CHANGES IN LIPID BINDING AND PROTEIN EXTRACTABILITY DURING DOUGH MIXING IN PRESENCE OF SURFACTANTS[1]

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ABSTRACT

Mixing accelerated binding of free flour lipids and of added surfactants: sodium stearoyl 2-lactylate (SSL), calcium stearoyl 2-lactylate (CSL), and ethoxylated monoglycerides (EMG). At each mixing stage (arrival, peak, departure, and twice the departure on a farinograph), less bound lipids were extracted by water saturated *n*-butanol from doughs containing the three surfactants than from control doughs. Both mixing and surfactants at the 0.5% level significantly affected protein extractability as well as the amount of lipids bound to proteins in 0.05*N* acetic acid extracts (A). At each mixing stage, the presence of the ionic surfactants (SSL and CSL) decreased protein extractability and the amount of lipids bound to (A). The nonionic surfactant (EMG), did not decrease, and rather stabilized the acid-soluble proteins in (A) by accelerating the binding of some lipid components (*e.g.*, glycolipids) to (A).

Surface-active agents (surfactants) widely used in the baking industry as "dough conditioners" have received increased attention because of their use to improve high-protein breads (1,2,3,4). In breadmaking, small amounts of glycolipids and their related compounds alleviate the adverse effects of protein-rich foodstuffs used for fortification (5,6).

Previously, we studied the changes in binding and distribution of flour lipids during dough mixing (7). Because surfactants and flour lipids have similar chemical and physical characteristics, conceivably, the added surfactants might affect the interaction between flour lipids and the other wheat flour constituents.

We studied the combined effects of mixing and surfactants on lipid binding in total dough system and in protein extracts. We also studied dough mixing's effect on surfactant binding.

MATERIAL AND METHODS

Materials

Flour sample used for this study was as described previously (7). The surfactants used were sodium stearoyl 2-lactylate (Emplex or SSL), calcium stearoyl 2-lactylate (Verv or CSL), and ethoxylated monoglycerides (EMG) obtained from Patco Products, Kansas City, Missouri. The reference lipids, reference sugars, organic and inorganic solvents and other chemicals used for this study were as described previously (7). The reference surfactants were those already mentioned.

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Preparation of Dough Samples

Two sets of dough samples were prepared using two farinographs; one with 50-g mixing bowl and the other one with 300-g mixing bowl. Differences in water absorption and mixing time required to reach same consistency and mixing stage between two farinographs were within 1% and a half-minute. Water absorptions for optimum consistency of control, 0.5%-SSL, 0.5%-CSL, and 0.5%-EMG doughs were 57.4, 56.7, 57.0, and 56.2%, respectively. Dry powdery SSL or CSL was mixed with wheat flour by hand-shaking for 2 min, in a closed mason jar whereas, semisolid oily EMG was blended with wheat flour in a mortar. They were further blended for 2 min in a farinograph mixing bowl before distilled water was added. Doughs were mixed to various stages on a farinograph as follows:

	Mixing stage			
Dough	*I (Arrival)*	*II (Peak)*	*III (Departure)*	*IV (2 × Departure)*
	min			
Control	1.8	4.3	7.1	14.2
0.5% SSL	1.5	2.5	10.0	20.0
0.5% CSL	1.5	4.0	8.3	16.6
0.5% EMG	2.5	5.5	10.0	20.0

Doughs were frozen immediately, lyophilized, and ground as described previously (7). Moisture content of the lyophilized and ground doughs was determined according to Method 14.004 of Official Methods of Analysis (8).

Extraction of Proteins

The extracting and lyophilizing procedure was described in "Acetic Acid Soluble Fraction" in a companion article (7). Nitrogen content was determined by the micro-Kjeldahl method according to Method 42.014-42.016 of Official Methods of Analysis (8) and protein content was calculated using 5.7 as conversion factor.

Extraction of Lipids and Surfactants

Procedures for extracting free and bound lipids were as described previously (7). Same solvents also extracted surfactants together with lipids.

Thin-Layer Chromatography (TLC)

The 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 more polar components including SSL and CSL.

