EFFECTS OF SOLVENT EXTRACTION ON LIPID COMPOSITION, MIXING TIME, AND BREAD LOAF VOLUME[1]

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ABSTRACT

Wheat-flour lipids were extracted with each of nine solvents, petroleum ether, n-hexane, n-heptane, benzene, chloroform, acetone, water-saturated 1-butanol, methanol, and 95% ethanol. Nonpolar solvents extracted substantially less lipids than the more polar solvents. Lipids extracted with nonpolar solvents contained less polar lipids than those extracted with polar solvents. Generally, as extracted bound or total lipids increased, mixing time increased and loaf volume decreased for reconstituted flours. Extracting lipids with each of the three alcohols reduced to zero or greatly impaired the gas-retaining capacity of gluten protein.

Extracting lipids from wheat and milled products depends not only on the type of solvent used but also on the moisture content and particle size of the material. In dough, the gluten proteins form a lipoprotein complex that is impermeable to some lipid solvents. Various polar organic solvents, in a mixture with water, effectively disrupt the lipoprotein complex and extract additional lipids. Several workers have reported effective solvent systems to extract lipids from dough and gluten. They include water-saturated 1-butanol (1), a mixture of chloroform-methanol-water (2), a mixture of water-acetone (3), and a benzene-ethanol-water mixture (4).

The extensive investigations of the role of wheat flour and other lipids in breadmaking have been summarized recently (5,6). Additional data obtained during our previously reported investigations are presented here because we believe they point to further related research. Those data concern the characterization of lipids extracted from wheat flours with various solvents and the effects of those solvents on breadmaking properties of reconstituted flours. Reconstituted flour refers to solvent-extracted flour to which the solvent-free extract was added back.

MATERIALS AND METHODS

Flour

The straight-grade flour, designated "Regional Baking Standard (RBS-66A)," was milled from a composite of several wheat varieties that were harvested at many locations throughout the southern, central, and northern Great Plains in

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1965. The RBS flour had a protein content of 12.7% (14% moisture basis), good loaf volume potential, and medium mixing time (4 min) and oxidation requirement (30 ppm $KBrO_3$).

Analytical Procedures

Protein and moisture were determined as described in AACC Approved Methods (7). The baking procedure described by Finney and Barmore (8,9,10) and Finney (11) and adapted by Shogren *et al.* (12) for 10 g of flour was used. The standard deviation for the average of duplicate loaf volumes was 1.75 cc. Gassing powers were determined on 10 g flour at 30° C with gauge-type pressure meters and with the same formula used in baking, except that shortening was omitted and water absorption was increased to 100%.

Preparing Lipid Fractions

All lipid preparations were from RBS flour or its gluten proteins. All solvents, of analytical grade, were distilled before use. Lipids that were extracted with benzene, chloroform, acetone, water-saturated 1-butanol, methanol, and 95% ethanol were purified, after evaporating the solvent, by redissolving in petroleum ether and centrifuging. Solvents in extracts were evaporated under vacuum below 40° C. Lipids were extracted with petroleum ether (bp 35° to 60° C), n-hexane, n-heptane, and acetone in a Soxhlet apparatus. For extraction with benzene, chloroform, water-saturated 1-butanol, methanol, and ethanol, we used the room-temperature extraction procedure and a Stein mill (13). The extracted flours, except for the flour treated with butanol, were dried in air until the solvent's odor was not detected. Residual butanol, detectable after addition of water, could not be removed from the flour by air-drying, vacuum distillation, or lyophilization of the frozen flour-water slurry. The deleterious effects on flour of water-saturated 1-butanol were eliminated by the previously described lipid-extracting (of protein) and reconstituting techniques (14). Nonpolar and polar lipids were determined gravimetrically after fractionating total lipids on silicic acid columns as described by Daftary and Pomeranz (15). Total lipids were reconstituted with the corresponding solvent-extracted flour by mixing in a Stein mill. The low moisture contents of the extracted flours were increased to their original levels in our equilibration cabinet.

Thin-Layer Chromatography

Extracted lipids (100 μg) were characterized by thin-layer chromatography. The lipids were separated with chloroform-methanol-water (65:35:4) on glass plates coated with silica gel G. Plates were sprayed with a saturated solution of $K_2Cr_2O_7$ in 70% (v/v) aqueous sulfuric acid and charred at 150° C for 30 min. The plates were photographed under ultraviolet light.

RESULTS AND DISCUSSION

Effects of nine solvents on lipids extracted and on nonpolar and polar fractions separated from each extract are summarized in Table I. The nonpolar solvents (petroleum ether, n-hexane, and n-heptane) extracted substantially fewer total lipids than the more polar solvents (benzene, chloroform, acetone, and especially water-saturated 1-butanol). Generally, as extracted lipids

increased, nonpolar components decreased and polar components increased. However, anhydrous methanol and 95% ethanol extracted relatively small to intermediate amounts of total lipids; yet, the extracts were rich in polar components.

Thin-layer chromatography (Fig. 1) indicated that lipids extracted with the nonpolar solvents (n-heptane, n-hexane, and petroleum ether) contained less of the polar lipids digalactosyl diglyceride and phosphatidyl choline than the lipids extracted with acetone, benzene, chloroform, 95% ethanol, and methanol.

Extracting free lipids (defined as those extracted with petroleum ether) had little effect on mixing time (Table II). As total and bound lipids increased (Table I, from n-hexane down to acetone), mixing times increased greatly from 4 to 14 min, and loaf volumes gradually decreased from 80 to 71 cc. Thus, increasing mixing time indicates a decreasing rate of protein hydration, which can be attributed to either increasing removal of bound lipids or to solvent effects on the proteins. Loaf volumes of the reconstituted flours, previously extracted with the nonpolar solvents petroleum ether, n-hexane, and n-heptane, were essentially equal to that of the control (80 cc). Solvent extraction increased baking absorption 1.0 to 4% (Table II), but did not affect oxidation requirement (30 ppm).

