DEFATTED AND RECONSTITUTED WHEAT FLOURS III. EFFECTS OF FLOUR MOISTURE CONTENT AND AQUEOUS BINARY AZEOTROPES ON FUNCTIONAL (BREADMAKING) PROPERTIES[1]

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ABSTRACT

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Lipid extractability increased when the water content of wheat flour was raised from 1.2 to 13.8% or when aqueous binary azeotropes were used as extractants instead of pure organic solvents (hexane, acetone, and 2-propanol). Rheological dough properties (mixograph) and baking characteristics of the defatted and reconstituted flours were affected by moisture content of either flour samples or the solvent systems. Adverse effects on rheological and breadmaking properties were smallest for hexane and largest for 2-propanol. The adverse effects increased with water content of the flour, especially from 7.2 to 13.8%, or by addition of water to the solvent.

To demonstrate the role of flour lipids, including bound lipids, in breakmaking it is necessary to establish conditions that maximize extraction of the lipids and yet minimize damage to the functional properties of the wheat flour. Native flour lipids bound to flour constituents, such as proteins or starch, are not easily extractable with organic solvents alone or with mixtures of several solvents. Addition of a limited amount of water to the extracting solvent system, however, substantially increased extractability of lipids due to disrupting the lipoprotein complexes (1–3).

When flours are wetted and doughs are mixed, lipids extractable from flour with nonpolar solvents become bound (3); about one-half to two-thirds of wheat flour free lipids (some free nonpolar and practically all free polar components) become bound in dough or gluten (4–6). During dry-mixing of petroleum ether-defatted flour, practically no added flour nonpolar lipids were bound, but substantial amounts of flour free polar lipids were bound in flour with a moisture as low as 4.4%; the binding increased with increasing flour moisture content (7).

The molecules of water in flour are considered to be associated with specific chemical groups in starch, proteins, gums, and other constituents (8). Especially important is the bonding between proteins and lipids (9). Daniels (10) recently reviewed the effects of water in wheat flour dough. The adsorption isotherm of flour exhibits a sigmoid relationship between water activity (Aw) and moisture content (MC) (11). Water is bound at the low moisture region (MC $<$ 14%, Aw $<$ 0.7); not bound and free to condense as a liquid phase at moisture levels between 14 and 23%; and free in the true liquid phase and separates from the flour particles only in the high moisture region (MC $>$ 23%, Aw $>$ 0.95). Simply

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raising the MC of a flour accelerated lipid binding in work-free systems to which no mixing was applied (12,13). When MC of flour was between 20 and 45%, petroleum ether-extractable free lipids decreased, polar solvent-extractable bound lipids increased, and the sum of free and bound lipids decreased (14). The extractability of lipids may be different, however, at low moisture levels (MC < 14%, Aw < 0.7) where water is bound.

Little is known about the effects of water in extractants or in flour samples on the functionality of the reconstituted flours in breadmaking. Therefore, we extracted lipids in a Soxhlet apparatus from lyophilized flours with aqueous binary azeotropes of organic solvents to study the effects of water in flours and in extractants on lipid extractability. We used a reconstituting technique to determine the effects of water in flours or in extractants on rheological dough properties and breadmaking functionality.

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 (both 14% moisture basis) and had a good loaf volume potential and medium mixing and oxidation requirements. Organic solvents were analytical reagent grade.

Analytical Procedures

Protein, ash, and moisture contents were determined by AACC Approved Methods 46-11, 08-01, and 44-15A, respectively (15). The 10 g baking procedure has been described elsewhere (16). Bakes were replicated three times. The loaves were cooled to 25° C, and loaf volumes were determined by dwarf rapeseed displacement. Loaves were cut and their crumb grains were evaluated as follows: S, satisfactory; Q, questionable; and U, unsatisfactory. Mixograms of 10 g flour were also determined (17).

Lyophilizing Flours

Flours were lyophilized to the desired moisture levels. The MC of about 150 g of flour was reduced from 13.8 to 7.2% in about 30 hr and to 1.2% in about 72 hr.

