Functional Properties of Surfactants in Breadmaking. III. Effects of Surfactants and Soy Flour on Lipid Binding in Breads[1]

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ABSTRACT

Lipid binding progressively increased during dough mixing and baking; over three fourths of the free wheat flour lipids were bound during breadmaking. Over 90% of free flour glycolipids and phospholipids (PL) and 66% of glycerides were bound during the entire bread-making process. Extractability of total (free plus bound) lipids of certain classes changed during breadmaking. Free plus bound trigalactosyldiglycerides or PL (mainly lysophosphatidylcholine) were extracted most from bread and least from flour. Extractability of digalactosyldiglycerides decreased during baking; about one half of them became so tightly bound that they were inextractable with water-saturated *n*-butanol. Surfactants, ethoxylated monoglycerides (EMG) alone or in combination with distilled monoglycerides and sodium stearoyl-2-lactylate (SSL), suppressed lipid binding by displacing some lipid components on binding sites in breads. Similarly, surfactants lowered extractability of certain bread lipid components by complexing with them. Nonionic EMG and/or distilled monoglycerides showed stronger displacing effects and weaker complexing effects than the anionic SSL did. However, EMG or SSL did not displace lipids in the presence of soy flour because soy proteins supplied sufficient binding sites for both native lipids and the added surfactants. Surfactants accommodated the soy protein in the gluten matrix through new association by sharing and enhancing the role of native lipids, especially glycolipids and PL, in formation of complexes that involve multiple interactions. Such accommodations, as depicted by proposed models, presumably can overcome the adverse effects of soy flour and produce acceptable protein-enriched bread.

Studies have demonstrated that small amounts of polar lipids or lipid-related surfactants can counteract the adverse effects of high-protein nonwheat products, such as soy flour, on functional properties in breadmaking (Finney and Shogren 1971; Pomeranz et al 1969a, 1969b; Tsen et al 1971). Modes of action by which surfactants counteract the deleterious effects of protein fortification have not been fully explained. Chung and Tsen (1975d, 1977) reported the effects of surfactants on interactions among native flour lipids and proteins and/or starch in optimally mixed doughs.

The present study reports the effects of surfactants on lipid binding in breads with or without soy flour added. Models, based on previous studies and the present investigation are used to explain the overall functionalities of surfactants as dough conditioners and bread improvers.

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MATERIALS AND METHODS

Materials

The wheat and soy flours were obtained from Archer-Daniels-Midland Milling Co., Kansas City, MO. The wheat flour (Commander III), milled from a blend of hard red spring and winter wheats, was an untreated straight-grade flour and contained 12.9% protein (N × 5.7), 0.43% ash, and 13.7% moisture. The hexane-defatted soy flour (Ardex 550) contained 52.7% protein (N × 6.25), 6.90% ash, and 8.3% moisture.

Sodium stearoyl-2-lactylate (SSL) and ethoxylated monoglycerides (EMG) were from Patco Products, Kansas City, MO. Distilled monoglycerides (DMG), was Myverol, type 18-40, from Eastman Chemical Products Inc., Kingsport, TN. Reference lipids were from Applied Science Laboratories Inc., State College, PA. Organic solvents were analytical reagent grade, and solutions were prepared from analytical reagent-grade compounds.

Baking Procedure

Two sets of breads were baked according to the "K-State process" described by Tsen and Tang (1971) without shortening added. One set included regular wheat flour breads; the other set included high-protein breads baked from wheat flour fortified with soy flour in a weight ratio of 100 to 12. Both sets were baked with no surfactant (control), with 0.5% EMG, with 0.5% EMG and 0.25% DMG, with 0.25% EMG and 0.25% DMG, and with 0.5% SSL. The surfactant level was percent of flour weight. In this report, the

term "regular bread" refers to bread baked from wheat flour only and "high-protein bread" or "soy bread" refers to bread baked from the wheat-soy (W+S) blend. Loaf weight and volume were measured immediately after baking. Specific loaf volume was averaged from flour replicates. The breads were lyophilized, ground in a Stein Mill (Fred Stein Laboratories, Atchinson, KS) for 30 sec, sieved to pass a 60-mesh screen, and stored at −18°C between experiments.

Analytical Methods

Protein, ash, and moisture contents were determined by AACC methods. Nitrogen contents of protein extracts were determined by the AOAC micro-Kjeldahl method; protein content was calculated using conversion factors N × 5.7, 6.25, and 5.76 for wheat, soy, and the blend of W+S flours respectively.