Extracting flour with water-saturated 1-butanol greatly increased mixing time and impaired loaf volume (53 cc) as anticipated, but the premix method (14) restored loaf volume (82 cc) to that of the control (80 cc), and demonstrated that the removal of bound lipids and the accompanying long mixing time (13.5 min) did not cause an irreversible change. The premixed dough from the reconstituted flour that contained glutenin (100–5C) extracted with water-saturated 1-butanol had, as expected (16, p. 132), the shortest mixing time.

Doughs from flours treated with methanol and 95% ethanol could not be mixed to conventional optimum consistency and appeared to have infinitely long mixing times, which we attributed to the doughs' having essentially no functional gluten protein (17). Their loaf volumes of 25 and 24 cc, respectively, indicated that the gluten had zero gas-retaining capacity. Treating flour with water-

TABLE I
Effects of Solvents on Lipid Extraction and Composition

Solvent	Lipids Extracted		Lipid Composition[a]	
	Total %	Bound[b] %	Nonpolar %	Polar %
Petroleum ether	0.84	0.00	64.9	32.9
n-Hexane	0.96	0.12	59.2	36.9
n-Heptane	1.01	0.17	57.9	39.1
Benzene	1.28[c]	0.44	48.1	47.3
Chloroform	1.32[c]	0.48	45.8	48.2
Acetone	1.38[c]	0.54	43.7	54.5
1-Butanol (water-saturated)	1.50[c]	0.66	41.0	55.0
Methanol	1.04[c]		44.5	52.0
95% Ethanol	1.16[c]		46.7	48.6

[a]As % recovered from silicic acid column.
[b]Total lipids minus those extracted with petroleum ether (0.84).
[c]Redissolved in petroleum ether.

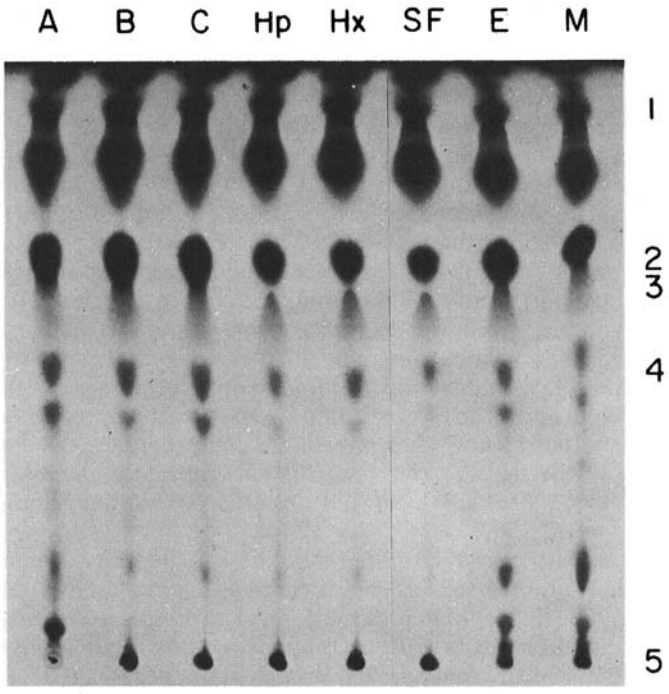

Fig. 1. Thin-layer chromatography of 100 μg lipids extracted from a composite wheat flour. From left to right: lipids extracted with acetone (A), benzene (B), chloroform (C), n-heptane (Hp), n-hexane (Hx), petroleum ether (Skelly F, SF), 95% ethanol (E), and methanol (M). Spots are tentatively identified as: 1) Monogalactosyl diglycerides, 2) Digalactosyl diglycerides, 3) Phosphatidylethanolamine, 4) Phosphatidyl choline, and 5) Lipoproteins.

TABLE II
Effects of Lipid Extraction on Breadmaking Quality of Reconstituted Flours

Solvent	Breadmaking Characteristics of Reconstituted Flours		
	Mixing time min	Absorption %	Loaf volume cc
None	4	62	80
Petroleum ether	4-1/8	66	82
n-Hexane	5-3/8	64	79
n-Heptane	6-3/4	64	77
Benzene	7-3/8	65	75
Chloroform	10-1/2	65	74
Acetone	14	63	71
1-Butanol[a]	13-1/2	63.5	53
1-Butanol[a], premix	2-3/4	63.5	82
Methanol	Infinite	65	25
95% Ethanol	Infinite	63	24

[a]Water-saturated.

saturated 1-butanol decreased gassing power (after 4 hr of fermentation) to 100 mm Hg (485 mm Hg for the control). Extracting with chloroform, acetone, and 95% ethanol reduced gassing power readings only to 465, 453, and 401 mm Hg, respectively.

Baking properties indicate that some solvents had little or no adverse effect on wheat-flour components, but that others may modify flour components adversely and irreversibly. Some solvents had relatively small effects on gassing power and loaf volume (Table II), compared to the large effects of water-saturated butanol. Those data indicate that it might be possible to use some of them (especially benzene, chloroform, and acetone) to extract flour lipids without irreversibly damaging breadmaking properties, particularly since the deleterious effects of water-saturated butanol are reversible (14). Use of those solvents might require fractionating the flour before extraction, proper rehydration, use of appropriate dough development procedures, or a combination of those techniques.

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