BINDING OF CRUMB SOFTENERS AND DOUGH STRENGTHENERS DURING BREADMAKING[1]

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ABSTRACT

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Studies were conducted to determine whether preferential binding of crumb softeners and dough strengtheners occurs at various stages of the sponge-and-dough breadmaking process. The additives studied were sodium stearoyl-2-lactylate, succinylated monoglycerides, and monoglycerides. The obtained results indicated that very little binding by the major flour components occurred at the sponge stage. During dough-mixing, the additives were firmly bound by the gluten proteins. In bread, the additive was strongly bound to the starch. To gain some insight on how the translocation occurred during baking, model system studies were conducted between the proteins and the additive as related to temperature. The effect of both temperature and pH on the starch-additive complex formation was also studied. Further studies showed that a crumb softener formed a complex with both the amylose and amylopectin fractions of starch in bread. In addition to the additives, the triglycerides, free fatty acids, and lysolecithin were also found bound to the starch.

Food additives are employed by the baking industry to attain certain desirable properties in the finished product (1,2). For example, crumb softeners such as the monoglycerides (GMS) are used by bakers to produce the soft type of white bread. Other fatty adjuncts such as the ethoxylated GMS are used as dough strengtheners to improve dough processing characteristics. A third group of additives used by the baker seems to exert its effect both as a dough strengthener and as a crumb softener. An example of this group is either sodium stearoyl-2-lactylate (SSL) or succinylated monoglycerides (SMG). After many years of usage, little is really known about how such additives exert their effect in a dough system. Inadequate methodology has been a major obstacle to a clear understanding of the mechanism involved. An additive is often composed of a multicomponent reaction mixture, rather than a single component, thus rendering the analysis very difficult. Furthermore, the additive is usually added to a dough system at levels less than 1%. Further complications arise when the additives need to be measured in the presence of both added fat and native flour lipids.

The principal purpose of this study was to follow the path taken by the additive from the sponge stage to the finished product to obtain some understanding of how this material exerts its effect during breadmaking.

MATERIALS AND METHODS

Commercial baker's patent flour was used in this study. Solvents used were of a reagent grade.

The additives used were SSL, SMG, and GMS, all in granular form.

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Thin-Layer Chromatography (tlc)

A slurry, made up of 30.0 g of silica gel (no binder) (Quantum Industries) + 10.0 g of kieselguhr G (Merck) + 100 ml of distilled water, was deposited on 20× 20-cm glass plates with a Desaga applicator (0.5 mm). The tlc plates were air-dried overnight before use. The following solvent systems were used to separate the additives:

1. SSL: benzene-methanol-acetic acid = 98:1.5:0.5, v/v/v.
2. SMG and GMS: benzene-acetic acid = 85:15, v/v.

Sample Preparation

The additives were added at 0.50% level (flour basis) at the beginning of sponge make-up. Test samples were obtained immediately after the sponge period, at the end of dough-mixing and the final bread. The samples were freeze-dried, then refrigerated until needed for the binding studies. The extent of binding of SSL, SMG, and GMS was determined on the water-solubles, gluten proteins, and starch fraction of each sample.

Quantitative Recovery of the Additive from Bread

One gram of freeze-dried bread (solids), 0.050 g of papain (Worthington), and 0.050 g of a bacterial α-amylase (Wallerstein) were suspended in 10 ml of water (adjusted to pH 6.50), then incubated at 60°–62°C for 15 min, followed by 15 min at 70°–75°C. The enzymatic digest was cooled to ambient temperature, then 10 ml of chloroform was added and thoroughly mixed. All lipids were extracted with the wrist-action shaker for 30 min, followed by centrifugation at 14,000 relative centrifugal force (R.C.F.) for 10 min.

Extraction of the Water-Solubles

Two grams of the test sample was extracted with 20 ml of water at ambient temperature, using the wrist-action shaker for 30 min. The suspension was centrifuged at 14,000 R.C.F. for 10 min. Five milliliters of the supernatant (water-solubles) was withdrawn and transferred into a tube. Five milliliters of chloroform was added and the additive associated with the water-solubles was extracted with the wrist-action shaker. Twenty-five microliters of the lipid extract was analyzed by tlc.

