DISTRIBUTION OF LIPIDS IN ACID-SOLUBLE PROTEIN COMPONENTS AS AFFECTED BY DOUGH-MIXING AND SURFACTANTS[1]

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ABSTRACT

When dough was mixed to optimum development time on a farinograph and proteins were extracted with 0.05 *N* acetic acid, mixing increased most the acid-extractability of glutenin proteins (I), followed in decreasing order by the nonprotein nitrogenous fraction (IV), the albumin proteins (III), and the gliadin proteins (II). Mixing greatly increased lipids associated with the glutenin fraction. Most lipids were in I, followed by IV, II, and III. The type of lipids associated with proteins depended on the protein components; they were mainly nonpolar in II and III and both polar and nonpolar in I and IV. Less proteins were extracted by acid from the ionic surfactant (sodium and calcium stearoyl-2-lactylate) doughs than from the control dough mainly due to a decrease in acid-soluble glutenin proteins. Less lipids were associated with the acid-extractable protein components of the ionic surfactant doughs than with components of the control dough. Dough mixed with nonionic surfactant ethoxylated monoglycerides contained slightly more I and substantially more lipids associated with I than the control dough.

Lipid-protein interactions in the complex wheat-flour dough system attract increasing interest because both the proteins and lipids govern breadmaking quality of flour. Lipids associated with gluten washed from wheat-flour doughs have been quantitatively measured (1–4), but little is known about lipids associated with acid-soluble proteins of wheat flour and its dough. The amount of lipids bound to acid-soluble proteins has been altered significantly by mixing and by adding surfactants to doughs (5,6). We continued investigations on such interactions by separating the acid-soluble proteins by gel filtration. Quantitative data on proteins and lipid components associated with the proteins should provide a better understanding of biochemical changes which take place during mixing in the presence of surfactants.

MATERIALS AND METHODS

Materials

The flour, surfactants, reference materials, and chemical reagents used in this study were the same as previously described (5,6).

Preparation of Dough Samples

Two sets of dough samples were prepared using two farinographs, one with a 50-g mixing bowl and the other one with a 300-g mixing bowl (6). Control dough and doughs treated with 0.5% sodium stearoyl-2-lactylate (SSL), calcium

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stearoyl-2-lactylate (CSL), or ethoxylated monoglycerides (EMG) were mixed in farinographs to optimum development times, immediately frozen, lyophilized, ground, and stored at −18°C. Moisture content of the lyophilized doughs was determined according to AOAC Method 14.004 (7).

Extraction of Proteins

Proteins were extracted with 0.05*N* acetic acid and lyophilized as previously described (5,6). Nitrogen content was determined by AOAC micro-Kjeldahl method 42.014-42.016 (7), and protein content was calculated using 5.7 as conversion factor.

Gel Filtration of Protein Extracts

The gel filtration procedure of Tsen (8) was used with small modifications: Bio-Gel P-150 (100–200 mesh, Bio-Rad Laboratories, Richmond, Calif.) was used as the gel filtration medium. A suspension was made by adding the dry gel (16 g) into an excess (800 ml) of 0.02*N* sodium acetate buffer of pH 3.8.

Lyophilized acetic acid extracts were redissolved in 0.05*N* acetic acid to give concentrations of 60 mg proteins per 10 ml. The column (2.5×45 cm, Pharmacia Fine Chemicals, Uppsala, Sweden) was attached to a vibration-free support of an Isco Fraction Collector (Model 326) and connected with a buffer reservoir via a metering pump (Beckman Model 746). The absorbance of the column effluent was monitored at 280 nm and recorded by an automatic uv analyzer (LKB). Fractions of the eluant were collected according to positions of elution peaks; four fractions of each sample were lyophilized.

Each sample was chromatographed six times. The fractions obtained from six runs were combined to give four major fractions per sample for further studies such as determining proteins and extracting lipids on a microgram scale.

