Limited Extraction of Soybeans with Hexane

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Soybean flakes were extracted with lower than optimal quantities of hexane in the laboratory and comparisons were made of oil yield, phospholipids yield and phospholipids composition as a function of the volume of hexane. Although the oil yield and the total amount of phosphorus were significantly decreased with limited volumes of hexane, the distribution of phospholipid components remained essentially the same. Because the oil obtained by this limited solvent extraction contained less phosphatides, it is expected to be more easily processed by conventional techniques.

The extraction of soybean flakes with hexane has been studied extensively, and most investigations have been aimed at maximizing the yield of crude oil by exhaustive extraction at high solvent/flake ratios. Previous evidence (1) indicates that phosphatides are concentrated in the last solvent-extracted material from soybeans, and that this material contributes to refining loss in oil processing. Crude oil extracted at high solvent-feed ratio had an elevated phosphorus content and a higher refining loss than the oil obtained at lower solvent-feed ratio. No information was presented on phospholipid distribution in extracted oils.

Depending on markets and economic conditions, the gums and soap stocks of the oil refinery usually are added back to the meal. Therefore, by decreasing the yield of extracted oil it may be possible also to decrease the level of phosphorus in the oil and retain a major part of the gums and by-products in the meal. In this paper we report the effect of limited extraction on the distribution of polar and nonpolar lipids from soybeans. The distribution of phospholipids in the extracted oil also was investigated by high pressure liquid chromatography (HPLC). The oils obtained by limited solvent extraction are expected to be more easily processed.

EXPERIMENTAL

All solvents used were reagent-grade suitable for HPLC. Standards used for characterization were phosphatidic acid (PA) from Supelco Inc. (Bellefonte, Pennsylvania) and phosphatidylcholine (PC) and phosphatidylethanolamine (PE) from Sigma Chemical Co. (St. Louis, Missouri). Two lots of fresh seed-grade soybeans, Amsoy-71 (1982 crop lot 1) and Century (1983 crop lot 2), were cracked and dehulled. The meats were sealed and stored in plastic bags at −17 C.

Extraction of soybeans. 250-g quantities of soybean meats were conditioned by steaming for 2 min on a metal screen. Each sample (bulk temperature 82–86 C, moisture 16–23%) was then flaked on a 12 × 6 in. roll mill with a 0.003 in. gap setting (Wolf Mfg. Co., Chambersburg, Pennsylvania). Each flaked sample was transferred to a side-arm soxhlet apparatus (No. 3885, Corning Glass Works, Corning, New York) and extracted at 25 C with n-hexane. The meal was partially extracted by pouring solvent on top of the column and siphoning miscella from the bottom. The different volumes of hexane per weight of flakes used were 3.5, 7 and 14, representing 875, 1750 and 3500 ml, respectively. More complete extraction of each meal was obtained by filling the solvent reservoir through the flake chamber and continuing the extraction by refluxing for two cycles, using 34 volumes of hexane per weight of flakes, representing 8536 ml. Each extract was concentrated and the solvent removed by a rotary evaporator (50 C, 10–20 Torr).

Degumming of crude oils. 15- or 30-g batches of oil were degummed by adding 2% water, stirring mechanically for 15 min at 60 C, allowing to stand 1 hr, and centrifuging at room temperature (2,000 × g for 20 min). The resulting clear oils were analyzed for phosphorus colorimetrically (2).

Fractionation of crude oil. One-gram portions of crude oils (10% solution in petroleum ether/diethylether, 95/5) were applied to silicic acid columns ("Sep-Pak," Waters Associates, Milford, Massachusetts) and neutral lipids were eluted with 10 ml additional solvent. The more polar lipids were next eluted with 20 ml diethylether and then with 10 ml methanol.