Extraction of the Additive

One gram of either dough or bread solids was transferred to three separate tubes, then treated as follows:

I. *Unreacted and Loosely Bound Additive.* Ten milliliters of chloroform was added to the test samples and extracted for 30 min with the wrist-action shaker, then centrifuged at 14,000 R.C.F. for 10 min. Five microliters (sponge), and 10 μl (other samples) of the extract were analyzed by tlc.

II. *Additive Strongly Bound by the Proteins.* One gram of the test samples and 0.050 g of pepsin were suspended in 10 ml of 0.08*N* H_2SO_4 (pH 1.55–1.75) and incubated at 40°C overnight. Ten milliliters of chloroform was added and the pepsin-released additive was extracted for 30 min with the wrist-action shaker. Centrifugation and tlc analysis were described previously (1).

III. *Additive Strongly Bound by the Starch.* One gram of the test sample and 0.050 g of protease-free bacterial α-amylase were suspended in 10 ml of water

(adjusted to pH 6.50) and incubated at 70°–75°C overnight. Ten milliliters of chloroform was used to extract the additive bound to the starch, as described in II. The amount of additive strongly bound to either the proteins or the starch was determined by subtracting I from either II or III, respectively.

Quantitation of the Additive

Lipids and additives separated by tlc were oxidized with 50% H_2SO_4 for 30 min at 200°C. The indexes, representing various fatty adjuncts, were quantitated with the Photovolt Densitometer, using a response setting of 6 and filter No. 610. Both known concentrations of SSL, SMG, and GMS and unknowns were deposited on the same tlc plate (3).

Degree of Binding as a Function of Mixing

Patent bread flour was mixed in a farinograph with 0.50% SMG at the optimum absorption. At the end of 0.5, 1, 3, 5, and 10 min, the farinograph was stopped and 5.00 g of dough was removed and extracted immediately with 20 ml of chloroform on the shaker.

Effect of Heating on the Binding of the Additive by the Proteins

Gluten and starch were isolated from flour by the Batter process, then freeze-dried. Thirty grams of gluten, 0.15 g of the additive, and 30 ml of water were mixed on the Swanson mixograph for 5 min. The gluten ball was hand-shaped, then heated at 130°C for 15 min. At the end of the heat-treatment, the gluten ball was cooled to ambient temperature and triturated, followed by sequential extraction with 100 ml of chloroform three times, then 100 ml of methanol three times. The strongly bound additive was obtained after digesting the gluten with pepsin (as described in II), followed by chloroform extraction (100 ml, three times).

Effect of Temperature on Starch-Additive Complex Formation

One milliliter of a 0.50% additive solution (chloroform) was transferred to a test tube and evaporated to dryness. Then 2.56 g of wheat starch and 20 ml of the dilute amylograph buffer (pH 5.35) were added to the additive, and thoroughly mixed with a glass stirring rod. A series of tubes was prepared as previously described, then transferred to a water bath. The glass rods were kept in each tube. The temperature of the bath was raised from the ambient to the desired range, *i.e.*, 30°–32°, 40°–42°, 50°–52°, ... 90°–92°C. At each temperature, the test samples were held for 15 min with occasional stirring. At the end of the specified period, the tubes were removed from the water bath and quickly cooled to ambient temperature. Five milliliters of chloroform were added to each tube and the unreacted additive was extracted (30 min) with the shaker, followed by tlc analysis. The bath temperature was raised to the next predetermined range and the same procedure was followed. The samples (separate tubes) were treated in the same manner at each temperature range from 30° to 90°C.