Extraction of Lipids on Microgram Scale

The method of Pomeranz and Chung (9) was modified slightly. Fractions I, II, and III from the 0.05*N* acetic acid extracts, each containing 25 mg of proteins, were introduced into a tissue homogenizer (inner diameter 1 cm and height 12.5 cm). The close-fitting, ground-glass pestle of the homogenizer was connected to a power stirrer (Wilkins-Anderson Co., Chicago, Ill.). Lipids were extracted three times with 2 ml of chloroform-methanol (2:1, v/v) by repeated downward and upward movements of the pestle, which was rotated at the same time by a power stirrer for 10, 3, and 1 min for the first, second, and third extractions, respectively. The combined extracts were evaporated under reduced pressure after centrifugation at 29,600×*g* for 10 min. The dried extracts were redissolved in petroleum ether. The ether extracts were recentrifuged, evaporated, and dried in vials. Amounts of purified extracts were too small to be weighed precisely with an ordinary analytical balance. The extracts were dissolved in 1 ml of chloroform-methanol (2:1, v/v) and 20 μl of each extract was applied on a thin-layer plate. Fifty milligrams of the fourth fraction (nonprotein) obtained by gel filtration was used for extracting lipids.

Thin-Layer Chromatography (tlc)

Glass plates (20 × 20 cm) were coated with a 250-μm layer of silica gel G and

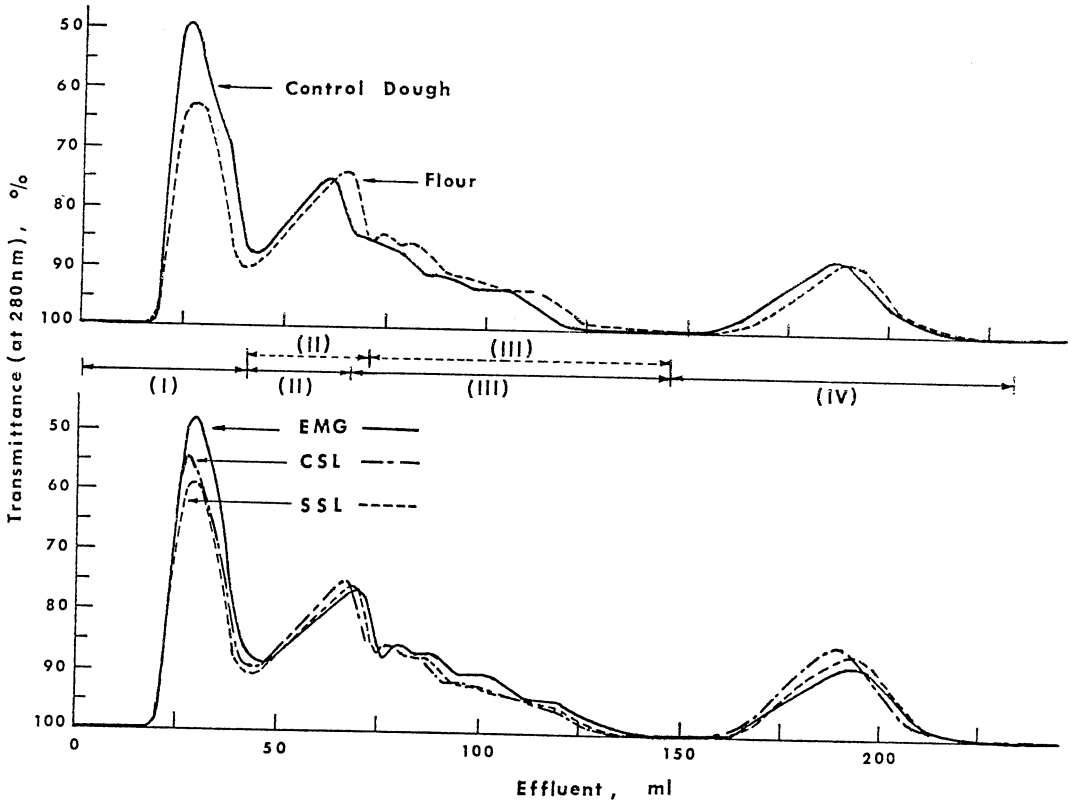

Fig. 1. Gel filtration of 0.05*N* acetic acid extracts of flour, lyophilized wheat flour-water dough, and doughs containing 0.5% sodium stearoyl-2-lactylate (SSL), calcium stearoyl-2-lactylate (CSL), and ethoxylated monoglycerides (EMG), mixed to optimum dough development times on a farinograph.

