DEFATTED AND RECONSTITUTED WHEAT FLOURS. I. EFFECTS OF SOLVENT AND SOXHLET TYPES ON FUNCTIONAL (BREADMAKING) PROPERTIES[1]

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ABSTRACT

Cereal Chem. 54(3): 454-465

Lipid extractability increased with solubility parameter in the series Skelly B, benzene, acetone, and 2-propanol, and was higher in a regular than in a vacuum Soxhlet. Extraction temperature was lowered 12° to 18°C by pressure reduction with a vacuum pump connected to the condenser of a modified Soxhlet. Chromatography by silicic acid column and thin-layer indicated that the increase in total extractable lipids was primarily from an increase in extracted polar lipids. Rheological properties and baking characteristics of dough were little affected, whether the flour was extracted in a regular or vacuum Soxhlet with Skelly B. Deleterious effects were small for flours treated with benzene and acetone, and substantial for flour treated with 2-propanol. The adverse effects were lowered by extraction of the flour in a vacuum Soxhlet. Extraction of lipids by 2-propanol was effective in a vacuum Soxhlet, and damage to functional breadmaking properties of the flour was much less than to those of a flour extracted in a regular Soxhlet.

The important role of wheat-flour lipids in breadmaking and the rheological properties of dough has been reported (1–3). MacRitchie and Gras (4), however, have suggested that lipids do not contribute significantly to rheological properties of doughs. Also, they found that curves of loaf volume as a function of lipid content showed minima at lipid contents intermediate to those of the defatted and whole flours. Extractability and functionality of lipids from wheat and milled products depend primarily on the type of solvent, moisture content of wheat products and/or of solvent, and particle size of the material (1). Little is known about the effects of the extracting conditions (temperature and pressure) on functionality of reconstituted flours in breadmaking.

The Soxhlet apparatus is used widely to extract lipids from biological samples. The extracting chamber of the conventional Soxhlet is positioned between a boiling flask and a condenser. A commercially available, modified Soxhlet apparatus, unlike the conventional one, has an extracting chamber positioned on the side. Temperature in the extracting chamber largely depends on the boiling point of the solvent and its condensation rate. The condenser may be connected to a vacuum pump for reduction of pressure and, hence, boiling point of solvent, and temperature in the extracting chamber. We compared lipids extracted in a modified vacuum Soxhlet and in a regular Soxhlet with two common hydrocarbon solvents (Skelly B and benzene), a ketone solvent (acetone), and an alcohol solvent (2-propanol). We made the comparison because we were in search of conditions which maximize extraction of lipids and minimize damage

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to functional properties of the reconstituted flour. It is necessary to establish the best procedure for lipid extraction, without altering the nonlipid components, to study the role of free and bound flour lipids in breadmaking. The effects of extracting conditions on functionality were assessed by the rheological and breadmaking properties of the flours.

MATERIALS AND METHODS

Materials

Regional Baking Standard (RBS-74)—an untreated, straight-grade flour—was experimentally milled (Allis) from a composite grist of many hard red winter wheat varieties harvested at many locations throughout the Great Plains in 1973. The flour contained 12.4% protein (N × 5.7) and 0.41% ash (14% mb), and had good loaf volume potential and medium mixing and oxidation requirements.

Reference lipids were from Applied Science Laboratories, Inc., State College, Pa. Silicic acid for chromatography of lipids was from Mallinckrodt, New York, N.Y. Organic solvents were analytical reagent grade, and solutions were prepared from analytical reagent-grade compounds.

Analytical Procedures

Protein, ash, and moisture contents were determined by AACC Approved Methods (5). The baking procedure described by Finney and Barmore (6–8) and Finney (9), as adapted by Shogren *et al.* (10), for 10 g (14% mb) flour was used. The breadmaking formula was the same as described by Shogren *et al.* (10), except that 10 ppm potassium bromate and 100 ppm ascorbic acid were used as oxidizing agents. Shortening was a commercial vegetable product, partly hydrogenated, mp 41° C. The standard deviation for the average of duplicate loaf volumes was 1.35 cc. After loaf volumes of breads cooled to room temperature were determined by dwarf rapeseed displacement, loaves were cut and their crumb grains and textures were evaluated. This code was employed: S, satisfactory; Q, questionable; and U, unsatisfactory. Mixograms of 10-g flour-water doughs were determined according to Finney and Shogren (11).