Effect of pH on Starch-Additive Complex Formation

One milliliter of a 0.50% additive solution (chloroform) was evaporated to dryness; 2.56 g of starch and 20 ml of water were intimately mixed. The pH of the starch suspension was adjusted to 3.95, 5.15, 6.20, and 7.35 with either 1 *N* H_2SO_4

or 1*N* KOH. Sample blanks and various tubes were placed in a water bath and slowly heated to 60° C, with occasional stirring. The starch suspension, at various pH values, was kept at 60°–62° C for 15 min; thorough mixing with glass stirring rods followed every 5 min. The tubes were then cooled to ambient temperature.

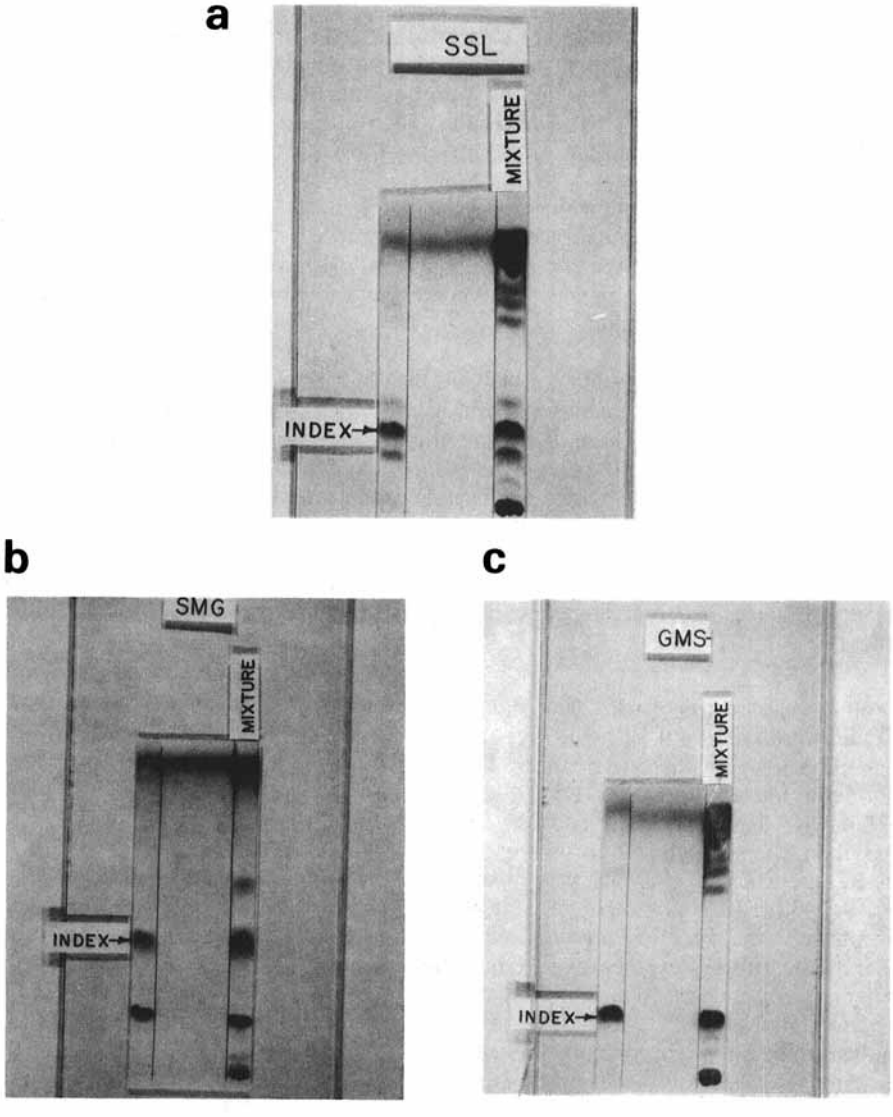

Fig. 1. Separation of a) SSL, b) SMG, and c) GMS indexes. The left lane represents a separation of the key compound from the reaction mixture of each additive. The right lane exhibits the separation of the key compound from a mixture of the additive, the added fat, and the native flour lipids.

Five milliliters of chloroform was added to each tube and the unreacted SSL, SMG, or GMS was extracted with the shaker for 30 min, then analyzed by tlc.