activated (5). The solvent system, used for one-dimensional ascending development, was a mixture of hexane-diethyl ether-methanol (80:20:1, v/v/v, solvent I) for steryl esters, triglycerides, and diglycerides, and a mixture of chloroform-methanol-water (65:25:4, v/v/v, solvent II) for more polar components including SSL and CSL. The quantitative determination of lipid components and surfactants separated and charred after spraying with a H_2SO_4–$K_2Cr_2O_7$ solution was carried out with 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.) as described previously (5,6). Peak areas of quadruplicated chromatograms were converted to weight by using the least square regression equation of the corresponding standard lipids; variation between plates was corrected by spotting the bound lipids extracted from a lyophilized dough optimally mixed as an internal standard (5).

RESULTS AND DISCUSSION

Distribution of Protein Components

The gel filtration patterns of the acid-soluble proteins obtained from flour and lyophilized doughs are shown in Fig. 1. All samples contained four major uv-absorbing fractions (I, II, III, and IV). The elution profiles are similar to those reported by Tsen (8) and Aidoo (10). Approximate molecular-weight ranges reported by Aidoo (10) were above 160,000 for fraction I, 45,000 to 160,000 for fraction II, 12,400 to 45,000 for fraction III, and 1,450 to 12,400 for fraction IV. Molecular weights of the first three fractions correspond to those of glutenins, gliadins, and albumins; molecular weight of the nonprotein nitrogenous fraction was somewhat higher than reported by Tsen (8) and Meredith and Wren (11). Mixing the dough with or without surfactants affected heights and relative sizes of fraction peaks, but not the elution profile in agreement with Tsen (8,12) and Aidoo (10).

Table I shows the protein distribution in acid extracts computed from the weight and protein content of the fractions. Figure 1 and the upper portion of Table I show distribution of proteins with the total amount of proteins kept constant (60 mg); relative size of peaks II and III decreased because mixing increased peak I. As protein extractability differed among samples, the distribution was also computed as per cent of total proteins in the sample (lower portion of Table I).

More proteins were extracted in the four fractions of the acid-soluble proteins from the control dough than from its flour. Extractable proteins increased most (50%) in the glutenin fraction I, next (24%) in the nonprotein fraction IV, (6%) in the albumin fraction III, and (5%) in the gliadin fraction II. Micro-Kjeldahl-N assays confirmed data of Tsen (8) on the basis of elution patterns determined by absorbance at 280 nm that the increase in the protein extractability during dough-mixing arose mainly from an increase of component I (primarily glutenin), rather than from decrease in the other three components, because the other three fractions also increased during dough-mixing.

Holme reported (13) that wheat-flour proteins consist of 85% gluten (dough-forming proteins) and 15% nongluten (non-dough-forming proteins). Gluten contains about 47% glutenin and 53% gliadin (14) which comprise 40 and 45%,

TABLE I
Protein Distribution in Acetic Acid Extracts of Flour and Lyophilized Doughs (Control, 0.5% SSL, CSL, and EMG)[a] Mixed to Optimum Consistency

Sample	Fraction[b] I	II	III	IV
	% of acid-soluble proteins			
Flour	32.0	48.3	17.2	2.5
Doughs				
Control	40.2	41.9	15.3	2.6
SSL	32.3	46.0	17.2	4.5
CSL	36.1	44.6	15.5	3.8
EMG	43.3	38.0	15.2	3.5