Extracting Proteins, Lipids, and Surfactants

Flour or lyophilized and ground bread samples (20 g, db) were extracted with 240 ml of 0.05*N* acetic acid by shaking for 30 min and then centrifuged (Chung and Tsen 1975a). The lyophilized extracts were used for determining N content and for extracting lipids and surfactants.

Procedures for extraction, purification, and quantitative (densitometric) determination of lipid classes and surfactants by thin-layer chromatography (TLC) were as described previously (Chung and Tsen 1975a, 1975b). Lipids or surfactants extracted with petroleum ether were defined as free and those with water-saturated *n*-butanol (WSB), following petroleum ether extraction, as bound. Free total or bound total lipids are defined as the unfractionated lipid mixture. Protein or lipid extractions and quantitative TLC assays were replicated four times.

Analysis of Data

To simplify the presentation of the results, the sum of triglycerides (TG), diglycerides, and monoglycerides were reported under the glyceride heading; the sum of digalactosyldiglycerides (DGDG) and trigalactosyldiglycerides under the glycolipid (GL) heading and the sum of phosphatidylethanolamines, phosphatidylcholines, phosphatidylserines, phosphatidylinositols, and lysophospholipids under the phospholipid (PL) heading.

Analysis of variance (ANOVA) and tests for Fisher's least significant difference (LSD) were computed to study the significance of surfactant effects, soy flour effects, and their interaction, and also the significant difference of mean value (Fryer 1966). The differences in mean values greater than the LSD values listed in the tables were significantly different at the 0.05% level.

TABLE I
Specific Loaf Volume[a] of Regular[b] and High-Protein[c] Breads

Surfactant[d] Levels (% flour)	Specific Loaf Volume (cc/g)	
	Regular	High-Protein
None (control)	6.70 a	3.87 e
0.5% EMG	7.39 b	6.36 f
0.5% EMG + 0.25% DMG	7.55 bc	6.28 f
0.25% EMG + 0.25% DMG	7.69 cd	6.01 g
0.5% SSL	7.84 d	5.68 h
LSD value ($\alpha = 0.05$)	0.19	0.17

[a] Values are averages of four replicates (overall standard deviation, 0.12 cc/g). Values followed by the same letter are not significantly different at the 0.05 level based on the LSD value.
[b] Breads baked from wheat flour.
[c] Breads baked from a wheat-soy (100:12) flour blend.
[d] EMG = ethoxylated monoglycerides, DMG = distilled monoglycerides, SSL = sodium stearoyl-2-lactylate.

RESULTS

Specific Loaf Volume

Surfactants significantly increased specific loaf volumes (loaf volume/loaf weight) of both regular or high-protein breads containing no shortening (Table I), based on ANOVA and the LSD test. Anionic SSL was a better loaf volume improver than was nonionic EMG or a combination of 0.5% EMG and 0.25% DMG

TABLE II
Free Lipids and Surfactants Extracted from 10 g (db) of Ingredient Flours and Lyophilized Bread

Sample[a] Surfactant[b] Levels (% flour)	Total Extracts[c] by PE (mg/10g, db)	Lipid Class[d] (mg/10g, db)					
		Steryl Esters	Glycercerides	FFA + MGDG[e]	Glycolipids	Phospholipids	Surfactant
Flour							
W	109.0	11.9	51.8	17.0	17.3	10.9	0
S	85.7	···	56.0	3.0	5.0	22.0	0
W + S	107.0	10.6	52.1	14.3	14.9	12.3	0
Bread							
Without surfactant (control)							
W	24.0	4.4	17.5	0.6	0.2	1.5	0
W + S	32.8	5.3	24.5	1.0	···	1.7	0
With surfactant							
0.5% EMG							
W	58.6	6.7	29.2	1.1	3.4	5.9	12.4
W + S	40.8	2.9	24.3	0.8	1.5	2.3	8.9
0.5% EMG + 0.25% DMG							
W	66.0	4.4	33.9	1.7	3.3	5.9	17.3
W + S	60.6	3.1	36.7	2.0	2.9	5.5	10.4
0.25% + 0.25% DMG							
W	52.4	4.6	33.4	1.1	2.3	3.3	7.6
W + S	44.6	3.9	34.6	0.7	2.0	2.4	1.1
0.5% SSL							
W	29.0	3.0	21.0	0.3	···	0.8	3.8
W + S	27.4	2.7	21.1	0.2	···	0.6	2.8
LSD value ($\alpha = 0.05$)	3.3	NS[f]	3.0	0.5	1.1	2.1	2.2