Extracting Lipids

Lipids were extracted from 10 g (moisture-free basis) flour wrapped with filter paper in a cellulose extraction thimble (Whatman, 33 mm × 80 mm) with 175 ml of solvent (hexane, benzene, acetone, 2-propanol, or the aqueous binary azeotrope of hexane, acetone, or 2-propanol) in a Soxhlet apparatus. Extraction time was 16 hr with hexane, acetone, and their azeotropes, and 48 hr with benzene, 2-propanol, and the azeotrope of 2-propanol. Extraction times were selected for maximum extraction of lipids at a condensation rate of 2 or 3 drops per second. Lipid extractions were replicated 12 times. Solvent was evaporated from the lipid extract at reduced pressure below 40° C. The dried extracts, except for the hexane extract, were redissolved in petroleum ether (three times with 25 ml). Purified lipids were obtained in the supernatant from a residue, which was

discarded, by centrifugation at 20,384 × *g* for 10 min at 4° C. The defatted flours were air dried at room temperature in a hood until the solvent odors were not detected—about 24 hr for acetone and hexane and 48 hr for the other solvents and all azeotropes. The flours were sifted thru a 100-mesh sieve (149 μm openings); the portion retained on the sieve, if any, was ground by a micro Wiley mill to pass a 60-mesh sieve. The combined, sifted, defatted flours were stored at 4° C.

Reconstituting Flour Lipids and Defatted Flours

The defatted flour for each extractant was composited from ten replicates, and each composite was blended with the corresponding lipids in a Stein mill for 1 min. The low MC of the reconstituted flours or untreated control lyophilized flours was increased to about 13 to 14% in an equilibration cabinet as described previously (18). Mixograph, mixing time, water absorption, and breadmaking tests were all made on rehydrated reconstituted flours or rehydrated untreated lyophilized flours.

RESULTS AND DISCUSSION

Flour Lipids

Lipid extractability generally increased with the MC of the flour. Less lipids were extracted with pure hexane, acetone, and 2-propanol than with their azeotropes (Table I). Analysis of variance (ANOVA) and Fisher's Least

TABLE I
Flour Lipids (mg per 10 g flour, db) Extracted With Four Solvents and Their Aqueous Binary Azeotropes

Extracting Solvent Solvent/Water (% Composition)[c]	bp[c] (° C)	Lipids (mg)[a] Extracted From Flours With MC[b] (%)		
		1.2	7.2	13.8
Hexane				
100:0	69.0	87	91	99
94.4:5.6	61.6	95	94	100
Benzene				
100:0	80.1	116	119	125
91.1:8.9	69.4	...	...	...
Acetone				
100:0	56.5	121	128	125
88.5:11.5	56.1	129	129	132
2-Propanol				
100:0	82.3	143	149	150
87.8:12.2	80.4	167	172	173

[a]Averages of 12 extractions; overall standard deviation: 3.3 mg.
[b]MC = moisture content.
[c]Data from Handbook of Chemistry and Physics (1963).

Significant Difference (LSD) were used to determine statistical significance of all effects (19). MC in flour, extracting solvent (S), and MC × S interaction were all significant at the 0.01 level for total amounts of extracted lipids.

For comparisons between two treatments, effects were significant at the 0.05 level if differences in extracted lipids were larger than 2.7 mg, and at the 0.01 level if larger than 3.5 mg. Lipid extractability with hexane, its azeotrope, and benzene increased more when flour moisture was increased from 7.2 to 13.8% than when flour moisture was increased from 1.2 to 7.2%. When 2-propanol and its azeotrope were used, however, increases in moisture in the low moisture region affected lipid extractability more significantly than did moisture increases in the 7.2 to 13.8% range. The presence of water in hexane increased lipid extractability most for flour with 1.2% moisture and least for flour with 13.8% moisture. The presence of water in 2-propanol increased lipid extractability about equally from flours at the three moisture levels.

Lipids could not be extracted with the benzene azeotrope. Although forming an azeotrope, benzene and water are immiscible and thus formed two layers. During refluxing, water formed a layer of dough around the flour sample in the extractor that benzene could not penetrate. The hexane azeotrope also formed two layers, but we accomplished the extractions presumably because less water was in the hexane azeotrope than in the benzene azeotrope.