Isolation and Fractionation of Starch from Bread

Five grams of freeze-dried bread (solids) was digested with 0.250 g of pepsin at 40°C (previously described). The sample was extracted three times with 25 ml of chloroform, followed by several washings with acetone, then air-dried. The sample was then suspended in 200 ml of 0.10*N* ammonium hydroxide, heated to 90°–92°C, and maintained at this temperature for 15 min. The test sample was quickly cooled to ambient temperature, then centrifuged at 14,000 R.C.F. for 10

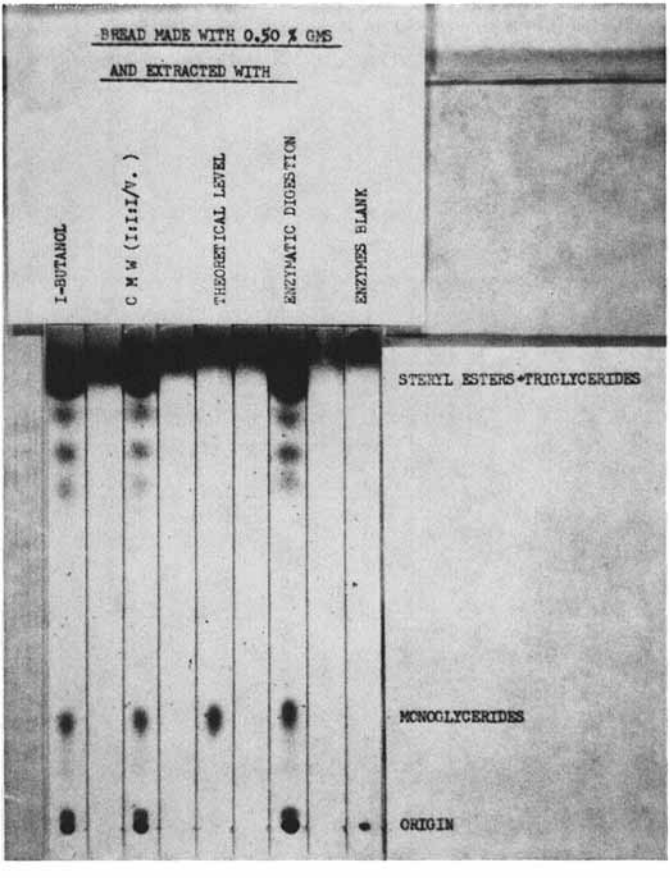

Fig. 2a. Illustration of the adopted extraction procedure of the additive from bread. The extreme left lane represents GMS extracted with 1-butanol. Lane 2 is GMS extracted with chloroform-methanol-water (1:1:1, v/v/v). Lane 3 illustrates the actual level (theoretical) added in the sponge. Lane 4 shows a quantitative recovery of GMS after predigestion of bread with both papain and bacterial α-amylase. The extreme right lane shows the lipids associated with the enzymes.

min. Five separate extractions were made on the residue to a total volume of 1000 ml. The obtained supernatant was rich in amylose, while the residue was rich in amylopectin. The amylopectin was also suspended in 1000 ml of 0.10*N* ammonium hydroxide. Both the amylose and amylopectin suspensions were adjusted to pH 6.00–7.00, then 0.100 g of bacterial α-amylase was added, thoroughly mixed, and digestion allowed to follow at 70°–75°C, overnight. Each suspension was transferred to a separatory funnel. Total lipids associated with the amylose and amylopectin fractions of starch (bread) were extracted three times with 500 ml of chloroform. The chloroform extract was filtered through Whatman No. 1 filter paper, then evaporated to dryness with the Flash Evaporator at less than 40°C. The obtained lipids were dissolved in chloroform, then analyzed by tlc.