Quantitative TLC

Lipids were determined as described previously (7). R_f values in solvent system II and color intensity of SSL and CSL charred after spraying with H_2SO_4-$K_2Cr_2O_7$ were the same. Both SSL and CSL presumably contained an impurity which had an R_f value equal to that of free fatty acids. The major active components in SSL and CSL separated on the TLC plate between phosphatidylethanolamines and phosphatidylcholines. In determining the

amounts of SSL and CSL in extracts of the SSL or the CSL doughs, the amounts of free fatty acids associated with those surfactants were allowed for. EMG could not be determined by TLC densitometry as it stayed at the origin or traveled to the solvent front line in solvent systems I and II, respectively. EMG was, therefore, calculated by difference between the amount applied to the plate and total amount of separated lipid components.

RESULTS AND DISCUSSION

For comparing combined effects of mixing and surfactants we included data from the control system reported in a companion report (7).

Changes in Extractability of Lipids and Surfactants

Figures 1 through 3 were obtained from Table I. Amount of petroleum ether (PE)-extract (free) and water saturated n-butanol (WSB) extract (bound) given in Table I was average value of four extracts—duplicated extractions of each set of doughs mixed in farinograph with 50-g and 300-g mixing bowl. Amounts of lipids and surfactants expressed as weight percentage of extract was obtained from average of quadruplicated chromatographs. Amounts of PE extracts (lipids + surfactants) in the surfactant-doughs decreased with increase in mixing

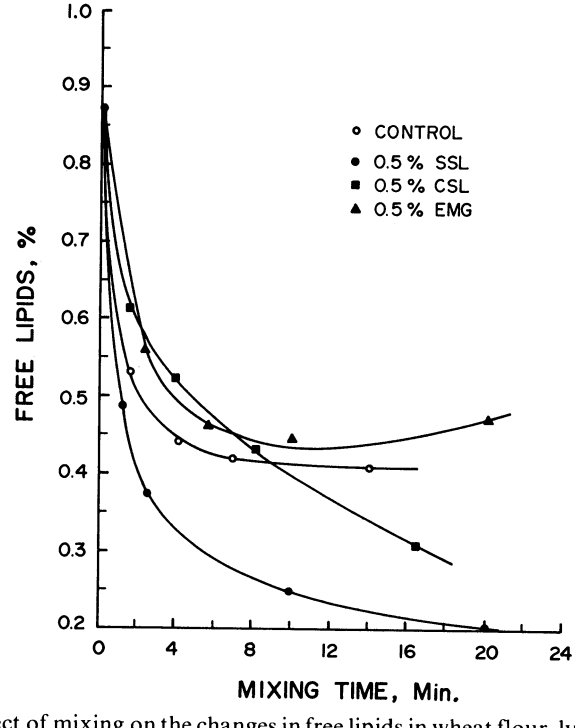

Fig. 1. The effect of mixing on the changes in free lipids in wheat flour, lyophilized control dough, and doughs containing 0.5% surfactants (SSL, CSL, and EMG), expressed as percentage of sample on dry basis.

time. Amounts of WSB extracts increased with increasing mixing time in the SSL and the CSL doughs, and were maximum at optimum mixing time in the EMG dough.

PE extracted less free lipids from the SSL and CSL doughs as mixing time was increased (Fig. 1). Decrease was greater from the SSL dough than from the CSL dough. In the EMG dough system, free lipids decreased rapidly up to optimum dough development stage (5.5 min) and increased slightly after dough was completely broken down. The control and the EMG dough systems show the same general pattern. More free lipids were extracted from the EMG and CSL doughs than from the control dough at all mixing stages except for stage IV (16.6 min) of the CSL dough. At all four mixing stages, less free lipids were extracted from the SSL dough than from the control dough.

Regardless of mixing time, WSB extracted less bound lipids, after PE extraction, from lyophilized doughs containing surfactants than from the control doughs (Fig. 2). The extractable-bound lipids increased as mixing time increased. Overmixing affected the extractability of bound lipids in the nonionic surfactant (EMG) dough system differently than in the ionic surfactants (SSL and CSL) dough systems. Mixing of the control dough and the EMG dough to stage II increased bound lipids; thereafter, bound lipids decreased more in the EMG dough than in the control dough.