Extraction of Flour Lipids

Two extractors were used: a regular large Soxhlet and a modified Soxhlet extraction apparatus (Corning 3885; VWR Scientific catalogue #27608-000), the condenser of which was connected to a vacuum pump. The condensation rate was 1 to 2 drops/sec.

In the regular Soxhlet, lipids were extracted from 500 g RBS-74 flour with about 2.8 liters of organic solvent. After 24 hr of extraction, the flours were air-dried for 24 hr (sifted through a 100-mesh sieve after acetone and 2-propanol extraction) and repacked in a cellulose thimble for further extraction. Total extraction time was 48 hr with Skelly B and benzene, and 72 hr with acetone and 2-propanol. Extraction times were selected for maximum extraction of lipids at condensation rates described above. Extraction time varies with condensation rate, as described in AACC Approved Methods (5).

In the modified Soxhlet, lipids were extracted from 1000 g RBS-74 flour (two thimbles in a chamber, each thimble containing 500 g flour) with about 2.8 liters of organic solvent. Every 48 hr, the defatted flour was air-dried for 24 hr and repacked in thimbles. Total extraction times were twice those with the regular Soxhlet. Pressure in the modified Soxhlet was equivalent to 9 to 10 in. of mercury for each of the four solvents. Temperatures of the extracting chambers were:

	Acetone, °C	*Skelly B, °C*	*Benzene, °C*	*2-Propanol, °C*
Regular Soxhlet	40	42	45	50
Vacuum Soxhlet	28	29	27	32

The defatted flours spread in thin layers on trays were air-dried at room temperature in a hood until the odors of organic solvents were not detected: about 24 hr for acetone and Skelly B and 48 hr for benzene and 2-propanol. The flours were sifted through 100-mesh sieve (149-μm opening), and then stored at 4° C.

Solvents of the lipid extracts were evaporated at reduced pressure below 40° C. Lipids in each dry extract were re-extracted twice with 150 ml and then once with 100 ml petroleum ether (bp 35° to 60° C). The combined petroleum ether extracts were centrifuged at 20,384 $\times g$ for 10 min at 4° C, and the supernatant was diluted to 500 ml. Two 5-ml aliquots were evaporated and dried at 65° C for determination of lipid contents. The remaining extract was evaporated at reduced pressure below 30° C, and the lipids were stored at −18° C.

Fractionation of Flour Lipids

Flour lipids (about 350 mg) were fractionated by silicic acid column chromatography (12) into nonpolar and polar lipids with chloroform and methanol, respectively, as eluting solvents. Complete elution was determined by thin-layer chromatography (tlc). Lipid fractionations were replicated at least twice. Total recovery from silicic acid column fractionation ranged from 89.7 to 98.4%; average recovery was 94.1%.

Thin-Layer Chromatography

Glass plates (20 × 20 cm) were coated with a 250-μm layer of silica gel G, and the plates were activated for 3 hr at 130° C. The solvent systems for one-dimensional ascending development were: hexane-diethyl ether-methanol (80:20:1, v/v/v, solvent system I) for nonpolar lipids; and chloroform-methanol-water (65:25:4, v/v/v, solvent system II) for polar lipids. Plates were sprayed with a 0.6% $K_2Cr_2O_7$ solution in 55% H_2SO_4 and heated for 25 min at 180° C (13). The plates were photographed under uv light. Specific sprays were 0.2% α-naphthol in ethanol followed by a light spray with 95% H_2SO_4 for glycolipids (14), and molybdenum blue reagent for phospholipids (15).

Reconstitution of Flour Lipids and Defatted Flour

The defatted flours (75 g, db) were reconstituted with appropriate amounts of lipids in a Stein mill for 1 min. The moisture contents of the reconstituted flours were raised to about 14% by the placement of small beakers of water (with wicks) in the center of flours (4- to 5-mm layer) in closed containers. The reconstituted flours were used for mixograph and baking studies.

