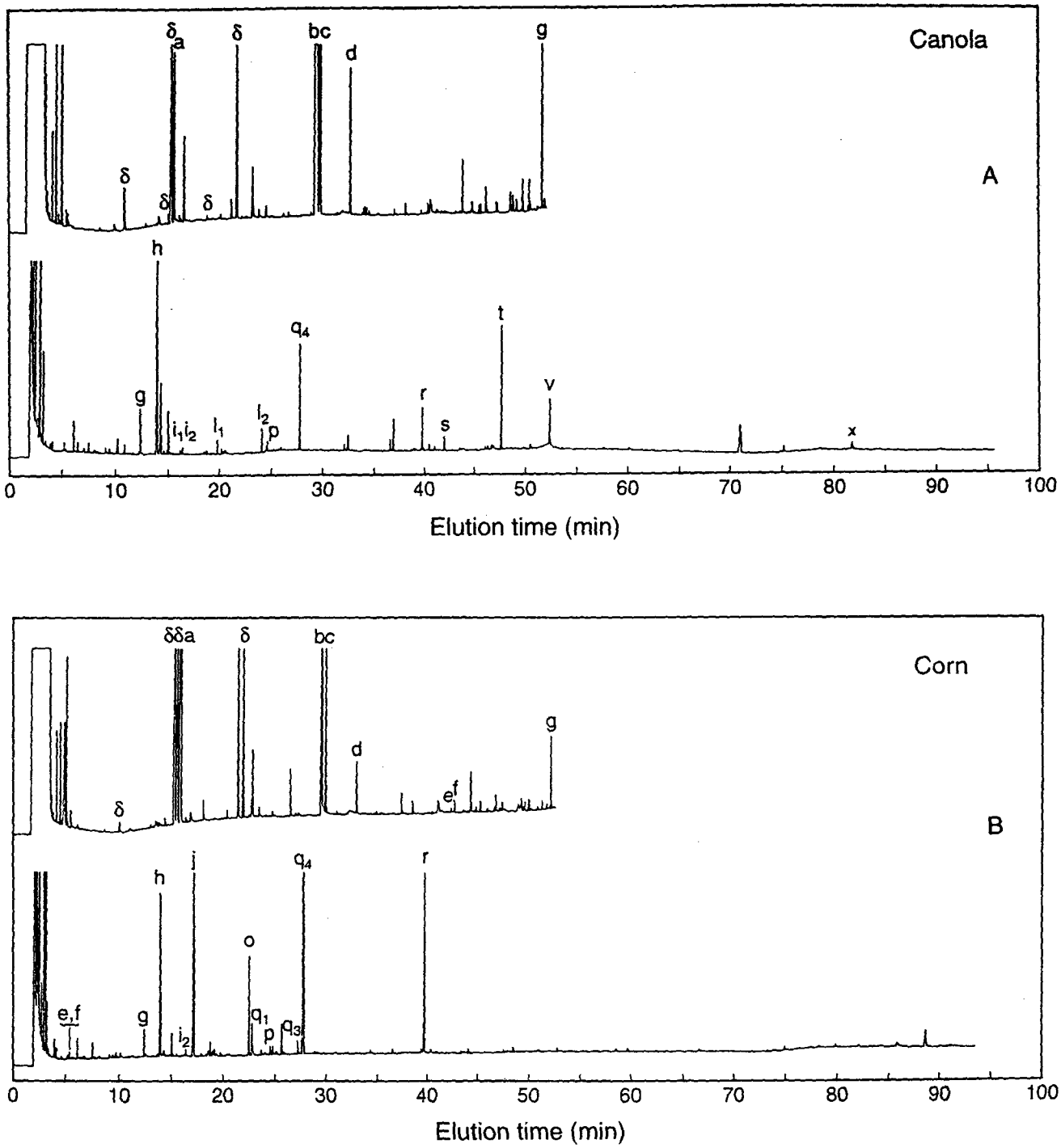