As mixing time increased, surfactants were extracted less by PE and more by

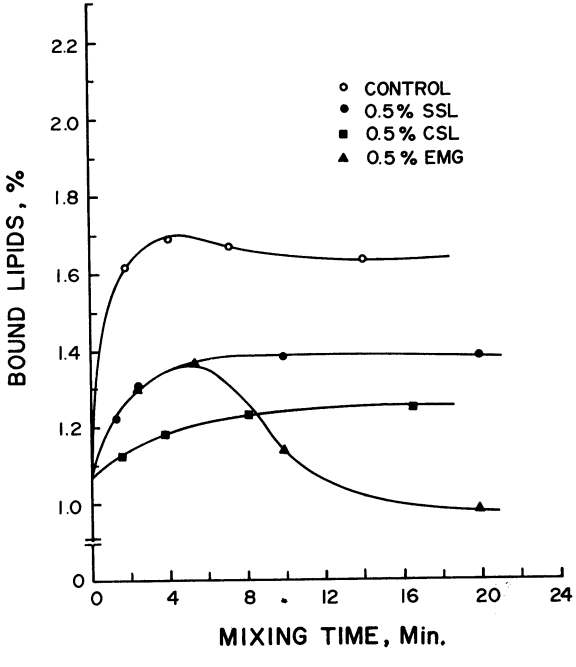

Fig. 2. The effect of mixing on the changes in bound lipids in wheat flour, lyophilized control dough, and doughs containing 0.5% surfactants (SSL, CSL, and EMG), expressed as percentage of sample on dry basis.

WSB (Fig. 3), indicating that the added surfactants were bound to flour constituents during dough mixing. It is interesting to note a difference in binding abilities among surfactants, especially between SSL and CSL. SSL showed the strongest and EMG the weakest binding ability.

Analysis of variance showed that amounts of bound lipids were affected (α= 0.01, data not shown) by the length of mixing, the surfactant (SSL, CSL, and EMG), and their interaction. That analysis indicated that a comparison of mixing stages depended primarily upon which of the surfactants was used.

Composition of Extracted Lipids

In all tables concerning the lipid components, each component is given as in weight per cent of the starting sample (dry basis), rather than per cent of the extracted lipids, because amounts of lipids extracted from samples varied. Tables were obtained using average values of quadruplicated chromatograms.

In the surfactant doughs, all free nonpolar lipids, except diglycerides, decreased with increasing mixing stage (Table II). Changes in free polar lipids (Table III) varied with the surfactants. Free polar lipids were not present in the control dough (7) and also the SSL and CSL doughs contained no detectable amount of polar components, except digalactosyl diglycerides which were absent at later mixing stages of both doughs. In the EMG doughs, digalactosyl diglycerides, phosphatidylethanolamines, and phosphatidylcholines increased

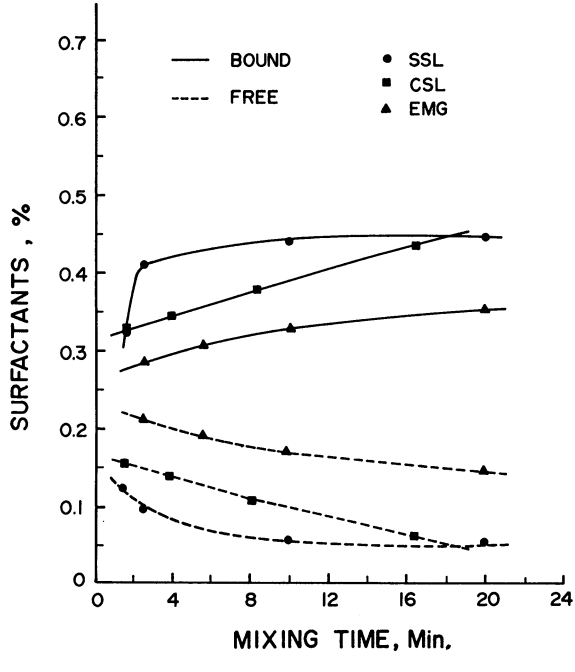

Fig. 3. The amount of surfactants extracted by petroleum ether (free) and water saturated *n*-butanol (bound) in lyophilized doughs containing 0.5% surfactants (SSL, CSL, and EMG) at various mixing times, expressed as percentage of sample on dry basis.