RESULTS AND DISCUSSION

Flour Lipids

Lipid extractability by the two Soxhlet methods increased with solubility parameter of the solvent (Table I). Solubility parameter is defined as the square root of cohesive energy density (16):

$$\delta = \left(\frac{\Delta E}{V}\right)^{1/2} = \left(\frac{\Delta H - P\Delta V}{V}\right)^{1/2}$$

where δ is the solubility parameter, ΔE the internal energy, V the molar volume, ΔH the heat of vaporization, and P the pressure (17). Each solvent extracted more lipids in the regular Soxhlet than in the modified vacuum Soxhlet. Analysis of variance (ANOVA) and tests for Fisher's Least Significant Difference (LSD) showed that Soxhlet type, solvent, and Soxhlet × solvent interaction were all significant at the 0.01 level for total amounts of extracted lipids.

ANOVA and LSD showed that amounts of nonpolar lipids were not affected significantly by Soxhlet, solvent, or Soxhlet × solvent interaction (Table II). Amounts of polar lipids, however, were affected by Soxhlet type and by solvent

TABLE I
Total Flour Lipids Extracted with Four Solvents by Regular and Vacuum Soxhlets

Extracting Solvent	Solvent Solubility Parameter[a]	Solvent bp °C	Total Lipids (%, db)[b] Extracted by	
			Regular Soxhlet	Vacuum Soxhlet
Skelly B	7.27[c]	66.7	1.03	0.91
Benzene	9.16	80.1	1.12	1.07
Acetone	9.62	56.5	1.17	1.14
2-Propanol	11.44	82.3	1.43	1.34

[a] Values at 25° C according to Hoy (17).
[b] Averages of two extractions; overall standard deviation: 0.004.
[c] Solubility parameter of hexane.

TABLE II
Nonpolar and Polar Lipids (g/100 g flour, db) Fractionated by Silicic Acid Column Chromatography

Extracting Solvent	Nonpolar Lipids (g)[a] from		Polar Lipids (g)[a] from	
	Regular Soxhlet	Vacuum Soxhlet	Regular Soxhlet	Vacuum Soxhlet
Skelly B	0.640	0.607	0.317	0.251
Benzene	0.613	0.651	0.392	0.350
Acetone	0.648	0.647	0.505	0.475
2-Propanol	0.645	0.666	0.653	0.616

[a] Averages of two fractionations; overall standard deviation: 0.016.

at the 0.01 level. The amount of polar lipids extracted increased with solubility parameter of the solvent, and increases in lipids extracted by different solvent were mainly from increases in polar lipids.

The nonpolar lipids both quantitatively and qualitatively were not affected by the solvent or Soxhlet types used (Fig. 1). Triglycerides were major components of nonpolar lipids as determined by tlc.

