CHANGES IN LIPID BINDING AND DISTRIBUTION DURING DOUGH MIXING[1]

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ABSTRACT

Effects of mixing on lipid binding and distribution were studied by extracting free and bound lipids from wheat flour, lyophilized doughs mixed different times (arrival, peak, departure, and twice the departure time on a farinograph), and various fractions of samples (flour and lyophilized doughs). Samples were fractionated by solubilizing in dilute acetic acid and by centrifugation. Composition of the extracted lipids was studied by thin-layer chromatography and densitometry. Dough mixing affected lipid binding. The initial mixing stage was most critical for lipid binding. Half of flour free lipids (some free nonpolar and all free polar components) became bound when dough was optimally mixed (peak time on a farinograph). Extractability of bound lipid components by water saturated n-butanol depended on their polarities and mixing times. Distribution of proteins and lipids in various fractions was affected by dough mixing. Bound lipid components were distributed differently in sample fractions depending on their polarities. Nonpolar bound lipids were distributed mainly in acetic acid-soluble fractions rich in acid-soluble proteins and highly polar bound lipid components were mainly in acid-insoluble starch-lipid-protein fraction. Lipid components with intermediate polarities became evenly distributed in dough fractions during dough mixing.

The mixing property of dough is one of very important parameters in breadmaking. When flours are wetted and doughs are mixed, a decrease in the size of protein aggregate that takes place is reflected by an increase in protein extractability (1,2), and lipids normally extractable from flour with nonpolar solvents become bound (3). About two-thirds of the free lipids become bound during dough mixing (4). Readily extractable free lipid shows a drop in proportion as an immediate consequence of adding water to flour (5) even without rigorous mechanical work applied (6). Lipid binding increases with the rate of dough development (7) and lipid distribution is markedly affected by the atmosphere in the dough mixing chamber (8,9). Several proposed models have demonstrated lipid-protein interaction in flour, gluten, dough, and bread: starch-lecithin-adhesive protein complex (10); the bimolecular phospholipid layer in gluten (11); the gliadin-glycolipid-glutenin complex (12); the starch-glycolipid-gluten system (13); and phospholipid-metal-protein chelate formation (14). Also, the presence of a lipid-protein complex in gluten has been studied quantitatively (12,15). However, more compositional studies on lipids associated with dough proteins are needed to explore the role of lipids during dough formation. The present study has been undertaken to examine the association of lipids with proteins in dough fractions and the changes in extractability of

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various lipid components during dough mixing.

MATERIALS AND METHODS

Materials

An untreated sample of a typical hard red winter wheat, Scout, from the 1969 crop grown at experimental plots in Kansas was experimentally milled on Miag Multomat with approximately 72% extraction. Straight-grade flour sample contained 11.8% proteins (N × 5.7) and 0.45% ash on dry basis. Proteins, ash, and moisture were determined as described in the AACC Approved Methods (16); Method 46-11, Method 08-01, and Method 44-15A, respectively.

The reference lipids were purchased from Applied Science Laboratories, Inc., State College, Pa. The standard sugars, d(+) raffinose and sucrose, were obtained from Pfanstiehl Chemical Company, Waukegan, Ill., and J. T. Baker Chemical Company, Phillipsburg, N.J., respectively.

All organic and inorganic solvents were analytical reagent grades. All solutions were prepared from analytical reagent-grade compounds and deionized water or proper solvents. Distilled water was used for mixing doughs in a farinograph.

Preparing Dough Samples

Two sets of dough samples were prepared using two farinographs; one with 50-g mixing bowl (I), and the other one with 300-g mixing bowl (II). Farinogram characteristics are as follows:

	Farinograph	
	I	II
	% of flour (14.0% mb)	
Water absorption	58.4	57.4
Mixing stage	min	
Arrival time	1.8	1.8
Peak time (optimum)	4.3	4.3
Departure time	7.2	7.0
2 × departure	14.4	14.0

Doughs were mixed at arrival, peak, departure, and twice the departure times on farinographs (using 50-g and 300-g mixing bowls at 60 rpm and 30°C), immediately frozen, and lyophilized. The lyophilized doughs were repeatedly ground in a Stein Mill (Fred Stein Laboratories, Atchison, Kans.) for 30 sec, sieved to pass a 60-mesh screen, and stored at −18°C between experiments. Moisture content of the lyophilized doughs was determined according to Method 14.004 of Official Methods of Analysis (17).

Fractionating Samples

Before fractionating studies were carried out, free lipids, bound lipids, and acid-soluble proteins (2.5-g sample on dry basis with 30 ml 0.05*N* acetic acid) were extracted from both sets of dough samples. Average data of duplicated extraction from each set of doughs were in good agreement for both lipids and

proteins. Because larger amounts of lyophilized doughs were required for fractionating studies, only one set of dough samples mixed on farinograph II with 300-g mixing bowl was used.

