EFFECTS OF SURFACTANTS ON GLUTENIN AND GLIADIN DURING DOUGH MIXING AND IN MODEL SYSTEM

by

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INTRODUCTION

The mixing property of dough is one of the most important parameters in the assessment of flour quality. It has recently become more important with the introduction of the continuous breakmaking processes in which dough development is achieved primarily by high speed mixing. For any breakmaking process, using a particular flour and formula, there is an optimum mixing for dough development. Too little or too much mixing will give inferior results. Accordingly, it is obvious that the function of dough mixing is much more complex than just the physical blending of ingredients. There are many changes in dough components to produce the overall effect of mixing. The key position in these changes is occupied by flour proteins which undergo constant changes during mixing (79, 80, 86).

Surfactants have been widely used in the baking industry as "dough conditioners" to improve baking performance of flour. Recently, surfactants have received more attention because they can improve the quality of protein-fortified baked products by alleviating the adverse effects of protein-rich additives. The mechanisms of improving action of surfactants in wheat dough are, however, still obscure (30, 66, 67, 90, 91)

The present study has been conducted to examine the changes in flour proteins during mixing, with or without surfactants and can be summarized as follows:

The scanning electron microscope has been used in studying the structure of wheat kernel, flour, dough, glutenin, and starch, but

little information is available on the structural changes of glutenins and gliadins during mixing, with or without surfactants.

Dough mixing decreases the size of particles or aggregates of the matrix protein and thereby increases solubility. This observation needs confirmation by further study of polyacrylamide gel electrophoresis and gel filtration on wheat proteins.

The electrophoretic patterns of glutenins and gliadins are well known, but little information is available on the combined effects of mixing and surfactants on glutenins and gliadins in dough systems, and on the interaction between surfactants and glutenins or gliadins in a model system.

REVIEW OF LITERATURE

Wheat Flour Proteins

The protein of wheat flour, together with starch, lipid, and other components, forms a three-dimensional network that exhibits elastic and viscous properties, distinguishing wheat from other cereal grains (64). Wheat protein which is so important to the structure and physical properties of dough has been under steady investigation since the early part of the century (28, 65).

Wheat protein is composed of a water-soluble and heat-coagulable leucosin (albumin); a neutral salt-soluble globulin; a poorly defined protease; gliadin, a prolamine which is soluble in 70 per cent ethanol; and glutenin, a glutenin soluble in dilute acid and alkali (62). The

last two fractions, glutenin and gliadin, mainly govern the quality of wheat flour (38, 40).

Dough is a highly complex chemical system. The main chemical bonds involved are intramolecular and intermolecular disulfide bonds, ionic bonds, Van der Waals force, interpeptide hydrogen bonds, side chain hydrogen bonds. While covalent and ionic bonds primarily increase cohesiveness of dough, dipole-, hydrogen-, and hydrophobic bonds contribute to elasticity. The amide groups of protein are also partially responsible for the cohesive and elastic characteristics (4, 13, 25, 28, 76, 97, 99).

In large measure, the rheological behavior of dough is dependent on the physical and chemical properties of gluten (65, 76). The non-gluten protein will not be dealt with here because it only plays a minor role in the formation of dough structure (65).

The gluten is traditionally isolated as a wet elastic mass by kneading a dough in water to remove starch. Gluten and its fractions are soluble only in aqueous media of very low ionic strength, since this protein contains less amount of ionic functional groups, and high contents of non-polar groups. Gluten is heterogeneous mixture consisting of high molecular weight and low molecular weight protein-fractions (39, 46). The gluten protein absorbs water, but not as much as the soluble protein. Some sections of gluten protein chain coil up like a spring to form helical segments (95, 100). Gluten protein represents 80-90 per cent of total flour protein. It possesses a markedly different amino acid composition than the albumin and globulin (39).