Polar lipids separated by solvent system II produced different

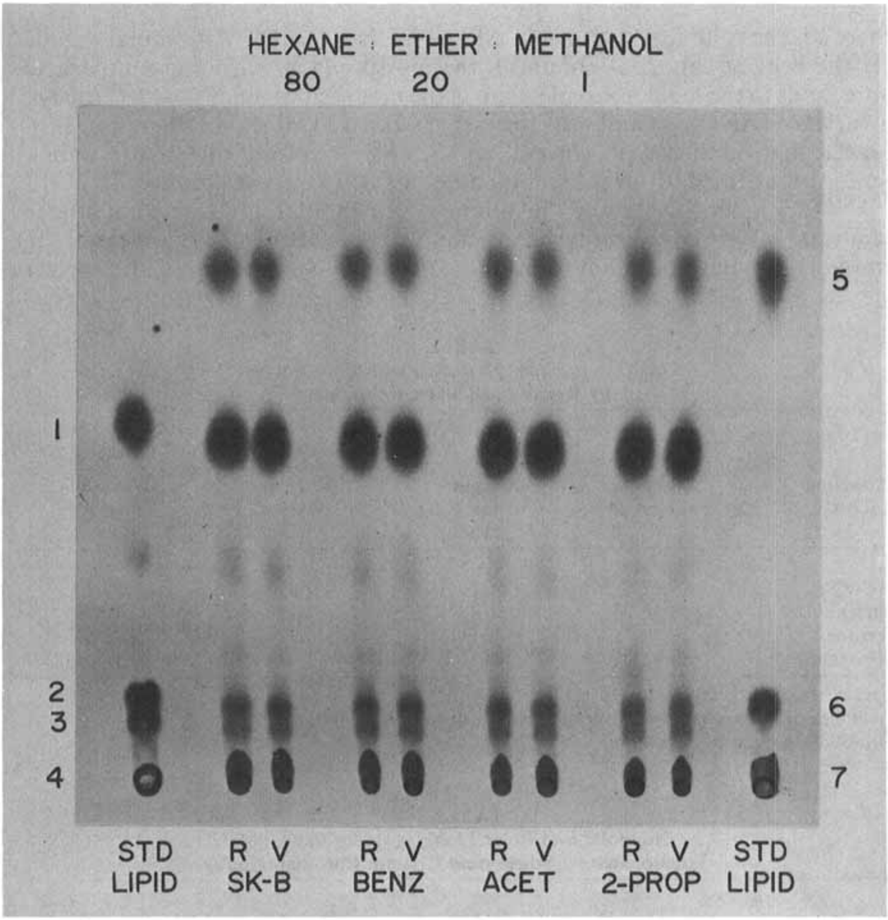

Fig. 1. Thin-layer chromatogram of nonpolar, standard, and wheat-flour lipids developed with hexane-diethyl ether-methanol (80:20:1), charred with a 0.6% $K_2Cr_2O_7$ solution in 55% H_2SO_4, and photographed under uv light. From left to right, standard lipids, lipids extracted with Skelly B (SK-B), benzene (BENZ), acetone (ACET), and 2-propanol (2-PROP), and standard lipids. (R) and (V) denote lipids extracted in a regular and a vacuum Soxhlet, respectively. Standard lipids were: 1) triolein; 2) 1,3 diolein; 3) 1,2 diolein; 4) monolein; 5) β-sitosteryl palmitate; 6) β-sitosterol; and 7) oleic acid. Amounts applied were 15 μg of each standard lipid and 100 μg of flour lipids.

chromatograms, depending on extracting solvent and Soxhlet apparatus (Fig. 2). Components with R_f values of 0.78, between those of monogalactosyl diglyceride and digalactosyl diglyceride, stained purple with α-naphthol and thus appeared to contain glycolipids. Components with R_f values near the R_f value of lysophosphatidylcholine stained gray-purple with α-naphthol and presumably contained sucrose and raffinose (18). Components containing sucrose and raffinose were essentially absent in polar lipids extracted with Skelly B or benzene. More galactolipids and other sugar-containing compounds were extracted by acetone and 2-propanol with the regular Soxhlet than with the