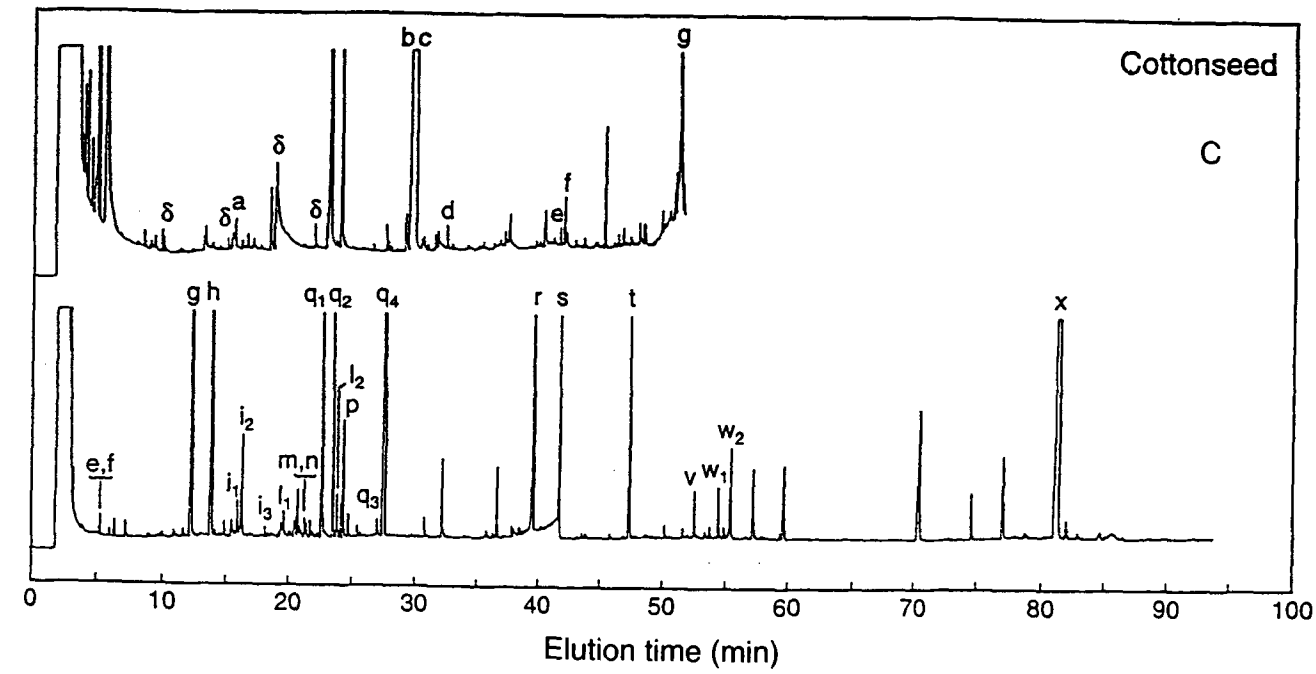