As shown in Fig. 1, 20-g (dry basis) samples of flour or lyophilized and ground doughs were dispersed in 240 ml 0.05*N* acetic acid and shaken for 30 min on a mechanical shaker (Eberbach Company, Ann Arbor, Mich.) at room temperature. Suspensions were centrifuged for 30 min at 4° C at 30050 × *g* using a Beckman Model J-21 refrigerated centrifuge with a fixed-angle rotor, JA-14. The supernatant solutions were collected.

The residues were centrifuged for additional 30 min until they fractionated into three layers. The residues were cut longitudinally to three to four pieces so that they were narrow enough to be transferred easily through centrifuge bottle neck to a watch glass; the top gelatinous layer was separated by scraping off with a spatula and collected; the middle layer—a tan portion and a major fraction of residue—was obtained by washing the bottom layer, a white starch portion, using a small stream of distilled water.

Each sample was fractionated six times. Because the amount of top gelatinous layer of residue was very small, the separated fractions of three runs were combined together, lyophilized, and further dried in a vacuum desiccator over P_2O_5 for 48 hr at room temperature.

The lyophilized supernatants were termed: "acetic acid – soluble fraction" (A); top layer of residue, "gelatinous fraction" (B); and middle layer of residue "starch-lipid-protein fraction" (C). The bottom layer of residue termed "starch fraction" (D) was washed away. Because we were mainly interested in lipid binding to proteins, we did not save fraction (D), which contained almost no proteins.

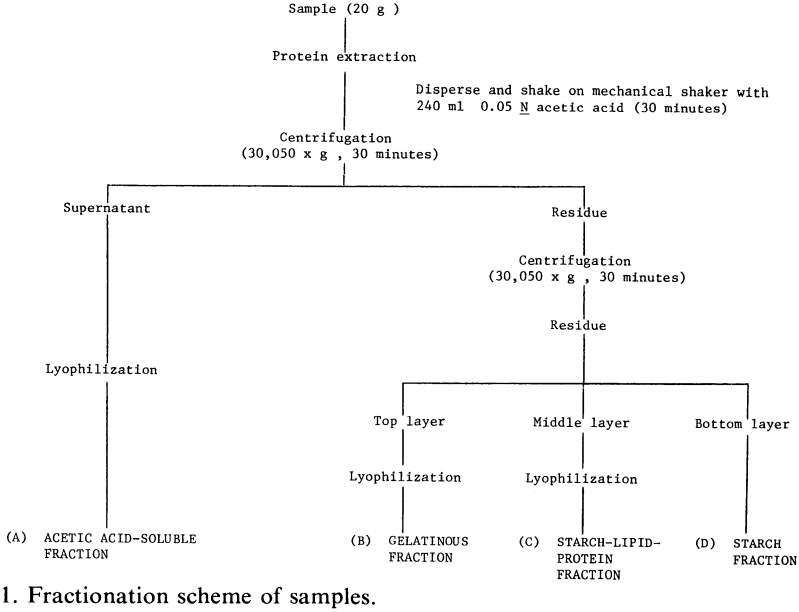

Fig. 1. Fractionation scheme of samples.

Fractions (A) and (B) were uniformly ground in a mortar and (C) was repeatedly ground in a Stein Mill for 30 sec to sieve through a 60-mesh screen. As fraction (A) was too fluffy to determine moisture content, and the amount of (B) was insufficient for moisture determination, we did not make corrections for moisture variations of dried dough fractions.

Determination of Proteins

Nitrogen content was determined by micro-Kjeldahl method according to Method 42.014-42.016 of Official Methods of Analysis (17) and protein content was calculated using 5.7 as conversion factor.

Extracting Lipids

Free lipids were extracted for 12 hr from 5 g (dry basis) of sample (flour and lyophilized doughs) with 100 ml petroleum ether (bp 35° to 60° C) in a Goldfish extractor. Similarly, free lipids were extracted from fractions (A), (B), and (C) of flour or lyophilized doughs containing respectively, 1.0, 0.5, and 0.25 g proteins.

Bound lipids were obtained by treating the petroleum ether (PE) extracted material three times with 50 ml water-saturated n-butanol (WSB) in a Stein Mill. The first and second extractions were three times for 2 min at 1-min intervals; the third was only once for 2 min. The rest of the drying and reextraction procedure was as described by Pomeranz *et al.* (18).

Thin-Layer Chromatography (TLC)

Glass plates (20 × 20 × 0.38 cm) were coated with a 250-μ layer of silica gel G (E. Merck, A. G., Darmstadt, Germany) using a commercial spreader (Quickfit Instrumentation, England). The plates were activated for 3 hr at 130° C.

