QUANTITATIVE STUDIES OF WHEAT-FLOUR LIPIDS EXTRACTED WITH VARIOUS SOLVENTS BY AN ELUTION METHOD[1]

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ABSTRACT

Untreated, slurried-and-dried, and hexane-Soxhlet-extracted flours and flour fractions mixed with diatomaceous earth were packed in columns and lipids were eluted with various solvents. Among 15 solvents studied, water-saturated 1-butanol (BW) generally gave maximum lipid yields, but a 2-propanol:fluorocarbon:water mixture gave comparable results. Hexane elution gave about 20% less and chloroform elution about 20% more lipid than hexane Soxhlet extraction. Most alcohol-containing solvent systems did not give higher yields than 95% ethanol or chloroform alone. Nonpolar solvents generally eluted more lipids from soft wheat than from hard wheat flours, but polar solvents eluted about the same amounts from soft and hard wheat flours. Nonpolar solvents extracted less lipid from flours suspended in water and dried, but as polarities of solvents were increased, yields approached those of untreated flours. Lipids from hexane-extracted flours also increased with solvent polarity. When flours were eluted with a sequence of hexane, chloroform, and BW, a higher proportion of total lipid was extracted by hexane and chloroform from soft than hard wheat flours, but total yields were comparable. Among fractions from nonextracted flour, almost 90% of total ethanol-extractable lipid was in the gluten (7.68% lipid). Gluten from hexane-Soxhlet-extracted flour contained 1.60% lipid and contributed 63.6% of the total. Results suggest that elution of lipids by use of standardized columns and procedures is a practical method for comparative studies of solvents, flours, and flour treatments.

Extensive data have shown that wheat-flour lipid yield and composition are functions of extraction conditions. Common nonpolar lipid solvents may extract less than half as much lipid as highly polar organic solvent systems, and yields are maximum only when water is incorporated into the solvent. Solvents of intermediate polarities give corresponding recoveries (1–3). These effects are generally ascribed to lipid binding, but solubility, permeability, and other factors may influence extraction. Because of this variability, free, bound, and total lipids must be defined in terms of extraction conditions.

Free flour lipids are usually extracted with hydrocarbons or diethyl ether, and bound lipids with water-saturated 1-butanol (BW) (1,4). Lipids can be extracted by refluxing boiling one-component solvents (as in Soxhlet and Goldfisch extractions), but reflux is not satisfactory with multiple-component systems (unless azeotropic). Therefore, extractions of flours with butanol-water and similar systems usually involve repeated suspension and filtration or centrifugation (5–7). Lipid extraction by filtration of solvent at ambient temperature through a flour bed has also been used for applications ranging from

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micro-scale analyses to large-scale preparative extractions (3,7–9). This process, usually referred to as percolation, is also involved in extraction by reflux, but it is more universally applicable than reflux because it is adaptable to any solvent system.

The primary objective of this study was to determine the practicality of a standardized percolation technique for routine extraction of lipids from flour and flour fractions by applying the technique to a comparative study of solvents, flours, and flour treatments. Since reflux methods are also based on the general process of percolation, the more specific term elution will be used in this study to refer to extraction by controlled washing from columns under standardized conditions. Quantitative results will be presented here; qualitative data will be presented in another report.

MATERIALS AND METHODS

Flours and Flour Fractions

Kansas hard red winter wheat flour was obtained from the Hard Winter Wheat Quality Laboratory at Manhattan, KS. All other flours were milled in the Wooster Laboratory. Flours designated as slurried were suspended in two parts water and immediately frozen and freeze-dried to effect lipid binding characteristic of flour-water suspensions. Flours designated as hexane-extracted were extracted with hexane in a Soxhlet extractor for 24 hr. Extracts were filtered, made up to volume, and aliquots were evaporated, dried 1 hr at 100°C and weighed to determine lipid.

Flours were fractionated by a conventional wet fractionation procedure (10), and water-soluble solids, tailings, and gluten were freeze-dried; starch was air-dried.

Solvents

Hexane was redistilled from ligroin (bp 63°–75°C) or from technical-grade hexane; other solvents were reagent grade and were not redistilled. Solvent mixtures are expressed as ratios of volumes.