TABLE II
Water Absorption (14% mb) of Reconstituted, Solvent-Extracted Flours

	Water Absorption (%)[a]					
	Solvent Extracted From Flours With Original MC[b] (%)					
Extracting Solvent[c]	1.2	7.2	13.8	1.2	7.2	13.8
	Mixograph			Bake[d]		
None (control)	63.1	63.1	63.1	63.6	63.6	63.6
Hexane						
Pure	64.1	64.1	64.1	64.6	64.1	64.1
Azeotrope	58.1	59.4	57.1	68.1	67.4	66.1
Benzene						
Pure	64.1	64.1	60.1	66.6	67.1	68.1
Acetone						
Pure	64.1	63.1	62.1	65.6	65.1	64.1
Azeotrope	61.1	59.1	58.1	67.1	67.1	66.1
2-Propanol						
Pure	63.1	59.1	58.1	69.1	67.1	63.1
Azeotrope	70.1	69.1	66.1	68.1	68.1	67.1

[a]Averages of three replicates for baking absorption; overall standard deviation, 0.68.
[b]MC = moisture content.
[c]Azeotropes: aqueous binary solvent system with % composition given in Table I.
[d]With 3.0% shortening added.

Water Absorption and Mixing Requirements

Flour treatment (solvent extraction), dough composition, and consistency changes during fermentation affected optimum baking absorptions. Water absorptions (14% mb) of bakes with shortening were generally higher than mixograph absorptions (Table II). Water absorptions of bakes without shortening were, on the average, 2 percentage points higher than with shortening added (data not given). Lyophilization did not affect mixograph or baking absorptions of untreated control flours as long as the low moisture sample was brought up to the normal moisture level (13 to 14%).

Mixograph absorptions decreased when flour moisture was increased and when the azeotrope, except that of 2-propanol, was used instead of the pure solvent. In general, however, flour moisture affected baking absorptions less than did mixograph absorptions. Also, baking absorptions were greater for flours extracted with the azeotropes than with the pure solvents.

The solvent, as shown previously (18,20), and both MC of flour and extractants affected mixograph and bake mixing times (Table III). Original MC, however, did not affect mixing times of untreated control flours, *i.e.*, the lyophilizing process did not affect the mixing times of control flours. Overall effects on bake mixing time of flour MC, S, and the interaction MC × S were significant at the 0.01 level. All data for 2-propanol and its azeotrope were excluded from statistical analyses because of the infinite mixing times for the 2-

TABLE III
Mixing Characteristics of Reconstituted, Solvent-Extracted Flours

	Mixing Time (min)[a]					
	Solvent Extracted From Flours With Original MC[b] (%)					
Extracting Solvent[c]	1.2	7.2	13.8	1.2	7.2	13.8
	Mixograph			Bake[d]		
None (control)	3 7/8	3 7/8	3 7/8	4.0	4.0	4.0
Hexane						
Pure	3 15/16	4.0	4 5/16	4 3/8	4 3/4	4 7/8
Azeotrope	5 7/8	5 7/8	6 1/2	7 3/16	6 3/4	7 9/16
Benzene						
Pure	4 1/2	4 7/8	8 3/4	4 3/4	5 3/4	11 3/4
Acetone						
Pure	4 1/2	4 1/2	5 5/8	5 1/2	4 3/4	6 7/16
Azeotropes	7 1/2	8.0	11 1/2	9 1/4	11 9/16	21 5/16
2-Propanol						
Pure	5 1/2	17 1/2	∞	7.0	29 7/8	2 (∞)[e]
Azeotrope	∞	∞	∞	2 (∞)[e]	2 (∞)[e]	2 (∞)[e]

[a]Averages of three replicates for bake mixing time; overall standard deviation, 0.47 ≃ 15/32 min.
[b]MC = moisture content.
[c]Azeotropes: aqueous binary solvent system with % composition given in Table I.
[d]With 3.0% shortening added.
[e]Mixed for 2 min to incorporate the ingredients.

propanol treated flour (13.8% flour moisture) and the azeotrope treated flours (1.2, 7.2, and 13.8% flour moistures). Fisher's LSD showed that differences in mixing times between two treatments were significant at the 0.05 level if they were larger than 1 min and at the 0.01 level if larger than 1 1/2 min. Only pure hexane treatment did not significantly affect the mixing times at all three flour moisture levels as compared with those for the controls. For the flours treated with hexane and with its azeotrope, MC of flours did not significantly affect mixing times. For the other solvent treatments, however, increases in flour MC increased significantly the mixing times of the reconstituted flours.

Figs. 1 and 2 show typical mixograms for treatments described in Table III.

