Functional (Breadmaking) and Biochemical Properties of Wheat Flour Components. V. Role of Total Extractable Lipids[1]

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ABSTRACT

Water-saturated butanol, used to extract bound lipids, formed a complex with starch and inhibited gas production. A reconstituted flour almost completely free of lipid (0.08%) was prepared by first extracting free lipids from the flour with petroleum ether, then washing out the gluten, solubilizing the gluten in 0.005N lactic acid, and centrifuging at 100,000 X g for 5 hr.; the lyophilized centrifugate was then extracted

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with water-saturated butanol. The reconstituted flour, after premixing to restore certain rheological properties, was used to study the comparative roles of free polar, free nonpolar, and bound polar lipids. Small amounts of free polar or bound polar lipids were detrimental to loaf volume, unless accompanied by nonpolar lipids or bound lipids in their native state. High amounts of bound polar or free polar lipids restored loaf volume. However, larger quantities of bound polar than free polar were required. Bound polar lipids in their native state performed differently in baking than when they were extracted and reconstituted.

The lipids in flour have been divided into two groups, "free" and "bound" (1). The free (approximately 60% of the total lipids) have been defined as those extractable by petroleum ether (Skelly F) or similar nonpolar solvents. The remaining bound lipids (practically all polar) have been extracted by a more polar solvent such as water-saturated butanol (WSB). When flour was mixed into a dough, all the free polar lipids and about 50% of the free nonpolar lipids became bound, and were no longer soluble in petroleum ether (2).

Free lipids have been fractionated further by silicic acid column chromatography into polar (25% of total free) and nonpolar (remaining 75%). The role of free lipids and their fractions has been studied extensively (3,4). Free polar lipids increased loaf volume substantially; the increase was smaller when bound polar lipids were added instead of free polar lipids. Nonpolar lipids decreased loaf volume and impaired crumb grain of bread from petroleum ether-extracted flours; the deleterious effects were counteracted by polar lipids. Preliminary work has shown that fractions rich in galactosyl glycerides are most effective in restoring loaf volume.

Attempts to extract bound lipids (5,6,7) without irreversibly damaging rheological properties, fermentation, and bread quality have been essentially unsuccessful.

The present study was undertaken to determine the flour fraction or fractions that were damaged by WSB; to investigate techniques of extracting all the lipids (or nearly all) without damaging the flour; and to compare the role of the free polar, free nonpolar, and bound polar lipids in baking when reconstituted with a completely defatted flour.

MATERIALS AND METHODS

Flour

The flour, designated as Regional Bake Standard (RBS), was milled from a composite of several wheat varieties that were harvested at many locations throughout the Southern, Central, and Northern Great Plains in 1966. The RBS flour had a protein content of 12.7%, good loaf-volume potential, and medium mixing time.

Analytical Procedures

Protein and moisture were determined as described in AACC Approved Methods (8). Gassing powers were determined on 10 g. flour at 30°C., with gage-type pressuremeters, and with the same formula employed in baking except that shortening was omitted and water-absorption was increased to 100%. The baking procedure described by Finney and Barmore (9,10,11) and Finney (12) was

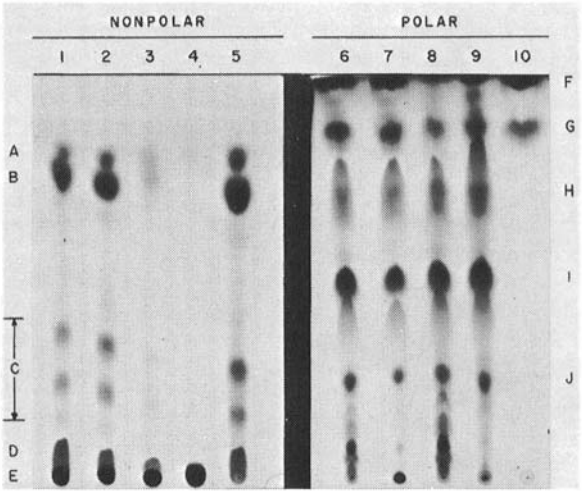


Fig. 1. Thin-layer chromatograms of lipid fractions. Patterns 1 and 6, total extractable lipids; 2 and 7, total free lipids; 3 and 8, free polar lipids; 4 and 9, bound polar lipids; 5 and 10, nonpolar lipids. Spots are tentatively identified as follows: A, hydrocarbons and steryl esters; B, triglycerides; C, diglycerides; D, free fatty acids; E, unresolved polar lipids; F, unresolved nonpolar lipids; G, monogalactosyl diglycerides (except in pattern 10, this spot did not react with Dragendorff's reagent); H, phosphatidyl ethanolamine; I, digalactosyl diglycerides; J, phosphatidyl choline.