FIG. 1. Gas chromatograms of TMS-derivatized neutralized acid waters. Upper: 50°C→150°C gradient. Lower: 150°C→250°C gradient. Peaks: δ: trimethylsilylation byproduct, a: lactic acid, b: phosphoric acid, c: glycerol, d: glyceric acid, e: threitol, f: erythritol, g: β-glycerophosphate; h: α-glycerophosphate, i_1: β-fructofuranose, i_2: β-fructopyanose, i_3: α-fructofuranose, j: pinitol, k_1: α-galactopyranose, k_2: β-galactopyranose, l_1: α-glucopyranose, l_2: β-glucopyranose, m: mannitol; n: sorbitol, o: *chiro*-inositol, p: gluconic acid, q_1-q_3: penta-TMS-*myo*-inositols, q_4: hexa-TMS-*myo*-inositol, r: *myo*-inositol-1-phosphate, s: *myo*-inositol-2-phosphate, t: sucrose, u_1, u_2: palatinose (1) and (2), v: α-glycerophospho-1-*myo*-inositol, w_1, w_2: melibiose (1) and (2), x: raffinose.



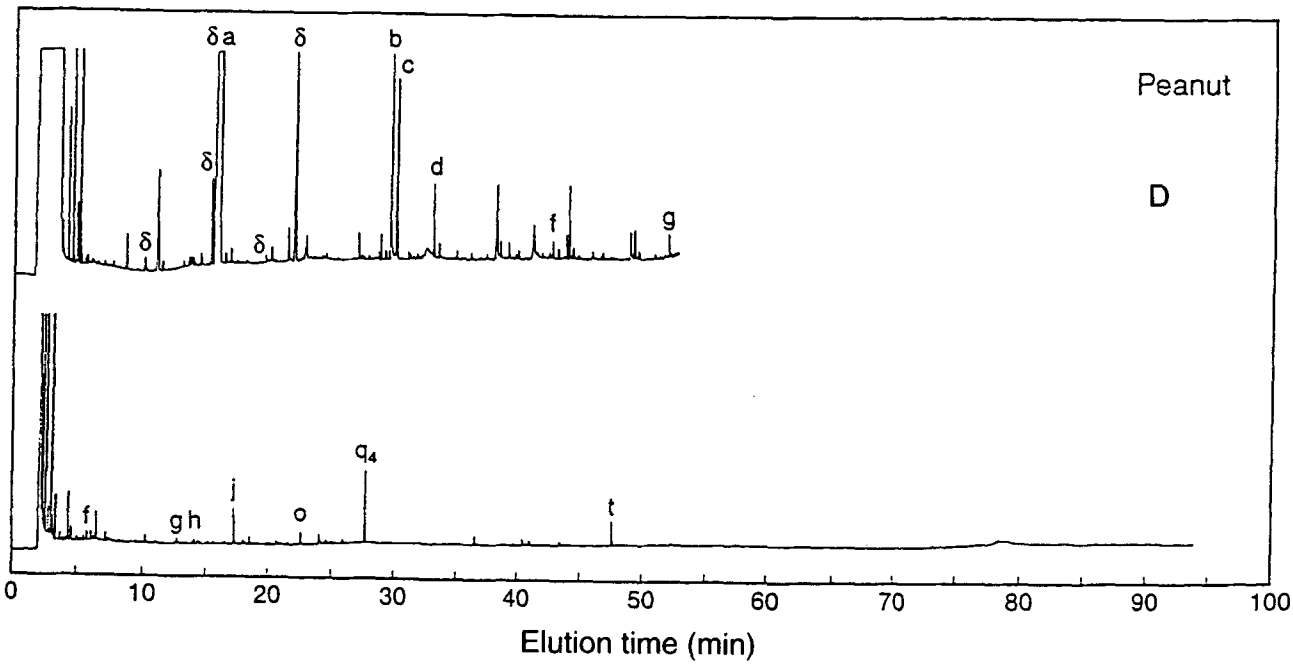
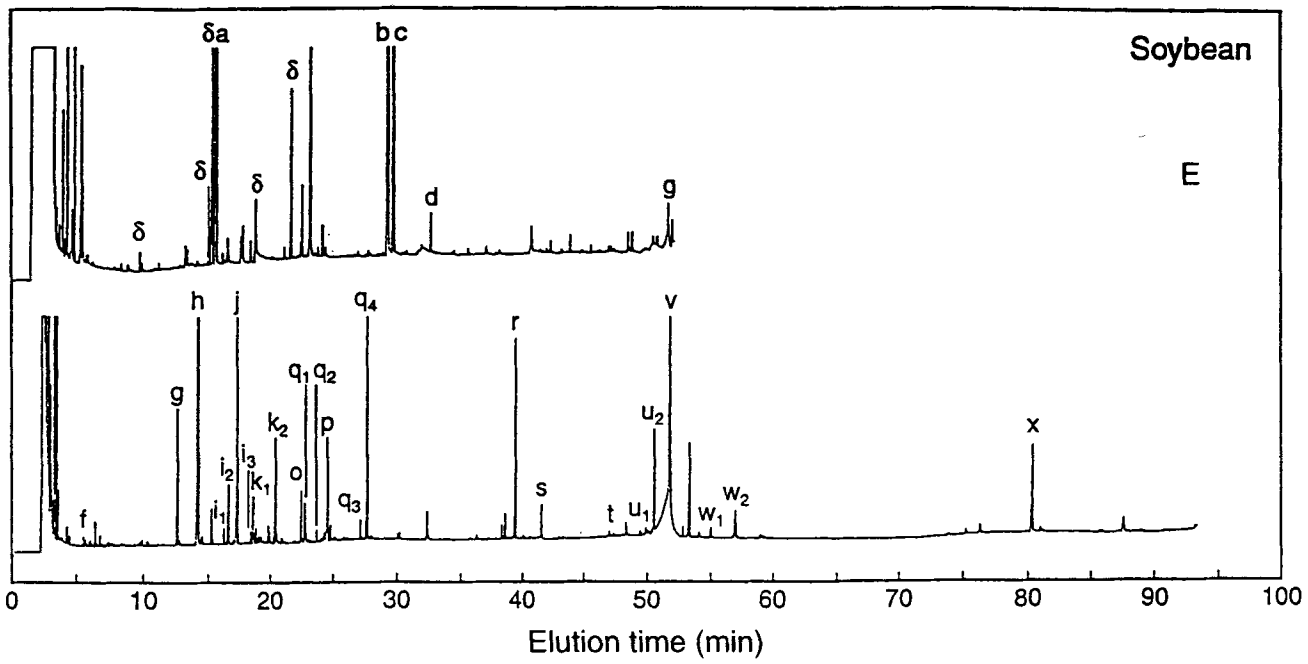


FIG. 1. (continued)