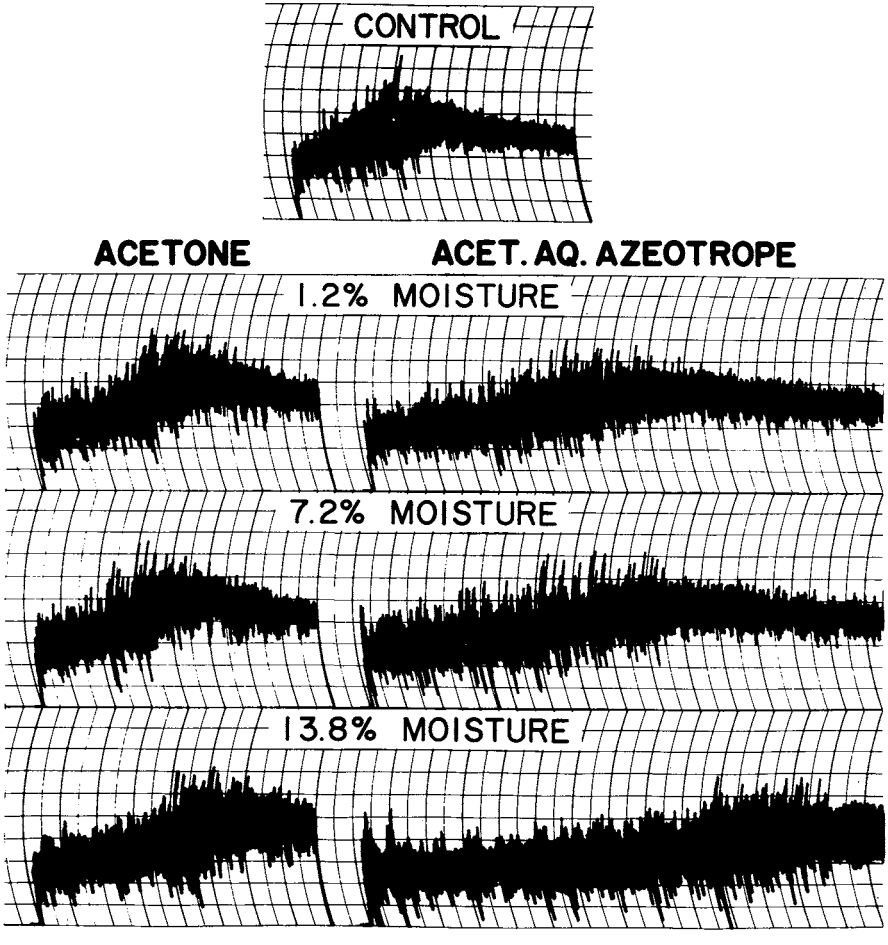

Fig. 1. Mixograms (10 g flour) of untreated control flour (RBS-74) and of solvent-treated and reconstituted flours. Lipids were extracted with acetone or acetone aqueous azeotrope from flours with 1.2, 7.2 or 13.8% moisture. Heavy-lined arcs are at 1-min intervals.

For flours treated with acetone and with its azeotrope, mixing times increased with increasing flour moistures; increased mixing times were significantly larger for the acetone azeotrope than for the pure solvent. Raising MC of flours from