Three models of dough structure have been proposed: (a) starch-lipid-adhesive protein complex in flour, (b) gliadin-glycolipid-glutenin complex, and (c) the Grosskreutz model. Grosskreutz examined dough by X-ray diffraction and electron microscope and concluded that gluten existed in the form of platelets of thickness equivalent to 7 nm. The lipoprotein structure is bound to the main protein platelets by hydrogen bonds or salt links (35, 36). Several binding forces may be involved in the interactions of lipid and protein. There are covalent, ionic, hydrogen, Van der Walls, hydrophobic bonds, and chelate formation (32, 68, 69, 97).

Ponte found that gliadin lipid contained 75 per cent polar lipid and 6 per cent triglycerides; glutenin lipid had 24 per cent and 58 per cent, respectively, of these components (70). Chung (16) found that gliadin contained mostly nonpolar lipid and very little polar lipid, glutenin contained equal amounts of polar and nonpolar lipids (74). Hoseney et al. found that free polar lipid (principally glycolipid) was bound to the gliadin protein by hydrophilic bonds and to the glutenin protein by hydrophobic bonds (69, 73, 97). The aromatic amino acids such as phenylalanine and tryrosine furnished hydrophobic functional groups to lipid (32). In unfractionated gluten, the lipid apparently is bound to glutenin and gliadin at the same time (42).

Interaction between starch granules and protein is evidenced by the adherence of starch granules to the surface of the protein fibril, which is clearly seen in scanning electron micrographs. A protein sheet interacting with starch granules is distributed throughout the protein matrix (5, 20, 80, 94). D'appolonia (21) indicated that all the gluten extracted from hard red spring wheat flour contained pentosan material. Also,

protein-starch combination could be detected by electrolytic conductance measurement (78). The nature of the starch and storage-protein interface differs between hard and soft wheat varieties (2).

Gluten can be separated into gliadin and glutenin fractions by the solubility of gliadin in 70 per cent ethyl alcohol (62, 63). The poor solubility of glutenin is due to its size and amino acid composition. A proper ratio of glutenin to gliadin is needed for the optimum loaf volume (8).

Glutenin was first described in 1820 by Taddei (8) as a protein in wheat flour not extractable by neutral solvent. Disulfide linkages in glutenin can occur either in intramolecular disulfide bonds that stabilize the compactly folded structure or in intermolecular disulfide bonds that bind glutenin subunits into large protein molecules. The polypeptide chains are partly folded into compact structures and also partly in the random-coil conformation (6, 8, 48, 56, 65). Because of their high molecular weight, shape, and amino acid composition which are favorable for hydrogen and hydrophobic bondings, glutenin molecules have relatively large surface areas suitable for molecular association (6, 41, 56). The elasticity can be destroyed by adding small amounts of disulfide-breaking reagents, e.g., cysteine, bisulfite or 2-mercaptoethanol (8, 56).

Glutenin is very heterogeneous with different molecular weight components ranging up to three million (19, 37, 46, 47). Glutenin appears to have an amino acid composition generally similar to that of gliadin, but there are individual differences. For example, glutenin has a much higher proportion of lysine, glycine and tryptophan, whereas

gliadin is rich in proline, cystine, phenylalanine, glutamic acid and glutamine. No significant differences in amino acid composition resulting from variety, or from strong wheats as against weak, are observed among glutenin or gliadin (6, 10, 25, 27, 28, 56).

Gliadin has tightly folded single polypeptide conformation stabilized by intramolecular disulfide bonds with molecular weight ranging from 17,000 to 216,000. Gliadin interacts with the other proteins and other constituents of dough, by means of secondary forces such as hydrogen, ionic, and polar bondings, contributing significantly to the cohesiveness and extensibility of dough (48, 51, 56, 74). It has been suggested that the role of gliadin is in controlling the loaf volume potential while glutenin is in controlling the mixing time (40, 41, 43). Deutsch (23) used the end group analysis technique and reported that wheat gliadin contained two end groups of phenylalanine. The chromatographic and electrophoretic patterns of gliadin from different varieties of wheat have been similar in general form, but no two have been identical. In general, varieties in different classes show a greater variation in gliadin components than varieties in the same class (45, 72).