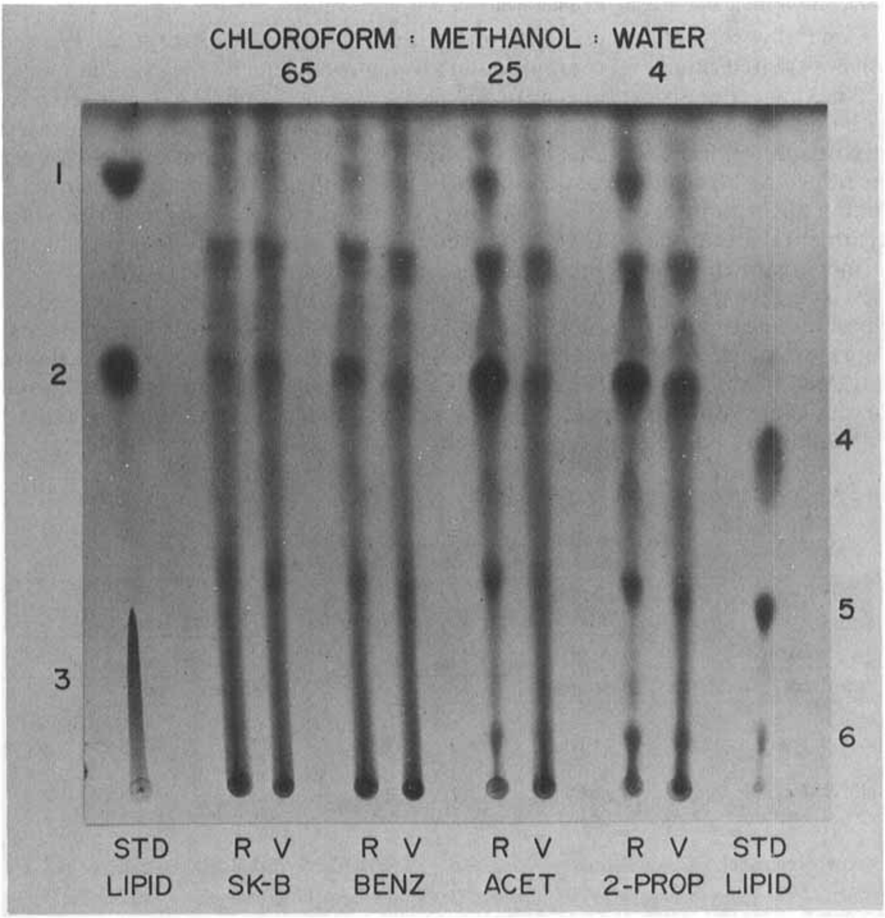

Fig. 2. Thin-layer chromatogram of polar, standard, and wheat-flour lipids developed with chloroform-methanol-water (65:25:4), charred with a 0.6% $K_2Cr_2O_7$ solution in 55% H_2SO_4, and photographed under uv light. The abbreviations in Fig. 2 indicate the same materials as in Fig. 1, except that the standard lipids were: 1) monogalactosyl diglyceride; 2) digalactosyl diglyceride; 3) phosphatidylserine; 4) phosphatidylethanolamine; 5) phosphatidylcholine; and 6) lysophosphatidylcholine.

vacuum Soxhlet. The molybdenum blue spray showed that no lysophosphatidylcholine was extracted with Skelly B or benzene, but it faintly indicated the presence of phosphatidylethanolamine and a component with an R_f value slightly larger than that of digalactosyl diglyceride. The component was probably N-acyl phosphatidylethanolamine (19,20). Streaks extending from origin stained blue, indicating phospholipids (presumably phosphatidylserine and its degradation products, as described by MacMurray and Morrison, ref. 20). The higher the solubility parameter of the extracting solvent, the larger the qualitative and quantitative differences between polar lipids extracted by the regular and the vacuum Soxhlets (Fig. 2).

Water Absorption and Mixing Requirements

Generally, solvents differed little in their effects on water absorption. For the solvent-treated flours, mixograph water absorptions were, on the average, 64.4% and baking water absorptions were, on the average, 65.3 and 67.4%, respectively, with and without added shortening (Table III). The solvent treatment increased mixograph water absorptions by 1 to 2% and baking absorptions by 2 to 4% over the respective absorptions of the control flour. For the 2-propanol-treated flours, baking absorptions were about 5% lower for the flour with the regular Soxhlet treatment than for the flour with the vacuum Soxhlet treatment. Apparently, the former treatment considerably damaged the proteins.

Mixograph and bake-mixing times were affected by both solvent and Soxhlet types: mixing time increased with solubility parameter of solvent; for all solvents, flours extracted in the regular Soxhlet had longer mixing times than flours extracted in the vacuum Soxhlet. Differences between baking and mixograph mixing times or differences in mixing times between Soxhlet types increased with solubility parameters of the solvents (Table III).

TABLE III
Water Absorption and Mixing Characteristics of 10 g Reconstituted, Solvent-Extracted Flours

	Water Absorption, %, 14% mb			Mixing Time, min		
		Baking[a]			Baking[a]	
Extracting Solvent : Soxhlet	Mixograph	0	3	Mixograph	0	3
None	63.1	64.9	63.1	3-7/8	3-7/8	4.0
Skelly B: Regular	64.1	67.1	65.4	4.0	4-7/8	4-7/8
Vacuum	64.1	67.1	65.1	4.0	4-1/4	4-1/2
Benzene: Regular	64.1	68.6	65.6	5-3/4	6-5/8	6-7/8
Vacuum	64.1	67.4	65.6	4-1/2	5.0	5-1/8
Acetone: Regular	65.1	68.6	66.4	7-1/2	8-1/2	8-5/8
Vacuum	65.1	67.1	65.1	5-7/8	6-1/8	7-1/4
2-Propanol: Regular	64.1	63.8	62.2	∞	2(∞)[b]	2(∞)[b]
Vacuum	64.1	69.1	67.1	10-1/2	12-3/8	12-1/2

[a] 0 and 3 = 0 and 3% shortening added.