[a] W = wheat; S = soy, W + S = wheat/soy (100:12) blend.
[b] EMG = ethoxylated monoglycerides, DMG = distilled monoglycerides, SSL = sodium stearoyl-2-lactylate.
[c] Values are averages of four extractions by petroleum ether (PE) with overall standard deviation of 2.01 mg.
[d] Values obtained by using amounts of total PE-extracts and averages of four determinations on composition (percent) of 50-μg extracts separated by thin-layer chromatography.
[e] FFA = Free fatty acids, MGDG = monogalactosyldiglycerides.
[f] NS = No significant effects of surfactant or soy and their interaction based on analysis of variance by two-way analysis.

for regular bread; the reverse trend was shown for high-protein breads. For the W+S blend, specific loaf volume decreased about 42%. However, loaf volume was restored almost to that of the regular bread control by adding surfactants. The average increase in specific loaf volume of high-protein bread was 61% from the addition of EMG alone or in combination with DMG and 47% from the addition of SSL. A similar improvement was reported by Pomeranz et al (1969a, 1969b), Finney and Shogren (1971), and Tsen et al (1971).

Lipid Binding During Breadmaking

Although the soy flour used in this study was commercially defatted with hexane, petroleum ether extracted significant amount of free total lipids (Table II). Soy flour free lipids contained almost no steryl esters (SE), much less free fatty acids and GL, and more PL that were rich in phosphatidylinositols than did wheat flour lipids; amounts of free glycerides were about the same for both wheat and soy flours. The quantity and composition of free lipids in the W+S blend were similar to those of wheat flour free lipids. Bound total lipids extracted with WSB in soy flour were almost twice the amount of wheat flour bound total lipids, mainly because of polar lipids, especially PL (Table III). The W+S blend contained substantially more bound PL than did wheat flour.

Over half of free total wheat flour lipids were not extractable from optimally mixed dough with petroleum ether as a result of lipid bindings (Chung and Tsen 1975a), and over three fourths of free total wheat flour lipids were no longer extractable from bread with petroleum ether (Table II). Over 90% of GL and PL and two thirds of SE and glycerides (80% of TG) were not extracted from bread with petroleum ether, indicating that those lipid classes had been bound during bread-making stages. Monoglycerides and DGDG were totally absent, and free fatty acids and monogalactosyldiglycerides were nearly absent in bread free lipids. Binding of wheat flour lipids during dough mixing and baking was reported by other researchers (Chiu and Pomeranz 1966, Daniels et al 1966, Mann and Morrison 1974). Free lipids of the W+S blend were also bound during breadmaking to produce high-protein breads (Table II); more free lipids, mainly TG, remained free during breadmaking for the W+S blend than for the wheat flour (Table II).

Amounts of WSB-extractable bound lipids increased during mixing (Chung and Tsen 1975a) and further increased during baking for both the regular and high-protein breads (Table III). Binding of each lipid class except SE increased during breadmaking. Extractability of lipids by petroleum ether and WSB depended on lipid classes; total (free plus bound) PL, mainly lysophosphatidylcholine (LPC), were extracted most from bread samples and least from flour. The results indicated that starch lipids, mostly LPC (Acker and Becker 1971, Morrison et al 1975), were extracted from bread with WSB at room temperature after the starch was gelatinized in bread. Starch lipids, those inside the starch granule, are not extractable from flour with WSB at room temperature but only with hot (90°–100°C) WSB (Acker and Becker 1971, Morrison et al 1975). Extractability of trigalactosyldiglycerides increased from 12 mg/10 g of wheat flour to 36 mg/10 g of bread. Extractability of total (free plus bound) DGDG decreased from 43 mg/10 g of wheat flour to 21 mg/10 g of bread; thus, about half of DGDG became too tightly bound to be extractable with WSB at room temperature.

Effects of Surfactants and Soy Flour on Lipid Binding in Breads

Free Lipids and Surfactants. Total amounts of petroleum-ether extracts (free lipids plus surfactants), free total lipids, and free surfactants were shown by ANOVA to be significantly affected by addition of surfactant or soy flour and by type of surfactant (Fig. 1 and Table II).