Mixing

The elementary function of mixing is the uniform blending of all the dough ingredients. Water is one of the major ingredients, accordingly, its distribution in dough is an important phase of mixing. Hydration of all the flour components is a prerequisite for proper dough formation (12). In addition to blending of ingredients, mixing must be

optimal to achieve the proper development of physical properties for subsequent handling and gas retention (13). If overmixed, the physical properties of the dough change quite drastically. The dough surface takes on a sheen and becomes sticky, and is difficult to handle. This overmixed, which usually produces a deleterious effect on the ultimate quality of the bread, is referred to as dough breakdown (44).

Mecham and coworkers (53, 54, 55) found that the amount of protein extracted from dough by dilute acetic acid increased with extended mixing. Rates of increase were different for flours of different mixing characteristics. To explain this increase in extractability, it was postulated that mixing decreased the size of protein aggregates in the flour particles. This was later confirmed by Tsen.

Tsen (86, 88) reported that the amount of flour protein that was extractable with 0.01 N acetic acid and the distribution of protein components in the extract were significantly altered by dough mixing. Mixing at higher speeds intensifies the increase of "glutenin" (first chromatographic peak) component. Because mixing, through its tearing and shearing, can break or separate the protein aggregates to form continuous protein films and a protein network, more protein would be accessible for interaction with solvent. The size of large protein aggregates of soft wheat flour may be smaller and more liable to dissociation than that of the hard wheat flour. So, the glutenin component of weak flour increases faster and is less tolerable to overmixing than strong wheat flour.

THIS BOOK CONTAINS NUMEROUS PAGES WITH DIAGRAMS THAT ARE CROOKED COMPARED TO THE REST OF THE INFORMATION ON THE PAGE.

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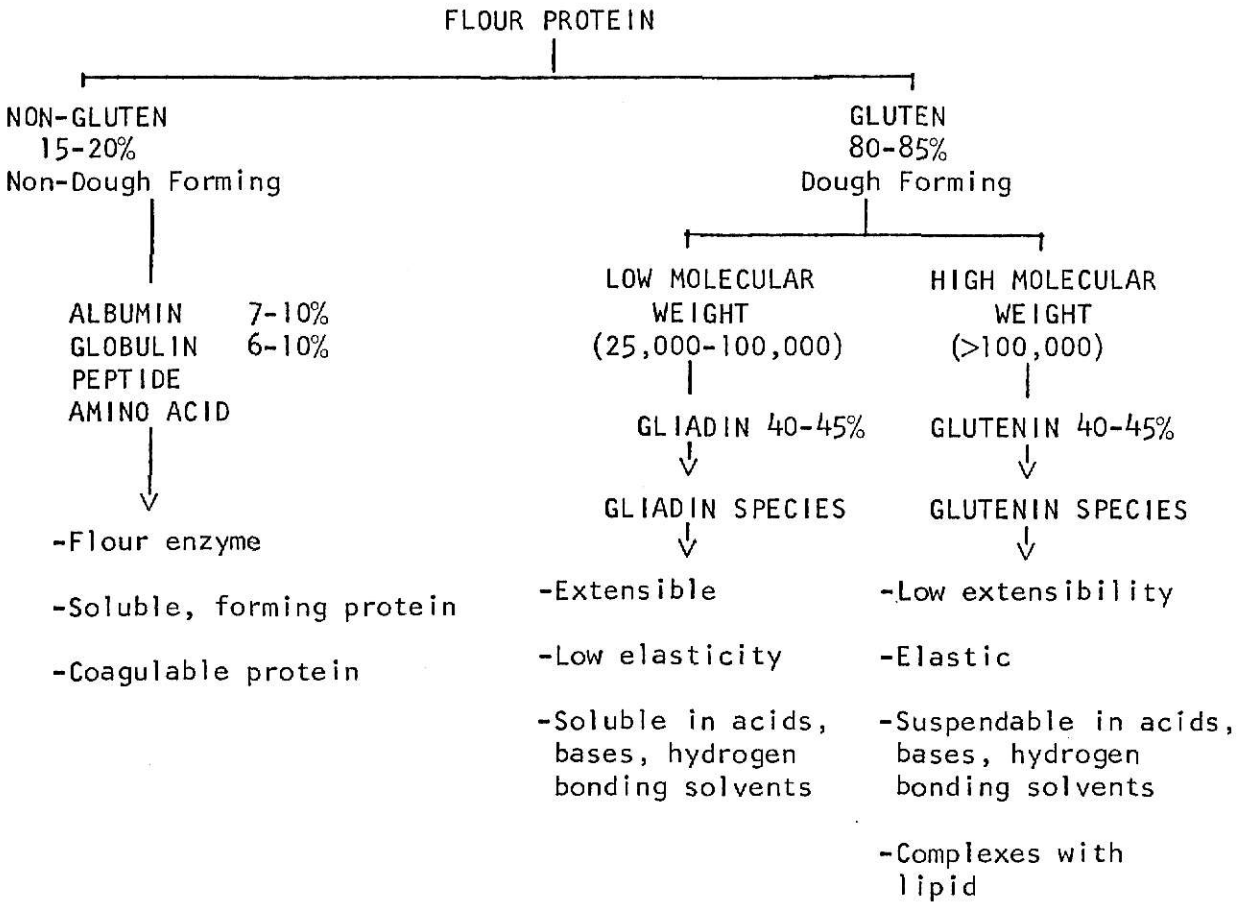
Fractionation