The chromatographic solvents used for one-dimensional ascending development were: a mixture of hexane-diethyl ether-methanol (80:20:1, v/v/v) (solvent system I) for steryl esters, tri- and diglycerides, and a mixture of chloroform-methanol-water (65:25:4, v/v/v) (solvent system II) for monoglycerides, free fatty acids, glycolipids, and phospholipids.

Lipids separated by TLC were tentatively identified by comparing the R_f values with those of lipids separated and visible by specific sprays, as reported in the literature (19,20,21,22) and confirmed by comparing the R_f values with those of pure standard compounds.

Quantitative TLC

A densitometer (Photovolt Multiplier Photometer Model 520-A) with a scanning stage, Model 52-C, and Varicord Variable Response Recorder, Model 42-B (Photovolt Corporation, New York, N.Y.) was used to quantitatively measure 50 μg lipids separated by TLC after they had been sprayed with $K_2Cr_2O_7$ solution in 55% H_2SO_4 and heated for 25 min at 180° C.

We had series of lipid determinations requiring several hundred plates. Quantitative TLC is generally known to be poor in reproducibility because of many factors involved, such as substantial variations between plates. In order to compensate for those variations as much as possible, the bound lipids extracted from a dough optimally mixed (peak time on a farinograph—4.3 min) were spotted on every plate as an internal standard. The peak area of each component

of the internal standard was averaged from several hundred plates, a correction factor for each component was calculated for each plate, and the peak areas of the other samples were corrected according to this correction factor for the entire series of lipid works. Each peak area on the recording chart was integrated twice using a Gelman planimeter. The average peak area of each component obtained

TABLE I
Constants in Equations in Form, Y = A + BX for Calibration Curves of Standard Compounds on TLC Developed in Two Solvent System[a]

Solvent System	Standard Compounds	A	B
I. Hexane-diethyl-ether-methanol (80:20:1)	Steryl esters[b]	0	15
	Triglycerides[c]	5.15	13.57
	Diglycerides[d]	10.63	8.47
	Monoglycerides[e]	1.82	5.26
	Free fatty acids[f]	1.50	9.48
II. Chloroform-methanol-water (65:25:4)	Monoglycerides[e] (2 to 10 μg)	0	18.00
	Free fatty acids[f]	13.20	15.65
	Monogalactosyl diglycerides	2.83	7.9
	Digalactosyl diglycerides	−24.40	17.97
	Phosphatidylethanolamines	−5.00	16.00
	Phosphatidylcholines	4.70	10.90
	Lysophosphatidyl cholines	−4.82	10.12
	Phosphatidyl serines	−2.30	15.52
	Phosphatidyl inositol	−8.00	12.11
	Sucrose (2 to 8 μg)	27.50	11.25

[a]Y is the peak area in square in. × 10^2, and X is the weight of standard compound in μg.
[b]β-Sitosteryl palmitate.
[c]Trilinolein and tripalmitin.
[d]1,2- and 1,3-dilinolein, 1,2- and 1,3-dipalmitin.
[e]Monolinolein and monopalmitin.
[f]Linoleic acid and palmitic acid.

TABLE II
Effect of Mixing on Changes in Lipid Binding

Farinogram	Mixing Time of Dough, min[a]	Extractable Lipids, Free[b]	Bound[b]	Total
			% of sample wt[c]	
	0	0.9	1.1	2.0
Arrival	1.8	0.5	1.6	2.1
Peak (optimum)	4.3	0.4	1.7	2.1
Departure	7.1	0.4	1.7	2.1
2 × departure	14.2	0.4	1.6	2.0

[a]Average of two sets of farinograph (I = 50-g and II = 300-g mixing bowl).
[b]Average value of four extracts (duplicated extractions of each set of doughs mixed in farinograph I and II).
[c]Flour and lyophilized dough on dry basis.

from quadruplicated chromatograms (duplicated chromatograms of two extractions) was converted to weight by using the least square regression equation of the corresponding standard lipids (23).

Calibration Curves of Standard Compounds

Various standard lipids were also chromatographed using the same solvent system. The density of charred spots was measured and the peak areas were integrated in the same way with the samples. When standard compounds were applied in various concentrations, developed, visualized, and measured for the density, a linear relationship was found, over a certain range, between quantity of the standard compound and the planimetrically determined peak area. Linearity was obtained between 2 μg and at least 20 to 24 μg for most of the standard lipids used. Each standard compound was spotted in increasing order of 2 μg to 18 μg to give nine spots on a plate. The value of the peak area was obtained from averaging quadruplicate analyses.

For standard sugar compounds, only sucrose was used in the range of lower concentration (2 to 8 μg), because of the low solubility of sucrose in a mixture of chloroform:methanol (2:1). Raffinose was almost insoluble in the chloroform-methanol mixture, which was the solvent used for dissolving dried lipid extracts and standard lipids.