TABLE I
Effect of Mixing on Extractability of Lipids and Surfactants in 5-g Sample[a] (Dry Basis)

Sample and Mixing Stage[b]	Free			Bound		
	Extract mg	Lipids % of Extract	Surf.[c] % of Extract	Extract mg	Lipids % of Extract	Surf.[c] % of Extract
Flour	43.8	100	0	53.3	100	0
Dough with 0.5% SSL added						
I	30.9	79	21	76.3	79	21
II (Optimum)	22.9	80	20	85.4	76	24
III	15.3	82	18	91.3	76	24
IV	13.0	78	22	91.1	76	24
Dough with 0.5% CSL added						
I	38.3	80	20	71.4	77	23
II (Optimum)	33.2	79	21	76.2	78	22
III	27.2	81	19	80.6	77	23
IV	18.5	83	17	83.8	74	26
Dough with 0.5% EMG added						
I	38.9	72	28	79.5	82	18
II (Optimum)	32.7	71	29	84.2	81	19
III	31.3	73	27	73.4	78	22
IV	31.1	77	23	67.4	74	26

[a]Flour and lyophilized doughs.
[b]Arrival (I), peak (II), departure (III), and twice the departure (IV) on a farinograph.
[c]Surfactants.

TABLE II
Free Nonpolar Lipids[a] Affected by Dough Mixing

Sample and Mixing Stage[b]	Steryl Esters	Glycerides			FFA + MGDG[c]
		Tri-	Di-	Mono-	
	% × 10^2 of sample (dry basis)				
Flour	11	31	6	4	15
Dough with 0.5% SSL added					
I	7	20	6	1	12
II (Optimum)	5	17	6	...	7
III	5	8	7	...	5
IV	6	4	7	...	4
Dough with 0.5% CSL added					
I	10	25	9	2	11
II (Optimum)	10	23	8	1	9
III	9	18	10	...	5
IV	8	12	8	...	3
Dough with 0.5% EMG added					
I	9	21	9	2	10
II (Optimum)	8	17	9	2	7
III	7	16	11	2	4
IV	6	16	14	2	3

[a]Lipid components, having polarity equal to or lower than free fatty acids.
[b]Arrival (I), peak (II), departure (III), and twice the departure (IV) on a farinograph.
[c]FFA = free fatty acids; MGDG = monogalactosyl diglycerides.

TABLE III
Free Polar Lipids[a] Affected by Dough Mixing

Sample and Mixing Stage[b]	Glycolipids		Phospholipids			
	DGDG	CS	PE	PC	LPC	PS
	% × 10² of sample (dry basis)					
Flour	16	...	5	...	...	...
Dough with 0.5% SSL added						
I	3	...	...	...	...	...
II (Optimum)	2	...	...	...	...	...
III	...	...	...	...	...	...
IV	...	...	...	...	...	...
Dough with 0.5% CSL added						
I	3	...	...	...	...	...
II (Optimum)	2	...	...	...	...	...
III	3	...	...	...	...	...
IV	...	...	...	...	...	...
Dough with 0.5% EMG added						
I	3	...	...	...	...	...
II (Optimum)	2	...	1	2	...	...
III	2	...	1	3	...	...
IV	3	...	1	3	...	...

[a]DGDG = digalactosyl diglycerides; CS = components containing sugars (sucrose and raffinose) PE = phosphatidylethanolamines; PC = phosphatidylcholines; LPC = lysophosphatidylcholines; PS = phosphatidyl serines.
[b]Arrival (I), peak (II), departure (III), and twice the departure (IV) on a farinograph.