Sample	Fraction[b] I	II	III	IV	Total Extractable Proteins
	% of total proteins				
Flour	21.5	32.5	11.6	1.7	67.3
Doughs					
Control	32.2	33.6	12.3	2.1	80.2
SSL	24.0	34.2	12.8	3.3	74.3
CSL	26.0	32.2	11.2	2.7	72.1
EMG	34.9	30.7	12.3	2.8	80.7

[a]SSL = sodium stearoyl-2-lactylate, CSL = calcium stearoyl-2-lactylate, EMG = ethoxylated monoglycerides.
[b]Fractions I, II, III, and IV contained mainly glutenins, gliadins, albumins, and nitrogenous nonprotein compounds, respectively. Fraction IV was calculated as proteins.

TABLE II
Distribution of Lipids in Acetic Acid-Soluble Protein Extracts from Flour and Lyophilized Doughs (Control, 0.5% SSL, CSL, and EMG)[a] Mixed to Optimum Consistency

Sample	Fraction[b] I		II		III		IV	
	mg[c]	%[d]	mg	%	mg	%	mg	%
Flour	7.5	40.5	4.5	25.5	0.7	3.5	5.7	31.0
Dough								
Control	33.2	42.1	10.1	13.2	4.4	5.8	29.8	38.9
SSL	20.2	52.7	4.4	11.4	1.3	3.2	12.3	32.7
CSL	19.1	34.6	6.1	10.9	3.5	7.8	25.8	46.7
EMG	54.6	56.6	8.6	8.9	1.6	1.7	31.7	32.8

[a]SSL = sodium stearoyl-2-lactylate, CSL = calcium stearoyl-2-lactylate, EMG = ethoxylated monoglycerides.
[b]Fractions I, II, III, and IV contained mainly glutenins, gliadins, albumins, and nitrogenous nonprotein compounds, respectively.
[c]Weight per 10 g of the original material (flour or dough, dry basis).
[d]% of total extracted.

respectively, of total flour proteins. Nongluten proteins consist of about 60% albumin, 40% globulin, and a small amount of peptides and amino acids. Therefore, 0.05*N* acetic acid extracted most of the nongluten protein fractions (III and IV), but only part of the gluten protein components (I and II) from both flour and dough. About one-third of gliadins remained in the acid-unextractable residues of both flour and control dough. About half of glutenins (19% of total proteins) remained in the flour residue and only one-fifth of glutenins (8% of total proteins) were unextracted from control dough. It is evident that dough-mixing resulted in dissociation (disaggregation) mainly of glutenins.

Surfactants affected mainly the glutenin fraction in the proteins extracted with acetic acid. The effects of surfactants on dough are summarized in Table I. The ionic surfactants, SSL and CSL, decreased the acid-extractable glutenin proteins (I) by 26 and 19%, respectively; the nonionic surfactant, EMG, increased fraction I 8% over that in the control dough. Apparently the ionic surfactants retarded disaggregation of the glutenin proteins and the nonionic surfactant enhanced disaggregation during dough-mixing. All three surfactants slightly increased the nonprotein nitrogenous fraction IV.

Lipids Associated with Protein Fractions

The amount of surfactant bound to the unfractionated acid-soluble proteins was 5×10^{-2}, 4×10^{-2}, and 11×10^{-2}% of lyophilized dough sample (dry basis) for SSL, CSL, and EMG, respectively (6). However, the ionic surfactants (SSL and CSL) from the extracts of the acid-soluble protein fractions were not detectable on tlc. The nonionic EMG could not be determined by tlc densitometry and was calculated by difference between the amount applied to the plate and total amount of separated lipid components (6). The amount of EMG associated with the acid-soluble protein fraction could not be calculated because the microgram amount of extract from the protein fraction was not determined gravimetrically.