For regular breads, significantly more free total lipids were extracted from breads containing nonionic EMG or its combination with DMG than from the control bread (Fig. 1), indicating that the nonionic surfactants displaced some wheat flour bound lipids in breads as they did in doughs (Chung and Tsen 1977). However, no significant differences were found in amount of free total lipids extracted from breads containing EMG or its combination with DMG. The displacement was nonselective

TABLE III
Bound Lipids and Surfactants Extracted from 10 g (db) of Ingredient Flours and Lyophilized Bread

Sample[a] Surfactant[b] Levels (% flour)	Purified Total Extracts[c] by WSB[c] (mg)	Lipid Class[d] (mg/10g, db)					
		Steryl Esters	Glycerides	FFA + MGDG[e]	Glycolipids	Phospholipids	Surfactant
Flour							
W	135.7	20.0	19.6	6.3	37.7	52.0	0
S	290.4	17.0	15.1	18.0	59.0	181.0	0
W + S	154.0	19.8	19.2	7.6	38.8	68.0	0
Bread							
Without surfactant (control)							
W	240.8 a	14.9	60.6	20.3	57.2	88.3	0
W + S	241.0 a	5.5	54.4	18.6	59.9	101.6	0
With surfactant							
0.5% EMG							
W	247.0 a	14.5	36.8	11.2	55.7	79.7	22.1
W + S	272.4 cd	5.4	48.7	13.9	63.5	107.0	25.6
0.5% EMG + 0.25% DMG							
W	232.8 ab	10.9	34.1	13.2	54.4	77.7	38.5
W + S	303.8 e	5.8	48.3	18.1	71.5	119.1	42.1
0.25% EMG + 0.25% DMG							
W	245.7 a	14.5	41.6	12.4	62.3	109.4	28.0
W + S	283.8 d	5.7	53.3	15.6	63.7	111.4	34.5
0.5% SSL							
W	219.0 b	19.2	46.2	12.2	41.0	67.5	25.8
W + S	257.0 ac	2.8	51.2	17.7	56.4	103.2	26.6
LSD value ($\alpha = 0.05$)	13.0	2.9	5.8	4.0	5.1	11.1	3.6

[a] W = wheat, S = soy, W + S = wheat/soy (100:12) blend.
[b] EMG = ethoxylated monoglycerides, DMG = distilled monoglycerides, SSL = sodium stearoyl-2-lactylate.
[c] Values are averages of four extractions by water saturated *n*-butanol (WSB), following petroleum ether extraction, with overall standard deviation of 8.1 mg. The values followed by the same letter are not significantly different at the 0.05 level based on the value of least significant difference.
[d] Values obtained by using amounts of purified total WSB-extracts and averages of four determinations on composition (percent) of 50-μg extracts separated by thin-layer chromatography.
[e] FFA = free fatty acids, MGDG = monogalactosyldiglycerides.

because extractability of each lipid class increased (Table II); the increase in quantity of displaced lipids was highest with glycerides (mainly TG), and the rate of increase was highest with glycolipids (mainly DGDG). Only glycerides were displaced by SSL, and the amounts of lipids from other classes were less than those of the regular bread control. Consequently, the increase in free total lipids extracted from breads containing anionic SSL was not significant (Fig. 1 and Table II). Krog (1975) also reported that surfactants bound to proteins displaced some flour lipids in dough.

For high-protein breads, more free lipids were extracted from bread containing a combination of EMG and DMG, slightly less from bread containing EMG alone, and significantly less from bread containing SSL than from the high-protein bread control (Fig. 1). The increase in free lipids from the high-protein breads containing a combination of EMG and DMG was mainly due to displacement of glycerides and GL, as in the case of regular breads (Table II); EMG alone displaced mainly GL; SSL displaced no lipid class and tended to accelerate binding of each lipid class in high protein bread (Table II).

More free lipids were extracted from the high-protein bread control than from the regular bread control (Fig. 1), indicating that the presence of soy flour suppressed the binding of glycerides (mainly TG, Table II). However, in the presence of surfactants, the soy flour did not suppress lipid binding; the amounts of free lipids extracted were about the same for the regular and soy breads containing SSL or combinations of EMG and DMG. For breads containing EMG alone, less free lipids were extracted from soy bread than from regular bread, indicating that soy flour accelerated flour lipid binding in the presence of 0.5% EMG. Amounts of total petroleum-ether extracts (free lipids plus free surfactants) were significantly less from the high-protein breads than from the regular breads containing EMG or its combination with DMG (Fig. 1, Table II).