The least square regression was used for converting peak area to weight of lipid component because of the following reasons: a) the calibration curve did not go through the origin; b) some lipid components were present in such small amounts that converting peak area into weight from the calibration curve on the graph paper was not very accurate; c) when the sample size was very large, it was much simpler and time saving to calculate the results using the least square regression equations.

The constants in the least square regression equations in the form, $Y = A + BX$, where Y is the peak area in square in. $\times 10^2$ and X is the weight of standard compound in μg for the calibration curves of standard compounds, are tabulated in Table I. Same amount of each lipid standard gives different densitogram area and even same standard gives different area depending on developing solvent system. For example, monoglycerides and free fatty acids give different constants in solvent systems I and II.

RESULTS

Effect of Mixing on Lipid Binding

The initial mixing stage (arrival time on a farinograph, 1.8 min) was most critical for lipid binding (Table II). When a dough was mixed to an optimum consistency (peak time on a farinograph, 4.3 min) more than a half of flour free lipids became bound. A prolonged mixing showed little effect on lipid binding and extractability. A similar result was reported by Chiu and Pomeranz (4).

Compositional Studies on Free and Bound Lipids

Figure 2 shows the thin-layer chromatogram of bound lipids extracted from wheat flour. Component "e" was identified as free fatty acids (FFA) and monogalactosyl diglycerides (MGDG) in solvent system (II). When densitogram peak area was converted into weight, the least square regression equation of FFA

was used instead of the corresponding equation of MGDG because amount of FFA present in wheat flour is about twice of the amount of MGDG (24) and the peak area of densitograms of FFA was more than twice of the peak area of MGDG (25). Components "g_1" and "g_2" were identified as phosphatidylethanolamines by spraying with ninhydrin reagent and molybdenum blue reagent. Probably, "g_1" was N-acyl phosphatidylethanolamine as identified by Clayton *et* al. (26) and MacMurray and Morrison (24). Component "g_2" was confirmed by pure standard phosphatidylethanolamine. Components "k_1" and "k_2" were identified as sucrose and raffinose, respectively, by comparing R_f values with standard sugars on both paper- and thin-layer chromatograms (27), and spraying

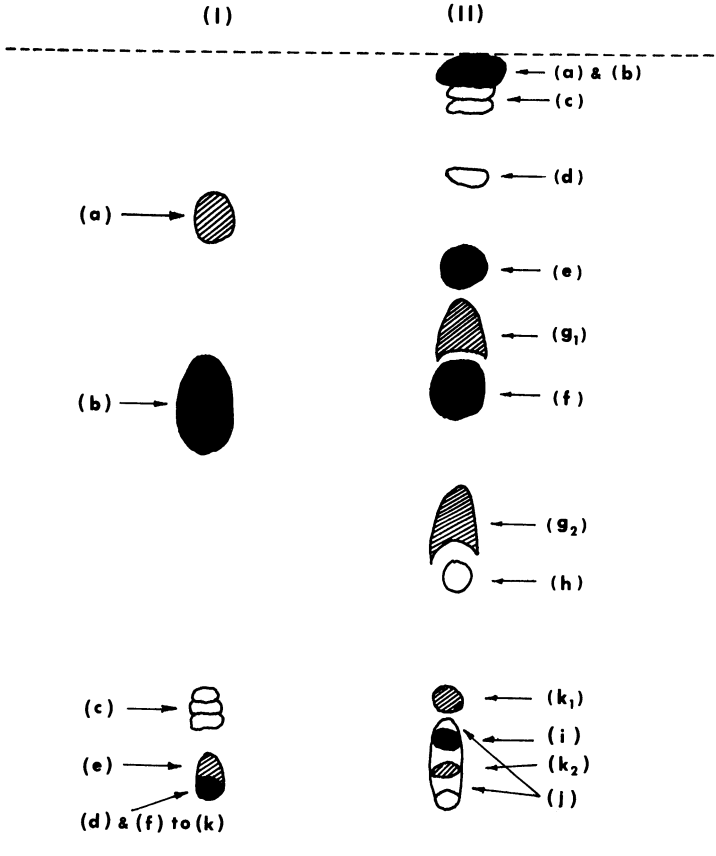

Fig. 2. Thin-layer chromatogram of bound lipids from wheat flour. Amount applied at each spot: 50 μg. Developed with (I): hexane-diethyl ether-methanol (80:20:1); and (II): chloroform-methanol-water (65:25:4), charred with potassium dichromate in 55% sulfuric acid. The darker the shading of spots, the higher the concentration. **a**) Steryl esters; **b**) triglycerides; **c**) 1,3- and 1,2-diglycerides; **d**) monoglycerides; **e**) free fatty acids in solvent (I), free fatty acids + monogalactosyl diglycerides in solvent (II); **f**) digalactosyl diglycerides; **g**) phosphatidylethanolamines; **h**) phosphatidyl cholines; **i**) lysophosphatidylcholines; **j**) phosphatidyl-serines; **k**) components containing sugars (sucrose and raffinose).

with α-naphthol reagent. It seems reasonable to consider those components as glycolipids rather than free sugars, because they were reextracted by petroleum ether after WSB extraction.