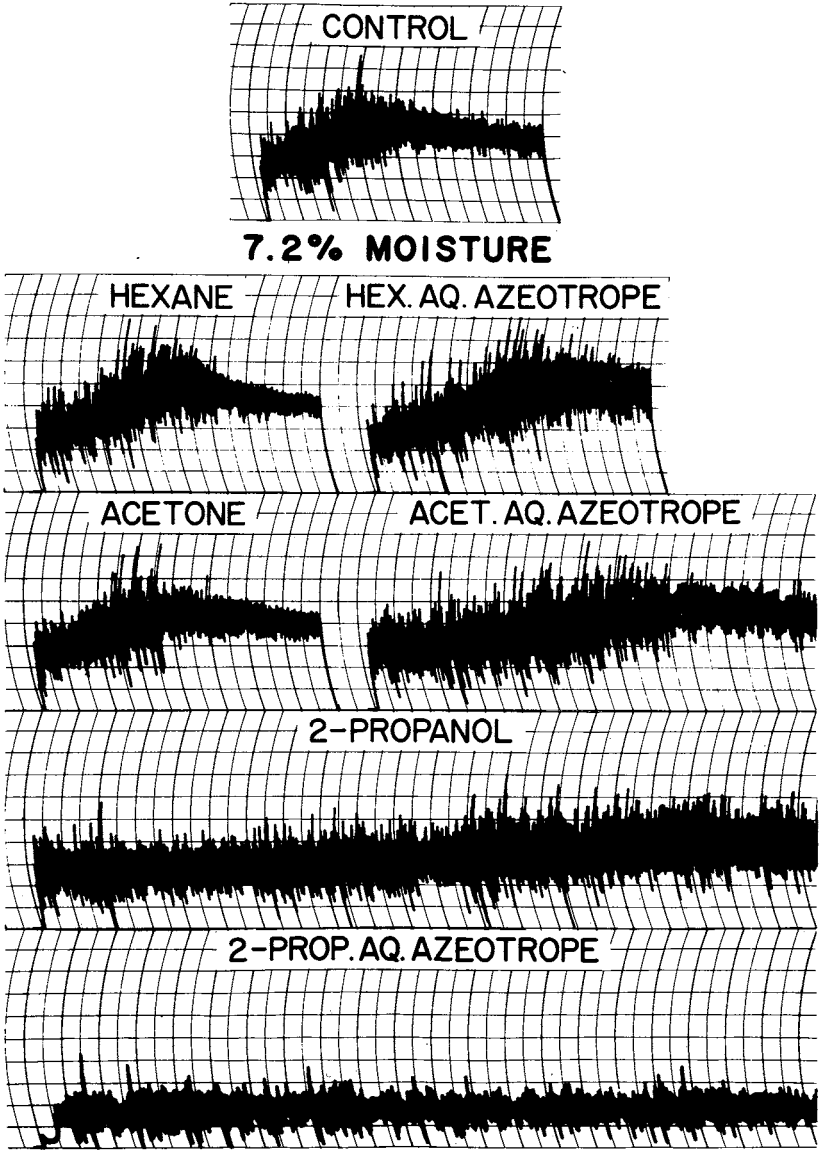

Fig. 2. Mixograms (10 g flour) of untreated control flour (RBS-74) and of solvent-treated and reconstituted flours. Lipids were extracted with hexane, acetone, 2-propanol, and their aqueous azeotropes from flour with 7.2% moisture. Heavy-lined arcs are at 1-min intervals.

1.2 to 13.8% prior to lipid extraction increased mixing times of reconstituted flours from 4 1/2 to 5 5/8 min for the acetone treatment and from 7 1/2 to 11 1/2 min for the acetone azeotrope treatment (Fig. 1). For flour with 7.2% MC prior to lipid extraction, treatments with pure solvents or their aqueous azeotropes significantly increased mixing times of the reconstituted flours (Fig. 2). Pure hexane or acetone treatment increased mixing time little, whereas pure 2-propanol treatment increased mixing time from 3 7/8 min, for the control flour, to 17 1/2 min. Adding water to solvents to form azeotropes drastically increased mixing times of the reconstituted flours from 4 to 5 7/8 min for the hexane series, 4 1/2 to 8 min for the acetone series, and 17 1/2 min to infinite mixing time for the 2-propanol series. Dough from flour treated with 2-propanol at 13.8% moisture, and doughs treated with the azeotrope at all three moisture levels were mixed for 2 min merely to incorporate the ingredients (Table III).

Loaf Volume and Crumb Grains

The lyophilization process affected loaf volumes of untreated control breads little when shortening was added and somewhat more when shortening was omitted (Table IV). ANOVA showed that flour MC, shortening (Sh), S, and all the interactions MC × Sh, MC × S, Sh × S, and MC × Sh × S had significant effects on loaf volume at the 0.01 level. Fisher's LSD test showed that the overall average loaf volume (with and without shortening) did not decrease significantly when flour MC was increased from 1.2 to 7.2% (average loaf volume decreased from 58.1 to 58.0 cc) but decreased significantly when the MC was increased from

TABLE IV
Loaf Volume of Bread Baked With 10 g Reconstituted, Solvent-Extracted Flours