The methanol fraction was evaporated to dryness, redissolved in chloroform and decanted to remove particles of silicic acid. Also, 5% solutions of these fractions were passed through 0.45 μm Millipore filters (Millipore Corp., Bedford, Massachusetts) for further clarification. Column fractions were examined by thin layer chromatography (TLC) using a mixture of chloroform/methanol/water (65/25/4) and visualized by charring with a sulfuric acid/water mixture (50/50). The column fractions eluted with petroleum ether/diethylether (95/5) and with diethylether, respectively, were shown to be free of polar components that are characteristically present in fractions eluted with methanol. Phosphorus was determined colorimetrically (2) in polar chromatographic fractions.

High pressure liquid chromatography (HPLC). In a modification of the method of Geurts van Kessel, et al. (3), methanol-eluted fractions were applied to silicic acid columns and separations were made of major phospholipid classes by stepwise rather than continuous gradient elution. A ternary mixture of n-hexane/2-propanol/water (6:8:0.3) was our equilibration solvent and first eluent, I. Second and final eluents contained the same ratio of hexane/2-propanol (6:8), and the water was increased to 0.75 and 1.4 parts, II and III. Samples of 0.3 to 0.7 mg in 10–20 μl volume of chloroform were injected onto an equilibrated column (Partisil - 10 M9/50, Whatman, Inc., Clifton, New Jersey) in an HPLC apparatus (Waters Associates, Milford, Massachusetts), and solvent flow was set at 3.0 ml/min. Stepwise elution with solvents I, II and III for 10, 50 and 60 min, respectively, was monitored with a UV detector at 206 nm (Schoeffel Instruments, Kratos, Inc., Westwood, New Jersey) using air as reference. Peak areas were integrated by computer. Major components (PA, PE, PC) were identified by comparing their Rf's on TLC and column chromatography with those of authentic standards.

RESULTS AND DISCUSSION

Soybeans were extracted with limited volumes of n-hexane to determine to what extent polar lipids can be

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TABLE 1

Yields and Distribution of Lipids from Soybeans Extracted with Different Volumes of Hexane

Volume factor of extractant	Oil extracted, %	Residual fat in meal, %	Phosphorus (%) in		Silicic acid chromatography[a]					Phospholipids[b] in methanol eluate by HPLC (%)		
			Crude oil	Degummed oil	P/E (95/5) wt%	Diethylether		Methanol				
						wt%	%P	wt%	%P	PE	PC	PA
Soybeans lot 1												
3.5	16.8	5.7	0.038	0.0007	96.8	2.2	0.009	0.41	2.49	9	19	3
7	18.6	3.4	0.041	0.0007	95.8	2.0	0.03	0.52	3.55	6	13	4
14	20.7	2.7	0.045	0.0007	95.6	1.9	0.01	0.49	3.55	9	13	4
34	22.8	1.5	0.078	0.0009	94.4	2.3	0.02	0.66	2.89	10	13	4
Soybeans lot 2												
3.5	19.1	5.7	0.031	0.0009	95.4	2.7	0.003	0.47	3.01	6	5	0
14	21.1	1.9	0.045	0.0013	95.1	2.6	0.01	0.57	2.02	5	6	0
34	23.4	0.84	0.069	0.0009	96.2	2.6	0.01	0.65	3.15	10	9	0

[a]P, petroleum ether; E, diethylether.

[b]PE, phosphatidylethanolamine; PC, phosphatidylcholine; PA, phosphatidic acid.

preferentially retained in the meal. Distribution of the lipid components extracted from soybean meats is presented in Table 1. As expected, increasing amounts of oil were extracted with increasing volumes of n-hexane. With 3.5 volumes of hexane per weight of flakes, the lipids recovered from two lots of flakes represented 17 and 19% of the flakes, respectively. A difference in the efficiency of draining was suggested because 19% yield of oil was achieved from lot 1 flakes with 7 volumes of hexane. On the other hand, more uniformity in yields was noted with extractions at 14 volumes (21%) and 34 volumes (23%). Different efficiencies of extraction with volume were noted also in the contents of residual fats (5-6% to 1-2%) as determined by 3-hr Soxhlet extraction with petroleum ether.