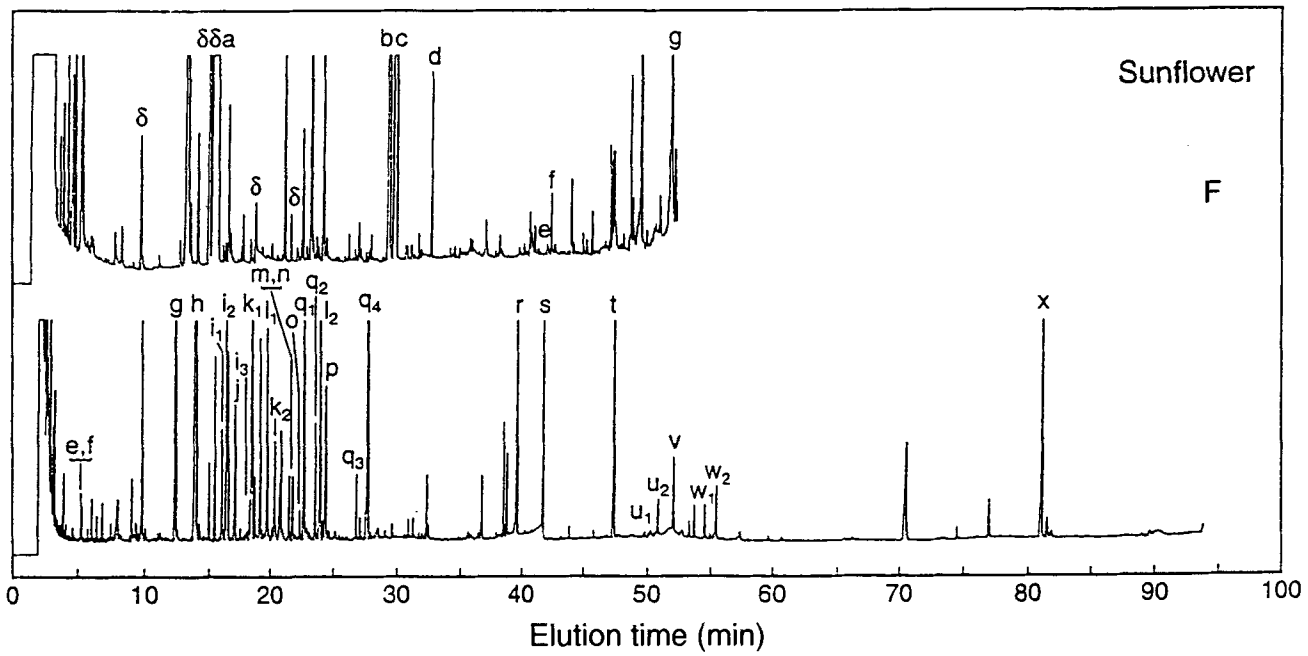


FIG. 1. (continued)

TABLE 3
Gas Chromatography Retention Times and Concentrations of the Low-Molecular Weight Components of Neutralized Acid Water

Component	Relative retention time		Concentration (g/L)					
	50°C→150°C[a]	150°→250°C[b]	Canola	Corn	Cottonseed	Peanut	Soybean	Sunflower
Lactic acid	0.53		0.01	0.88	0.03	0.27	0.71	3.08
Phosphoric acid	0.99		0.85	0.82	0.07	0.04	0.79	1.17
Glycerol	1.00		0.13	0.11	19.4	0.03	0.47	5.96
Glyceric acid	1.10		0.01	0.04	0.03	0.01	0.03	0.06
Threitol		0.19		trace	trace			trace
Erythritol		0.20		trace	trace	trace	trace	0.02
β-Glycerophosphate		0.45	0.03	0.11	6.17	0.01	0.40	3.47
α-Glycerophosphate		0.50	0.34	0.82	12.4	trace	0.55	14.0
β-Fructofuranose		0.58	trace		0.03		0.02	0.08
β-Fructopyranose		0.59	0.01	0.04	0.08		0.06	0.25
Pinitol		0.62		0.32		trace	0.21	0.06
α-Fructofuranose		0.66			0.01		0.01	0.03
α-Galactopyranose		0.67					0.04	0.90
α-Glucopyranose		0.71	0.03		0.18			0.21
β-Galactopyranose		0.74					0.09	0.41
Mannitol		0.78			trace			trace
Sorbitol		0.79			trace			trace
chiro-Inositol		0.81		0.05	0.01	trace	0.03	0.02
β-Glucopyranose		0.87	0.04		0.26			0.34
Gluconic acid		0.88	trace	0.02	0.18		0.15	0.25
myo-Inositol[c]		1.00	0.02	1.21	3.63	0.03	0.44	1.57
myo-Inositol-1-phosphate		1.43	0.03	0.81	6.31		0.75	4.10
myo-Inositol-2-phosphate		1.50	0.01		2.78		0.23	1.82
Sucrose		1.71	0.02		0.72	0.02	0.04	0.46
Palatinose (1)		1.81					0.02	0.02
Palatinose (2)		1.83					0.19	0.11
α-Glycerophospho-1-*myo*-inositol		1.87	0.04		0.20		1.18	0.27
Melibiose (1)		1.96			trace		trace	trace
Melibiose (2)		2.00			trace		trace	trace
Raffinose		2.92	0.01		2.31		0.14	0.29

[a]Retention times relative to trimethylsilyl (TMS)-glycerol (30.80 min).
[b]Retention times relative to hexa-TMS-*myo*-inositol (27.74 min).
[c]Penta-TMS-*myo*-inositol peaks were at 0.82, 0.85, and 0.98. All TMS-*myo*-inositol peaks have been summed.

The highest total concentratrations of low-molecular weight organic components were in the acid waters from cottonseed and sunflower, while the lowest were in peanut and canola acid waters, in rough agreement with the amounts of acid water carbohydrate found by proximate analysis (Table 2).

The high concentrations in the neutralized acid waters of α-glycerophosphate, β-glycerophosphate, *myo*-inositol-1-phosphate, *myo*-inositol-2-phosphate, and α-glycerophospho-1-*myo*-inositol excite interest. They appear not to be formed from phospholipid hydrolysis during soapstock acidification, given that extrapolation to pH 1.3–1.5 at 40°C of previously obtained first-order rate coefficients for phosphatidylcholine (18) suggests that even 1% hydrolysis of the fatty acid–glycerol bond, the most labile one in phosphatides, would require approximately 100 h, while exposure to those pH values of our soapstock samples during acidification and before neutralization was limited to 1–2 h, much of it at room temperature or below. However, hydrolysis of phosphatide bonds during NaOH extraction of crude vegetable oils cannot be excluded if sufficiently high temperatures, pH, and residence times were maintained.

