EFFECTS OF MIXING AND SURFACTANTS ON MICROSCOPIC STRUCTURE OF WHEAT GLUTENIN[1,2]

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ABSTRACT

Glutenin was extracted with 0.1*M* acetic acid, 3.0*M* urea, and 0.01*M* cetyltrimethyl ammonium bromide and later purified with SE-Sephadex C-50. Scanning electron microscopy data showed that the structure of glutenin that was isolated from flour differed from that of glutenin from mixed dough. With mixing, glutenin could be stretched and spread to form a sheet-like structure. Further mixing could reduce the size of the sheet-like structure. Glutenin appeared to bind with surfactants and form glutenin-surfactant complexes. The size of the complexes was smaller when the glutenin that was used for the interaction was isolated from flour or overmixed dough than when it was isolated from optimum mixed dough. Between the surfactants tested, sodium stearoyl-2 lactylate was more effective with glutenin than was sucrose monopalmitate-forming complexes.

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Mixing property of dough is one of the most important factors in assessing flour quality. Its importance increased with the introduction of the continuous mix processes, which develop doughs primarily by high-speed mixing.

Mecham *et al.* (1–3) found that protein extracted from dough by dilute acetic acid increased with extended mixing. Rates of increase varied with flours with different mixing characteristics. To explain the increase in extractability, they postulated that mixing decreased the size of protein aggregates in flour, which Tsen (4,5) later confirmed. He reported that dough mixing significantly altered the amount of flour protein that is extractable with 0.01*N* acetic acid and the distribution of protein components in the extract. The structural change in protein, however, particularly glutenin, has not been fully explored.

Surfactants have been widely used as dough conditioners in the baking industry to improve baking performance. Recently, surfactants have received more attention, because they can improve the quality of protein-fortified baked products by alleviating adverse effects of protein-rich additives on dough properties and baking quality (6–8). Sodium stearoyl-2 lactylate (SSL), an ionic surfactant, reportedly can increase mixing tolerance of dough to permit production of uniform, high-quality baked products over a wide range of processing and ingredient variations. SSL can increase the stability of dough containing 12 to 28% soy flour. It can also help stored breads to retain softness, and spare or replace shortening that is normally required in white bread or bread containing 12% soy flour (9,10). Sucrose monopalmitate (SMP) is nonionic (11). It has improving action in regular and high-protein breads despite SMP's not markedly affecting dough stability. The mechanism of action of the two surfactants with different ionic natures on glutenin during dough mixing is still obscure.

Recently Orth *et al.* (12,13) successfully used a scanning electron microscope

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(SEM) to study the structure of glutenin. Glutenin, a major component of wheat protein, is responsible in large part for dough-mixing properties and baking quality.

The purpose of this study was to examine structural changes of glutenin during mixing to provide some insight concerning the functionality of glutenin, and also to use SEM to study effects of SSL and SMP on structural changes of glutenin to understand better the improving action of surfactants.

MATERIALS AND METHODS

Materials

In this study, a hard red winter wheat (Bison), a soft red winter wheat (Logan), and two surfactants (SSL and SMP) were used. The wheats were milled on a Miag Multomat mill. The Bison flour contained 11.5% protein (N × 5.7 on a 14% moisture basis), 0.6% crude fat, and 0.41% ash. The Logan flour contained 10.5% protein, 0.8% crude fat, and 0.36% ash. SSL was obtained from the C. J. Patterson Company, Kansas City, MO, and SMP from Dai-Nippon Sugar Manufacturing Company, Ltd., Tokyo.

Sample Preparation

Dough samples were mixed from flour and water with or without 0.5% surfactant in a farinograph (50-g bowl) at 60 rpm and 30°C according to the constant-dough weight method (14). Their farinograph characteristics are presented in Table I to show the effect of the surfactants on dough properties.