Effect of Temperature on Binding of SSL in a Gluten + Starch System

A blend of 17.9 g of freeze-dried starch, 2.7 g of gluten (freeze-dried), and 0.103

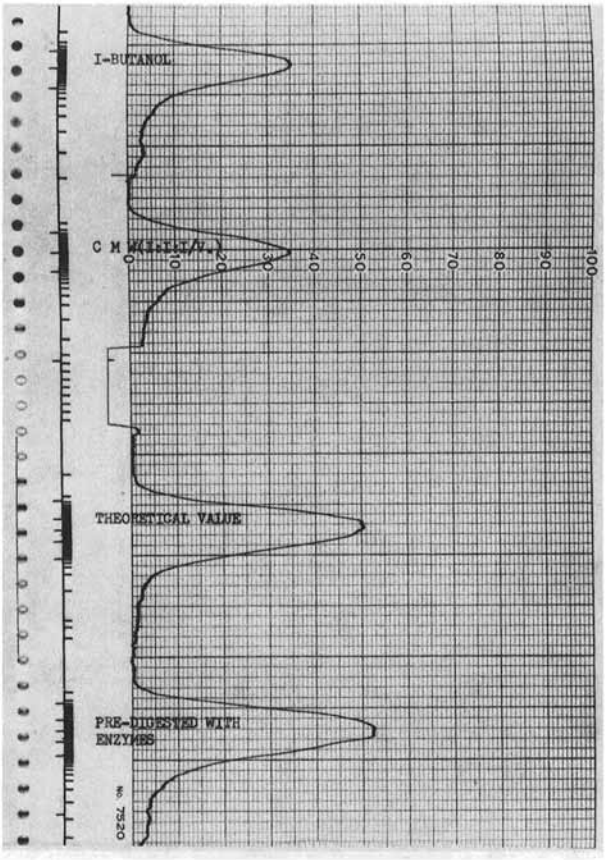

Fig. 2b. Densitometric scan of GMS spots.

TABLE I
Recovery of Additive from Bread

Additive	Theoretical Value μg	Obtained Value μg[a]	Recovery %	Standard Deviation	Coefficient of Variation
GMS	10.00	10.02	100.4	0.18	3.57

[a]Mean of quadruplicate determination obtained on different days.

TABLE II
% Additive Bound to Dough and Bread Components at Various Stages of Breadmaking[a]

Stage of Breadmaking	Unreacted and Loosely Bound	Water-Solubles	Proteins	Starch
SSL— Sponge	92.7	5.4	1.9	0
Dough	38.4	8.8	50.6	2.2
Bread	13.7	0	14.5	71.8
SMG—Sponge	92.3	7.4	0.3	0
Dough	29.8	7.8	56.6	5.8
Bread	28.4	0	10.3	61.3
GMS—Sponge	92.1	7.9	0	0
Dough	46.8	0	46.6	6.6
Bread	39.2	0	14.4	46.4

[a]Mean of triplicate determinations.

TABLE III
% Additive Released from Gluten after Heating at 130°C for 15 min[a]

Additive	Chloroform Extractables	Methanol Extractables	Chloroform Extractables after Protease Digestion
SSL	20.0	31.2	48.8
SMG	45.8	47.9	6.3
GMS	96.3	3.7	0

[a]Mean of triplicate determinations.

TABLE IV
% Additive Released by Gluten after Various Heating Periods at 130°C[a]

Heat Treatment at 130°C min	Chloroform Extractables		
	SSL	SMG	GMS
0	5.0	6.2	14.8
5	20.0	20.8	71.6
15	20.0	45.8	96.3
60	33.8	91.7	100.0

[a]Mean of triplicate determinations.

g (0.50%) of SSL was transferred to a Swanson mixograph. Twelve milliliters of dilute amylograph buffer (pH 5.31) was added to the blend and mixed for 5 min. One gram of dough was transferred to two sets of tubes, which contained 20 ml of the dilute amylograph buffer (pH 5.31). Glass stirring rods were used to ensure that the dough balls would move freely in water. Both sets of tubes were transferred to a water bath, then heated to 30°–32° C. Thereafter, the tubes were treated as described previously from 30° to 90° C. When all the samples were cooled to ambient temperature, the pH was lowered to 1.72 with 1 *N* H_2SO_4 (both sets). To one set of tubes, 0.050 g of pepsin was added, while no enzyme was added to the other set. Both sets were placed in a water bath at 40° C (overnight). Five milliliters of chloroform was added to each tube and extracted for 30 min with the shaker. The extracted surfactant was quantitated by tlc. The portion of the surfactant associated with the starch was obtained by difference. Pepsin + surfactant and starch + gluten (no additive) blanks were also run alongside the test samples.

