Interaction of Lipids with Proteins and Carbohydrates in Breadmaking[1]

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ABSTRACT

After reviewing the evidence for interaction in breadmaking of lipids with proteins and carbohydrates, theories on the shortening response are discussed. Recent studies show that the response cannot be explained entirely on the basis of physical phenomena. Both overall breadmaking quality (presumably related to gluten and its components) and the presence of wheat flour lipids are important in the shortening response.

INTRODUCTION

While the significance in breadmaking of lipid interaction with proteins and carbohydrates is widely accepted, it has been studied little for several reasons (1). They include the complexity of the system, the limited knowledge of the interacting components, and the paucity of methods to study such interactions. In recent years there has been renewed interest in studying the interaction and its significance. This review summarizes briefly previous work from our laboratories, some recent studies conducted by investigators in several laboratories, and some new work from the U.S. Grain Marketing Research Center.

THE INTERACTING COMPONENTS

Wheat flour contains about 70% starch, 12% proteins, 2% lipids, 2% pentosans, and 12% moisture.

Total wheat flour lipids contain about equal amounts of nonpolar and polar components. Wheat flour lipid composition is shown schematically in Figure 1 adapted from data reported by MacMurray and Morrison (2). Triglycerides (TG) are a major component of nonpolar lipids, digalactosyldiglycerides (DGDG) of glycolipids; and lysophosphatidylcholines (LPC) and phosphatidylcholines (PC) are major components of phospholipids (Fig. 1). Differences in solubility provide a convenient and useful means of separating wheat flour lipids into major categories: free and bound (Fig. 2). Free lipids can be extracted with nonpolar solvents such as ether or petroleum ether (PE). For extraction of bound (mainly to protein) lipids, polar solvents such as water-saturated butanol (WSB), or a mixture of chloroform-methanol-water are required. Lipids extracted by PE are arbitrarily defined as free and those by WSB, following PE extraction, as bound lipids.

The free lipids can be fractionated according to their elution from a silicic acid column. About 70% free lipids can be eluted with chloroform, and they form what is arbitrarily called the "nonpolar" fraction containing TG as a major component. The residual 30% free lipids can be eluted from the column with a more polar solvent, such as methanol, and comprise a mixture of free polar lipids. Among the free polar lipids, about two thirds are glycolipids containing DGDG as a major component, and one third are phospholipids with PC as a major component (3).

About 0.6-1.0% bound lipids can be extracted from flour with WSB after PE extraction. Bound lipids contain about 30% nonpolar and 70% polar lipids. Bound polar lipids are rich in phospholipids with LPC as a major phospholipid component (3). As glycolipids are important in breadmaking, it is important to distinguish clearly between two classes of polar lipids. Although the free polar lipids are richer in glycolipids than the bound polar lipids are, the actual amounts of both glycolipids and phospholipids are higher in bound polar than in free polar lipids (Fig. 2).

Recently the lipids of wheat starch have received new attention. Morrison et al. (4) divided flour lipids into those inside starch granules, which are true starch lipids, and all other lipids outside the starch granules, which are called non-starch lipids. Even polar solvents containing alcohol-water mixtures such as WSB extract mainly non-starch lipids at room temperature. Starch lipids can only be extracted efficiently with hot n-butanol-water (65:35) or hot WSB (4-7). Starch lipids are almost exclusively monoacyl lipids, and 86 to 94% of the total starch lipids are lysophospholipids, LPC as the chief constituent.

Gluten proteins comprise about 80% of the total flour proteins (8). Gluten proteins can be separated into two, approximately equal, fractions of gliadin (a mixture of prolamines soluble in 70% alcohol) and glutenin (a mixture of glutelins soluble in dilute acids and alkali). The starch content of wheat flour is, in general, inversely related to protein content (9). In flours below 80% extraction, the starch content ranges from about 65 to 70% (on an as-is