Bound Lipids and Surfactants. For regular breads, a smaller amount of bound lipids was extracted from breads containing surfactants than from the control (Fig. 2). Decreases in bound lipids were primarily caused by displacement of glycerides (mainly TG and diglycerides) by the nonionic surfactants on the binding sites (Table III); the amount of total WSB-extractable bound lipids plus bound surfactants, except for those of the bread containing SSL, was about equal to the WSB-extractable bound lipids of the control (Fig. 2, Table III). Amounts of bound GL and PL were significantly lower from the bread containing SSL than from the control (Table III), not because of the higher amounts of free GL and PL from the SSL-bread than from the control (Table II), but because total extractability of those lipid classes decreased in the presence of SSL. SSL was not as effective in displacing the bound flour lipids as were the nonionic EMG and/or DMG but formed a complex that was inextractable with WSB from the bread and similar to the complex in doughs (Chung and Tsen 1977).

In the high-protein breads, the total WSB-extract (bound lipids plus bound surfactants) was significantly higher in each bread containing surfactant than in the control, whereas amounts of bound lipids were about the same from all the breads except that containing a combination of 0.5% EMG and 0.25% DMG (Fig. 2). High-protein breads contained more of each class of bound lipids except SE than did regular breads irrespective of type and amount of surfactants added, although differences in amounts of some classes were not significant for the set of breads containing a combination of 0.25% EMG and 0.25% DMG (Table III). The results indicated that EMG or SSL did not displace lipids in the presence of soy flour because soy protein supplied enough binding sites for both native flour lipids and the added surfactants.

Changes in Protein Extractability During Breadmaking

Although soy flour contained 57.4% and the wheat flour only 15.3% protein (on a dry weight basis), the amount of acetic acid-extractable proteins from soy flour was only half that from wheat flour. Therefore, the protein extractability by 0.05*N* acetic acid was only 8.3% for soy flour, 62.8% for wheat flour, and 45.7% for the blended flour (Table IV). The lower extractability with acid of soy flour proteins than of wheat flour proteins was probably because of the lower isoelectric region of soy proteins (pH 4–5) than of wheat gluten proteins (pH 6–9).

Although extractability of wheat flour proteins increased during dough mixing (Chung and Tsen 1975a), it decreased from 62.8 to 14.4% (a 77% decrease) during baking. Similarly, extractability of proteins from the W+S blend decreased from 45.7 to 9.7% (a 79% decrease) during baking (Table IV). During mixing, the anionic SSL significantly decreased protein extractability, whereas nonionic EMG slightly increased it (Chung and Tsen 1975c, 1975d). During baking, however, both nonionic and anionic surfactants decreased protein extractability of the regular breads, whereas the surfactants had no significant effect on protein extractability of the high-protein breads.

After centrifugation, the acid-insoluble residue of flour or dough consisted of three layers: a top gelatinous layer, a middle tan-colored layer (starch-lipid-protein), and a bottom starch layer. For bread samples, a single tan acid-insoluble residue indicated formation of a complex mass of gelatinized starch, denatured protein, and lipids at elevated temperatures in baking.

Lipids and Surfactants in the Acid-Soluble Fraction

Significantly less lipids were extracted from the acid-soluble fraction of 10-g bread samples than from the corresponding fraction of 10-g flour samples (Table IV). Chung and Tsen (1975a)

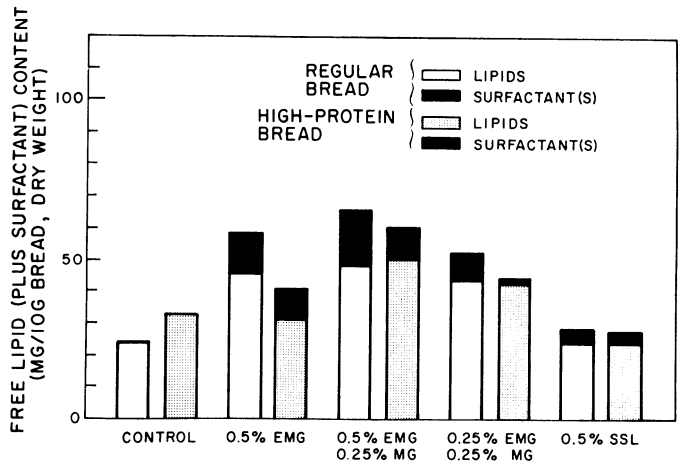

Fig. 1. Free lipid or lipid plus surfactant content (mg) extracted with petroleum ether from 10 g (dry weight) of lyophilized regular bread or high-protein bread. EMG = ethoxylated monoglycerides, MG = distilled monoglycerides, SSL = sodium stearoyl-2-lactylate.