TABLE IV
Bound Nonpolar Lipids[a] Affected by Dough Mixing

Sample and Mixing Stage[b]	Steryl Esters	Glycerides			FFA + MGDG[c]
		Tri-	Di-	Mono-	
	% × 10² of sample (dry basis)				
Flour	15	10	3	3	19
Dough with 0.5% SSL added					
I	5	13	5	5	9
II (Optimum)	6	15	4	6	7
III	6	18	5	5	4
IV	5	16	6	5	3
Dough with 0.5% CSL added					
I	4	9	4	5	12
II (Optimum)	4	13	5	5	10
III	4	18	6	5	8
IV	4	18	8	6	7
Dough with 0.5% EMG added					
I	6	8	4	2	14
II (Optimum)	6	8	5	6	12
III	5	13	6	4	10
IV	4	10	5	4	7

[a]Lipid components, having polarity equal to or lower than free fatty acids.
[b]Arrival (I), peak (II), departure (III), and twice the departure (IV) on a farinograph.
[c]FFA = free fatty acids; MGDG = monogalactosyl diglycerides.

TABLE V
Bound Polar Lipids[a] Affected by Dough Mixing

Sample and Mixing Stage[b]	Glycolipids		Phospholipids			
	DGDG	CS	PE	PC	LPC	PS
	% × 10^2 of sample (dry basis)					
Flour	22	4	6	4	14	6
Dough with 0.5% SSL added						
I	27	14	10	7	21	6
II (Optimum)	25	16	12	10	23	7
III	23	23	12	10	26	6
IV	22	24	10	10	28	9
Dough with 0.5% CSL added						
I	28	12	10	5	16	6
II (Optimum)	26	15	10	5	18	7
III	29	13	11	7	16	6
IV	27	13	11	7	17	7
Dough with 0.5% EMG added						
I	27	18	12	7	23	8
II (Optimum)	28	22	12	7	26	7
III	21	16	11	5	19	5
IV	19	15	9	4	17	5

[a]DGDG = digalactosyl diglycerides; CS = components containing sugars (sucrose and raffinose); PE = phosphatidylethanolamines; PC = phosphatidylcholines; LPC = lysophosphatidylcholines; PS = phosphatidyl serines.
[b]Arrival (I), peak (II), departure (III), and twice the departure (IV) on a farinograph.

TABLE VI
Protein Extractability Affected by Dough Mixing

Sample and Mixing Stage[a]	Acid-Sol. Fraction (A) % of sample[b]	Proteins (N × 5.7) % of (A)	Protein Extractability[c] % of total protein
Flour	12.9	61.7	67.3
Dough with 0.5% SSL added			
I	16.0	54.4	73.9
II (Optimum)	15.9	54.7	74.3
III	16.0	53.0	72.2
IV	14.8	52.4	66.0
Dough with 0.5% CSL added			
I	15.8	54.3	73.2
II (Optimum)	15.9	53.1	72.1
III	16.2	53.5	73.9
IV	17.0	53.0	72.2
Dough with 0.5% EMG added			
I	16.1	57.3	78.5
II (Optimum)	16.1	58.9	80.7
III	16.0	59.5	79.7
IV	15.5	57.8	76.6

[a]Arrival (I), peak (II), departure (III), and twice the departure (IV) on a farinograph.
[b]Flour and lyophilized dough on dry basis.
[c]Protein extractabilities in control doughs were 77.6, 80.2, 74.3, and 72.6% of total proteins at mixing stage I, II, III, and IV, respectively (7). Protein content of lyophilized doughs containing 0.5% surfactants was 11.7% (N × 5.7) on dry basis.