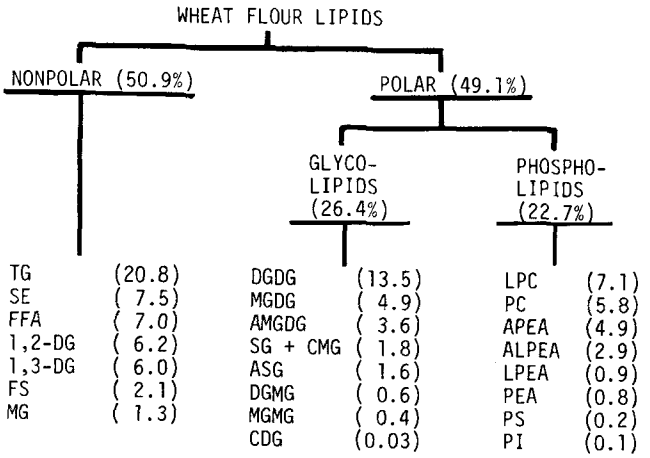

FIG. 1. Composition of total wheat flour lipids (extracted with water-saturated butanol). The abbreviations are: TG=triglycerides; SE=steryl esters; FFA=free fatty acids; 1,2-DG=1,2-diglycerides; 1,3-DG=1,3-diglycerides; FS=free sterols; MG=monoglycerides; DGDG=digalactosyldiglycerides; MGDG=monogalactosyldiglycerides; AMGDG=-0-acyl monogalactosyldiglycerides; SG=steryl glucoside; CMG=ceramide monoglycerides; ASG=6-0-acyl steryl glucosides; DGMG=digalactosylmonoglycerides; MGMG=monogalactosylmonoglycerides; CDG=ceramide diglycosides; LPC=lysophosphatidylcholines; PC=phoshatidylcholines; APEA=N-acylphosphatidylethanolamines; ALPEA=N-acyl lysophosphatidylethanolamines; LPEA=lysophosphatidylethanolamines; PEA=phosphatidylethanolamines; PS=phosphatidylserines; PI=phosphatidylinositols. (Adapted from Ref. 2).

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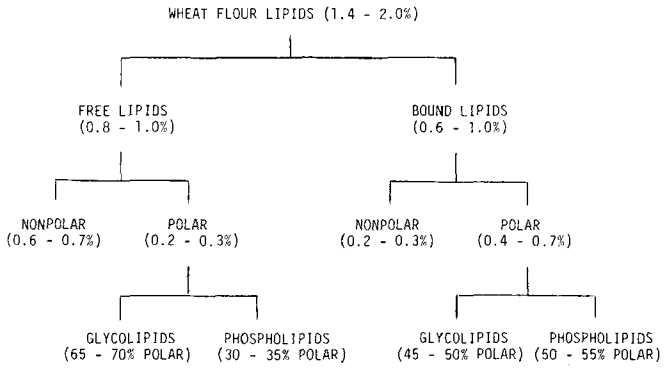

FIG. 2. Free and bound lipids in wheat flour.

moisture basis). The proportion of linear (amylose) to branched (amylopectin) fractions in the starch is ca. 1:3.

EVIDENCE OF INTERACTION

In washing out gluten, or in doughmaking, or in baking, one third to two thirds of the nonpolar and practically all polar components are bound and inextractable with PE (3,10). Extractability of lipids depends on several factors that include particle size, composition and age of wheat flour (11), dough composition (amounts and types of lipids) (12), water content in extractants and in flour samples (13-15), presence of shortening or surfactants (16,17), work input and atmosphere in dough mixing (13,18), and stage in bread production (19,20).

Available information indicates that in dough, interaction of glycolipids is mainly with gluten rather than with the soluble wheat flour proteins. This is of significance as gluten is the skeleton or framework of wheat flour dough and is responsible for gas retention that is required in the production of light, yeast-leavened products. In the baked bread, much of the interaction is with the starch. This is of significance as the starch governs, to a large extent, freshness retention of the baked bread (1).

Methods of study, bonds, and mechanisms of interaction between lipids and proteins and starch are summarized in Table I (from Ref. 21), and some of the proposed models are shown in Figure 3. Almost a quarter century ago, Hess (22) proposed, on the basis of X-ray electron microscope and optical measurements, a structural relationship of protein, lipids, and starch in wheat flour, in which wedge protein deposits are surrounded by a lipid layer, beyond which lie adhesive protein layers and corresponding starch granules. Hess and Mahl (23), proposed a model (Fig. 3a) in which the adhesive protein is bound to starch through a lecithin layer.

In 1957 Traub et al. (24) reported that a 46-Å spacing observed in X-ray studies of wheat and flour was due to phospholipids associated with protein fibers in the form of bimolecular leaflets. Then Grosskreutz (25) studied the structure of wheat gluten by electron microscopy and X-ray techniques and proposed a lipoprotein model involving a bimolecular lipid layer structure (the Danielli and Dawson-type membrane) as shown in Figure 3 (b). Grosskreutz showed that the proteins in moist gluten consist of folded, polypeptide chains in the α-helix configuration, arranged into flat platelets of the order of 70 Å thick. Extraction of the phospholipids did not affect the basic platelets but seriously impaired their ability to bond into sheets capable of sustaining large plastic deformation (25). X-ray evidence of the phospholipid structure in gluten favored the assumption that there exist well oriented bimolecular leaflets of the type found in myelin, that lipoprotein occupies about 2 to 5% of the elastic gluten structure, and that protein chains are bound to the outer edge of a phospholipid bimolecular leaflet array, probably by saltlike linkages between acidic groups of the phospholipid and the basic protein groups.