Tables III and IV present each component as in weight per cent of the starting sample (dry basis) rather than per cent of the extracted lipids, because amounts of free and bound lipids extracted from flour and doughs varied. They were computed using Table II and lipid composition (weight per cent of lipids) that was obtained by densitometric determination. The lipid components are reported separately for free and bound lipids of flour and doughs. If the sum of each component from free and bound lipids of flour is calculated as weight per cent of total extracted lipids (free lipids + bound lipids), it shows a general agreement with the results reported by MacMurray and Morrison (24) on total glycerides, glycolipids, and phospholipids, except for several trace components which could not be measured by TLC in a total sample of 50 μg.

Free lipids, except diglycerides, decreased with increase in mixing time. The free polar lipids of flour, of which three-quarters was glycolipids and one-quarter was phospholipids, were completely bound at the first stage of mixing.

Dough mixing also drastically decreased free fatty acids and monogalactosyl diglycerides among free lipid components. Graveland (25) also observed decreases in free phospholipids and free saccharide-containing lipids as to the polar lipids, and in free fatty acids and monoglycerides as to the nonpolar lipids when dough mixing occurred in air or oxygen.

Extractability of bound-lipid components by WSB depended on their polarity. Extractability of the most nonpolar steryl esters decreased, whereas extractabilities of the most polar phosphatidyl serines and sugar-containing components increased with an increase in mixing time. Glycerides, fatty acids, galactolipids, and lysophosphatidyl cholines were most extractable by WSB at the optimum mixing time (4.3 min).

TABLE III
Effect of Dough Mixing on the Distribution of Nonpolar Lipid Components[a] in Free and Bound Lipid Fractions

Dough Mixing Time min	Steryl Esters	Glycerides			FFA + MGDG[b]
		Tri-	Di-	Mono-	
		% × 10^2 of flour or lyophilized dough (dry basis)			
Free lipids					
0	11	31	6	4	15
1.8	12	29	7	1	5
4.3	10	25	7	1	2
7.1	8	26	7	1	1
14.2	9	23	7	1	1
Bound lipids					
0	15	10	3	3	19
1.8	9	19	4	6	23
4.3	7	19	6	6	15
7.1	6	11	5	5	13
14.2	5	11	5	5	10

[a]Lipid components having polarity equal to or lower than free fatty acids.
[b]FFA = free fatty acids; MGDG = monogalactosyl diglycerides.

TABLE IV
Effect of Dough Mixing on the Distribution of Polar Lipid Components[a] in Free and Bound Lipid Fractions

Dough Mixing Time min	Glycolipids		Phospholipids			
	DGDG	CS	PE	PC	LPC	PS
	% × 10² of flour or lyophilized dough (dry basis)					
Free lipids						
0	16	...	...	5	...	...
1.8	...	...	...	...	...	...
4.3	...	...	...	...	...	...
7.1	...	...	...	...	...	...
14.2	...	...	...	...	...	...
Bound lipids						
0	22	4	6	4	14	6
1.8	31	14	18	10	21	8
4.3	35	22	19	10	22	8
7.1	33	33	19	12	20	11
14.2	31	34	18	12	18	14

[a]DGDG = Digalactosyl diglycerides; CS = Components containing sugars (sucrose and raffinose); PE = Phosphatidylethanolamines; PC = Phosphatidylcholines; LPC = Lysophosphatidyl cholines; PS = Phosphatidyl serines.

TABLE V
Protein and Lipid Content[a] in Dough Fractions

Mixing Time min	Fractions[b] % of sample (db)	Proteins (N × 5.7)	Lipids		Bound Lipids per 1 g Proteins mg
			Free	Bound	
		% of lyophilized fractions			
A. Acid-soluble fraction					
0	12.9	61.7	0.3	0.9	14
1.8	15.3	59.7	0.4	3.2	54
4.3	15.8	59.7	0.4	2.6	43
7.0	15.3	57.3	0.4	2.4	42
14.0	15.2	56.1	0.3	2.1	38
B. Gelatinous fraction					
0	2.4	20.0	3.4	4.4	219
1.8	4.5	19.2	2.8	5.1	266
4.3	5.5	20.0	2.4	5.5	274
7.0	5.0	24.9	2.8	5.5	222
14.0	5.0	27.4	2.8	5.0	183
C. Starch-lipid-protein fraction					
0	62.4	4.7	0.3	1.3	275
1.8	29.3	4.9	0.4	1.9	380
4.3	19.6	5.4	0.5	2.0	378
7.0	25.8	5.5	0.4	2.1	377
14.0	28.4	5.8	0.4	2.1	369

[a]Average of duplicate extractions of proteins and lipids.
[b]Average of duplicates (each value was weight of the combined fractions obtained from three 20-g fractionations), expressed as per cent of flour or lyophilized dough on dry basis.