With increasing extractant volume, the total phosphorus in crude oil increased from 0.03% to 0.08%. After degumming, the phosphorus content varied only from 0.0007 to 0.0013% P. Thus, the amount of crude phosphatides recovered in the hydrated gums would be roughly proportional to the phosphorus content of the crude oil. Using a factor of 30 to convert phosphorus to phosphatide (2), we calculated the percentage of phosphatides (0.93 to 2.34%) and of non-phosphatides by difference (97.7 to 99.1%). Limiting the volume of extractant reduced the crude phosphatide more than the triglyceride portion of the crude oil. Thus, both the nature of lipid components and the yield of oil were dependent on extraction conditions.

To determine the relative distribution of lipids, crude oils were fractionated on short "Sep-Pak" silicic acid columns. The fraction eluted with petroleum ether-diethylether (95/5) contained primarily triglyceride and represented 94-97% (average 95.6 ± 0.8%) of the crude oil. The fraction eluted with diethylether contained compounds usually associated with the unsaponifiables and represented 2-3% (average 2.3 ± 0.3%) of the crude oil. The fraction eluted with methanol contained the major phospholipids and represented 0.4-0.7% (average 0.54 ± 0.09%) of the crude oil. The amount of triglyceride (94.4-96.8%) was slightly lower than that of the non-phosphatides as estimated by difference on the crude oil (97.7-99.1%). This difference averages about 1% more than the amount of the chromatographic fraction eluted with diethylether. To account for the phosphorus that was extracted initially, the eluates obtained with diethylether and methanol were analyzed for phosphorus. Phosphorus contents of the methanol eluates were at the low end of the range expected for crude phospholipids; 2.5-3.6% P from lot 1 and 2.0-3.2% P from lot 2 flakes. The diethylether eluates contained some phosphorus, which indicated that certain of the phosphorus-containing compounds were adsorbed only weakly onto the silicic acid column. Other workers have attributed such losses to the formation of reversed micelles of hydrated phospholipids (4). Such micelles result when polar head groups associated with traces of water form a central core that is stabilized from the bulk of the oil by nonpolar tail groups.

Separation of phospholipids in the methanol eluates by HPLC revealed that a variation occurred in the two lots of soybean flakes examined. From lot 1 extractions, the PE in the methanol eluates varied from 6 to 10%, PC from 13 to 19% and PA from 3 to 4%. From lot 2, the corresponding distribution was 5-10% PE, 5-9% PC and 0% PA. These ranges of distribution apparently were due to random variation and were unrelated to extraction volume.

The results of Arnold (1) showed that oils from extractions over a range of moderate volume were similar in their contents of neutral oil, nonsaponifiable material and phospholipid. Fractions of our oils that eluted from silicic acid with petroleum ether-diethylether (95/5) and with diethylether were likewise uniform in relative proportions. However, fractions eluted with methanol indicated difference over an extended range of extraction volumes.

In this work, limiting the volume of hexane to extract soybean flakes reduced both the yield of oil and its total phosphorus content. On the other hand, chromatographic separations showed that relative contents of neutral and polar lipids in the crude oils were changed little and the distribution of major phospholipids in the polar lipid re-

mained essentially the same. Additional work is needed to evaluate the ease of processing the crude oil obtained with limited volumes of hexane, and the nutritive value of the resulting meal for feeding.

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Oxidative Stability of Jojoba Wax

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The rates of autoxidation of crude, bleached and stripped jojoba wax were determined under conditions of accelerated oxidation (98 C). Oxidation of the raw yellow wax had a long induction period (50 hr) compared with the bleached wax (10–12 hr) or stripped wax (2 hr). These differences indicate the presence of a natural antioxidant in the crude wax. Addition of 0.02% butylated hydroxytoluene or butylated hydroxyanisole to the bleached wax restored and even improved its stability. Autoxidation of jojoba wax was also studied at room temperature. In the presence of light and air, the activity of the natural inhibitor was rapidly lost.