Hoseney et al. (26) found that free polar lipids (principally glycolipids) are bound to the gliadin proteins by hydrophilic bonds and to the glutenin proteins by hydrophobic bonds as it is shown in Figure 3 (c). In unfractionated gluten, the lipid apparently is bound to both protein groups at the same time. The simultaneous binding of polar lipids to gliadin and glutenin may contribute structurally to gas-retaining complexes in gluten.

Wehrli and Pomeranz (27) supported the model proposed by Hoseney et al. (26) by studying the interaction of glycolipids with wheat flour macromolecules. They investigated by infrared and nuclear magnetic resonance (NMR) spectroscopy complexes between galactolipids and raw starch, gelatinized starch, gliadin, and glutenin. Infrared spectroscopy indicated hydrogen bonds between glycolipids and gelatinized starch or gluten components, and Van der Waals bonds between glycolipids and gluten components. The NMR spectra showed an inhibition of the methylene signal of glycolipids (at 8.7 τ) by glutenin, indicating hydrophobic bonding (Table I).

Wehrli and Pomeranz (28) studied further interactions that take place in dough and bread containing both starch and gluten proteins. For that purpose, tritium-labeled galactosyldidecanoylglycerol was synthesized by a new procedure (29). Sections prepared from dough and bread containing the labeled galactolipids were studied by autoradiography. In the dough, the galactolipid was distributed in the gluten and, to a limited extent, in the starch; in the bread most of the galactolipid was in gelatinized (by oven heat) starch granules and formed a complex which seemed to be

TABLE I

Bonds in Glycolipid and Wheat Flour Macromolecule Complexes

Method of study	Type of bond between glycolipid and:		
	Starch	Gliadin	Glutenin
Solvent extraction of gluten proteins	—	Hydrogen	Hydrophobic
Lipid binding in starch dough	Hydrogen	—	—
Infrared	Hydrogen	Van der Waals, hydrogen	Van der Waals, hydrogen
Nuclear magnetic resonance	Hydrogen, some induced dipole interaction	—	Hydrophobic and hydrogen
Autoradiography	Strong interaction in bread	—	Interaction in dough
Baking test	Hydrophobic and hydrogen bonds are essential for improvement in breadmaking.		