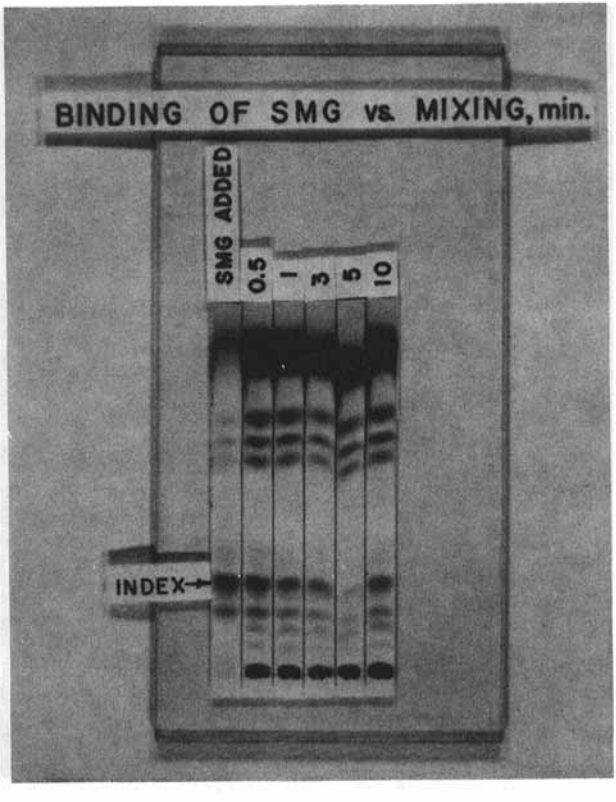

Fig. 3. Binding of SMG as a function of mixing time. Patent bread flour and 0.50% SMG were mixed in a farinograph at the optimum absorption. Dough samples were extracted with chloroform after 0.5, 1, 3, 5, and 10 min. The extractable SMG was analyzed by tlc.

RESULTS AND DISCUSSION

The tlc separations of the SSL, SMG, and GMS indexes are illustrated in Figs. 1a, b, and c. On each tlc plate, the left lane shows the separation of each additive. The right lane exhibits a separation of the key compound (index) from the added fat and the native flour lipids.

The tlc analysis of additives in complex lipid systems is easily carried out without difficulty; however, when either a dough or bread is analyzed, the procedure needs special attention. It is well known that lipids become increasingly bound during breadmaking, particularly the dough strengtheners and the crumb softeners. Since quantitative recovery of SSL, SMG, and GMS is crucial in this type of study, a discussion of the adopted extraction procedure is presented in Fig. 2a. The lipids shown in Fig. 2a are crude bread extracts. The extreme left lane shows the level of GMS recovered from bread when extracted with saturated 1-butanol. Similarly, in lane 2 chloroform-methanol-water (1:1:1, v/v/v) was used as the extractant. Lane 3 shows the actual level (theoretical) added in the sponge. It is obvious from Fig. 2a that organic solvents do not quantitatively extract GMS from bread. Since SSL, SMG, and GMS strongly