The procedure for preparing glutenin samples from doughs was essentially that of Orth and Bushuk (15). Dough prepared from 50 g of flour was washed under a gentle stream of distilled water until a gluten ball was obtained. The gluten ball was dispersed and dissolved in 200 ml of AUC solvent (0.1*M* acetic acid, 3.0*M* urea, 0.01*M* cetyltrimethyl ammonium bromide) by overnight magnetic stirring. The solution was centrifuged at 20,000 × *g* for 30 min and the supernatant was made 70% (v/v) in ethanol and adjusted to pH 6.4 by the dropwise addition of 1.0*N* NaOH. The resulting precipitate was allowed to settle

TABLE I
Farinograph Characteristics of Dough Prepared From Wheat Flour With or Without Surfactants

Flour	Surfactant	Water Absorption (%)	Arrival Time (min)	Peak Time (min)	Departure Time (min)	Dough Stability (min)	Mixing Tolerance Index (BU)
Bison							
	Control	58.2	2.0	8.0	17.0	15.0	30
	SSL	58.4	2.0	9.5	21.0	19.0	4
	SMP	59.6	2.0	7.5	17.5	15.5	25
Logan							
	Control	56.4	0.5	1.0	5.0	4.5	86
	SSL	56.5	0.5	1.25	4.5	4.0	64
	SMP	55.8	0.75	1.5	5.0	4.25	90

overnight and separated by centrifugation (20,000 × *g* for 30 min). The precipitate (crude glutenin) was dispersed in 0.01*M* acetic acid, dialyzed against distilled water for five days, and freeze-dried. The freeze-dried glutenin was dispersed by overnight magnetic stirring in 250 times its weight of AUC solvent containing half its weight of SE-Sephadex C-50, and centrifuged at 20,000 × *g* for 30 min. The supernatant was dialyzed against distilled water for five days and freeze-dried to yield purified glutenin. The fractionation and purification were all conducted in a cold room at 4° C (15,16).

To prepare flour glutenin, 50 g of flour was first wetted with water (the amount of water used was according to the flour's absorption—29.1 ml for Bison flour and 28.2 ml for Logan flour), then dispersed with 200 ml of AUC solvent, and finally purified by the above-mentioned procedure.

SEM

For studying the effect of surfactants on the glutenin structure, 0.25 mg of SSL or SMP was added to a model system containing 50 mg of purified and freeze-dried glutenin dispersed in 10 ml of AUC solvent; the mixture was allowed to stand overnight. To prepare a homogeneous, representative sample for SEM examination, we placed an SEM circular stub covered with double-faced tape in a vial, filled the vial with 10 ml of glutenin solution or glutenin-surfactant mixture in a dialyzing tube, and dialyzed with distilled water for five days. After the dialysis, the purified sample in the vial was freeze-dried at 30° C for 24 hr. The excessive dried sample sticking over the tape on the stub was blown off and then coated with 60% gold and 40% palladium. The mounted specimens were examined in an ETEC Autoscan SEM at an accelerating potential of 20 kV and photographed on Polaroid 55 R/N type film.

RESULTS

Effects of Mixing on Wheat Glutenin

Figure 1 shows the changes in appearance of glutenin that was isolated from Bison flour and from flour-water dough mixed for different periods. Structure of the freeze-dried glutenin that is isolated from the flour (Fig. 1a) differs greatly from the structure of glutenin that is isolated from mixed dough (Fig. 1b-e). Flour glutenin is mostly fibrous. With mixing, glutenin fibers (Fig. 1a) appear to associate together rapidly to form various sheet- or film-like structures (Fig. 1b). This indicates that glutenin first undergoes rapid association with mixing to form a protein network (17).

Mixing seems to have two opposite actions on dough: One is to associate the original glutenin to form a sheet- or film-like structure (protein network), the other is to dissociate such structures. Probably the opposite actions reach a steady state (equilibrium) when the dough attains stability (Fig. 1c and d) of glutenins that are isolated from doughs mixed for 5 min (arrival time) and 21 min (departure time).

With prolonged mixing, the protein network (sheets or films) appears to break down gradually, as shown by increasing numbers of short fibrous structure that indicate the reduction of the size of glutenin structure (Fig. 1e and f). This observation supports the postulation that mixing can decrease the size of protein aggregates in flour (2,4,18,19).