The five phosphatide fragments are rather expensive in laboratory-scale quantities, and it may be economically beneficial to recover α- and β-glycerophosphate for use as raw materials, and the other three as laboratory chemicals. They can be separated by strong-base ion-exchange chromatography at pH 6 (Fink-Winter, R.J,. and P.J. Reilly, unpublished results).

It is instructive to compare GC analysis of TMS-derivatized acid water from cottonseed soapstock with that of TMS-derivatized cottonseed soapstock itself (Dowd, M.K., unpublished results). Soapstock acidification separates the more water-soluble material, chiefly salts, sugars and sugar alcohols, glycerol, phosphoric acid, phosphatide fragments, and shorter fatty acids, into the acid water fraction, while longer fatty acids, mono-, di-, and triglycerides, and sterols preferentially migrate to the organic fraction. GC of unseparated cottonseed soapstock detected the seven largest peaks found by GC of cottonseed acid water: phosphoric acid, glycerol, α- and β-glycerophosphate, *myo*-inositol, sucrose, and raffinose. It did not detect identifiable amounts of the smaller peaks found in the acid water that are likely to be found in the soapstock: glyceric acid, threitol, erythritol, fructose, glucose,

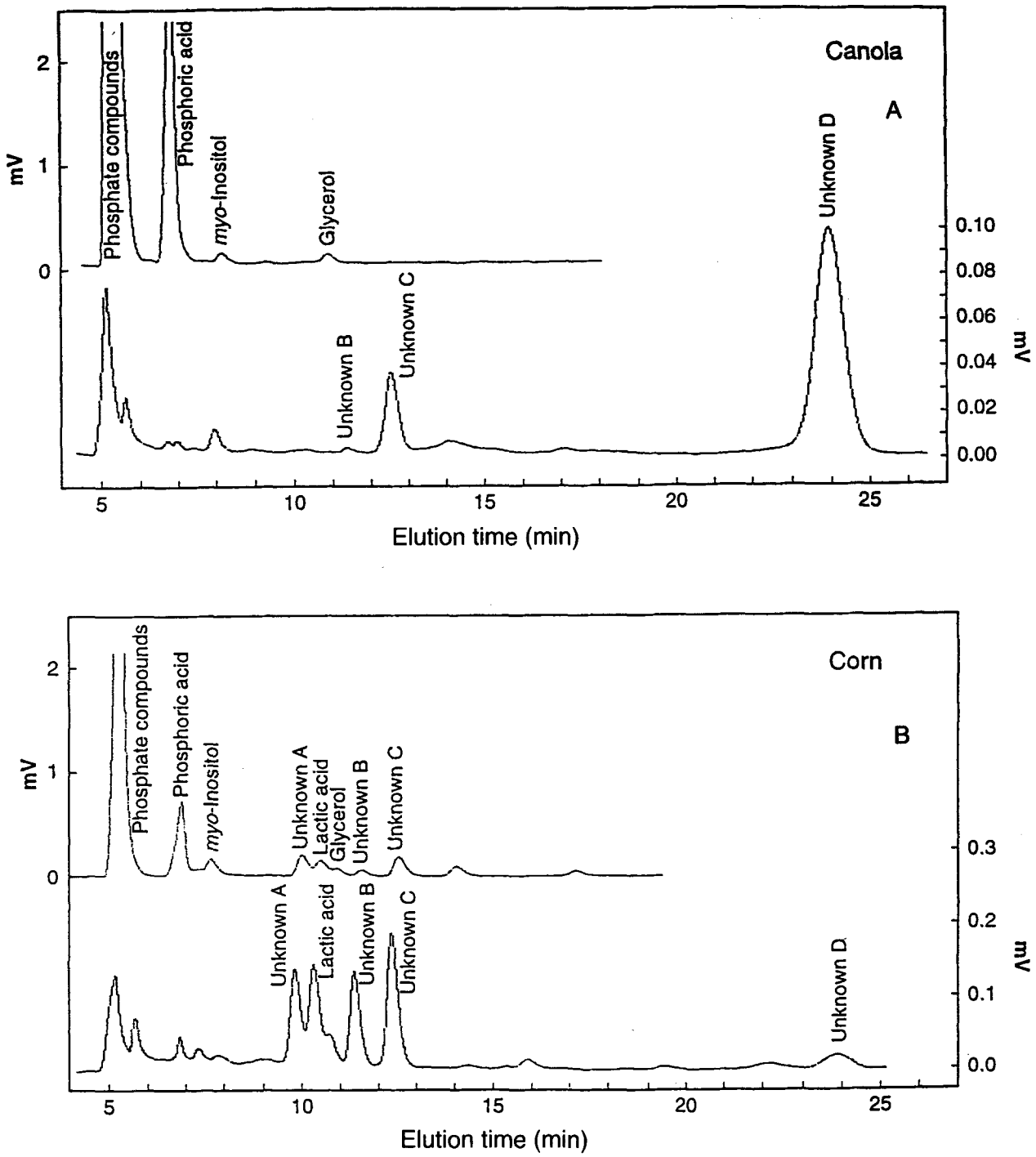
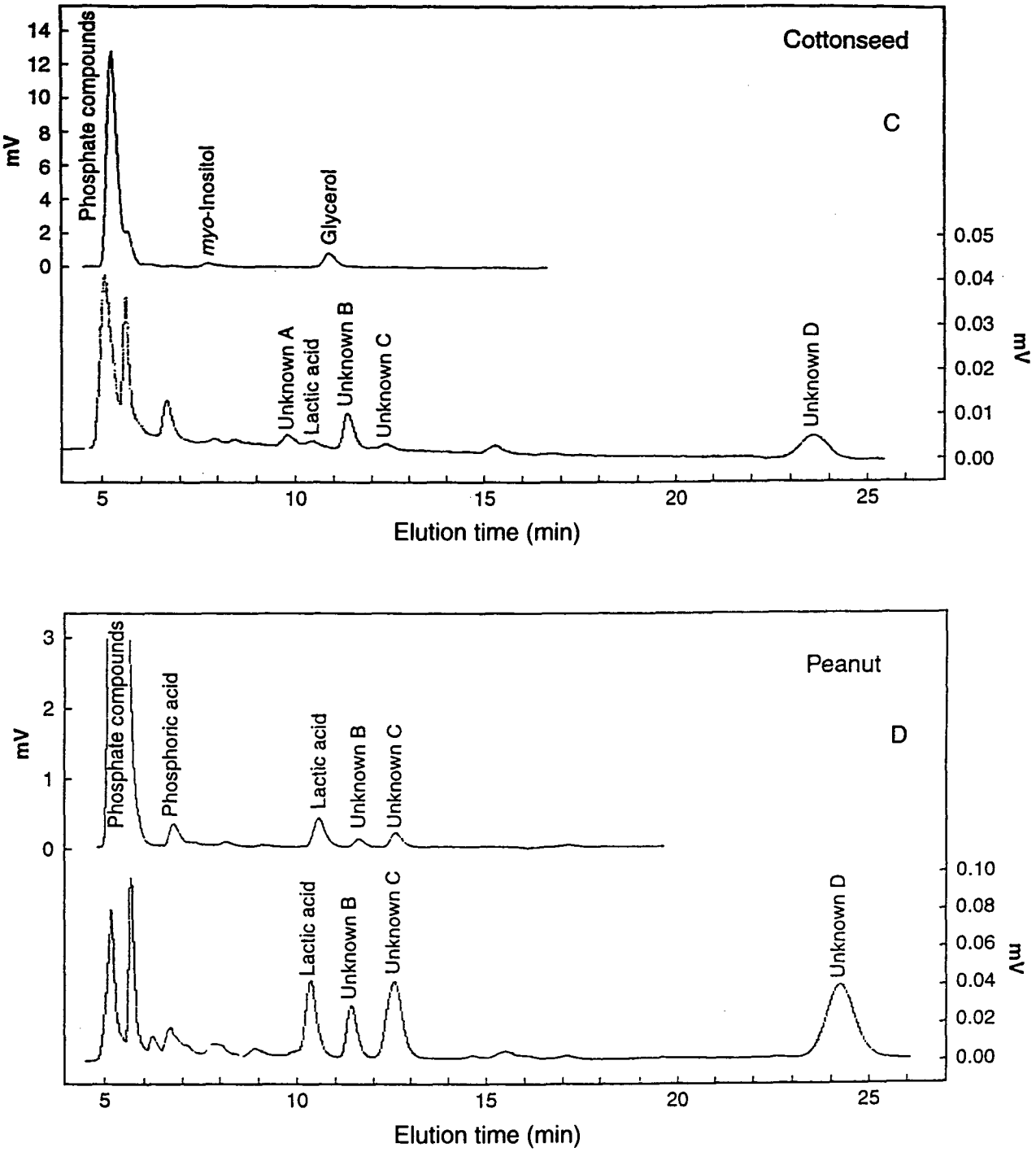


FIG. 2. HPLC chromatograms of neutralized acid waters. Upper: RI detection. Lower: UV detection.