Elution of Lipids from Flours

Columns were glass tubes, 27 mm i.d. × 150 mm with outlet tubes 11 mm × 100 mm without stopcocks. Six columns were attached to a rack with spring clamps for simultaneous elution of six samples, and glass wool was packed in the outlet tubes to support the bed. Diatomaceous earth (Celite 545) was then slurried in hexane and transferred to the columns to give a settled bed about 1 cm deep in the bottom of the column. Duplicate 10-g samples of flours were mixed with 5 g Celite 545 and slurried in 50 ml hexane. The suspensions were transferred to the columns and the columns were allowed to drain under gravity (with collection of effluent) until the solvent level approached the top of the sample. (Samples were added to columns as hexane suspensions, regardless of elution solvent, to avoid separation of flour from diatomaceous earth by flotation of flour). The elution solvent (150 ml) was then added in 50-ml portions; each increment was allowed to drain to the top of the sample before the next increment was added. Flow rates varied among flours and solvents but were generally 1–2 ml/min. After the last

increment had drained, pressure was applied to the top of the column to force out solvent remaining in the bed, and the effluents (including suspension effluents) were evaporated under an air stream. Residues from hexane, chloroform, carbon tetrachloride, and Freon TF (Freon 113; 1,1,2-trichloro-1,2,2-trifluoroethane) elutions were dried 1 hr at 100°C and weighed directly to determine lipids. Residues from other solvents and solvent mixtures were dissolved in chloroform:methanol (2:1) and taken up on 5 g Celite 545. The powder was placed in an air stream and stirred to remove solvent and extracted for 6 hr with hexane on a Goldfisch extractor. Hexane was evaporated from the extract and the residue was weighed after 1 hr at 100°C.

Sequential Elution of Flour Lipids

Flour (50 g) was mixed with 30 g Celite 545 and hexane was added to give a thick slurry. The suspension was transferred to a glass column, 30 mm i.d. × 300 mm, with a stopcock and containing a Celite-545 bed supported on a glass-wool plug. After the suspension had settled, the column was drained to the top of the sample, with collection of effluent. Additional hexane (250 ml) was added and allowed to drain, followed by 250 ml BW in sequence. Each solvent was added as the preceding solvent drained to the top of the sample and effluents were collected separately. Hexane and chloroform were allowed to flow under gravity, but BW was forced through under air pressure to maintain flow at about 5 ml/min. Pressure was maintained until all free BW was forced from the bed. The three effluents were evaporated to dryness in the air stream of a hood, and residues from hexane and chloroform elutions were dried 1 hr at 100°C and weighed. The residue from the BW elution was dissolved in chloroform:methanol (2:1) and taken up on Celite 545 and extracted with hexane on a Goldfisch extractor for 6 hr. The extract was evaporated, dried 1 hr at 100°C, and weighed.

Elution of Lipids from Flour Fractions

Duplicate 2.5-g samples of flour fractions were ground in a mortar with 2.5 g Celite 545 and slurried in 50 ml hexane. The suspensions were transferred to columns, 22 mm i.d. × 100 mm with outlet tubes 7 mm i.d. × 80 mm, containing Celite-545 beds supported on glass wool. The columns were allowed to drain until the hexane level approached the top of the sample, and the elution solvent was added in three increments (50 ml total). Effluents were evaporated and lipids were determined by taking residues up on Celite 545 and extracting with hexane on a Goldfisch extractor as described above.

RESULTS AND DISCUSSION

Fig. 1 shows lipid yields (per cent dry weight basis) from a hexane Soxhlet extraction of a soft red wheat flour (blend of varieties) and from elutions of the flour with 15 different solvents. Soxhlets gave 1.07% lipid, about 20% more than the yield from elution with hexane (0.87). Freon TF and carbon tetrachloride were comparable to hexane as elution solvents, but the yield from chloroform (1.21%) was almost 20% greater than that from hexane Soxhlet extraction and 39% greater than that from hexane elution. Ethanol gave 1.31% and BW gave

1.78%, the highest yield from any solvent studied. 2-Propanol:Freon TF:water, 18:12:5, gave 1.65%, approaching the yield from BW, and was included (as PFW) in subsequent comparative studies of flours. The remaining solvent combinations did not appear to offer any advantages over 95% ethanol or chloroform. About half of the solvents studied yielded about 1.30% lipid, which suggested that a binding threshold must be overcome to give higher yields.

