FUNCTIONAL PROPERTIES OF SURFACTANTS IN BREADMAKING. II. COMPOSITION OF LIPIDS ASSOCIATED WITH DOUGHS CONTAINING VARIOUS LEVELS OF SURFACTANTS[1]

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Cereal Chem. 54(4): 857–864

ABSTRACT

In doughs mixed to optimum consistency, anionic sodium stearoyl-2-lactylate (SSL) and calcium stearoyl-2-lactylate (CSL) displaced some digalactosyl diglycerides (DGDG), and substantial amounts of free fatty acids (FFA) together with monogalactosyl diglycerides (MGDG). Nonionic ethoxylated monoglycerides (EMG) displaced some indigenous phosphatidylethanolamines, phosphatidylcholines, DGDG, FFA, and MGDG, but displaced monoglycerides only at high levels (1.0 and 2.0%). Binding of glycolipids and phospholipids to acetic acid-soluble proteins by EMG increased most at the 0.5% level. Both SSL and CSL appeared to accelerate binding of DGDG and phospholipids, mainly lysophosphatidylcholines, to form complexes of starch-lipid-protein, which were not extractable with 0.05*N* acetic acid.

A companion paper (1) reported that surfactants, sodium stearoyl-2-lactylate (SSL), calcium stearoyl-2-lactylate (CSL), and ethoxylated monoglycerides (EMG), suppressed lipid binding to some extent, and also complexed with dough constituents including lipids. During dough mixing, binding of certain classes of lipids to dough fractions (2) and to different protein components (3) varied with different surfactants added at 0.5% of dough on dry basis. Binding of individual lipids likely can be affected by amounts of surfactants added.

We report here effects of types and levels of surfactants on binding and extractability of lipid components associated with unfractionated and separated individual fractions of optimally mixed doughs. Such quantitative data should provide a better understanding of how surfactants function in doughs.

MATERIALS AND METHODS

Flour sample, surfactants, reference materials, and chemical reagents used were the same as previously described (2,4). Preparation of dough samples, fractionation of lyophilized dough with 0.05*N* acetic acid, extraction of lipids and surfactants, and quantitative determination of flour lipid components and surfactants by thin-layer chromatography (tlc) and densitometry were as described previously (1–4).

Because lipid extractability and distribution of dough fractions varied with type and level of surfactant (1), each lipid component is given as a percentage of the starting lyophilized dough sample (dry basis) rather than as a percentage of the extracted lipids. Tables show average values of quadruplicated chromatograms (two extracts, each chromatographed twice). Sum of tri-, di-,

[1]Contribution No. 534-J, Department of Grain Science and Industry, Kansas Agricultural Experiment Station, Manhattan. This work constitutes part of the dissertation submitted by O. K. Chung to the Graduate Faculty of Kansas State University in partial fulfillment of requirements for the Ph.D. degree. Presented at the 58th Annual Meeting, St. Louis, Mo., Nov. 1973.

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and monoglycerides (TG, DG, and MG) were reported as glycerides; free fatty acids (FFA) and monogalactosyl diglycerides (MGDG) were grouped together because of similar R_f values on tlc developed with mixture of chloroform-methanol-water (65:25:4, v/v/v); digalactosyl diglycerides (DGDG) and components containing sucrose and raffinose (CS) with low R_f values near the R_f value of lysophosphatidylcholines (LPC) were reported as glycolipids; and phosphatidylethanolamines (PEA), phosphatidylcholines (PC), LPC, and phosphatidylserines (PS) were reported as total phospholipids. Lipids or surfactants extracted by petroleum ether (PE) were defined as free and those extracted by water saturated 1-butanol (WSB), following PE extraction, as bound.

RESULTS AND DISCUSSION

Composition of Lipids Extracted from Unfractionated Doughs

Some glycolipids (DGDG) and substantial amounts of FFA with MGDG were displaced by anionic SSL and CSL (upper portion of Table I). CSL (2.0% level) also displaced steryl esters (SE) and glycerides (mainly TG). At any level, more free lipids were extracted from the CSL dough than from the SSL dough: 0.42% FFA + MGDG were extracted with PE from the CSL (2.0%) dough but only 0.26% FFA + MGDG from the SSL (2.0%) dough. Therefore, CSL displaced native flour lipids more than SSL did in binding to the other flour constituents during dough mixing. Nonionic EMG (0.5%) displaced some phospholipids (PEA and PC) in addition to DGDG and FFA + MGDG (5). At higher levels (1.0 and 2.0%), EMG also displaced glycerides (MG). In general, EMG displaced more classes of flour lipids than the anionic SSL or CSL did.