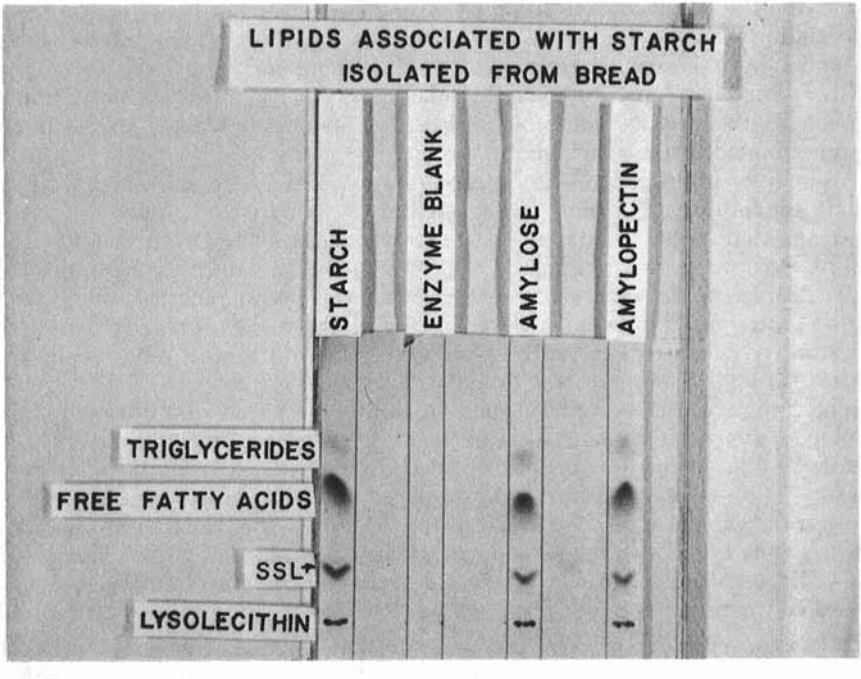

Fig. 4. Lipids bound to the starch component of bread. Freeze-dried bread was digested with pepsin, then extracted with chloroform. The residual starch was fractionated into amylose and amylopectin with 0.10*N* ammonium hydroxide (90°–92°C). The obtained starch fractions were digested with a protease free bacterial α-amylase to release all firmly bound lipids.

interact with the proteins and the starch components, it was thought that predigesting the bread with both a protease (papain) and an amylase (bacterial α-amylase), followed by solvent extraction, should lead to quantitative recovery of the additive from bread. Results obtained from this study are shown in lane 4. It is obvious from both the spot density and the densitometric scan (Fig. 2b) that quantitative recovery of GMS was attained. The lane on the extreme right represents the enzyme blank. Table I demonstrates the feasibility of the enzyme treatment of bread made with 0.50% GMS. Predigestion of a baked product with a protease and an amylase offers the researcher an excellent way to recover quantitatively all lipids associated with either a dough or baked products.

After having ascertained that the additives could be quantitatively recovered from the test system, a study was conducted to determine the extent of binding of SSL, SMG, and GMS by the water-solubles, proteins, and starch components of flour, at various stages of the sponge dough process. Results of this study appear in Table II. It is evident that the dough strengtheners, as well as the crumb softener, did not associate with the flour components at the sponge stage. After dough-mixing (dough stage), SSL, SMG, and GMS formed a strong bond with the proteins.

In bread, the additive was found strongly bound to the starch, rather than to the proteins.

The data in Table II suggest that, in the sponge dough process, very little starch-additive complexing took place from the beginning of sponge make-up to the end of dough-mixing. However, at baking temperatures, SSL, SMG, and GMS formed a strong complex with the starch. Results further show that a portion of the original amount employed remained unreacted or relatively loosely bonded to the flour components.

A closer examination of the data reported in Table II reveals that the first significant indication of binding occurred at the dough stage. Figure 3 further illustrates that the extent of binding was largely dependent on the time of dough-mixing. As mixing time increased to optimum, the extractability of the unreacted SMG decreased. This pattern suggests that SMG became increasingly bound during gluten development; however, as the dough was overmixed, binding of the additive decreased. Recently, Chung and Tsen (4,5) also reported on the changes in lipid binding during dough-mixing.