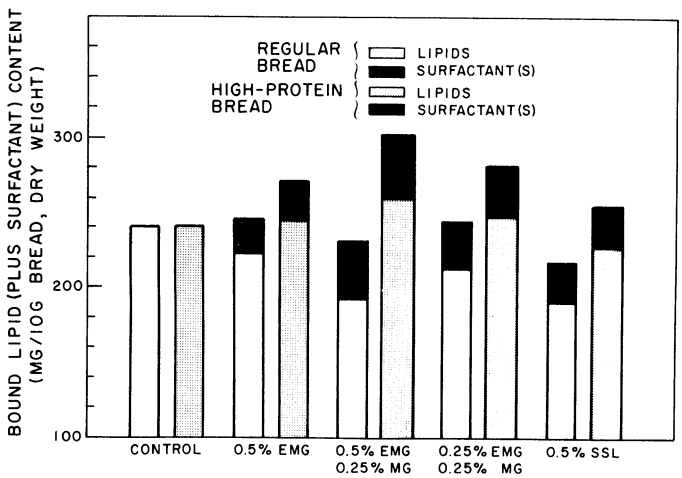

Fig. 2. Bound lipid or lipid plus surfactant content (mg) extracted with water-saturated *n*-butanol (following petroleum ether extraction) from 10 g (dry weight) of lyophilized regular bread or high-protein bread. EMG = ethoxylated monoglycerides, MG = distilled monoglycerides, SSL = sodium stearoyl-2-lactylate.

previously reported that about three times as much lipid was bound to acid-soluble proteins of the optimally mixed dough than to acid-soluble wheat flour proteins. However, about the same amounts of lipids were bound to 1 g of the acid-soluble proteins of both flour and bread (Table IV). Thus, baking appears to decrease the soluble protein-lipid complex, whereas mixing accelerates lipid binding to the soluble proteins. Although effects between EMG and SSL on lipid binding to acid-soluble proteins were significantly different in dough (Chung and Tsen 1975b, 1975c), they were not significantly different in breads. Effects of soy flour on lipid binding were significant, however; the acid-soluble fractions of the soy breads contained less free lipids and more bound surfactants than did the same fractions of the regular breads. The results indicate that surfactants displaced little, if any, wheat flour lipids on the binding sites of the soluble proteins of the breads (Table IV) and that any major surfactant effect probably took place on the acid-insoluble fraction of bread containing a complex mass of gelatinized starch, denatured protein, and lipids.

No measurable amount of any surfactant in free lipids was extracted by petroleum ether from the acid-soluble fraction of the bread. TLC results (not shown) indicated that the free lipids contained only nonpolar components and the bound lipids contained both nonpolar and polar components. Unlike total bound lipids in bread (Table III), the lipids bound to the acid-soluble bread proteins were rich in nonpolar lipids; over 80% of bound lipids for the entire set of regular breads and high-protein control breads were nonpolar, and 63%, on the average, for the high-protein breads containing surfactants were nonpolar. The result indicates that larger proportions of polar lipids than of nonpolar lipids were bound to the acid-insoluble fraction of bread.

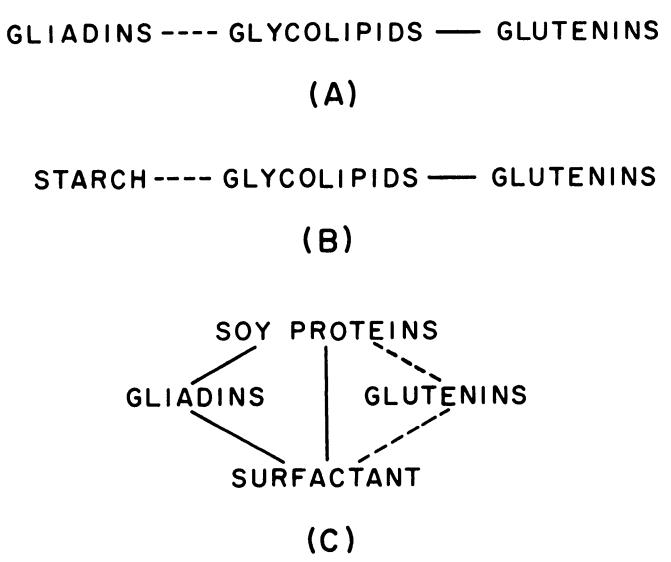

Fig. 3. Models of complexes formed in breadmaking: **A,** proposed by Hoseney et al (1970); **B,** proposed by Wehrli (1969); **C,** proposed by Aidoo (1972). ——— = hydrophobic bond, — — — = hydrophilic bond.