Less of each lipid class, bound to unfractionated doughs, was extracted with WSB from the surfactant doughs than from the control dough (lower portion of Table I). The sum of each class of free and bound lipids of the surfactant-doughs was, in general, about equal to or less than the sum of the same lipids extracted from control dough with PE and WSB. One exception was for FFA + MGDG from the doughs containing very high levels of surfactants.

WSB extractability of some lipid classes of the surfactant doughs seems to decrease for two reasons: 1) lipids which were displaced by surfactants in binding to flour-dough components and which were not, thus, involved in interactions with proteins or starch were extracted with PE prior to WSB extraction and consequently not extracted with WSB; and 2) some lipids, presumably, were complexed with dough fractions so tightly in the presence of surfactants that WSB did not extract them. Some lipids and surfactants bound to starch could have been discarded with starch fraction (D) (4). However, DeStefanis *et al.* (6) demonstrated that very little complex was formed between starch and SSL in the presence of proteins between 30° and 50°C, while the complex formation was very rapid above 50°C. Yet, in our dough system at room temperature, total recovered surfactant was considerably less than added surfactants, especially at high treatment levels. Although WSB is considered to be one of the most efficient extractants in maximizing lipid extraction at room temperature (7,8), WSB cannot completely extract lipids. Some residual lipids were found by an acid hydrolysis method, after extracting with WSB (9,10). Most of the lipids not extracted by WSB seem to be located within starch granule (7, 11–13).

SSL and CSL showed different effects on extractability of glycolipids and

phospholipids: 1) extractability of glycolipids (components containing sucrose and raffinose) decreased substantially by 0.25% SSL, from 0.56% (control dough) to 0.39% (0.25% SSL), and then increased with increasing SSL, to 0.59% (2.0% SSL), but decreased with increasing CSL, to 0.28% (2.0% CSL), and 2) less

TABLE I
Lipids (% × 100 of Dough, Dry Basis) in Unfractionated Lyophilized Doughs

Surfactant[a]	Steryl Esters	Glycerides	FFA + MGDG[b]	Glyco-lipids	Phospho-lipids	Surfactant[c]
			Free Lipids			
Control = 0	10	32	2	...	...	0
SSL						
0.25	8	26	5	...	...	3
0.50	5	23	7	2	...	9
1.00	7	22	15	2	...	21
2.00	12	19	26	2	...	44
CSL						
0.25	9	28	9	1	...	6
0.50	10	32	9	2	...	14
1.00	10	34	24	8	...	34
2.00	19	38	42	7	...	80
EMG						
0.25	7	31	7	...	...	10
0.50	8	28	7	2	2	19
1.00	9	41	9	8	7	51
2.00	9	39	33	13	11	110
			Bound Lipids			
Control = 0	7	32	15	56	59	0
SSL						
0.25	5	25	7	39	56	21
0.50	6	25	7	41	52	41
1.00	6	23	5	47	48	65
2.00	4	11	...	59	54	120
CSL						
0.25	6	27	13	41	39	18
0.50	4	23	10	41	40	34
1.00	5	22	10	32	42	44
2.00	5	19	9	28	47	50
EMG						
0.25	6	20	13	56	51	15
0.50	6	18	12	50	52	31
1.00	5	13	7	44	42	32
2.00	6	9	6	38	39	31

[a]SSL = sodium stearoyl-2-lactylate, CSL = calcium stearoyl-2-lactylate, EMG = ethoxylated monoglycerides. Surfactant level was % of dough (dry basis).
[b]FFA = free fatty acids, MGDG = monogalactosyl diglycerides.
[c]See companion paper by O. K. Chung and C. C. Tsen, Cereal Chem. 52: 832 (1975).

TABLE II
Protein and Lipid + Surfactant Contents in Dough Fractions

Surfactant[a]	Fraction[b] % of Dough, db	Protein N × 5.7	Lipids + Surfactant	
			Free	Bound
		% of lyophilized dough fraction		
		Fraction (A)		
Control = 0	15.8	59.7	0.40	2.59
SSL				
0.25	15.2	57.5	0.18	2.20
0.50	15.9	54.7	0.18	1.76
2.00	11.5	41.9	0.07	0.23
CSL				
0.25	15.0	56.3	0.14	2.25
0.50	15.9	53.1	0.26	1.91
2.00	14.8	44.9	0.30	1.25
EMG				
0.25	15.7	60.5	1.13	3.32
0.50	16.0	58.9	1.73	3.88
2.00	15.9	55.4	8.84	2.95
		Fraction (B)		
Control = 0	5.5	20.0	1.73	5.47
SSL				
0.25	4.4	14.6	2.09	3.95
0.50	2.9	15.9	3.72	4.97
2.00	0	...	...	...
CSL				
0.25	4.8	19.5	2.81	4.75
0.50	4.3	19.5	3.47	4.86
2.00	3.0	21.3	6.43	6.13
EMG				
0.25	3.8	26.7	3.55	5.55
0.50	3.7	25.3	3.92	5.68
2.00	3.4	22.0	3.76	4.79
		Fraction (C)		
Control = 0	19.6	5.4	0.46	2.05
SSL				
0.25	39.4	5.7	0.61	1.94
0.50	44.4	5.7	0.90	2.16
2.00	61.1	7.4	1.45	2.44
CSL				
0.25	37.0	6.1	0.77	1.76
0.50	33.6	7.2	1.06	2.16
2.00	34.9	10.0	2.61	3.27
EMG				
0.25	21.8	5.4	0.54	2.18
0.50	23.8	5.4	0.55	1.96
2.00	30.6	5.5	0.39	0.91