Elution flow rates were not precisely regulated and generally depended on viscosity and degree of flour swelling. Elutions tended to be slower with BW than with other solvents, and subsequent experiments suggested that the high yields from BW were partially due to prolonged contact with the flour. Flow rates of solvents other than BW were retarded somewhat by extending the Celite column beds 2 cm down into the outlet tubes; columns now in use are equipped with stopcocks.

Data from hexane Soxhlet extraction of six flours and from elutions of these flours with six solvents are shown in Table I. Lipid yields from elutions of these flours after hexane Soxhlet extraction and after treatment with water are also given. Solvents were hexane, chloroform, 95% ethanol, chloroform:ethanol:water (CEW) (20:10:1), PFW (18:12:5), and BW. In addition, one flour was extracted with chloroform:methanol:water (CMW) (20:10:1), a common combination for dissolving highly polar lipids.

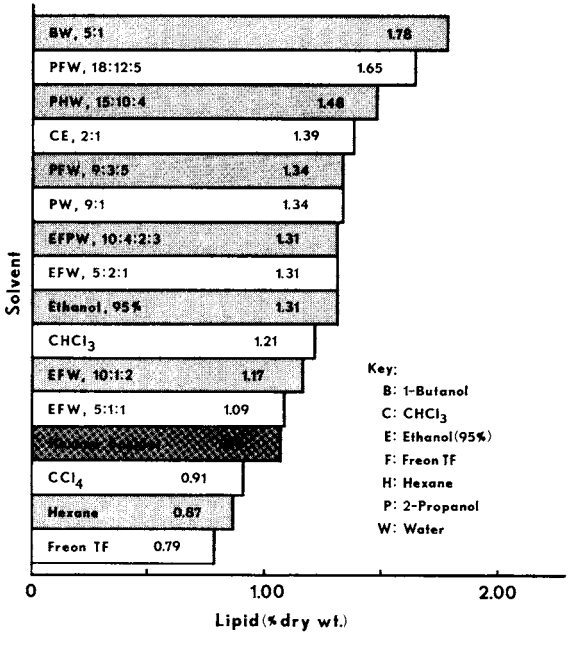

Fig. 1. Lipid yields from elutions of a soft red wheat flour (blend of varieties) with 15 different solvents compared with yield from hexane Soxhlet extraction. All solvent mixtures are expressed as volume ratios.

Data in Table I from untreated soft wheat flours generally agree with results from the solvent study (Fig. 1), but yields from the hard wheat flours varied somewhat from the patterns. Hexane Soxhlet yields were 1.00–1.07% (dry weight basis) from soft wheat flours and 0.95–0.97% from hard wheat flours; lipid yields from hexane elutions were 16–34% lower, and were lowest for Kansas hard red winter wheat flour. Chloroform elutions of soft wheat flours gave 1.16–1.24% lipid, 13–24% more than yields from Soxhlet extraction with hexane. Yields from chloroform elutions of hard wheat flours ranged from 0.83 to 1.16%, representing 1 and 20% increases over yields from hexane Soxhlet extractions of the two Eastern hard wheat flours, but a 14% decrease for Kansas hard red winter wheat flour. Ethanol elutions of soft wheat flours gave only slightly higher yields than chloroform, but increments were substantially greater from hard wheat flours (more than 35% higher from Kansas hard red winter wheat flour). BW elution yielded maximum lipid from every flour except Kansas hard red winter wheat flour, which gave maximum yield with PFW. Yields from PFW elutions of the other five untreated flours were slightly less than those from

TABLE I
Lipid Yields from Elution of Different Wheat Flours with Various Solvents before and after Hexane Extraction and Exposure to Moisture (% of Flour, Dry Weight)