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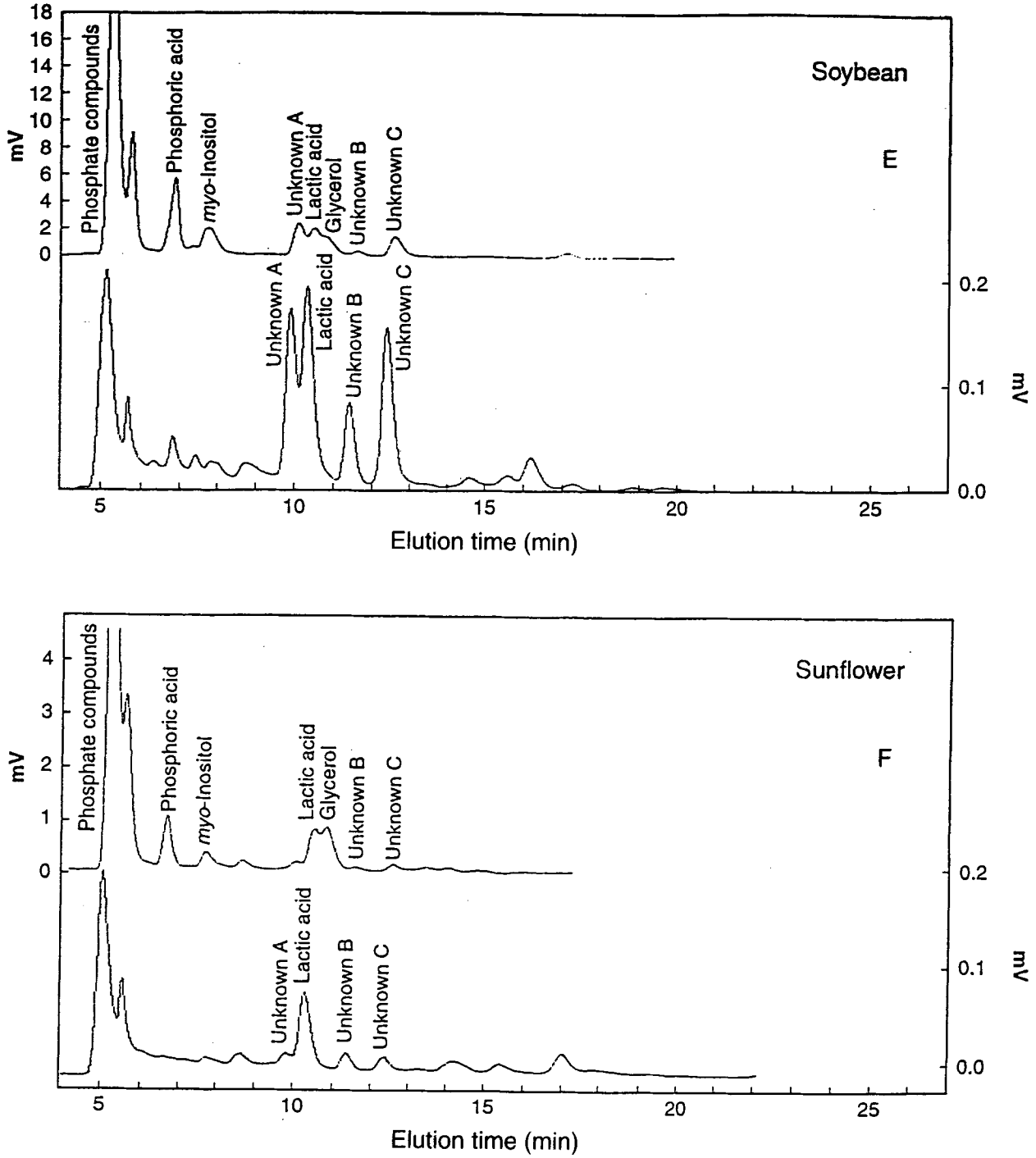

FIG. 2. (continued)

mannitol, sorbitol, gluconic acid, *myo*-inositol-1- and *myo*-inositol-2-phosphate, α-glycerophospho-1-*myo*-inositol, and melibiose, suggesting that soapstock acidification to produce acid water and GC analysis of the latter is an efficient way to find minor soapstock components. Conversely, cottonseed acid water did not contain detectable amounts of any of the fatty acids and mono-, di-, and triglycerides found in cottonseed soapstock, although some of the same fatty acids (lauric, myristic, palmitoleic, palmitic, oleic, and stearic) were found in a coconut acid water sample (11).

HPLC. Strong-acid ion-exchange HPLC was employed to find more volatile organic compounds than could be detected by GC because a number of such compounds were strongly retarded by this type of HPLC (9,10). In addition, HPLC could be used to produce larger amounts of unidentified components found by GC, to be submitted to MS. Chromatograms of the six acid waters are shown in Figure 2. Retention times of their peaks were compared with those of all components found by GC here and in the following article (11), as well as with those of strongly retarded components found earlier (9,10).

Without confirming evidence, such as MS, all peak identifications are more tentative than with gas chromatograms. However, three different comparisons of peak size can be made: (i) between HPLC peaks of the same retention time in different samples and their putative corresponding GC peaks, (ii) between different HPLC peaks in the same sample and their putative corresponding GC peaks, and (iii) between the same peak in the same sample detected by differential refractometry (RI) and ultraviolet absorption (UV), because the two detection methods can yield different response factors depending on component chemistry. Based on these comparisons, four concentrated components that were identified by GC/MS, phosphoric acid, *myo*-inositol, lactic acid, and glycerol, were also separated by HPLC. The various phosphatide fragments were detectable by RI only. They all eluted at about 5.5 min, and when present in sufficiently high quantities, they overwhelmed the negative peak corresponding to the water introduced with the sample. No other component separated by GC was clearly identified in the HPLC chromatograms.