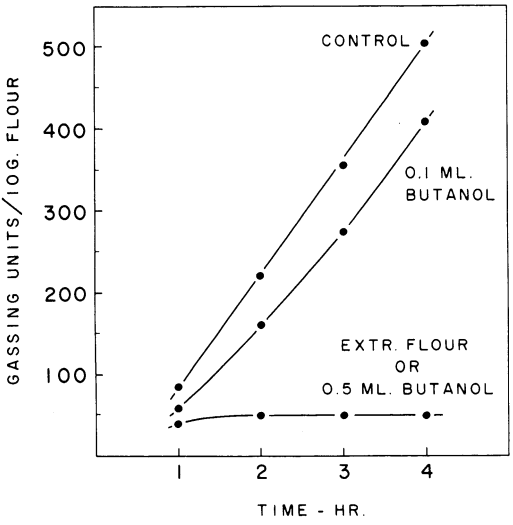
adapted by Shogren et al. (13) for 10 g. of flour. The standard deviation for the average of duplicate loaf volumes was 1.75 cc.

Preparation of Lipid Fractions

All flour lipid preparations used in this study were from RBS flour. All extracts were evaporated under vacuum below 40°C. Lipids that were extracted with water-saturated n-butanol were purified by dissolving in petroleum ether. Total free lipids (TF) were extracted by petroleum ether (b.p. 35° to 60°C.) in a Soxhlet apparatus. Total lipids (T) were extracted in a Stein mill with WSB as described previously (14). Nonpolar lipids (NP) and polar free lipids (PF) were obtained by fractionation of the total free lipids on silicic acid columns as described by Daftary and Pomeranz (1). Polar bound lipids (PB) were extracted with WSB from flour that had been extracted twice with petroleum ether. The lipid fractions were characterized by thin-layer chromatography (Fig. 1). Lipids were reconstituted with the other flour components by means of mixing in a mortar.

Thin-Layer Chromatography

Thin-layer chromatography was performed on 100 γ of lipids. One plate was developed with chloroform to determine nonpolar lipids. A second plate was developed with chloroform-methanol-water (65:35:4) to determine polar lipids. Plates were sprayed with a saturated solution of $K_2Cr_2O_7$ in 70% (v./v.) of aqueous sulfuric acid and charred at 150°C. for 30 min. The plates were photographed under ultraviolet light.



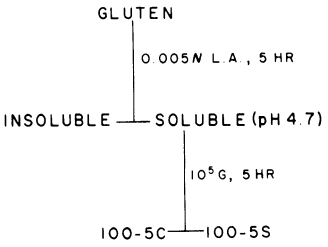


Fig. 2 (left). Effect on gas production of extracting RBS flour with WSB, and of adding 0.1 and 0.5 ml. of WSB to unextracted flour.

Fig. 3 (right). Scheme for fractionating gluten protein.

RESULTS AND DISCUSSION

Flours extracted with WSB invariably retained a strong butanol odor, particularly when wetted. Doughs from those flours appeared "dead," and produced negligible gas during fermentation (Fig. 2). Additional gassing-power determinations, carried out on the control flour to which small amounts of WSB were added, attested to impaired fermentation. The residual butanol in the extracted flour could not be removed by vacuum distillation or by lyophilization of the frozen flour-water slurry. Since butanol has been shown to complex with starch (15), and since most of the bound lipid was found in the gluten fraction, the flour was fractionated into gluten and a mixture of starch and water-solubles. Thereafter, the lyophilized gluten was extracted with WSB. The extracted gluten was cut into small pieces, placed on a glass plate, and allowed to dry in air until the odor of butanol disappeared. The reconstituted flour that contained the extracted gluten and unextracted starch-plus-water-solubles was found to have normal gas production. Thus, the detrimental effect of the WSB on gas production was eliminated by not extracting the starch with butanol.