TABLE VII
Effect of Mixing on Lipid and Surfactant Binding in Acid-Soluble Fraction (A)

Sample and Mixing Stage[a]	Free	Bound			Lipids Bound to 1 g Proteins in (A)[c]
	Extract % of (A)[b]	Extract % of (A)[b]	Lipids % of Extract	Surf. % of Extract	mg
Flour	0.2	0.9	100	0	14
Dough with 0.5% SSL added					
I	0.2	2.5	86	14	39
II (Optimum)	0.2	1.8	83	17	26
III	0.1	1.2	82	18	18
IV	0.2	0.8	81	19	13
Dough with 0.5% CSL added					
I	0.2	1.7	88	12	28
II (Optimum)	0.3	1.9	88	12	31
III	0.3	1.7	86	14	28
IV	0.2	1.1	86	14	19
Dough with 0.5% EMG added					
I	1.8	3.5	82	18	50
II (Optimum)	1.7	3.9	82	18	55
III	1.5	3.8	81	19	52
IV	1.6	3.5	79	21	47

[a]Arrival (I), peak (II), departure (III), and twice the departure (IV) on a farinograph.
[b]Expressed as percentage of lyophilized 0.05 *N* acetic acid-soluble fraction.
[c]In control dough, lipids bound to 1 g proteins in (A) were 54, 43, 42, and 38 mg at mixing stage I, II, III, and IV, respectively (7).

TABLE VIII
Surfactants and Nonpolar Lipids[a] Bound to Acid-Soluble Proteins

Sample and Mixing Stage[b]	Surfactant	Steryl Esters	Glycerides			FFA + MGDG[c]
			Tri-	Di-	Mono-	
		% × 10^2 of sample (dry basis)				
Flour		2	2	1	1	1
Dough with 0.5% SSL added						
I	6	2	8	2	2	3
II (Optimum)	5	3	5	2	1	2
III	3	2	3	1	1	1
IV	2	2	3	1	1	1
Dough with 0.5% CSL added						
I	3	2	5	1	2	2
II (Optimum)	4	2	6	2	2	2
III	4	2	5	2	2	2
IV	3	2	4	1	1	1
Dough with 0.5% EMG added						
I	10	3	7	2	2	5
II (Optimum)	11	3	8	3	2	4
III	12	2	8	3	3	3
IV	12	2	6	3	2	3

[a]Lipid components, having polarity equal to or lower than free fatty acids.
[b]Arrival (I), peak (II), departure (III), and twice the departure (IV) on a farinograph.
[c]FFA = free fatty acids; MGDG = monogalactosyl diglycerides.

slightly at later mixing stages. Though less free lipids were extracted from the SSL dough than from the control doughs (Fig. 1), the SSL dough contained more free fatty acids, monogalactosyl and digalactosyl diglycerides than the control dough at same mixing stage. Apparently, those components were partially replaced by SSL. At a given mixing stage, the amount of each component, except steryl esters and triglycerides, was higher in the EMG doughs than in the control dough (7), indicating that EMG could replace various lipid components.

Table IV shows increases in the total of bound glycerides with increasing mixing stages, except for mixing stage IV. Substantially less steryl esters, free fatty acids, and monogalactosyl diglycerides were extracted with WSB after PE-extraction from lyophilized doughs than from flour. It was previously shown (7) that those components in control dough decreased during dough mixing. Decreases in WSB extractability of those components during mixing were more pronounced in the surfactant doughs than in the control dough.

The total of bound phospholipids increased in both SSL and CSL doughs with increasing mixing stages (Table V); they were maximum in the EMG doughs at optimum mixing stage. In the CSL doughs substantially smaller amounts of sugar-containing components, phosphatidylcholines, and lysophosphatidylcholines were extracted using WSB than in the SSL doughs at

TABLE IX
Polar Lipids[a] Bound to Acid-Soluble Proteins

Sample and Mixing Stage[b]	Glycolipids		Phospholipids			
	DGDG	CS	PE	PC	LPC	PS
			% × 10^2 of sample (dry basis)			
Flour	2	...	1	...	1	...
Dough with 0.5% SSL added						
I	7	3	4	1	2	2
II (Optimum)	4	2	2	1	1	1
III	3	1	2	1	1	1
IV	1	...	1	...	...	1
Dough with 0.5% CSL added						
I	5	1	3	1	1	1
II (Optimum)	6	1	3	1	1	1
III	5	1	2	1	1	1
IV	2	1	2	1	1	1
Dough with 0.5% EMG added						
I	13	4	5	2	2	2
II (Optimum)	15	5	5	2	3	2
III	14	5	5	2	2	2
IV	12	4	4	2	2	1