The structural change of Logan glutenin differs from that of Bison glutenin

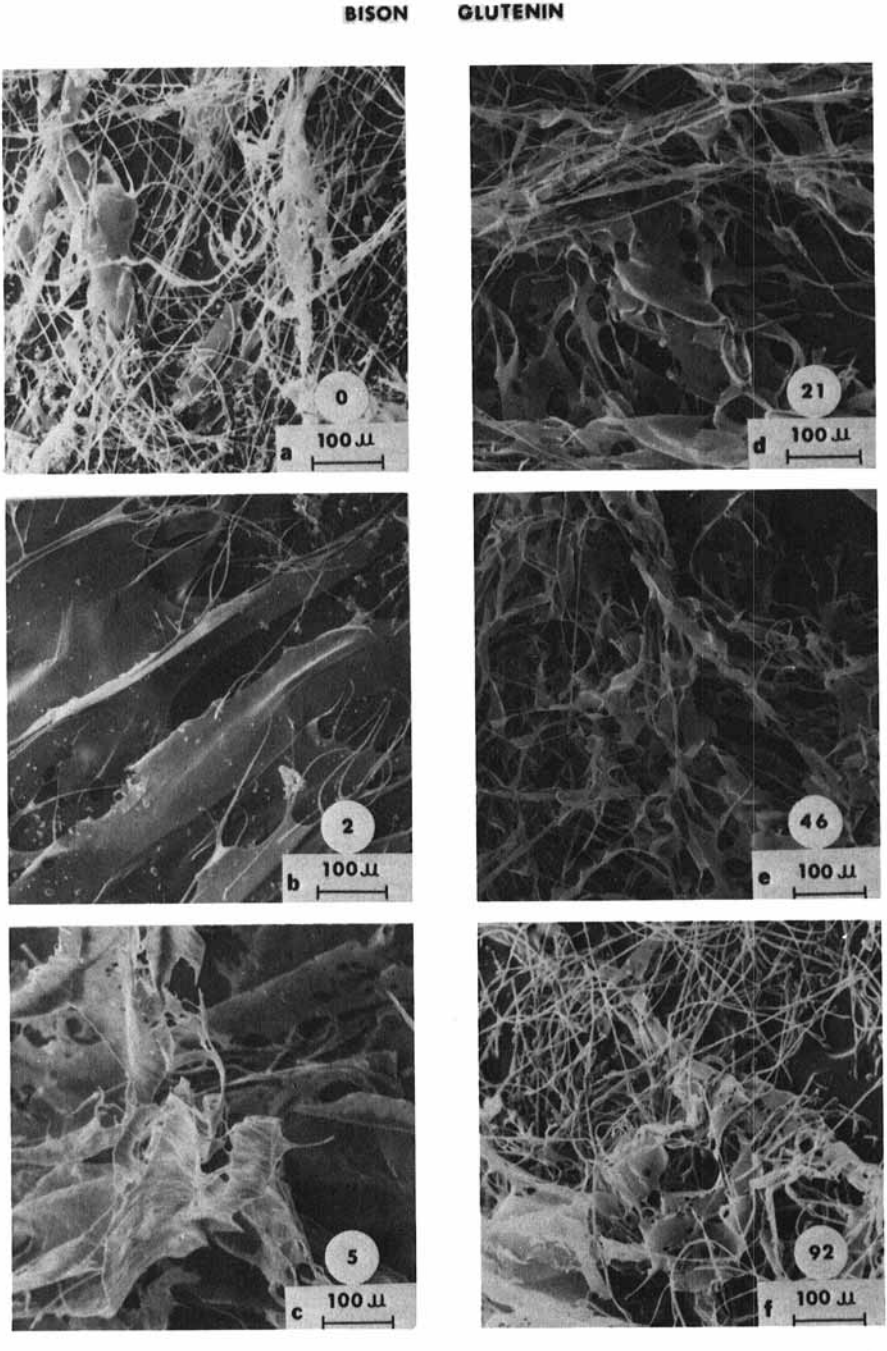

Fig. 1. SEM micrographs of glutenins isolated from Bison flour and flour-water doughs mixed for indicated periods.

(Fig. 1 and 2). Unlike Bison glutenin, Logan glutenin that is isolated from overmixed dough contains only a large sheet- or film-like structure, with no fibrous formation. The marked difference most likely results from variations in glutenin composition and structure between soft and hard wheat flours. Their farinograms also clearly show the difference (Table I). Logan wheat flour has shorter arrival and dough stability times than does Bison wheat flour (20).

The structure of glutenin that is isolated from Logan flour (Fig. 2a) differs from that isolated from Logan dough (Fig. 2b-d). Logan glutenin contains both fibrous and sheet-like structure. Logan glutenin appears to orient and associate itself more quickly than does Bison glutenin to form a protein network in the form of a sheet-like structure upon mixing. With overmixing, Logan dough's physical properties change drastically and become sticky; in this stage, Logan glutenin further dissociates into a smear-like structure.