Effect of Mixing on Distribution of Dough Fractions, Proteins, and WSB-Extractable Bound Lipids

Figure 3 was obtained from data given in Table V to show distribution of dough fractions on a cumulative basis against mixing time. The acid-soluble fraction (A) and gelatinous fraction (B) increased with mixing time until the optimum mixing time (4.3 min), then decreased slightly, and remained about constant. The starch-lipid-protein fraction (C) was responsible for a great change in weight of the combined fractions (A), (B), and (C). Fraction (C) decreased sharply until dough was mixed to optimum consistency (4.3 min) and gradually increased with overmixing. The upper area of curve corresponding fractions (A)+(B)+(C) shows a large increase in fraction (D) that must have been dissociated from (C) until 4.3 min and the reverse was taking place during overmixing to a smaller extent.

Figure 4 was obtained by converting protein content given in Table V to per cent of flour or lyophilized dough on dry basis and calculating distribution per cent based on total protein content in dry flour. More proteins were extracted with 0.05 *N* acetic acid from lyophilized doughs than from flour. Most proteins

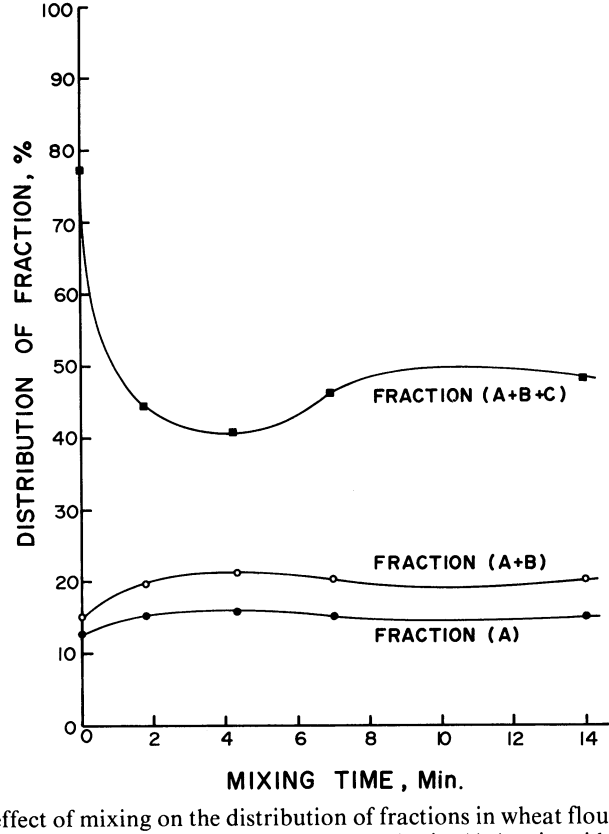

Fig. 3. The effect of mixing on the distribution of fractions in wheat flour and lyophilized doughs, expressed as percentage of sample on dry basis. **A**) Acetic acid-soluble fraction; **B**) gelatinous fraction; **C**) starch-lipid-protein fraction.

were extracted from lyophilized dough mixed to optimum consistency (4.3 min). The area between curve (A) and curve (A)+(B) represents protein distribution in fraction (B). Only 4% of total proteins were distributed in fraction (B) of flour, and 7 to 12% of total proteins were distributed in fraction (B) of doughs. About 25% of total proteins were distributed in fraction (C) of flour, and 9 to 14% of total proteins were distributed in fraction (C) of doughs. Least amount of proteins were distributed in fraction (C) when dough was mixed to optimum consistency (4.3 min). Very few proteins (2 to 4% of total proteins) were distributed in fraction (D). Though fraction (B) was richer in proteins than fraction (C) (Table V), less amounts of proteins were distributed in (B) than in (C) because (B) was a minor fraction compared with fraction (C).

Fraction (B) was richest in both free and bound lipids (Table V). Free lipid content was about same in both fractions (A) and (C). Bound lipid content was slightly higher in fraction (A) than in (C), except for flour sample. In all three fractions, more lipids were bound per 1 g proteins by dough mixing (Table V). Most lipids were bound to 1 g proteins in fraction (C), and least in fraction (A).