The jojoba shrub (*Simmondsia chinensis* [Link] Schneider) (1,2) yields dark brown, nut-like seeds from which a bright yellow liquid wax may be extracted (3,4). The major constituents of the wax are straight-chain esters of C20 and C22 δ-9 mono-unsaturated alcohols and carboxylic acids (5,6). The wax is free of rancidity (7) and stable to oxidation (8), but this property has not been investigated thoroughly. The growing interest in the development of this shrub as a cultivated oilseed crop to replace sperm whale oil (7) led us to study the stability of the wax to autoxidation. To satisfy requests by jojoba wax consumers in the cosmetic industry, we performed accelerated oxidation of the original crude wax as well as of the bleached and stripped waxes. We also determined the ability of butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) to protect bleached wax against oxidation.

EXPERIMENTAL PROCEDURES

Materials. Crude jojoba wax was obtained from the Apache Marketing Cooperative Association, San Carlos, Arizona. Bleached wax was prepared by treating the crude wax twice with 1% w/w commercial bleaching earth at 80 C. Stripped wax was obtained from crude wax by distilling off and discarding the fraction boiling up to 170 C at 1 mm Hg. BHT and BHA, purchased from Sigma Chemical Co., St. Louis, Missouri, were first dissolved in a small amount of wax by gentle warming. Then wax was added to obtain the required concentration.

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Apparatus. Oxidation was done in 250 ml gas washing bottles. Two hundred g of wax were used in each run. The bottles were immersed in a thermostatically controlled insulated water bath at 98 C. The accelerated oil oxidation procedure is described in AOCS tentative method Cd 12-57 (9). The refractive index of the wax was determined with an Abbe Refractometer, Bellingham and Stanley Model 60/HR, at 60 C.

Peroxide determination. The hydroperoxide content of 50 to 1000 mg of wax was determined by AOCS official method Cd 8-53 (10) using 0.001 and 0.01 N sodium thiosulfate solutions.

RESULTS AND DISCUSSION

The initial peroxide value of the crude jojoba wax was 17 meq/kg. After stripping or bleaching the wax, this value fell to almost zero. The changes in the peroxide values of the wax samples during accelerated oxidation are shown in Figure 1. In spite of its high initial peroxide value, the crude wax had a long induction period of

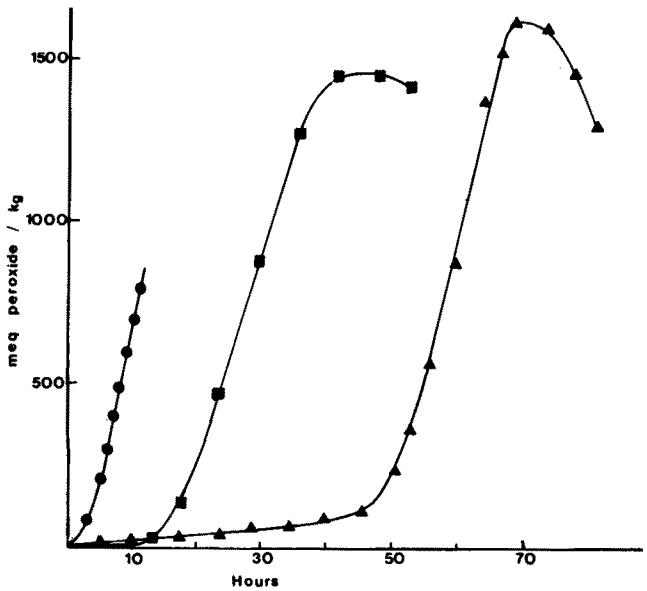

FIG. 1. Oxidation of jojoba wax by air under accelerated conditions (98 C). ▲, Crude wax; ●, stripped wax; ■, bleached wax.