Effects of Surfactants on Glutenin

To show clearly the effect of surfactant on glutenin structure, a model system

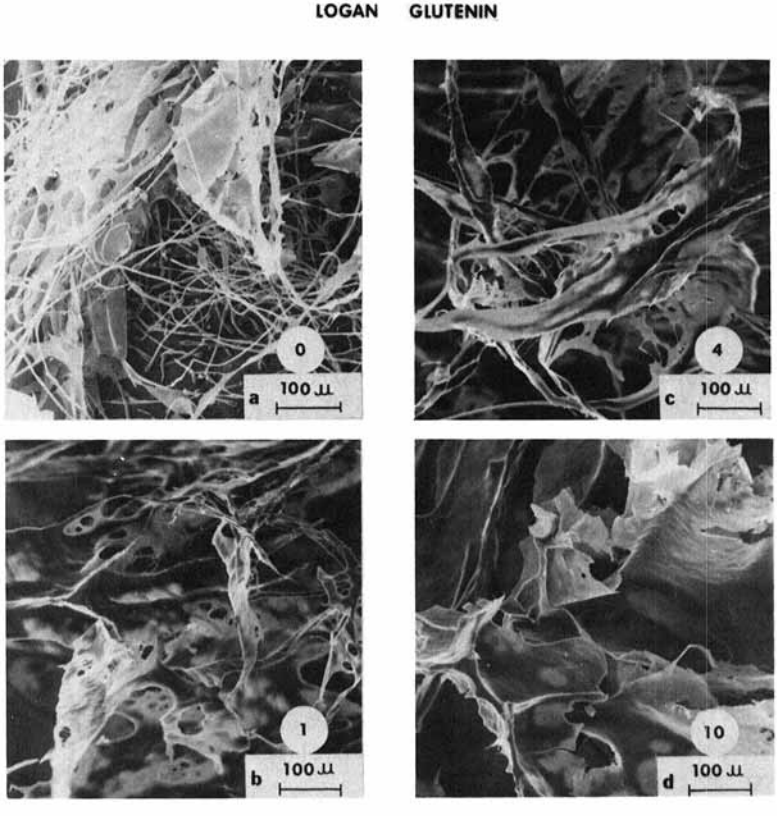

Fig. 2. SEM micrographs of glutenins isolated from Logan flour and flour-water doughs mixed for indicated periods.

was used in this study as described previously.

Figure 3 presents SEM micrographs that show the interaction between SSL and Bison glutenin. The glutenins shown in this figure are the same ones that are presented in Fig. 1, except that they were treated with 0.5% SSL (per cent based on protein weight). A comparison of Fig. 1 and 3 reveals that the original fibrous and sheet-like structures of Bison glutenin (Fig. 1) are transformed into a variety of granular and leaf-like structures with the SSL treatment. The transformation indicates the formation of glutenin-SSL complexes.

The complex structure was small with the glutenin that was isolated from Bison flour (Fig. 3a). Its size increased when glutenins that were used in the model system came from doughs mixed for 2, 5, 21, and 46 min. The change suggests that dough mixing can modify the configuration of glutenin to make the glutenin more accessible for the interaction with SSL (Fig. 3b-e). When glutenin that was isolated from overmixed dough was used, however, the complex's size was greatly reduced. The reduction indicates that the glutenin structure, once it is changed with prolonged mixing, becomes less available for the complex formation.

SEM micrographs presented in Fig. 4 show the interaction between surfactants SMP and Bison glutenin. Changing patterns with SSL and with SMP were similar. Like SSL, SMP forms complexes with glutenin that are more readily isolated from optimum-mixed than from overmixed dough.

Apparently SSL interacts more effectively with glutenin than does SMP, for SSL-glutenin complexes are larger than SMP-glutenin complexes (Fig. 3 and 4).

DISCUSSION

During dough mixing, the flour proteins are forced to change their structure to adapt to the new moist environment. Intermolecular adhesion would change during dough-making from hydrophobic at the beginning to interaction mainly between hydrogen and electrostatic bonds. The work input during mixing draws out the concatenations, so that they interlace the dough in all directions. If mixing continues, the individual polypeptide chains in the oriented concatenations participate. Overmixing produces a weak, sloppy dough, which having had its glutenin degree of polymerization drastically reduced and having lost many of its secondary cross-links, behaves like a viscous liquid (20,21).