		Solvent[b]							
		Soxhlet	Elution						
Flour	Treatment[a]	Hexane	Hexane	$CHCl_3$	EtOH 95%	CMW	CEW	PFW	BW
Soft White Blend A	None	1.01	0.77	1.16	1.25	0.91	1.27	1.51	1.59
	Hexane extd.		0.00	0.14	0.36	0.16		0.37	0.54
	Slurried		0.55	0.82	1.22	0.93		1.35	1.45
Soft White Blend B	None	1.00	0.82	1.24	1.29		1.32	1.43	1.53
	Hexane extd.		0.01	0.18	0.35			0.48	0.53
Soft Red Blend	None	1.07	0.87	1.21	1.31		1.39	1.65	1.78
	Hexane extd.		0.00	0.14	0.28			0.57	0.67
	Slurried		0.64	0.93	1.30			1.44	1.78
Hard Red Blend Wooster	None	0.97	0.81	1.16	1.35		1.33	1.35	1.54
	Hexane extd.		0.03	0.21	0.37			0.53	0.71
	Slurried		0.56	0.76	1.12			1.44	1.51
Hard Red Blend Kansas hard red wheat	None	0.96	0.63	0.83	1.14		1.19	1.51	1.46
	Hexane extd.		0.00	0.12	0.32			0.50	0.62
	Slurried		0.29	0.52	1.00			1.22	1.51
Comanche Hard red Wooster	None	0.95	0.70	0.94	1.17		1.31	1.32	1.42
	Hexane extd.		0.01	0.07	0.34			0.45	0.65

[a]Hexane extd: Hexane Soxhlet extracted; slurried: suspended in water and freeze-dried.
[b]CMW: chloroform:methanol:water, 20:10:1 (v:v:v); PFW: 2-propanol:Freon-113:water, 18:12:5 (v:v:v); BW: water-saturated 1-butanol; and CEW: chloroform:ethanol:water, 40:19:1 (v:v:v).

BW. The chloroform:alcohol:water mixtures, CMW and CEW, extracted considerably less lipid than BW, although CEW has been reported to be a satisfactory substitute for BW for extraction of bound flour lipids (9).

Yields of lipid were negligible from hexane elutions of hexane-Soxhlet-extracted flours but were substantial from chloroform elutions (0.07–0.21%). Yields were 0.28–0.37% from 95% ethanol, 0.37–0.57% from PFW, and 0.53–0.71% from BW (Table I).

Hexane-eluted lipids from five of the six water-treated (slurried) flours were about 70% of the yields from untreated flours, but were less than 50% from Kansas hard red wheat flour. As polarities of the eluting solvents increased, lipid recoveries from freeze-dried preslurried flours approached yields from untreated flours, and yields from BW elutions were about equal for slurried flours and untreated flours. Overall, the soft red and Kansas hard red wheat flours represented the extremes in lipid yields. The results indicate that PFW is comparable to BW for extraction of lipids from untreated flours, but BW extracted more lipid from flours in which binding had been induced by water treatment.

Lipid yields from stepwise elutions of soft white, soft red, and hard red wheat flours with hexane, chloroform, and BW are shown in Fig. 2, together with hexane Soxhlet yields. Lipid yields from the hexane elutions followed patterns noted previously: soft red flour gave 0.83%, soft white flour 0.76%, and hard red flour 0.58%. Subsequent elution with chloroform resulted in increments equal to about one-third of the yields from the hexane elutions (0.28, 0.31, and 0.25%, respectively). The remaining lipids, eluted with BW, were 0.47 and 0.53%, respectively, for the soft white and soft red flours, and 0.67% for the hard wheat

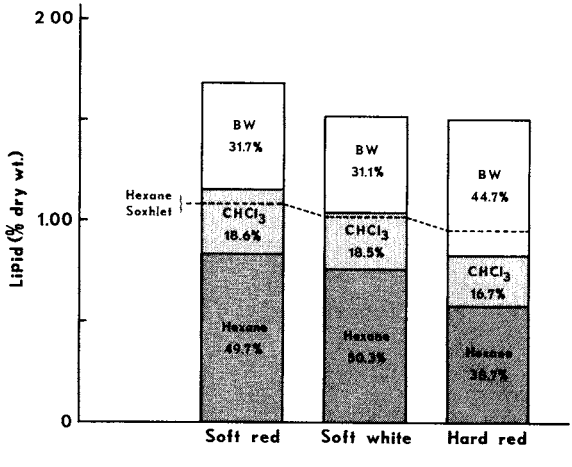

Fig. 2. Lipid yields from soft red, soft white, and hard red winter wheat flours from sequential elutions with hexane, chloroform and water-saturated 1-butanol (BW). Mixtures of 50 g flour and 30 g diatomaceous earth were eluted from columns 30 mm i.d. × 300 mm with 250 ml each of hexane, chloroform, and BW in sequence.

flour. Total lipids eluted from the soft white and hard red flours were about the same (1.51 and 1.50%, respectively), and only slightly higher from the soft red flour (1.68%). When expressed as proportions of total lipids, eluted lipids from the soft wheat flours were almost identical, hexane extracted about 50%, chloroform 19%, and BW 31%. However, hexane extracted about 39%, chloroform 17%, and BW 45% of total lipids from the hard red wheat flour.