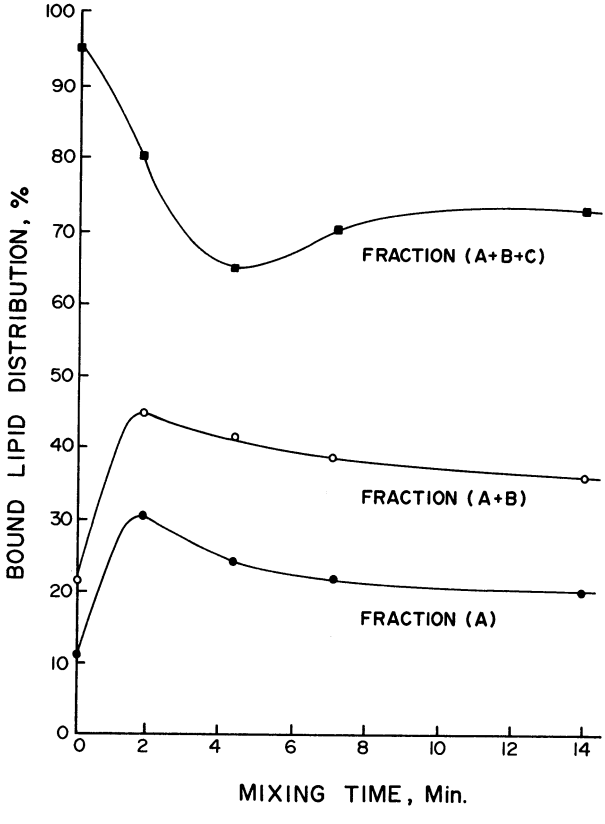

Fig. 4. The effect of mixing on the protein distribution in wheat flour and lyophilized doughs, expressed as percentage of total proteins. **A)** Acetic acid-soluble fraction; **B)** gelatinous fraction; **C)** starch-lipid-protein fraction.

Figure 5 was obtained by converting bound lipid content given in Table V to per cent of flour or lyophilized dough on dry basis and calculating distribution per cent based on bound lipid content of unfractionated samples given in Table II. In flour, over 70% of WSB-extractable bound lipids were distributed in fraction (C), and over 20% were distributed, about equally, in (A) and (B). Dough mixing significantly changed bound lipid distribution. Bound lipids distributed in (A) and (B) were most at initial mixing (1.8 min). Least bound lipids were distributed in (C) when dough was mixed to optimum consistency (4.3 min). The curve corresponding fractions (A)+(B)+(C) gives a minimum distribution at 4.3 min, indicating that most bound lipids must have been distributed in starch fraction (D) when dough was mixed to optimum consistency (4.3 min).

Compositional Studies on Bound Lipids Extracted from Dough Fractions

Tables VI and VII were computed using data given in Table V and lipid

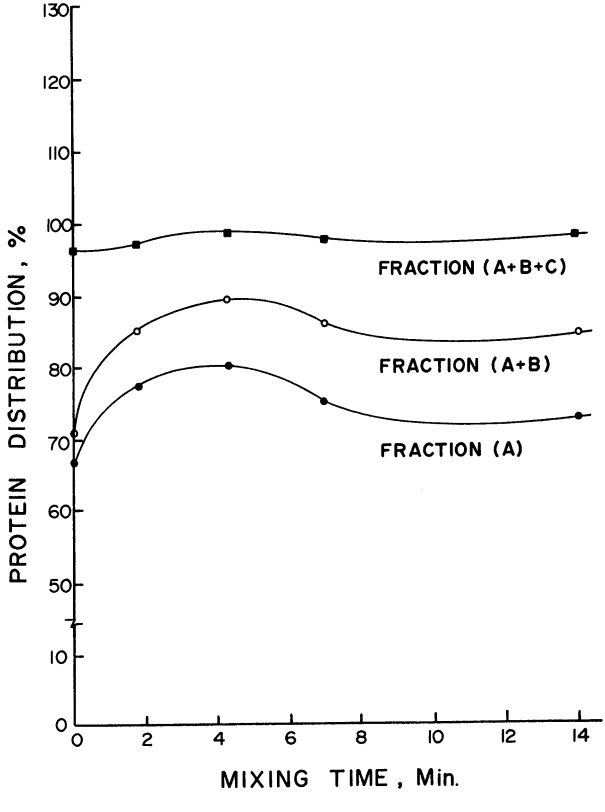

Fig. 5. The effect of mixing on the WSB-extractable bound lipid distribution in wheat flour and lyophilized doughs, expressed as percentage of WSB-extractable bound lipids of unfractionated samples. **A)** Acetic acid-soluble fraction; **B)** gelatinous fraction; **C)** starch-lipid-protein fraction.

composition (weight per cent of bound lipids) that was obtained by densitometric determination.