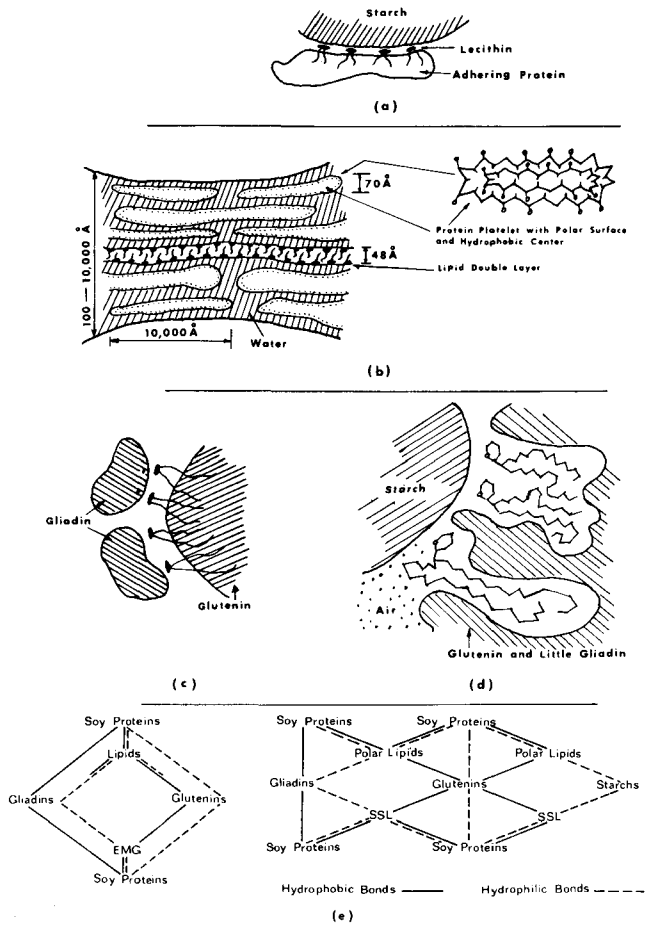

FIG. 3. Proposed models of the complex formed in breadmaking. (a) Starch-lipid-adhesive protein complex in flour by Hess and Mahl (23). (b) Lipoprotein model by Grosskreutz (25). (c) Gliadin-glycolipid-glutenin complex by Hoseney et al. (26). (d) Starch-glycolipid-gluten complex by Wehrli (21). (e) Models of surfactants (EMG: ethoxylated monoglycerides and SSL: sodium stearoyl-2-lactylate) and lipid binding to wheat- and soy-flour proteins by Chung (36).

responsible for the improved retention of freshness in bread baked with glycolipids. Wehrli (21) proposed that the mechanism of the effect of glycolipids on loaf volume involved improving gas retention by sealing the gas cells; presumably by complexing between the swelling starch and the coagulating proteins as shown in Figure 3 (d).

Chung and Tsen studied by solvent extraction, interactions between wheat flour lipids and proteins in relation to other flour constituents during dough mixing (3,30) and the effects of surfactants on lipid binding to various fractions in dough (30-33) and bread (34) baked with or without soy flour. Surfactants competed with native flour lipids on the binding sites of wheat flour dough constituents and suppressed lipid binding. The main reactive sites were in acid-soluble protein fractions for nonionic ethoxylated monoglycerides (EMG), and in starch-lipid-protein fractions (that were insoluble in 0.05N acetic acid) for anionic sodium stearoyl-2-lactylate (SSL). When soy flour was added to wheat flour, soy protein suppressed the interaction between flour lipids and surfactants by supplying sufficient binding sites for both flour lipids and surfactants, so that a new association occurred between lipids or the added surfactants and soy flour proteins. In wheat flour dough containing both a surfactant and soy flour, multiple interactions took place forming two major protein complexes; i.e. glutenin-soy protein-gliadin complex and glutenin-surfactant-gliadin complex (35). Therefore, the major role of nonionic EMG was to interact principally with proteins (along with flour lipids) to form a stable "Protein Complex." The role of anionic SSL was to complex strongly with glutenins and starch (along with the flour polar lipids serving as cross-linking agents) and to interact between glutenins and gliadins to form the stable aggregates of "Protein Complex-Starch Complex." Both surfactants could accommodate soy proteins in a gluten matrix through a new association in a manner depicted by models proposed by Chung (36) and shown in Figure 3 (e); such an accommodation, presumably, could overcome the adverse effects of soy flour in the production of acceptable protein-enriched bread.

DeStefanis et al. (37) recently reported that little binding of the surfactants (SSL, succinylated monoglycerides, and monoglycerides) by the major flour components occurred at the sponge stage. The additives were firmly bound to the gluten proteins during dough mixing and strongly bound to the starch by complexing with both the amylose and amylopectin fractions in bread. Based on a study of model systems, they concluded that two concurrent phenomena occurred during baking: (a) the bonds between the gluten proteins and the additives became increasingly weak (protein denaturation) as the dough temperature increased; and (b) as starch gelatinized above 50 C, the additives weakly bonded to proteins readily formed a strong complex with starch, thus allowing a translocation to occur from the proteins to the starch. In addition to the additives, TG, FFA, and LPC were also bound to the starch.

THE SHORTENING EFFECT

Interaction between lipids and wheat flour macromolecules came up in recent years in studies on the mechanism of the "shortening effect"; i.e., the increase in loaf volume and improvement of crumb grain from the addition of 1 to 3% shortening or hardened vegetable fat. The shortening effect was reviewed recently by Bell et al. (16), who visualized two mechanisms of shortening effects: chemical and physical. The chemical effect would involve lipid oxidation; the mechanism was considered to be inoperative, or at least insignificant, in breadmaking. The following physical effects were reviewed: lubrication, sealing, foam formation, involvement of hydrogen and hydrophobic bonds, and delayed carbon dioxide release. Bell et al. (16) have shown that the rate of carbon dioxide release was faster in doughs baked without than in doughs baked with shortening. Fat in dough increased gas retention in the initial stage of rapid expansion. The final loaf volume depends on the permeability of the dough to carbon dioxide in the earliest stages of baking. They suggested that the difference in carbon dioxide release might explain the shortening response. They postulated that physical mechanisms account for the increased loaf volume on adding shortening to the dough. Increased loaf volume results when sufficient solid shortening components remain free in the dough. The free components are especially important in the initial stage of rapid dough expansion in the so-called "oven spring." The free shortening components, presumably, facilitate the production of oriented structures in dough. The structures persist even when the temperature exceeds the melting point of the fat. Those structures also seem to favor gas retention in the early stages of baking.