As shown in Table II, most lipids were in fraction I, followed in a decreasing order by IV, II, and III, except for the CSL dough. Mixing increased relative amounts of lipids associated with protein components six, four, and two times, respectively, for fractions III and IV, I, and II. Absolute amounts of lipids increased during dough-mixing most in I, next in IV, and least in III. The ionic surfactants decreased the lipids associated with all four fractions. The nonionic surfactant increased lipids greatly in I and slightly in IV, but decreased the associated lipids in II and III compared with those from the control dough.

Each lipid component in Tables III and IV is given as percent of the original material (flour or dough, dry basis), because amounts of proteins extracted from flour or dough, of protein components fractionated from acid extracts, and of lipids extracted from protein components varied. Lipids associated with gliadins and albumins from all samples contained mainly nonpolar lipids, polarities of which were equal to, or less than, polarities of free fatty acids (Table III). The associated lipids contained only trace amounts of polar components which could not be measured by tlc (Table IV). The glutenin proteins I and the nonprotein fraction IV contained polar lipids as well as nonpolar lipids. An appreciable amount of lipids in IV could result partially from free lipids in the acid-soluble extracts. A substantially larger amount of steryl esters was extracted from three fractions (I, II, and IV) than from the unfractionated acid-soluble proteins previously reported (6).

Effect of Mixing. Dough-mixing accelerated binding of nonpolar lipids to all acid-soluble protein components (Table III), and of polar lipids mainly to glutenins and slightly to nonproteins (Table IV). Major components which increased during dough-mixing were triglycerides and steryl esters among nonpolar lipids and digalactosyl diglycerides among polar lipids.

Polar lipids might have been bound to glutenins and gliadins, respectively, mainly hydrophobically and hydrophilically in a way similar to that described by Ponte *et al.* (3) and Hoseney *et al.* (4). Nonpolar lipids were, apparently, bound mainly hydrophobically to all the protein components because the acid buffer solution used in fractionating proteins likely would rupture hydrophilic bonds but not hydrophobic bonds.

TABLE III

Nonpolar Lipids[a] (% × 10^2 of Sample, Dry Basis) Associated with Acid-Soluble Protein Fractions (I: Glutenins, II: Gliadins, III: Albumins, and IV: Nitrogenous Nonprotein Compounds)

Source and Protein Fraction		Steryl Esters	Glycerides			FFA + MGDG[b]
			Tri-	Di-	Mono-	
Flour						
I	(4.5)[c]	1.5	1.2	0.9	...	0.9
II	(4.8)	2.6	1.0	0.4	...	0.8
III	(0.6)	0.3	0.2	...	...	0.1
IV	(4.5)	2.6	0.3	...	1.0	0.6
Dough Control						
I	(18.3)	6.1	6.5	2.5	1.7	1.5
II	(9.3)	5.0	3.3	...	...	1.0
III	(4.4)	2.1	1.7	0.4	...	0.2
IV	(25.3)	21.3	1.9	1.3	...	0.8
0.5% SSL						
I	(7.1)	2.4	2.0	0.6	1.2	0.9
II	(4.3)	2.2	1.6	...	...	0.5
III	(2.3)	0.8	1.4	...	...	0.1
IV	(12.4)	9.1	1.7	0.7	...	0.9
0.5% CSL						
I	(8.9)	1.0	3.8	1.2	1.6	1.3
II	(6.1)	3.2	2.0	...	...	0.9
III	(3.4)	1.5	1.0	0.3	...	0.6
IV	(19.3)	13.9	2.6	0.8	...	2.0
0.5% EMG						
I	(27.4)	6.4	7.9	2.6	4.2	6.3
II	(8.5)	3.9	3.5	...	...	1.1
III	(1.6)	0.9	0.7	...	...	...
IV	(24.7)	17.9	5.3	...	...	1.5

[a]Lipid components having polarity equal to or lower than free fatty acids.
[b]FFA = free fatty acids, MGDG = monogalactosyl diglycerides.
[c]The value in parentheses is total sum of nonpolar lipids, expressed as % × 10^2 of sample (dry basis).