From the SEM data, we postulate that glutenin can associate and dissociate itself, depending on the mixing conditions. Fibrous glutenin seems to have the ability to associate itself to form a sheet-like structure during mixing. This sheet-like structure appears to be the major component of the protein network in dough. With overmixing, the sheet-like structure becomes smaller and some is transformed into short fibers for Bison glutenin, while for Logan glutenin, the structure appears to smear and readily rupture. The change in glutenin structure as presented in Fig. 1 and 2 demonstrates how glutenin acts as a major component in building the protein network in dough. These micrographs also support the suggestions that the concatenation of protein chains and the reduction of polymerization of glutenin takes place with mixing and overmixing, respectively.

One of the major actions of surfactants as dough conditioners is to form a surfactant-glutenin complex. Both SSL and SMP surfactants can form such complexes as shown in Fig. 3 and 4. Different surfactants are expected to have

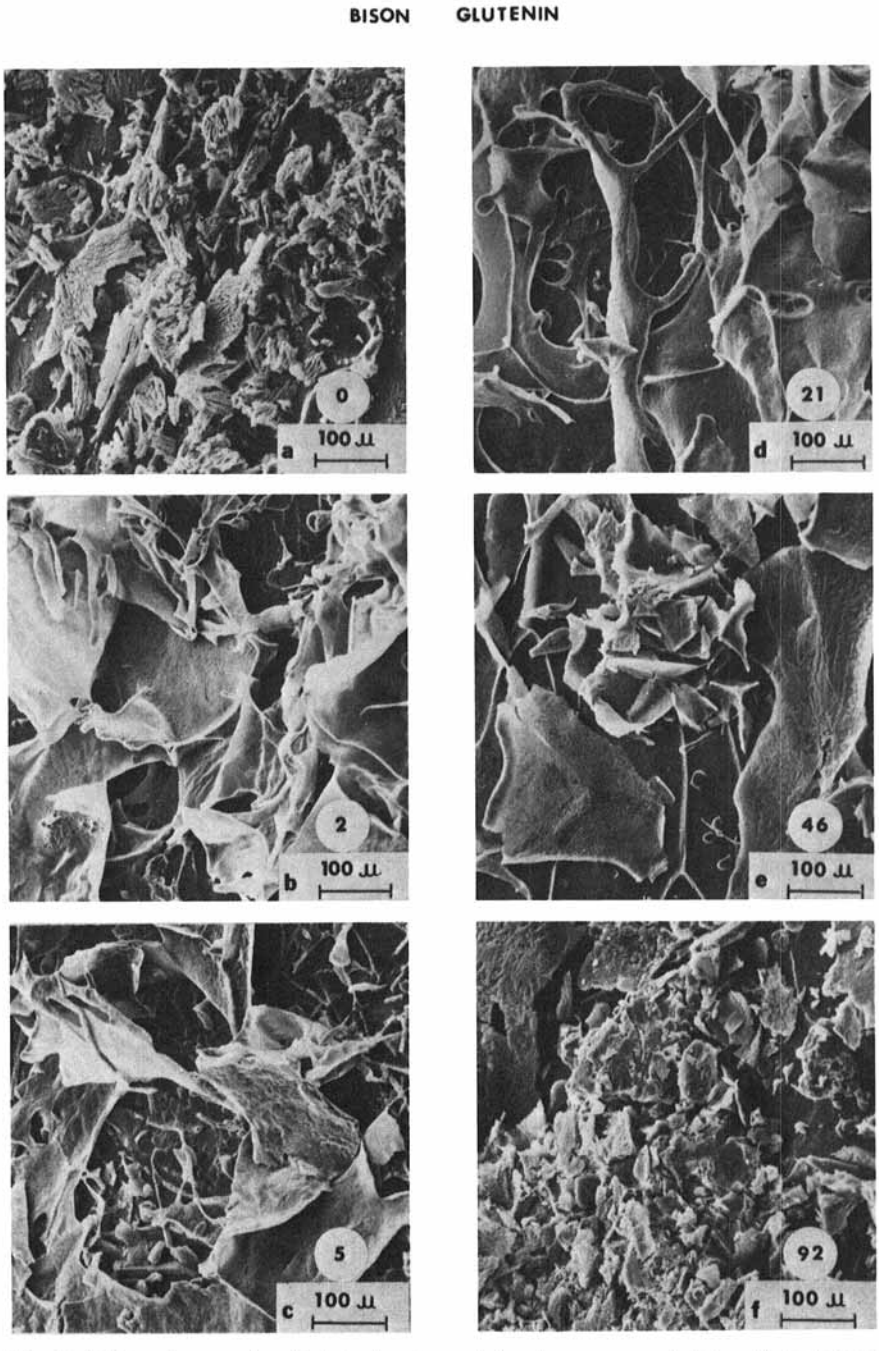

Fig. 3. SEM micrographs of glutenins in model system represent interactions between SSL and glutenins isolated from Bison flour and flour-water doughs mixed for indicated periods.

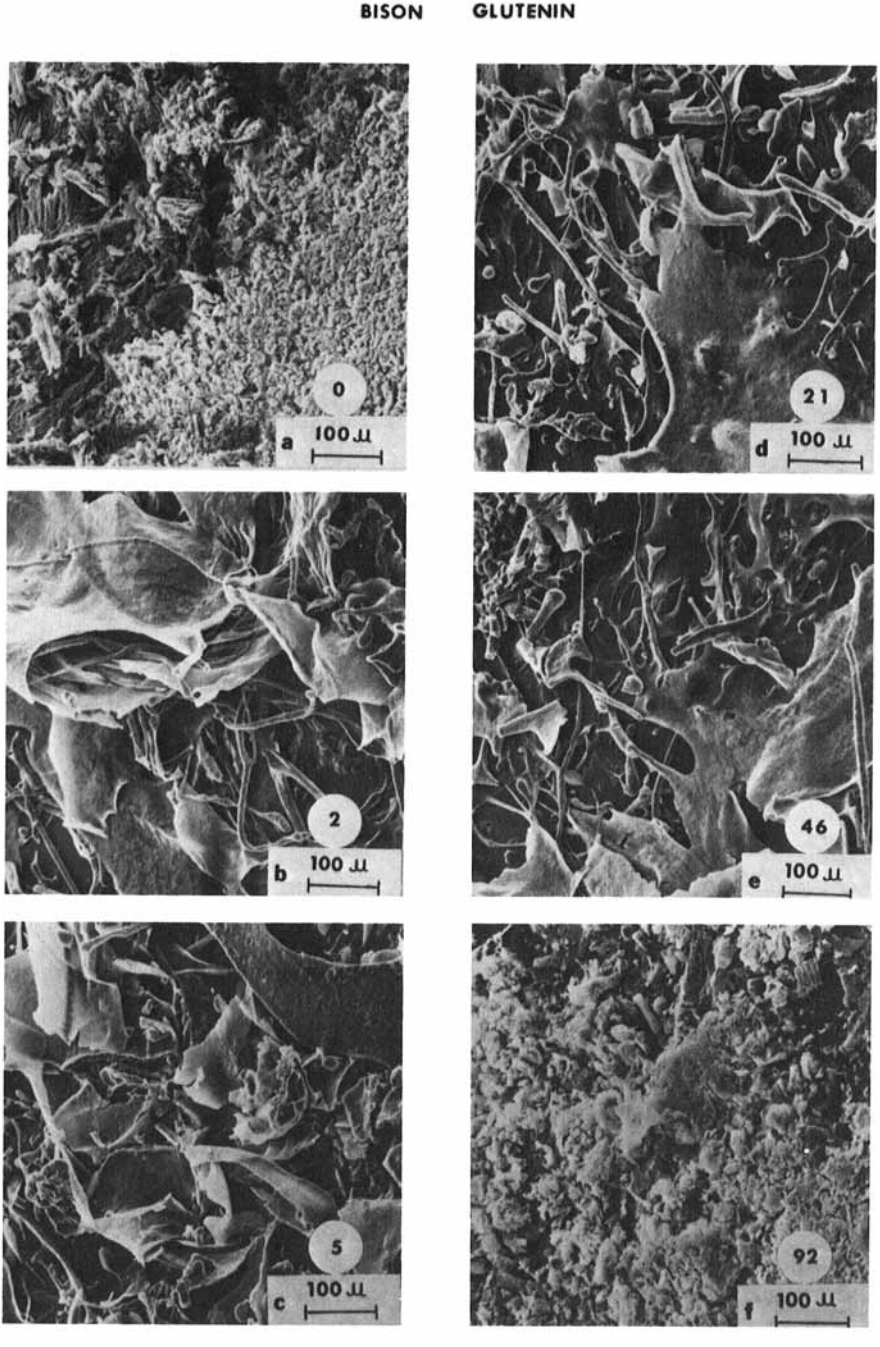

Fig. 4. SEM micrographs of glutenins in model system represent interactions between SMP and glutenins isolated from Bison flour and flour-water doughs mixed for indicated periods.