[b] Mixed for 2 min to incorporate the ingredients.

Duplicate water absorptions varied up to 0.5% and duplicate mixing times up to 1/8 min; average duplicate absorptions varied 0.15% and average mixing times 1/24 min.

Some typical mixograms, described in Table III, are shown in Fig. 3. Mixograph mixing time of the control flour was 3-7/8 min; it increased to 5-7/8 min and 7.5 min, respectively, for flours extracted with acetone in the vacuum Soxhlet and in the regular Soxhlet. Extraction with 2-propanol in the vacuum Soxhlet increased mixing time to 10.5 min. Flour treated with 2-propanol in the regular Soxhlet had an infinite mixing time, as even mixing for 28 min with 10% water below optimum to speed up rate of dough development produced no point of minimum mobility; the dough had essentially no functional gluten protein (21). Consequently, dough from this flour was mixed for 2 min merely to incorporate the ingredients (Table III).

Addition of shortening, which generally decreased water absorption 1.5 to 2%, slightly increased mixing times.

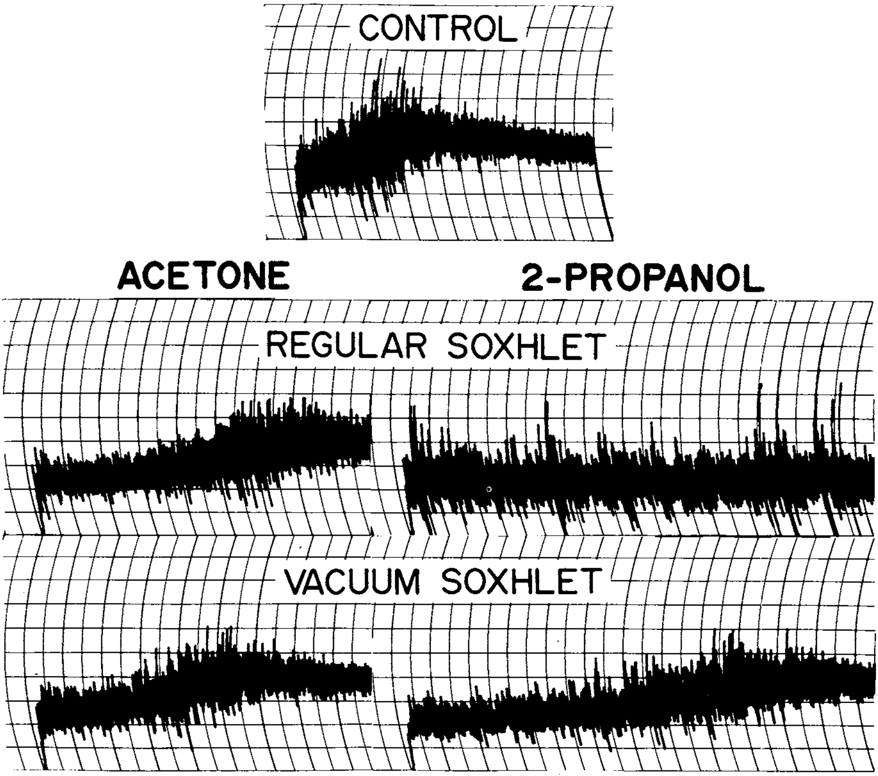

Fig. 3. Mixograms (10 g flour) of untreated control flour (RBS-74) and of reconstituted flours extracted with acetone in a regular and a vacuum Soxhlet, and with 2-propanol in a regular and a vacuum Soxhlet apparatus. Arcs are at 1-min intervals.

Loaf Volumes and Crumb Grains

Loaf volumes of breads without shortening, baked from reconstituted Skelly B- and acetone-extracted flours, were 68.2 to 69.0 cc as compared with 64.7 cc for the control bread (Table IV). That is of interest, because bread baked from petroleum-ether-defatted flour with no added shortening had a better crumb and often a higher loaf volume than that with added shortening (22,23). The nature of the "improving effect" of extraction with the hydrocarbon and ketone solvents is being investigated. Ponte *et al.* (24) first observed that adding 0.65% hexane or 0.43% heptane to the dough substantially improved bread crumb grain, structure, and color. However, the improving effect was exerted only in doughs containing shortening. The effect was cancelled if the solvent was allowed to evaporate before dough-mixing. As compared with the loaf volume of control bread without shortening, that of bread prepared similarly from the benzene-treated flour was smaller if the extraction had been in a regular Soxhlet and larger if it had been in a vacuum Soxhlet (Table IV).