In a previous publication (5) we have denoted hydrophobic bonds as — and hydrophilic bonds as ... . During dough development, hydrophilic bonds in the acid-insoluble residue (Glutenins—Polar Lipids...Starch) could have been cleaved by mixing with water and (Glutenins—Polar Lipids...) might have interacted with acid-soluble gliadins to form the "Protein Complex," *i.e.* (Glutenins—Polar Lipids...Gliadins), which were extractable with 0.05*N* acetic acid. Another likely association would be between free lipids and proteins as previously reported (5); free nonpolar lipids were bound to all protein components hydrophobically and free polar lipids would be bound, respectively, to glutenins and gliadins mainly hydrophobically and hydrophilically to form an additional acid-soluble "Protein Complex."

TABLE IV

Polar Lipids[a] (% × 10^2 of Sample, Dry Basis) Associated with Acid-Soluble Protein Fractions (I: Glutenins, II: Gliadins, III: Albumins, and IV: Nitrogenous Nonprotein Compounds)

Source and Protein Fraction		Glycolipids		Phospholipids			
		DGDG	CS	PE	PC	LPC	PS
Flour							
I	(3.0)[b]	1.6	...	1.4	...	...	...
II	(—)	...	...	...	...	...	...
III	(—)	...	...	...	...	...	...
IV	(1.2)	...	0.3	...	...	0.5	0.4
Dough Control							
I	(15.0)	8.7	...	4.2	1.6	...	0.5
II	(0.9)	0.9	...	...	...	...	...
III	(—)	...	...	...	...	...	...
IV	(4.5)	...	1.4	0.5	...	2.1	0.5
0.5% SSL							
I	(13.1)	5.0	2.1	2.0	1.2	1.7	1.1
II	(—)	...	...	...	...	...	...
III	(—)	...	...	...	...	...	...
IV	(—)	...	...	...	...	...	...
0.5% CSL							
I	(10.2)	5.5	0.1	2.6	1.3	0.1	0.6
II	(—)	...	...	...	...	...	...
III	(—)	...	...	...	...	...	...
IV	(6.6)	...	2.1	0.7	...	2.7	1.1
0.5% EMG							
I	(27.3)	15.1	0.6	6.0	2.5	0.6	2.5
II	(—)	...	...	...	...	...	...
III	(—)	...	...	...	...	...	...
IV	(7.0)	...	4.1	0.4	...	2.5	...

[a]DGDG = digalactosyl diglycerides, CS = components containing sugars (sucrose and raffinose), PE = phosphatidyl ethanolamines, PC = phosphatidyl cholines, LPC = lysophosphatidyl cholines, PS = phosphatidyl serines.

[b]The value in parentheses is total sum of polar lipids, expressed as % × 10^2 of sample (dry basis).

Effect of Surfactants. The ionic surfactants reduced binding of most of nonpolar lipid components to all acid-soluble protein components (Table III), and the components with intermediate polarities (digalactosyl diglycerides, phosphatidyl-ethanolamines and -cholines; Table IV). They slightly increased binding of lipid components with higher polarities (lysophosphatidyl cholines, phosphatidyl serines, and sugar-containing components; Table IV). The main difference in effects of SSL and CSL was that in nonprotein fraction IV of the CSL dough, slightly more polar lipids were present than in fraction IV of the control dough, whereas no polar lipids were present in fraction IV of the control dough nor the SSL dough. Because the ionic surfactants decreased both acid-soluble glutenins and their bound lipids, those surfactants possibly interacted with glutenins and lipids to form an acid-insoluble complex.

The nonionic surfactant enhanced binding of both polar and nonpolar lipids to glutenins (Tables III and IV). Galactosyl diglycerides (mono- and di-), free fatty acids, and monoglycerides were affected by EMG more than the other lipid components. The increase in galactolipids bound to glutenins could be significant with regard to the effect exerted by EMG, because galactolipids could simultaneously bind to both glutenins and gliadins to form a "Protein Complex" (4).

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