If a crumb softener becomes intimately bound by the proteins during dough-mixing, a question arises as to how the additive exerts its softening effect in bread. To understand how the translocation of the additive from the proteins to the starch occurs, it is necessary to know the effect of temperature on the protein-additive bonds. Table III describes this study. The data indicate that SSL formed a very strong bond with the proteins, while GMS formed a relatively weak one. Also, 48.8% of the original level of SSL used and 6.3% SMG were very firmly bound to the proteins.

Data appearing in Table IV demonstrate that the extent of heat-treatment influenced the amount of additive released by the proteins. Results indicate that SSL, SMG, and GMS must have occupied different bonding sites during dough-mixing. Again, SSL formed a strong bond while GMS did not. These data are in agreement with baking experience, where SSL behaves as a dough strengthener while GMS does not.

To further appreciate how the translocation occurs during baking, it is

necessary to study the rate of complex formation between the starch and the additive as a function of temperature. Data obtained from this study appear in Table V. Results show that little complexing took place between 30° and 50°C. Above 50°C, starch-additive complex formation was increasingly rapid. It is important to note that complex formation is also pH-dependent. Results appearing in Table VI show that SSL and SMG formed a complex with the starch at a faster rate in an alkaline medium.

TABLE V
Effect of Temperature on Starch-Additive Complex Formation

Reaction Temperature °C	% Additive Bound (pH 5.35)[a]		
	SSL	SMG	GMS
30	8.1	0	0
40	35.1	0	0
50	40.6	10.0	12.0
60	45.9	70.0	12.0
70	62.2	100.0	72.0
80	94.6	100.0	83.1
90	100.0	100.0	92.0

[a]Mean of triplicate determinations.

TABLE VI
Effect of pH on Starch-Additive Complex Formation

pH	% Additive Bound (15 min at 60°C)[a]		
	SSL	SMG	GMS
3.95	0	50.0	0
5.15	50.0	68.7	9.5
6.20	75.0	81.2	9.5
7.35	87.5	87.5	9.5

[a]Mean of triplicate determinations.

TABLE VII
Effect of Temperature on Binding of SSL in a Gluten + Starch System

Temperature °C	Unreacted and Loosely Bound %	Gluten %	Starch %
30	22.0	64.0	14.0
40	20.0	66.0	14.0
50	22.0	62.0	16.0
60	20.0	6.0	74.0
70	16.0	6.0	78.0
80	12.0	8.0	80.0
90	12.0	2.0	86.0

Previous studies were conducted with simple model systems consisting of either starch or proteins and the additive; the question is whether the same patterns would be obtained if both the proteins and starch were concurrently present in the test system. Table VII shows that very little SSL formed a complex with the starch between 30° and 50°C, while formation was very rapid above 50°C. The opposite trend is observed for the proteins. Data obtained from various model system studies and from bread strongly suggest that the additive (SSL, SMG, or GMS) tends to remain either unreacted or loosely bound to the flour components during the sponge period. At the dough stage, the additive becomes strongly bound to the gluten proteins. During baking, two concurrent phenomena occur: a) the bonds between the gluten proteins and the additive become increasingly weak (protein denaturation) as the dough temperature increases; and b) as starch gelatinizes above 50°C, the weakly bonded (proteins) additive readily forms a strong complex with the former, thus allowing a translocation to occur from the proteins to the starch. It would seem that the relative effectiveness of SSL, SMG, or GMS as a crumb softener is largely dependent upon how well the additive is dispersed in a dough system before starch gelatinization takes place.

The additive's ability to produce a softer bread has long been ascribed to its capacity to complex with the starch component of flour (6–8). In general, most of the reported studies were conducted with model systems, often consisting of amylose and additive only. Whether the same complex is formed in bread has not been clearly shown. To further our understanding in this matter, the starch fraction of bread was separated into amylose and amylopectin and then examined by tlc to determine what lipid classes became strongly bound to these fractions. A qualitative illustration of the lipids obtained is shown in Fig. 4. It is evident that during baking, SSL not only complexed with the amylose but also with the amylopectin. In addition to SSL, the other lipid classes associated with the starch component of bread were triglycerides, free fatty acids, and lysolecithin.

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