	Loaf Volume (cc)[a]					
	Solvent Extracted From Flours With Original MC[b] (%)					
Extracting Solvent[c]	1.2	7.2	13.8	1.2	7.2	13.8
	No Shortening Added			3.0% Shortening Added		
None (control)	62.3	66.0	64.8	79.8	81.8	81.0
Hexane						
Pure	60.7	64.3	61.7	73.0	81.8	79.9
Azeotrope	45.9	51.0	45.0	57.8	66.7	63.2
Benzene						
Pure	61.0	62.2	52.8	69.5	71.5	64.5
Acetone						
Pure	63.2	67.0	57.8	75.5	75.3	73.8
Azeotrope	52.5	47.0	43.0	64.8	64.3	64.8
2-Propanol						
Pure	52.5	42.9	24.5	68.5	58.0	25.7
Azeotrope	21.5	20.5	20.5	21.7	20.5	20.7

[a]Averages of three replicates; overall standard deviation, 0.95 cc.
[b]MC = moisture content.
[c]Azeotropes: aqueous binary solvent system with % composition given in Table I.

7.2 to 13.8% (average loaf volume decreased from 58.0 to 52.7 cc). Differences in loaf volumes between two treatments were significant at the 0.05 level if larger than 1.78 cc and the 0.01 level if larger than 2.37 cc. For the untreated control flours or flours treated with hexane, its azeotrope, benzene, or acetone, loaf volumes were largest when the MC of the original flour was 7.2%. The trend disappeared for the breads baked with flours treated with acetone azeotrope or 2-propanol and its azeotrope; probably stronger solvent effects masked the

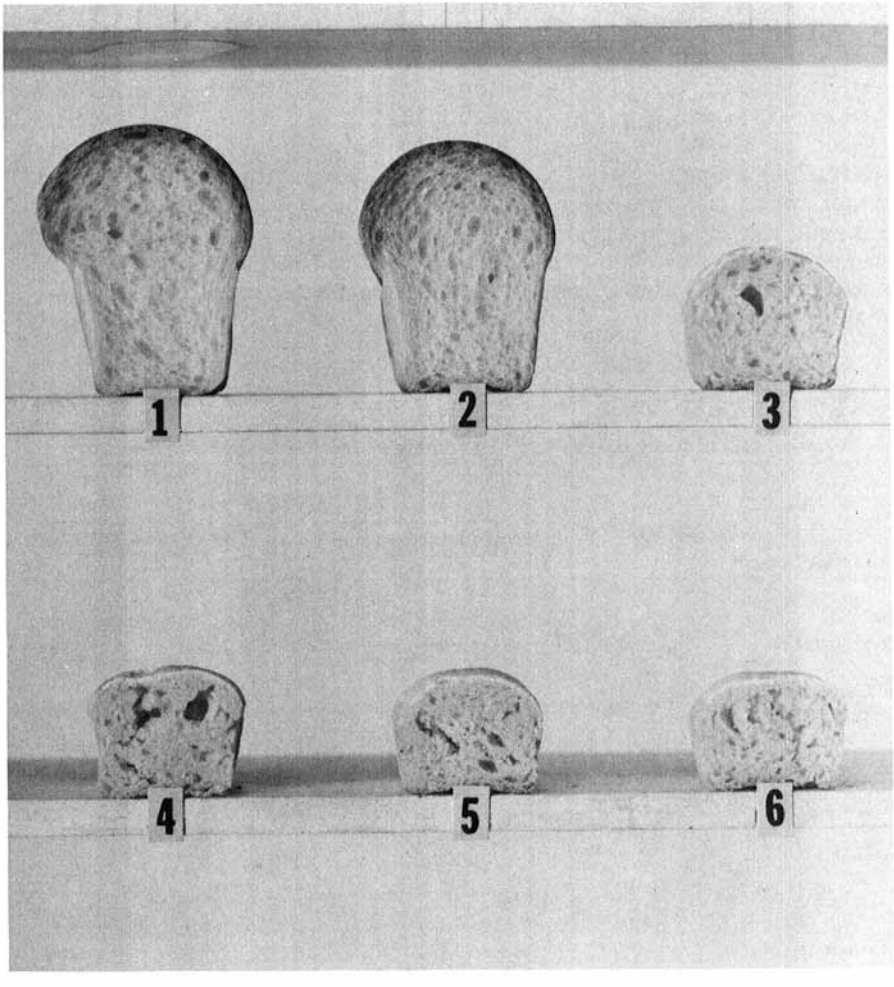

Fig. 3. Bread (10 g reconstituted flours baked with 3% shortening) made from flours extracted with 2-propanol (top row) and with 2-propanol aqueous azeotrope (bottom row) and then reconstituted. Breads 1 and 4, flour with 1.2% moisture; 2 and 5, flour with 7.2% moisture; 3 and 6, flour with 13.8% moisture.

particular effects of 7.2% moisture.