[a]DGDG = digalactosyl diglycerides; CS = components containing sugars (sucrose and raffinose); PE = phosphatidylethanolamines; PC = phosphatidylcholines; LPC = lysophosphatidylcholines; PS = phosphatidyl serines.
[b]Arrival (I), peak (II), departure (III), and twice the departure (IV) on a farinograph.

same mixing stages. Substantial decreases in extractable phospholipids from the CSL doughs could be partially due to Ca^{++} which complexed the soluble proteins that could interact further with phospholipids and stearoyl 2-lactylate to form highly stable, insoluble ternary complexes as described by Fullington (9). Overmixing of the SSL and EMG doughs decreased amounts of glycolipids extracted with WSB, but not amounts of components containing sucrose and raffinose in the SSL dough. The CSL dough did not show significant mixing effects on binding of glycolipids.

Changes in Protein Extractability

Protein extractability in all doughs was affected most by the initial mixing stage (Table VI); the reported values are averages of replicated assays. Protein extractability in the SSL and CSL doughs was lower than in the control doughs at same mixing stages. Compared with control doughs, slightly more proteins were extracted in the EMG doughs at arrival and development stages (I and II) and much more in highly overmixed doughs (III and IV). In the SSL doughs, protein extractability decreased markedly at overmixing indicating strong SSL binding to the acid-insoluble proteins. Farinograph studies showed increases in dough stabilities of the surfactant doughs. Their tolerance to overmixing might have been, at least partly, due to acid-soluble proteins for the EMG doughs and to acid-insoluble proteins for the SSL doughs.

Lipids and Surfactants Bound to Acid-Soluble Proteins

Mixing did not affect amounts of PE extracts (free lipids and free surfactants) in 0.05*N* acetic acid-soluble fractions (A) of the SSL and CSL doughs (Table VII). Values in Table VII were average values of replicated assays. Substantially larger amounts of PE extracts were obtained from (A) of the EMG doughs than from (A) of flour or the SSL and CSL doughs.

Amounts of lipids bound to 1 g acid-soluble proteins increased significantly with initial dough mixing stage in the surfactant doughs as it increased in a control dough. At prolonged mixing stages (III and IV) less lipids were bound to acid-soluble proteins than at initial and optimum mixing stages. At same mixing stages amounts of lipids bound to 1 g acid-soluble proteins were less in the ionic SSL and CSL doughs, and more in the nonionic EMG doughs than in the control doughs.

Mixing time affected differently binding of surfactants to acid-soluble fractions (A) than total dough components shown in Fig. 3 (Table VIII). Though SSL was highly bound during the development stage (II) in the total dough system, the amount of SSL bound to (A) decreased as mixing time increased. This indicated that the longer the mixing time, the greater the SSL binding to the acid-insoluble portion, most likely to starch and insoluble proteins. Whereas CSL binding was linearly related to the mixing time in the total dough system, CSL binding to (A) was not much affected by mixing time. In both systems, EMG binding slightly increased as mixing time increased. About one-third of WSB extractable EMG was bound to (A) at all mixing stages.

As shown in Tables VIII and IX, increase in each lipid component bound to (A) was greatest during the first stage of mixing regardless of surfactant added. Each component bound to (A) decreased with prolonged mixing (stage III and IV); decreases in bound lipid components were shown more pronouncedly in the

SSL and CSL doughs than in the control doughs (7) and the EMG doughs. The EMG dough seemed to be more stable at overmixing stages than a control dough, because acid-soluble proteins and their bound lipids in the EMG dough were less affected by overmixing. The nonionic EMG accelerated binding of most of lipid components, especially both galactolipids and sugar-containing components. EMG seemingly has strong affinity for the sugar moiety of glycolipids, probably because of EMG hydrophilic ethylene oxide molecules and the hydrophilic nature of the sugar moiety in glycolipids.

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