[a]SSL = sodium stearoyl-2-lactylate, CSL = calcium stearoyl-2-lactylate, EMG = ethoxylated monoglycerides. Surfactant level was % of dough (dry basis).
[b]See companion paper by O. K. Chung and C. C. Tsen, Cereal Chem. 52: 832 (1975).

phospholipids were extracted with WSB from the CSL doughs than from the SSL doughs, probably because insoluble ternary complexes formed between Ca^{++} and the soluble proteins and phospholipids as described by Fullington (14).

Effect of Surfactants on Changes in Protein and Lipid + Surfactant Contents in Dough Fractions

Data for 1.0% supplementation level of surfactants are not given in Tables II and III: the values lie between the values for 0.5% and 2.0% supplementation level, although they are closer to data at 0.5% than at 2.0%.

For acid-soluble fraction (A), anionic SSL or CSL decreased the amount of fraction (A), protein content, and the free and bound lipid + surfactant contents in fraction (A) (upper portion of Table II). Although free lipid + CSL content was less from fraction (A) of the CSL doughs at all levels than from fraction (A) of the control dough, free lipid + CSL content increased, in general, as CSL increased. Although nonionic EMG had little effect on yield of fraction (A), EMG resulted in slightly decreased protein content and substantially increased free lipid + EMG content. More bound lipids + EMG were obtained from fraction (A) of all EMG doughs than from fraction (A) of the control dough.

The gelatinous fraction (B), top layer of the acetic acid-insoluble residue, comprised only 5.5% of control dough but it was richest in lipids (1.7% free lipids and 5.5% bound lipids) and relatively rich in proteins (middle portion of Table II). As protein was extracted from the lyophilized dough by a single suspension in 0.05*N* acetic acid followed by centrifugation at 30,000 × *g*, some acid-soluble protein portion would have been retained in fraction (B). We assumed equal amount of residual soluble proteins retained in fraction (B) of the control and the surfactant doughs.

Amounts of fraction (B) generally decreased as any of the three surfactants increased, but most with SSL. No fraction (B) was obtained from the SSL-dough at 2.0% SSL. SSL reduced protein content of fraction (B) from 20.0% (control dough) to 14.9%, on average of 0.25, 0.50, and 1.00% SSL. CSL had very little effect (20.2%, on average of all four levels), and EMG increased it (24.9%, on average of all four levels). Substantially more free lipids + surfactants were obtained from fraction (B) of the surfactant doughs than the control dough. Less bound lipids + SSL were obtained from fraction (B) of the SSL doughs (4.68%, on average of three levels) than the control dough (5.47%). About the same amounts of bound lipids + surfactants were obtained from fraction (B) of the CSL doughs (5.29%, on average of all four levels) and the EMG doughs (5.59%, on average of all four levels) as the control dough.

The starch-lipid-protein fraction (C), the tannish middle layer of the acetic acid-insoluble residue, comprised about 20% of control dough. It was rich in starch, relatively poor in proteins (5.4%), and slightly richer in bound lipids (2.05%) than the unfractionated control dough (1.70%). Anionic SSL or CSL substantially increased amounts of fraction (C), protein contents, and amounts of free and bound lipids + surfactants (lower portion of Table II). Nonionic EMG also increased amount of fraction (C), had no effect on amounts of proteins and little effect on amount of free lipids + EMG, but decreased amount of bound lipids + EMG. Fractions (C) of the CSL doughs were richer in proteins than fractions (C) of the SSL doughs, probably because CSL has less complexing ability than SSL with starch.