Generally, solvent treatment adversely affected baking characteristics of reconstituted flours. The adverse effects were more pronounced when extractants contained water (*i.e.*, in aqueous azeotropes) or when flour samples contained 13.8% moisture rather than 1.2% prior to lipid extraction with solvents other than hexane or its azeotrope. The most notable effect of flour moisture was to decrease loaf volumes of breads made from 2-propanol treated flours (Table IV, Fig. 3). Propanol treatment significantly impaired bread volume and crumb grain (Table V). Breads 1, 2, and 3 in the top row of Fig. 3 were all made from flours treated by 2-propanol. They differed only in initial MC of flours prior to lipid extraction. As flour moisture increased from 1.2 to 13.8%, loaf volume of bread decreased from 68.5 to 25.7 cc and crumb grain became extremely poor. When water was added to propanol, however, flour moisture had no additional deleterious effects (breads in the bottow row of Fig. 3). The propanol azeotrope treatment permanently damaged functionality.

When the MC of flour prior to lipid extraction was 7.2%, solvent effects on breadmaking properties were as shown in Fig. 4. The hexane treatment (bread 1) did not impair loaf volume or crumb grain (Tables IV and V, Fig. 4); acetone treatment (bread 2) reduced loaf volume by 6.5 cc but crumb grain was satisfactory; and 2-propanol treatment (bread 3) had a significant adverse effect on loaf volume and crumb grain. Water in the extractant reduced loaf volumes for all three solvent systems: from 81.8 to 66.7 cc for the hexane system (breads 1

TABLE V
Crumb Grain of Bread Baked With 10 g Reconstituted, Solvent-Extracted Flours

	Crumb Grain[a]					
	Solvent Extracted From Flours With Original MC[b] (%)					
Extracting Solvent[c]	1.2	7.2	13.8	1.2	7.2	13.8
	No Shortening Added			3.0% Shortening Added		
None (control)	Q-U	Q-U	Q-U	S	S	S
Hexane						
Pure	U	U	U	S	S	S
Azeotrope	U^2	U^2	U^3	Q-U	Q-S	Q-U
Benzene						
Pure	U	U	U^2	Q-S	Q-S	Q
Acetone						
Pure	Q-U	Q-U	U	S	S	S-Q
Azeotrope	U^2	U^2	U^3	Q	Q	Q-U
2-Propanol						
Pure	U^2	U^3	U^7	Q	Q-U	U^6
Azeotrope	U^8	U^8	U^8	U^7	U^7	U^7

[a]S = satisfactory; Q = questionable; U = unsatisfactory (the higher the number, the poorer the crumb grain).
[b]MC = moisture content.
[c]Azeotrope: aqueous binary solvent system with % composition given in Table I.

and 4), from 75.3 to 64.3 cc for the acetone system (breads 2 and 5), and from 58.0 to 20.5 cc for the 2-propanol system. Poor crumb grain accompanied decreases in loaf volumes. Adverse effects varied with the solvents, both pure and as azeotropes. For flour at any moisture level, the adverse effects of solvents generally increased with increased solubility parameter: the square root of cohesive energy density (21,22) of the solvent (18).

COMMENTS AND CONCLUSIONS

The biochemical phenomena that take place during baking are complex, even in investigations of untreated flours. Complexities increase with flour treatment, especially if the treatment involves combinations and interactions of organic solvents and water. Water is the most important solvent in dough and bread systems, yet relatively little attention has been paid to it. Increases in lipid