Dough mixing increased bound lipid components in fractions (A) and (B), and decreased in (C). Lipid components with less polarity (steryl esters, tri- and diglycerides) were bound mainly to the acid-soluble fraction (A) of doughs; whereas those with higher polarity (PC, LPC, PS, and CS) were bound more to fraction (C) than to the other two fractions (A) and (B). Those with intermediate polarity (monoglycerides, FFA, MGDG, DGDG, and PE) were bound more evenly to both fractions (A) and (C) of doughs.

GENERAL DISCUSSION

Dilute acetic acid could extract about 13% of flour components of which 62% were proteins. Acid-soluble proteins were about two-thirds of total proteins. A major portion of acid-insoluble residue was fraction (C), that was almost two-thirds of flour. Fraction (C) decreased drastically, and accordingly, the other fractions increased until dough was mixed to optimum consistency. Presumably, some components were dissociated from fraction (C) during dough mixing. From protein distribution and WSB-extractable bound lipid distribution changes during mixing, we consider that lipids and proteins were dissociated from (C), and distributed in fractions (A) and (B) and lipids and starch, most

TABLE VI
Nonpolar Components[a] of Bound Lipids Associated with Flour and Dough Fractions

Sample Fraction	Mixing Time min	Steryl Esters	Glycerides			FFA+ MGDG[b]
			Tri-	Di-	Mono-	
		% × 10^2 of flour or lyophilized dough (dry basis)				
A. Acetic acid-soluble fraction						
	0	2	2	1	...	1
	1.8	5	16	2	2	4
	4.3	3	11	3	2	2
	7.0	3	8	2	2	2
	14.0	3	9	1	1	2
B. Gelatinous fraction						
	0	...	...	...	...	1
	1.8	1	1	...	1	1
	4.3	1	1	1	1	2
	7.0	1	1	1	1	1
	14.0	1	1	1	1	1
C. Starch-lipid-protein fraction						
	0	2	2	...	3	4
	1.8	1	1	...	2	3
	4.3	1	1	...	2	2
	7.0	1	1	...	2	3
	14.0	1	2	...	2	3

[a]Lipid components having polarity equal to or lower than free fatty acids.
[b]FFA = free fatty acids; MGDG = monogalactosyl diglycerides.

TABLE VII
Polar Components[a] of Bound Lipids Associated with Flour and Dough Fractions

Dough Fraction	Mixing Time min	Glycolipids		Phospholipids			
		DGDG	CS	PE	PC	LPC	PS
		% × 10^2 of flour or lyophilized dough (dry basis)					
A. Acetic acid-soluble fraction							
	0	2	...	1	...	1	...
	1.8	8	2	4	1	3	3
	4.3	9	1	4	2	2	1
	7.0	8	2	4	1	3	1
	14.0	6	2	4	1	2	2
B. Gelatinous fraction							
	0	2	3	2	1	1	...
	1.8	5	8	3	1	1	1
	4.3	6	11	4	1	2	1
	7.0	7	9	3	1	2	1
	14.0	6	9	2	...	2	1
C. Starch-lipid-protein fraction							
	0	16	21	12	5	12	6
	1.8	11	17	5	3	8	5
	4.3	8	11	4	2	6	3
	7.0	10	17	6	3	7	1
	14.0	11	18	7	3	9	5

[a]DGDG = digalactosyl diglycerides; CS = Components containing sugars (sucrose and raffinose); PE = Phosphatidylethanolamines; PC = Phosphatidylcholines; LPC = Lysophosphatidyl cholines; PS = Phosphatidyl serines.

likely might have been dissociated from (C) and distributed in fraction (D). Amounts of fraction (C) and bound lipids in (C) increased slightly with overmixing (departure and twice the departure time on a farinograph) and their increases would have come mainly from fraction (D). We conjecture possible formation of complexes between starch and proteins linked by polar lipids when wheat flour or lyophilized wheat-flour dough was suspended in dilute acid. Such complexes were formed to the least extent in lyophilized dough sample mixed to optimum consistency (4.3 min). Dough mixing could have disrupted some bonds between proteins and lipids, or lipids and starch; (starch-polar lipids-proteins) could have been disrupted to give (starch-polar lipids) or (polar lipids-proteins).

More than half of flour free lipids become bound during dough mixing. Compositional studies on bound lipids in various fractions showed that major portion of flour free lipids became bound to acid-soluble fraction, very likely acid-soluble proteins.

Lipid binding seemed dependent on polarities of lipid components. More nonpolar lipids were bound to acid-soluble proteins and more polar lipids were bound to acid-insoluble proteins and starch. Lipid components with intermediate polarities showed intermediate affinity toward acid-soluble proteins or acid-insoluble proteins and starch. Among those components, galactolipids were most abundant in wheat flour lipids. The bread-improving effects of free polar lipids, mostly galactolipids, studied by several investigators

(28,29,30) may well be associated with their intermediate polarity for interacting with the two major dough components—starch and proteins.

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