TABLE III
Lipids (% × 100 of Dough, Dry Basis) Bound to Dough Fractions

Surfactant[a]	Steryl Esters	Glycerides	FFA + MGDG[b]	Glyco-lipids	Phospho-lipids	Surfactant[c]
			Fraction (A)			
Control = 0	3	16	2	11	9	0
SSL						
0.25	3	10	2	8	6	4
0.50	3	7	2	6	6	5
2.00	1	1	1	...	...	1
CSL						
0.25	3	10	4	7	7	3
0.50	2	9	2	7	7	4
2.00	1	4	1	3	3	6
EMG						
0.25	2	12	2	15	11	9
0.50	3	14	4	19	12	10
2.00	2	3	1	16	11	15
			Fraction (B)			
Control = 0	1	2	2	17	8	0
SSL						
0.25	1	2	...	8	3	3
0.50	1	1	...	6	3	3
2.00	0	0	0	0	0	0
CSL						
0.25	1	2	1	11	6	4
0.50	1	2	...	9	5	4
2.00	...	1	...	6	4	7
EMG						
0.25	1	2	1	10	7	...
0.50	1	2	1	10	6	2
2.00	1	1	...	6	6	3
			Fraction (C)			
Control = 0	1	2	2	19	16	0
SSL						
0.25	3	4	5	28	26	10
0.50	14	4	3	28	33	15
2.00	12	2	...	31	57	53
CSL						
0.25	2	4	2	25	26	5
0.50	2	4	1	27	28	10
2.00	18	1	...	17	25	42
EMG						
0.25	2	2	3	19	15	7
0.50	2	3	4	17	15	6
2.00	4	2	3	6	6	7

[a]SSL = sodium stearoyl-2-lactylate, CSL = calcium stearoyl-2-lactylate, EMG = ethoxylated monoglycerides. Surfactant level was % of dough (dry basis).
[b]FFA = free fatty acids, MGDG = monogalactosyl diglycerides.
[c]See companion paper by O. K. Chung and C. C. Tsen, Cereal Chem. 52: 832 (1975).

Composition of Lipids Bound to Dough Fractions

Increasing anionic SSL or CSL decreased each lipid class bound to fraction (A); decreases were more pronounced with SSL than with CSL (upper portion of Table III). CSL bound to fraction (A) slightly increased as CSL supplementation increased; the reverse was true with SSL. On the other hand, nonionic EMG increased binding of glycolipids and phospholipids to fraction (A). Maximum amounts of glycolipids (DGDG, CS, MGDG), FFA, and phospholipids (PEA) were bound to fraction (A) at 0.5% EMG. Substantially less triglycerides were bound to fraction (A) at higher levels than at lower levels of EMG, possibly because EMG displaced some triglycerides.

In fraction (B), glycolipids (both DGDG and CS) substantially decreased as all three surfactants increased; decreases were most pronounced with SSL and slightly less with EMG than with CSL (middle portion of Table III). Phospholipids (mainly PEA and LPC) decreased with anionic SSL and CSL more than with nonionic EMG.

Large amounts of glycolipids (DGDG) and phospholipids (mainly LPC) were associated with fractions (C) of the SSL or CSL doughs (lower portion of Table III). Amounts of extractable bound phospholipids increased with increasing SSL, but varied little as CSL was increased. Perhaps highly stable, insoluble ternary complexes of Ca^{++}, proteins, and phospholipids (14) were formed in fractions (C) of the CSL doughs, so that the complexed portion of phospholipids would not be extractable with WSB.

Nonionic EMG, in general, had little effect on lipid binding in fraction (C) at low levels but at 2.0% substantially decreased glycolipids (DGDG and CS) and phospholipids. Large amounts of steryl esters were extracted from the surfactant doughs, especially the anionic ones. We can offer no explanation for that.

Fractionation of doughs into acid-soluble and -insoluble fractions does not represent 'normal' conditions of dough and perhaps the data presented here could be somewhat distorted by low pH, especially for extraction of lipids from lyophilized fraction (A). The pH value of WSB was about 6.5 whereas pH value of 50 ml WSB + 2 g fraction (A) was about 6.1. Complex formation between lipids or surfactants and other dough constituents such as proteins, starch, and carbohydrates could be dependent on many factors. Starch-SSL complex was affected by both temperature and pH of the system (6).

CONCLUSIONS

In unfractionated dough, the anionic SSL and CSL displaced some glycolipids and substantial amounts of FFA. CSL exerted greater replacing effect than SSL did at all four levels. Nonionic EMG displaced some phospholipids in addition to DGDG, MGDG, and FFA.

SSL and CSL seemed to enhance polar lipid binding (DGDG and phospholipids, mainly LPC) in forming complexes of starch-lipid-protein in fraction (C). Unlike the anionic SSL or CSL, the nonionic EMG appeared to reduce binding of polar lipids in fraction (C) and enhance binding of glycolipids and phospholipids to acetic acid-soluble proteins in fraction (A). Also EMG displaced native flour lipids in binding to proteins in fraction (A). The displacing effects of EMG increased with increasing EMG but binding of flour polar lipids was most enhanced by EMG at 0.5%.

Acknowledgments

We acknowledge financial support from C. J. Patterson Co., Kansas City, Mo., and partial support from Southern Bakers Association through the W. E. Long Merit Award for Excellence in Research to O. K. Chung.

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[Received October 14, 1976. Accepted February 25, 1977]