ANOVA showed that Soxhlet type, shortening, solvent, and several interactions—Soxhlet × solvent, shortening × solvent, and Soxhlet × shortening × solvent—affected loaf volume at the 0.01 level. Furthermore, tests for Fisher's LSD showed that differences in loaf volumes between two treatments were significant at the 0.05 level if they were larger than 3.56 cc, and at the 0.01 level if they were larger than 4.13 cc. Therefore, Soxhlet type significantly affected loaf volumes of the benzene-treated flours without shortening added, loaf volumes of the Skelly B-treated flours with shortening, and loaf volumes of the 2-propanol-treated flours with or without shortening added. For the no-shortening series, loaf volumes for flours treated with all four solvents by the vacuum Soxhlet or treated with acetone and 2-propanol by the regular Soxhlet were significantly different from the untreated control. When shortening was added, treatment

TABLE IV
Loaf Volume and Crumb Grain of Bread Baked with 10 g Reconstituted, Solvent-Extracted Flours

Extracting	Loaf Volume, cc		Crumb Grain[a]	
Solvent : Soxhlet	No shortening	3% shortening	No shortening	3% shortening
None	64.7	77.7	Q-U	S
Skelly B: Regular	68.2	76.5	Q	S
Vacuum	69.0	80.5	Q	S
Benzene: Regular	62.0	74.0	Q-U	S-Q
Vacuum	68.4	74.0	Q	S-Q
Acetone: Regular	68.4	74.0	Q-U	S-Q
Vacuum	68.9	75.0	Q	S-Q
2-Propanol: Regular	25.9	25.8	U^6	U^6
Vacuum	52.2	65.3	U^3	Q

[a] S = Satisfactory; Q = questionable; U = unsatisfactory (the higher the number, the poorer the crumb grain).

with 2-propanol by both Soxhlets and with acetone by the regular Soxhlet significantly decreased loaf volumes. Crumb grains of breads from Skelly B-, benzene-, or acetone-treated flours were equal to, or better than, those of the control bread. Breads with 3% shortening showed that Skelly B was the only solvent that did not impair baking properties of the flour. Benzene and acetone treatments decreased loaf volume by 3 to 4 cc and slightly impaired crumb grain. Similar results were reported by Finney *et al.* (25). The improving effect (for no-shortening doughs) of extraction with Skelly B, acetone, and with benzene (vacuum extraction) was cancelled in bread baked with shortening.

Functional properties were destroyed in the flour treated with 2-propanol in the regular Soxhlet. Loaf volume was only 25.8 to 25.9 cc, and crumb grain was extremely poor (Table IV and Fig. 4). These results indicated that the gluten had practically no gas-retaining capacity. Loaf volumes of the breads baked with flour that had been extracted with 2-propanol in the vacuum Soxhlet were not quite as low; but they were lower than those of the controls. All crumb grains of breads from 2-propanol-treated flours were inferior to those of the controls (Table IV and Fig. 4). However, the detrimental effects of 2-propanol on