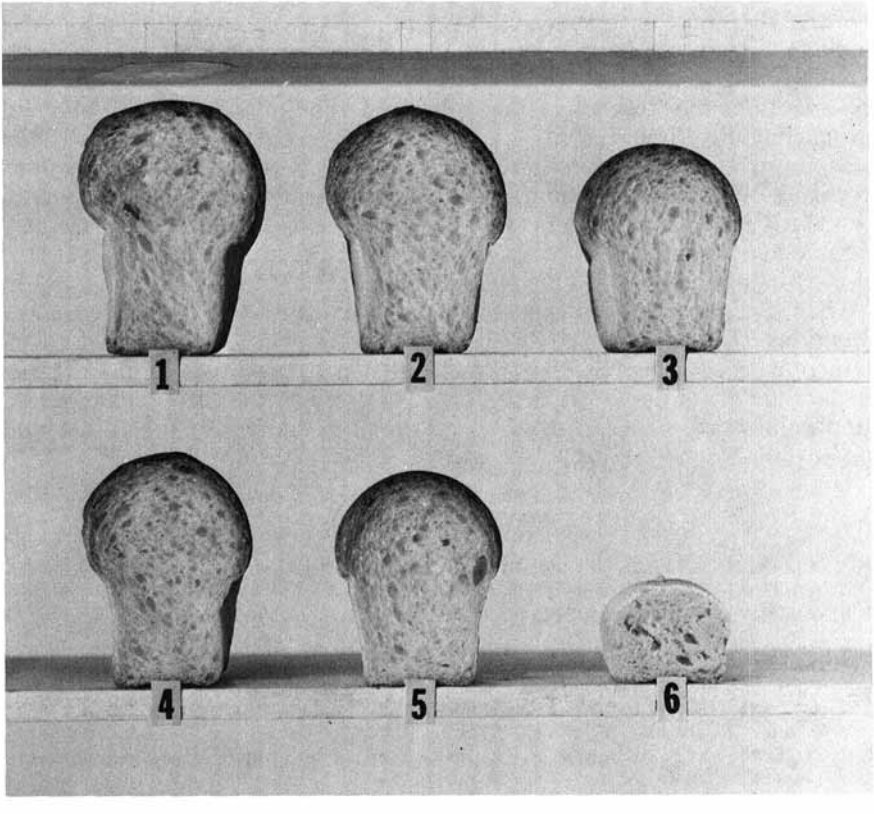

Fig. 4. Bread (10 g reconstituted flours baked with 3% shortening) made from flour (7.2% moisture) extracted with pure solvents (top row) and with their aqueous azeotropes (bottom row) and then reconstituted. Breads 1 and 4, hexane; 2 and 5, acetone; 3 and 6, 2-propanol.

extractability with increases in the MC of wheat flour from 1.2 to 13.8% or by use of aqueous binary azeotropes may have limited practical applications. Our work demonstrates the profound effects of relatively low moisture levels either in the flour or in solvent systems on the rheological and breadmaking properties of the reconstituted flours. Adverse effects on functionalities of the reconstituted flours were smallest for hexane and largest for 2-propanol, and increased with increasing MC of the flour, especially for the 2-propanol treatment.

Lyophilization of control flour, with subsequent restoration to the normal moisture range (13 to 14%) prior to mixograph or bake studies, did not significantly affect dough mixing time and consistency. Lyophilization somewhat affected loaf volume, however, without noticeably changing crumb grain. Lyophilizing flours to 1.2% MC removes water that is tightly bound as a monolayer (10,11) and disturbs the binding structure of flour components. Although we rehydrated the lyophilized flours to normal moisture levels, the binding capacity of the flour components was apparently not completely restored. This might be attributed, at least in part, to the lower loaf volume of breads baked from flour lyophilized to a 1.2% MC.

When flours were treated with solvents, removing flour lipids and then reconstituting lipids affected interaction of flour components, depending on the quantity and nature of lipids involved. Many combined effects were present—flour moisture effects on lipid extractability and on loaf volume of untreated control flour, effects on extraction of combining solvent and water, and the interaction between those effects on lipid extractability and functional properties of reconstituted flours. The loaf volume and crumb grain of a bread are the net result of many processes and interactions. Some of those processes and interactions may have, at times, opposite effects on the net result. Certain effects could be masked if others were dominant.

When functionalities of flour lipids are to be demonstrated, lipid extraction conditions become critical. Careful consideration should be given to the extraction apparatus (18,20), extractant, extraction time (20), extraction temperature (18,20), and probably, the nature of the flour. The MC of a flour sample alone may significantly affect the amounts and types of extracted lipids and govern the end use properties of the reconstituted flours.

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