Four significant unknowns, separated by HPLC, were studied further. Unknowns B and C appeared in all six acid waters, A was in all but canola and peanut, and D was in all but soybean and sunflower. A, B, and C were detected by both UV and RI, while D appeared only by UV.

A, B, and C generated no GC peaks after collection, drying, and derivatization. C could be acetic acid, based on its HPLC retention time, and considering that, its TMS derivative is too volatile to be separated by GC.

D was separated by high-temperature GC into two peaks with retention times of 35.26 and 39.12 min, which did not correspond with GC peaks from the original derivatized acid waters. Both peaks were detected by EIMS but only the second was detected by CIMS. EIMS strongly suggested that the earlier peak from D was dehydroabietic acid, for which no standard could be found. Its system of conjugated double bonds suggests that substantial UV peaks for D could occur without being accompanied by RI peaks. The molecular weight of the later peak was 386 Da, and EIMS strongly suggested that it was a saturated hydrocarbon. However, the molecular weight does not agree with this, the closest value being 380 Da for $C_{27}H_{56}$.

In summary, we have identified virtually all major low-molecular weight organic components of the acid waters from soapstocks of six major vegetable oils. Five components, all phosphatide fragments, are candidates for further utilization.

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REFERENCES

1. Howard, B., *Oils and Oilseeds to 1996: The New Patterns of Supply and Demand.* Special Report No. M703, The Economist Intelligence Unit, London, 1993, p. 29.
2. Crauer, L.S., Continuous Treatment of Refinery Waste Waters, *J. Am. Oil Chem. Soc. 47:*210A, 212A, 235A (1970).
3. Beal, R.E., V.E. Sohns, and H. Menge, Treatment of Soybean Oil Soapstock to Reduce Pollution, *Ibid. 49:*447–450 (1972).
4. Beal, R.E., L.L. Lauderback, and J.R. Ford, Soybean Soapstock Utilization: Fatty Acid Adducts with Ethylene and 1-Butene, *Ibid. 52:*400–403 (1975).
5. Ba, A., R. Ratomahenina, J. Graille, and P. Galzy, Etude de la Croissance de Quelques Souches de Levures sur les Sous-produits du Raffinage de l'Huile d'Arachide: Essais de Valorisation de Pâtes de Neutralisation, *Oleagineux 36:*439–445 (1981).
6. Woerfel, J. B., Alternatives for Processing of Soapstock, *J. Am. Oil Chem. Soc. 60:*262A–265A (1983).
7. Hesseltine, C.W., and S. Koritala, Screening of Industrial Micro-organisms for Growth on Soybean Soapstock, *Proc. Biochem.*, pp. 9–12 (February 1987).
8. Carlson, K.F., Fats and Oils Processing, *INFORM 2:*1046–1048, 1050, 1052, 1054–1056, 1058, 1060 (1991).
9. Dowd, M.K., P.J. Reilly, and W.S. Trahanovsky, Low Molecular Weight Organic Composition of Ethanol Stillage from Corn, *Cereal Chem. 70:*204–209 (1993).
10. Dowd, M.K., S.L. Johansen, L. Cantarella, and P.J. Reilly, Low Molecular Weight Organic Composition of Ethanol Stillage from Sugar Cane Molasses, Citrus Waste, and Sweet Whey, *J. Agric. Food Chem. 42:*283–288 (1994).
11. Sivasothy, A., and P.J. Reilly, Low Molecular Weight Organic Composition of Acid Water from Coconut Oil Soapstock, *J. Am. Oil. Chem. Soc. 73:*1305–1309 (1996).
12. *Official Methods and Recommended Practices of the American Oil Chemists' Society,* edited by D. Firestone, 4th edn., Vol. 1, Champaign, 1989.
13. *Official Methods of Analysis of AOAC International,* edited by P. Cunniff, 16th edn., Vol. 1, Arlington, 1995.

14. Nikolov, Z.L., and P.J. Reilly, Isothermal Capillary Column Gas Chromatography of Trimethylsilyl Disaccharides, *J. Chromatogr. 254:*157–162 (1983).
15. Honig, D.H., J.J. Rackis, and D.J. Sessa, Isolation of α-D-Galactopyranoside and Pinitol from Hexane-Ethanol Extracted Soybeans, *J. Agric. Food Chem. 19:*543–546 (1971).
16. Phillips, D.V., and A.E. Smith, Soluble Carbohydrates in Soybean, *Can. J. Bot. 52:*2447–2452 (1974).
17. Phillips, D.V., D.E. Dougherty, and A.E. Smith, Cyclitols in Soybean, *J. Agric. Food Chem. 30:*456–458 (1982).
18. Grit, M., W.J.M. Underberg, and D.J.A. Crommelin, Hydrolysis of Saturated Soybean Phosphatidylcholine in Aqueous Liposome Dispersions, *J. Pharm. Sci. 82:*362–366 (1993).

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