The ground extracted gluten was reconstituted with unextracted starch and water-solubles, supplemented with lipids, and baked into bread (Table I). Mixing time (46 min.) was extremely long, the dough was bucky, and loaf volume was low. Thus, it appears that WSB had a detrimental effect on the gluten proteins.

Gluten was fractionated (Fig. 3) by being stirred in 0.005N lactic acid and centrifuged at 1,000 × g to remove insoluble components (trapped mixture of starch and about 5% of the total flour protein). The gluten soluble in 0.005N lactic

TABLE I. BAKING RESULTS FOR RECONSTITUTED RBS FLOURS, CERTAIN FRACTIONS OF WHICH WERE EXTRACTED WITH WATER-SATURATED BUTANOL (WSB)

Original or Reconstituted Flour	Mixing Time	Baking Absorption	$KBrO_3$ Requirement	Loaf Volume
	min.	%	p.p.m.	cc.
RBS Flour	3 3/4	65.0	30	83
RBS Gluten	3	65.0	30	82
RBS 100-5S 85, 100-5C 15	1 1/8	62.0	30	84
RBS Flour (extr. WSB) + 1.5% TL[a]	12 1/2	67.0	30	42
Gluten (extr. WSB) + 1.5% TL	46	73.0	30	70
100-5S 85, 100-5C (extr. WSB) 15	1	62.0	30	56
100-5S 85, 100-5C (extr. WSB) 15 + 1.5% TL	1 3/8	63.0	30	68
Premix[b] + 1.5% TL	2 3/4	63.0	30	82

[a] TL is an abbreviation for total lipids.

[b] [100-5S / 100-5C (extr. WSB) 25/75 + 100-5S] 85/15

acid (pH 4.7-soluble gluten) was centrifuged at 100,000 X g for 5 hr. The neutralized and lyophilized centrifugate (100-5C fraction) contained 21.0% total lipid, whereas the precipitated and lyophilized supernatant (100-5S fraction) contained only 1.25% total lipid. The detailed fractionating procedure has been described previously (16). Consequently, only the 100-5C fraction was extracted with WSB, air-dried, reconstituted with the 100-5S fraction and starch-plus-water-solubles, and baked into bread (Table I). A duplicate reconstituted flour was supplemented with total lipids. Although the loaf with flour lipids was 12 cc. higher in volume than the one without lipids (56 cc.), both were considerably below the control and had a mottled appearance, and their doughs were lumpy. Therefore, reconstitutions were carried out in a premix similar to the previously described technique used with the fraction insoluble in 70% ethyl alcohol (14). The 100-5C (extracted with WSB) and 100-5S (unextracted) were reconstituted at a ratio of 25:75 with starch and water-solubles, and mixed with water to optimum consistency. The resultant dough was frozen, lyophilized, and ground; and part of it was reconstituted with the 100-5S fraction to give an 85:15 ratio of 100-5S:100-5C. The final reconstitution with starch-plus-water-solubles and lipid was followed by a second mixing and baking into bread. A loaf fully comparable to the control was obtained (82 cc.; Table I). Thus, glutenin proteins (100-5C) extracted with WSB were rendered functional.

It appears that WSB can damage flour in the following ways: (a) it complexes with starch and stops gas production, and (b) it denatures the gliadin fraction of gluten protein.

TABLE II. EFFECT OF TOTAL (T), TOTAL FREE (TF), FREE POLAR (FP), NONPOLAR (NP), AND BOUND POLAR (BP) LIPIDS ON BREAD BAKED (WITH 3% SHORTENING) FROM RECONSTITUTED, PETROLEUM ETHER-DEFATTED, AND ALMOST COMPLETELY DEFATTED RBS FLOURS

Original or Reconstituted Flour	Mixing Time[a]	Loaf Volume
	min.	cc.
Flour (unextr.)	3 3/4	83
Petroleum Ether-Defatted		
Flour	4	70
Flour + 0.8% TF	4	83
Flour + 0.2% FP	4	82
Flour + 0.3% FP	4	84
Flour + 0.2% BP	4	71
Flour + 0.3% BP	4	72
Flour + 0.6% NP	4	70
Almost Completely Defatted		
Premix[b]	3	71
Premix + 1.5% T	2 5/8	81
Premix + 0.8% TF	2 3/8	81
Premix + 0.6% NP	2 1/2	70
Premix + 0.3% FP	2 3/4	65
Premix + 0.3% BP	2 3/4	64
Premix + 0.6% FP	2 3/4	80
Premix + 0.6% BP	2 1/2	71
Premix + 0.8% FP	2 5/8	81
Premix + 0.8% BP	2 3/8	80

[a]Baking absorption of the unextracted and petroleum ether-defatted flours was 65%, and of the almost completely defatted flours was 64%. Potassium bromate requirement was 30 p.p.m. for all flours.