SHORTENING RESPONSE AND INTERACTION OF FLOUR COMPONENTS

The delayed carbon dioxide release mechanism agrees with several well-established facts, such as the significance of free lipids and the critical "oven spring." It seems, however, that attributing the differences among wheat flours to

TABLE II

Characterization of Flours[a]

Flour	Flour yield (%)	Protein (%)	Ash (%)	Free lipids (%)		
				Total	Nonpolar	Polar
RBS-75	72.8	12.4	0.42	1.00	0.70	0.30
Shawnee	75.9	13.0	0.40	1.03	0.66	0.37
KS501097	73.4	13.4	0.43	0.93	0.69	0.24

[a]Four yield, protein and ash contents are expressed on 14% moisture basis. Free lipid (Skelly B extract) contents were averages of two replicates and expressed on dry flour weight basis.

TABLE III

Bake Absorption, Mixing Characteristics, Loaf Volume, and Crumb Grain of Bread Baked with or without 3% Shortening from Three Untreated or Skelly B Defatted Flours (10 g)[a]

Parameter	RBS-75		Shawnee		KS501097	
	Untreat.	Defat.	Untreat.	Defat.	Untreat.	Defat.
Bake absorption (%)						
Without shortening	69.8	73.5	67.3	73.0	65.8	69.1
With 3% shortening	68.3	70.6	65.3	71.5	64.4	67.6
Mixing time (min)						
Without shortening	4 3/4	5 3/8	4 7/8	7 5/8	7/8	1.0
With 3% shortening	4 7/8	5 1/8	5 3/8	7 1/4	7/8	7/8
Loaf volume (cc)						
Without shortening	64.5	71.0	73.3	71.8	51.6	46.3
With 3% shortening	81.0	67.5	91.7	71.7	47.3	53.2
Crumb grain[b]						
Without shortening	U	Q-U	Q-U	Q	U	U
With 3% shortening	S	U	S	Q	U	U

[a]Averages of three replicates; overall standard deviation: 0.4% for bake absorption, 1/8 min for mixing time, and 1.3 cc for loaf volume.
[b]S = Satisfactory; Q = Questionable; U = Unsatisfactory.

delayed carbon dioxide release alone is an oversimplification. Wheat flour lipids and their role in breadmaking have been the subject of several comprehensive reviews (1,38-41). It is well established that, in PE-defatted flours, nonpolar lipids are detrimental, and polar lipids, especially glycolipids, are effective improvers (42-51). That role is modified by the addition of shortening and/or surfactants. MacRitchie and Gras (47) emphasized that if baking formulations include shortening or other lipid additives, the effect of the natural flour lipid may be obscured. On the other hand, it is difficult to determine the effects of adding lipids to an untreated flour because of the presence of natural flour lipids. Recent studies conducted at our Center indicate that interactions among wheat flour components are important in the shortening response.

The results reported in this section were obtained on three experimentally milled flours (untreated or extracted with Skelly B). The flours are described in Table II. The flours were mixed according to the procedure described by Finney and Shogren (52) and baked by the procedure of Shogren et al. (53).

First, a flour composite (RBS-75) made from many varieties grown at many locations in the Great Plains, U.S., was prepared. The medium-long mixing time flour contained 12.4% protein and was of satisfactory breadmaking quality. When the flour (10 g) was baked into bread with 3% shortening, water absorption was 68.3%, mixing time 4 7/8 min, and loaf volume 81.0 cc (Table III). The significance of the free flour lipids in the shortening effect is shown in Figure 4. In bread baked with 3% shortening (top line), extracting free flour lipids reduced loaf volume from 81.0 to 67.5 cc; in bread baked without shortening (bottom line), however, extraction of free lipids actually increased loaf volume from 64.5 to 71.0 cc.