Table II shows lipid yields from 95% ethanol elutions of fractions isolated from a soft wheat flour (mixture of red and white varieties) and from the same flour after hexane Soxhlet extraction. Gluten contributed almost 90% of the total lipid from the nonextracted flour; water-soluble solids contributed 4.7%; and starch and tailings, 2.8% each.

Total lipid (from the sum of the fractions) was 1.07% of the dry weight of the flour. When fractions from the hexane-extracted flour were eluted, gluten contributed 63.6% of the total lipid, the tailings 24.2%, and the water-soluble solids 12.1%. No lipid was detected in the starch extract. Total extracted lipid was 0.33% of the flour dry weight. As a result of the hexane Soxhlet extraction, ethanol-extractable lipid in the gluten fell from 7.68% to 1.60%, and lipid in the water-soluble solids dropped from 1.92% to 1.24%. An apparent increase in tailings lipid (from 0.36% to 1.00%) was probably from the high gluten contamination of tailings from the hexane-extracted flour.

The results show that elution of flour lipids under defined conditions can be a useful technique for studies of these lipids and of the effects of treatments and solvents on lipid yield. Yields from elution with a particular solvent cannot be expected to attain yields from exhaustive high-temperature reflux extraction with the same solvent. However, elution permits extraction of any sample with

TABLE II
Distribution of 95%-Ethanol-Eluted Lipids among Fractions Isolated from a Soft Wheat Flour before and after Hexane Soxhlet Extraction

Fraction	Contribution of Fraction to Flour %	Lipid Content[a] %	Lipid/100 g Flour[a] g	Contribution to Total Flour Lipid %
Nonextracted				
Water-soluble solids	2.7	1.92	0.05	4.7
Tailings	8.0	0.36	0.03	2.8
Gluten	12.5	7.68	0.96	89.7
Starch	76.9	0.04	0.03	2.8
Total			1.07	
Hexane-extracted				
Water-soluble solids	2.9	1.24	0.04	12.1
Tailings	8.2	1.00	0.08	24.2
Gluten	13.2	1.60	0.21	63.6
Starch	75.8	0.00	0.00	0.0
Total			0.33	

[a]Dry weight basis.

any solvent and it does this under identical conditions. Yield will then be a function of sample and solvent. Satisfactory flow rates can be obtained by diluting samples with diatomaceous earth and by selecting columns of appropriate dimensions, but application of pressure may be necessary with solvents which are viscous and/or which cause flour swelling. The procedure can be scaled up or down and can be carried out at reduced temperatures to provide mild conditions. Several samples can be extracted simultaneously.

The secondary Goldfisch extraction of lipids eluted by polar solvents is a departure from conventional methods for separating lipids from nonlipids, *i.e.*, selective solubilization or partitioning (4–7). Diatomaceous earth is an inactive support that retains only highly polar compounds (11,12) and preliminary studies showed that flour lipids were quantitatively extracted from it by a short Goldfisch extraction with hexane or chloroform. The procedure does not necessarily save time, but it is precise and several samples can be analyzed at the same time. All data presented here are averages of duplicates or of a greater number of replications. Generally, results agreed within 5%, even with secondary (hexane Goldfisch) extractions.

The relative nature of the terms free and bound lipids has been emphasized by other workers (1) and is evident from the data reported here. If free lipid is arbitrarily defined as lipid extracted by hexane Soxhlet extraction and bound lipid as the increment extracted by BW (4), differences between soft and hard wheats appear minor. However, data based on elutions indicate some characteristic differences, particularly between the soft wheat flours and Kansas hard red winter wheat flour. Low-polarity solvents gave lower lipid yields from hard wheat than from soft wheat flours, but these differences diminished with increasing solvent polarity and levels of total lipids were about the same in soft and hard wheat flours. Generally, soft wheat flours yield slightly more lipid than hard wheat flours from hexane Soxhlet extraction (13). These differences may be due to varying degrees of binding, or to variations in accessibility of lipids to solvent.

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