different complexing ability. SSL appears to be more effective than is SMP in the complex formation. The formation of such complexes helps to explain why some surfactants, such as SSL, can enhance dough stability with mixing.

Literature Cited

1. MECHAM, D. K., SOKOL, H. A., and PENCE, J. W. Extractable protein and hydration characteristics of flours and doughs in dilute acid. Cereal Chem. 39: 81 (1962).
2. MECHAM, D. K. Changes in flour proteins during dough mixing. Baker's Dig. 38(2): 46 (1964).
3. MECHAM, D. K., COLE, E. G., and PENCE, J. W. Dough-mixing properties of crude and purified glutens. Cereal Chem. 42: 409 (1965).
4. TSEN, C. C. Changes in flour proteins during dough mixing. Cereal Chem. 44: 308 (1967).
5. TSEN, C. C. Effects of oxidizing and reducing agents on changes of flour proteins during dough mixing. Cereal Chem. 46: 435 (1969).
6. POMERANZ, Y., SHOGREN, M. D., and FINNEY, K. F. Improving breadmaking properties with glycolipids. II. Improving various protein-enriched products. Cereal Chem. 46: 512 (1969).
7. TSEN, C. C., HOOVER, W. J., and PHILLIPS, D. High-protein breads: Use of sodium stearoyl-2 lactylate and calcium stearoyl-2 lactylate in their production. Baker's Dig. 45(2): 20 (1971).
8. TSEN, C. C., and HOOVER, W. J. The shortening-sparing effect of sodium stearoyl-2 lactylate and clacium stearoyl-2 lactylate in bread baking. Baker's Dig. 45(3): 38 (1971).
9. TSEN, C. C., and TANG, R. T. K-state process for making high-protein breads. I. Soy flour bread. Baker's Dig. 45(5): 26 (1971).
10. TSEN, C. C., and HOOVER, W. J. High-protein bread from wheat flour fortified with full-fat soy flour. Cereal Chem. 50: 7 (1973).
11. MICH, C. Investigation on the preparation of ethoxylated sucrose monopalmitate and evaluation of its potential use for making high-protein bread. MS thesis, Kansas State University, Manhattan, 1973.
12. ORTH, R. A., DRONZEK, B. L., and BUSHUK, W. Studies of glutenin. IV. Microscopic structure and its relations to breadmaking quality. Cereal Chem. 50: 688 (1973).
13. ORTH, R. A., DRONZEK, B. L., and BUSHUK, W. Scanning electron microscopy of bread wheat proteins fractionated by gel filtration. Cereal Chem. 50: 696 (1973).
14. AMERICAN ASSOCIATION OF CEREAL CHEMISTS. Cereal Laboratory methods. The Association: St. Paul, MN (1962).
15. ORTH, R. A., and BUSHUK, W. Studies of glutenin. I. Comparison of preparative methods. Cereal Chem. 50: 106 (1973).
16. MEREDITH, O. B., and WREN, J. T. Determination of molecular-weight distribution in wheat-flour proteins by extraction and gel filtration in a dissociating medium. Cereal Chem. 43: 169 (1966).
17. BERNARDIN, J. E., and KASARDA, D. D. The microstructure of wheat protein fibrils. Cereal Chem. 50: 735 (1973).
18. EWART, J. A. D. A hypothesis for the structure and rheology of glutenin. J. Sci. Food Agr. 19: 617 (1968).
19. DRONZEK, B., and BUSHUK, W. A note on the formation of free radicals in dough during mixing. Cereal Chem. 45: 286 (1968).
20. GREENWOOD, C. T., and EWART, J. A. D. Hypothesis for the structure of glutenin in relation to rheological properties of gluten and dough. Cereal Chem. 52: 146 (1975).
21. EWART, J. A. D. Recent research and dough visco-elasticity. Baker's Dig. 46: 22 (1972).

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