The picture becomes even more complicated when we consider the shortening response and interaction effect in flours varying in breadmaking quality. Two additional flours used are described in Table II. The flour milled from the 'Shawnee' wheat and the flour milled from the experimental KS501097 contained comparable amounts of proteins; Shawnee contained more free polar lipids than KS501097 flour. Defatting the flours increased their water absorptions; the increase was reduced somewhat by the addition of 3% shortening (Table III). The increase in water absorption was accompanied, generally, by an increase in mixing time. Shawnee flour had a long mixing time and produced excellent bread (in loaf volume and crumb grain); KS501097 produced inferior bread (Table III and Fig. 5). The flours varied widely in their shortening response, in loaf volume, and crumb grain. Thus, the untreated two good breadmaking flours (RBS-75 and Shawnee) were improved by adding shortening; untreated KS501097 produced somewhat better bread when it was baked without, than with, shortening. For the defatted flours, however, the shortening response was reversed. Crumb grain was highly correlated with loaf volume, i.e., the higher the loaf volume the better the crumb grain.

Those studies show clearly that the shortening response is affected to a large extent by wheat flour quality which is governed by the inherent wheat flour components (presumably gluten proteins) and is modified by the removal of wheat flour lipids. Wehrli (21) suggested that since shortening, in the absence of glycolipids, had a detrimental effect on loaf volume, shortening probably interfered with the formation of a stable membrane between starch and proteins; such as membrane, presumably, requires that the starch surface is covered with glycolipids in a manner depicted in Figure 3 (d). Probably, a beneficial effect of short-

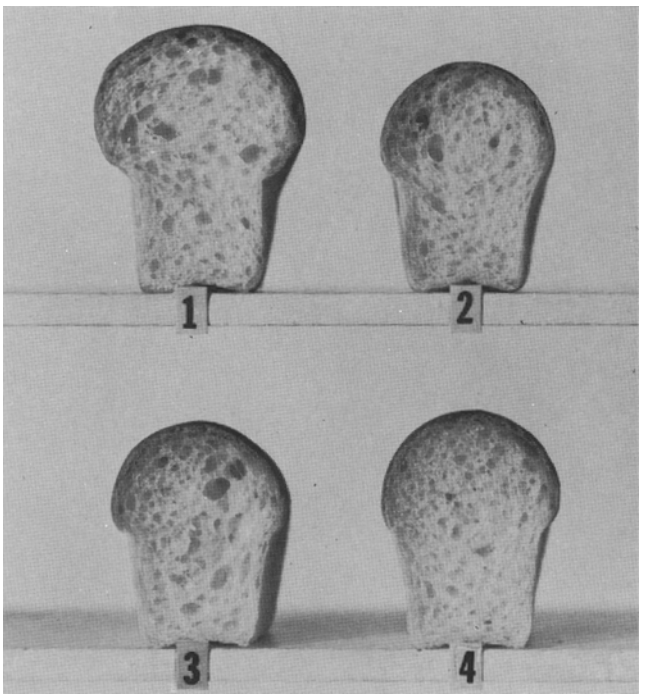

FIG. 4. Loaves baked with 3% shortening (top row) or without shortening (bottom row) from untreated (1 and 3) and Skelly-B extracted (2 and 4) RBS-75 flours.

ening depends on quantity and quality of gluten proteins and free lipids, especially polar glycolipids, in wheat flour. Studies underway in our laboratories indicate that the shortening response is further affected by the nature of wheat flour lipids that are removed.

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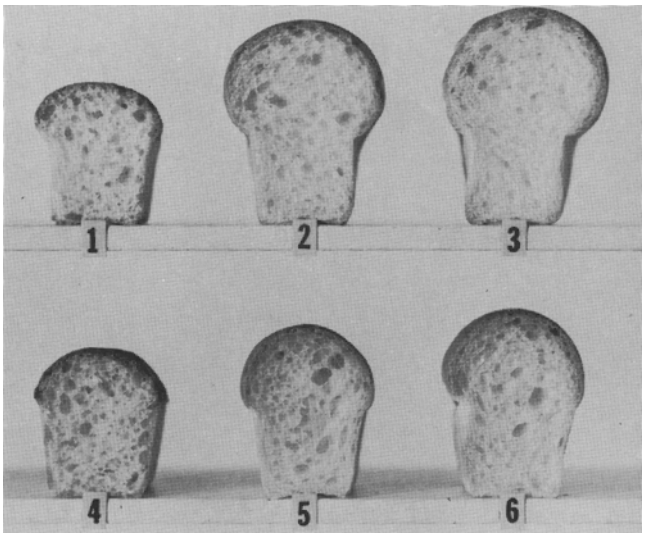

FIG. 5. Loaves baked with 3% shortening (top row) or without shortening (bottom row) from KS501097 (1 and 4), RBS-75 (2 and 5), and Shawnee (3 and 6) flours.

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