DISCUSSION

Interactions between native flour lipids and proteins have been studied by many researchers during the last two decades. Several models for such information were proposed. They include the starch-lipid-adhesive protein complex in flour by Hess and Mahl (1954), the lipoprotein model in gluten by Grosskreutz (1961), the gliadin-glycolipid-glutenin complex (Fig. 3A) by Hoseney et al (1970), and the starch-glycolipid-gluten complex (Fig. 3B) by Wehrli (1969). More recently Cumming and Tung (1975) reported a lipoprotein complex between free flour lipids and proteins during gluten development and pointed to the importance of the complex formation for protein-protein and protein-starch interactions.

Surfactants interact with gluten proteins to form a major glutenin-surfactant-gliadin complex (Aidoo and Tsen 1973a); similarly, surfactants such as SSL interact with gluten proteins in the Grosskreutz bilayer model (Stutz et al 1973); formation of a gluten-SSL complex was further visualized by scanning electron microscopy (Tu and Tsen 1978). Some surfactants interact with starch to form a water-insoluble helical complex with amylose

TABLE IV
Proteins and Lipids Extracted from the Acid-Soluble Fraction (A) Obtained from 10 g (db) of Ingredient Flours and Lyophilized Bread[a]

Sample[b] Surfactant[c] Levels (% flour)	Protein Extractability (% total protein)	Free Lipids	Bound Lipids and Surfactants (mg)			
			In A of 10-g Sample		In 1 g of Proteins in A of 10-g sample	
			Lipids	Surfactants	Lipids	Surfactants
Flour						
W	62.8	3.1	13.2	0	13.8	0
S	8.3	1.6	4.0	0	8.4	0
W + S	45.7	2.9	12.2	0	13.5	0
Bread						
Without surfactant (control)						
W	14.4	1.5	2.6	0	12.5	0
W + S	9.7	1.0	2.1	0	11.7	0
With surfactant						
0.5% EMG						
W	12.2	1.2	2.3	0.02	12.9	0.11
W + S	9.7	1.2	2.5	0.35	13.2	2.14
0.5% EMG + 0.25% DMG						
W	12.8	1.4	2.6	0.13	13.3	0.70
W + S	10.0	1.2	2.1	0.36	12.5	1.97
0.25% EMG + 0.25% DMG						
W	11.2	1.5	2.2	0.02	13.5	0.12
W + S	9.8	0.5	3.0	0.72	12.7	4.01
0.5% SSL						
W	13.5	1.8	3.1	0.14	15.2	0.72
W + S	9.9	0.5	2.5	0.18	12.7	0.99
LSD value ($\alpha = 0.05$)	0.4	0.2	0.4	0.19	1.9	1.10

[a] Values are averages of four determinations (overall standard deviation: 0.4% for protein extractability, 0.12 mg for free lipids, 0.31 mg for bound lipids in A of 10-g sample, and 1.37 mg for bound lipids per gram of proteins in A.
[b] W = wheat, S = soy, W + S = wheat/soy (100:12) blend.
[c] EMG = ethoxylated monoglycerides, DMG = distilled monoglycerides, SSL = sodium steroyl-2-lactylate.

(Krog 1971), and the ethoxylated surfactants such as EMG or polysorbate 60 form a water-soluble complex with amylose (Kim and Robinson 1979).

When soy flour was blended with wheat flour, soy proteins interacted with gliadin primarily hydrophobically and with glutenin primarily hydrophilically to form a gliadin-soy protein-glutenin complex that exerted deleterious effects on the wheat proteins and impaired their functional properties (Aidoo 1972, Aidoo and Tsen 1973b). In the presence of surfactants, however, the deleterious effects of the above complex were alleviated because both surfactants and soy proteins could react concurrently with gliadin and glutenin to form a complex involving multiple interactions, as shown in Fig. 3C.

Based on the models shown in Fig. 3, previous reports (Chung and Tsen 1975a, 1975b, 1975c, 1975d, 1977), and the present paper, the following models are proposed to explain the improving mechanism of the nonionic EMG and the anionic SSL in the macromolecular bread-making system (Fig. 4). When flour-water dough was optimally mixed and the lyophilized dough was dispersed in 0.05*N* acetic acid and centrifuged into the acid-soluble fraction and the three acid-insoluble fractions, EMG interacted with native flour lipids, mainly with acid-soluble proteins, to form a protein complex (Chung and Tsen 1975c, 1975d), shown in Fig. 4A. EMG accelerated the binding of flour lipids (particularly of GL) to acid-soluble proteins, although EMG displaced some bound lipids in the unfractionated dough (Chung and Tsen 1977) and bread. In the presence of soy flour, more EMG and flour lipids were bound because soy proteins increased binding sites (Fig. 4B).