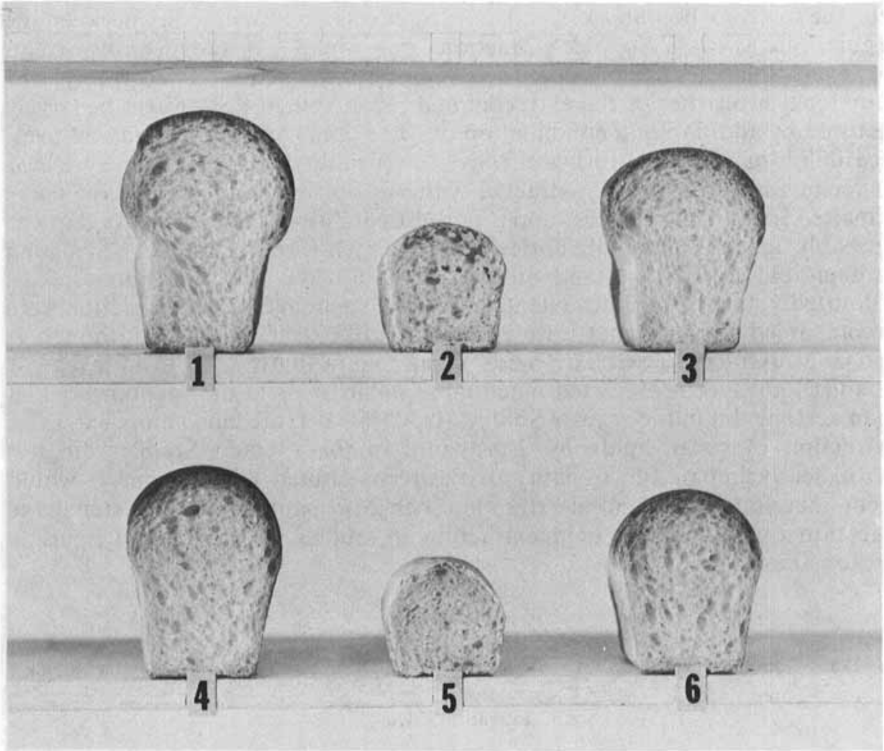

Fig. 4. Microloaves (10 g flour) baked with 3% shortening (1 to 3) and without shortening (4 to 6) from: 1 and 4, untreated flour; 2 and 5, reconstituted flour extracted with 2-propanol in a regular Soxhlet apparatus; and 3 and 6, reconstituted flour extracted with 2-propanol in a vacuum Soxhlet apparatus.

functional properties of the reconstituted flours were considerably reduced by use of the modified vacuum Soxhlet.

COMMENTS AND CONCLUSIONS

Lipid extractability was affected both by the solvent and by the type of Soxhlet apparatus used. The extraction chamber of the vacuum Soxhlet was cooler than that of the regular Soxhlet by 12° and 13°C, respectively, with acetone and Skelly B, and by 18°C with either benzene or 2-propanol. The large differences in chamber temperatures for benzene and 2-propanol strongly affected the functional properties of the flour in breadmaking, even though effects on extractability of total lipids and lipid composition apparently were minimal. Therefore, the higher temperatures of benzene and 2-propanol in the regular Soxhlet may have adversely affected flour components other than lipids, *i.e.*, proteins. Such possible effects are being investigated. As the solubility parameter of the extracting solvent increased, so did the amount of extracted lipids, as a result of increased extraction of polar lipids. Similarly, for a given solvent, more lipids were extracted in a regular than in a vacuum Soxhlet, presumably because more polar lipids were extracted at the higher temperature. For the hydrocarbon and ketone solvents, effects on functionality between the regular and vacuum Soxhlet extractions were small. For the alcohol solvent, however, the vacuum Soxhlet was substantially better. Conceivably, the functional properties of the extracted and reconstituted flour might be largely restored by addition of a combination of some lipids or lipid-related additives. Feasibility of such a restoring effect on a partially damaged dough system is under investigation. Flour extracted with alcohol in the regular Soxhlet was impaired irreversibly, at least under our test conditions. The extensive damage probably resulted from disruption of the gluten-forming capacity involving protein-lipid interactions and/or damage to other wheat-flour components. Admittedly, the reduced detrimental effects of vacuum Soxhlet extraction were accompanied by somewhat lowered amounts of extracted lipids. Although 2-propanol extracted less polar lipids in a vacuum (0.616%) than in a regular Soxhlet (0.653%), it extracted much more polar lipids in the vacuum Soxhlet than acetone did in the regular Soxhlet (0.505%). Yet, the much more extensive extraction of polar lipids by 2-propanol in the vacuum Soxhlet did not permanently impair functionality of the reconstituted wheat flour. It would seem, therefore, that vacuum extraction with 2-propanol is a correct step in the direction of maximizing lipid extraction in studies on the role of lipids in breadmaking.

Acknowledgments

Lerance C. Bolte milled the wheat samples; Ruth M. Jacobs fractionated the extracted lipids.

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[Received April 19, 1976. Accepted September 6, 1976]