[b] $\left[\frac{100\text{-}5S}{100\text{-}5C\ (\text{extr. WSB})}\ \frac{25}{75} + 100\text{-}5S\right]\frac{85}{15}$

The foregoing experiments indicate the feasibility of studying the role of the bound and other lipid fractions in baking by first extracting the total free lipids from flour with petroleum ether, and then extracting with WSB practically all the bound lipids from the 100-5C fraction. Thus, RBS flour was twice extracted with petroleum ether to remove all the free lipids. The ether-extracted flour was fractionated into crude gluten and a mixture of starch and water-solubles. The crude gluten was fractionated into 100-5S and 100-5C fractions which were lyophilized. The 100-5C fraction, after being extracted with WSB, was reconstituted with the 100-5S fraction, first at the 25:75 and then at the 85:15 ratio, and with starch and water-solubles by the premix technique previously described. The reconstituted flour contained only 0.08% lipid (principally polar lipids from starch, and extractable in WSB).

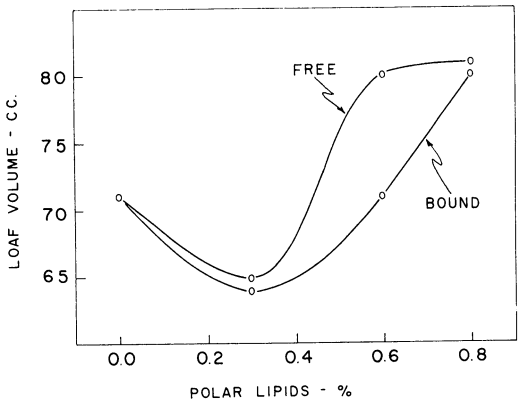


Fig. 4. Effect of free polar and bound polar lipids on bread baked (with 3% shortening) from reconstituted, almost completely defatted RBS flours.

The almost completely defatted, reconstituted flour (Premix in Table II) was mixed and baked into bread that had a loaf volume (71 cc.) comparable to that (70 cc.) of flour extracted with petroleum ether. Therefore, the 0.6% bound polar lipids (BP) in the petroleum ether-extracted flour did not appear to have a function in breadmaking. This was substantiated by baking the reconstituted premix with 0.8% total free lipids (TF); the resultant loaf volume (81 cc.) was comparable to that of the reconstituted premix with 1.5% total lipids (T).

However, when the petroleum ether-extracted flour (containing 0.6% bound polar lipids) was baked with 0.3% free polar lipids (FP), a loaf volume comparable to that of the control flour was obtained. The reconstituted premix, when baked with 0.3% free polar lipids, had a lower loaf volume (65 cc.) than that of the reconstituted premix baked without lipids. Thus, the 0.6% bound polar lipids apparently were functional when in their native state. When higher amounts of free polar lipids (0.6 and 0.8%) were reconstituted with the premix, loaf volumes essentially comparable to that of the control were obtained. Adding bound polar lipids to the reconstituted premix gave essentially the same volumes as those for the free polar lipids at the 0.3 and 0.8% levels, but much lower at the 0.6% level (Fig. 4). Adding 0.2% bound polar lipids to the petroleum ether-extracted flour gave loaf volume no higher than that of the extracted flour.

In completely defatted flours, small amounts of polar lipids, either free or bound, apparently are detrimental to loaf volume. However, small amounts of free polar lipids are beneficial when accompanied by nonpolar lipids (as in TF) or by polar lipids that are bound in their native state. Nonpolar lipids (at the 0.6% level), either by themselves or with bound polar lipids in their native state, have no effect on loaf volume of bread baked with shortening. Although a volume comparable to that of the control flour was obtained when 0.8% bound polar lipids were added to the reconstituted premix, adding 0.2% bound polar lipids to petroleum ether-extracted flour (containing 0.6% bound polar lipids) produced a volume of only 71 cc. Thus, bound polar lipids in their native state performed differently in baking than when they were extracted and reconstituted.

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