The anionic SSL accelerated binding of DGDG and PL (mainly LPC) to form a complex of starch-lipids-proteins (Fig. 4C); that complex was not extractable with 0.05*N* acetic acid (Chung and Tsen 1977). Formation of the insoluble complex could be, at least in part, attributed to the large difference in dough stability between SSL-dough and EMG-dough at the 2% level (Chung and Tsen 1975d). The leatherlike texture of bread containing a high level of SSL also could be caused by the excess amount of the above complex formation.[3] At low levels (up to 1%), however, the complexing ability of SSL would benefit the production of high-protein bread because SSL can accommodate soy proteins in a gluten matrix through new associations, as depicted by Fig. 4D.

Accommodations such as shown in Fig. 4B and D presumably can overcome the adverse effects of soy flour and produce acceptable protein-enriched bread. The proposed models, for macromolecular systems such as dough and bread that contain all the flour constituents, might deviate from the other models, which are based on interaction studies on the molecular level or on the macromolecular level only in separate starch or protein systems. The deviation may not be surprising. The proposed models in Fig. 4 apply primarily to dough mixing. The fact is generally accepted that free flour lipids are bound mainly to gluten proteins during dough mixing; the lipids (or surfactants) bound to proteins are translocated and bound mainly to starch during baking (De Stefanis et al 1977; Wehrli and Pomeranz 1970a, 1970b). At the elevated oven temperature, starch is gelatinized and proteins are denatured. Bread proteins differ from flour or dough gluten proteins, and bread starch differs from the native flour or dough starch. Therefore, only with extreme difficulty can a model expressing the multiple interactions among glutenins or gliadins and lipids be applied to a complex mass such as a bread system, which does not contain the original configuration of flour constituents. Nevertheless, surfactants displaced some native lipids bound to the constituents of regular breads. In high-protein breads, nonionic EMG and anionic SSL hardly displaced native bound wheat lipids and rather interacted with the native lipids, presumably due to the sufficient binding sites supplied by soy proteins. To accommodate a foreign protein such as soy protein into the gluten network, native lipids, especially GL and PL, were significantly involved in interactions among wheat proteins and between wheat and soy proteins. Surfactants such as EMG or SSL shared and also enhanced the role of native free polar lipids (present in flour in limited amounts) in the formation of complexes involving multiple interactions.

[3] Unpublished data.

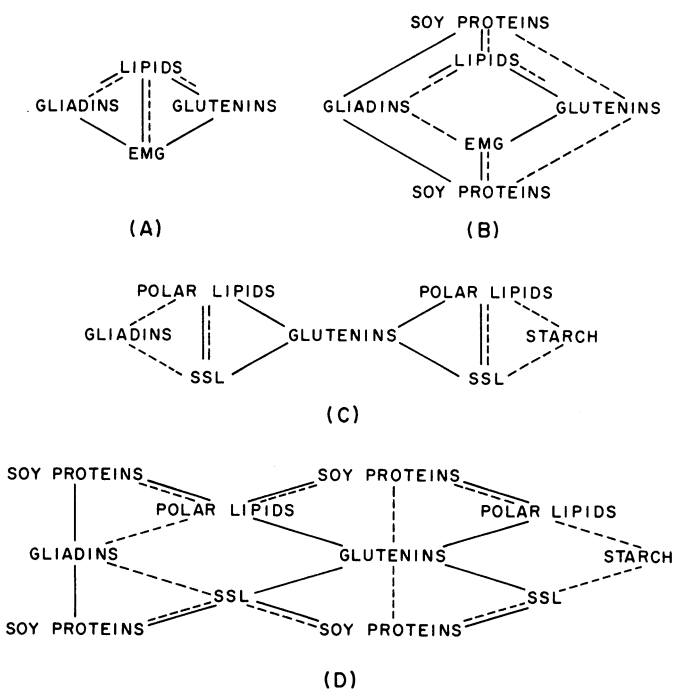

Fig. 4. Proposed models of complexes formed in breadmaking: **A**, ethoxylated monoglycerides (EMG) and lipid binding to wheat flour proteins; **B**, EMG and lipid binding to wheat and soy flour protein; **C**, sodium stearoyl-2-lactylate (SSL) and lipid binding to wheat flour proteins and starch; **D**, SSL and lipid binding to wheat and soy flour proteins and starch. ——— = hydrophobic bond, — — — = hydrophilic bond.

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