A schematic presentation of the main protein fractions of wheat flour is shown:



Several types of dispersing media have been used for the gluten protein, viz., alcohol, alkalies, acids, salt solutions, and urea. Each has its advantages.

Alcohol disperses a large portion of gluten--the so-called gliadin protein. Methyl alcohol has a far greater denaturing effect than ethyl alcohol. In general, as pH is increased for a given fraction, the amount

of glutenin decreases and that of gliadin increases. Alkali accomplishes the complete dispersion of all the gluten protein in a very short time. It has always a destructive irreversible alteration of protein, regardless of the concentration of alkali employed (9). Acetic acid presents the danger of hydrolysis (51, 71, 75). Gluten protein can be almost completely dispersed in neutral aqueous 5 M urea solution. Urea solution is a highly polar solvent, and its major action is to break hydrogen bonds; since urea is an amide compound, it may associate with polar groups on protein and thereby eliminate sites for protein hydrogen bonding (18, 39, 95).

Surfactants

Surface-active agents may be defined as materials which can modify the surface behavior of dispersing systems. Examples of surface-active agents other than emulsifiers are detergents, wetting agents, dispersing agents, and deemulsifiers (33).

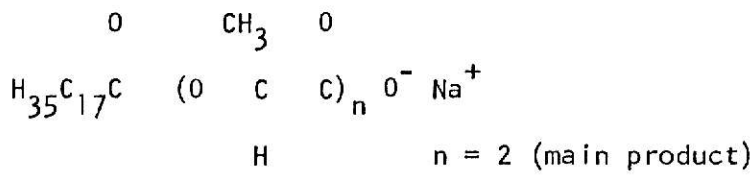
The properties and functional uses of surfactants in bakery products are anti-staling agent, dough conditioner, palatability improver, tenderness improver, and volume improver. Surfactants have a measurable improving effect on the quality of end products. Dough strengthening and volume improvement are apparently dependent upon the ability of certain surfactants to associate with protein, carbohydrate, and lipid of wheat flour. Surfactants retard crystallization by forming the helix with the starch and inhibiting moisture migration. But the mechanism by which they exert their improving action in wheat dough is still obscure (17, 37, 49, 52, 58).

Early in 1956, the compound stearoyl-2 lactylic acid was discovered to be an effective surfactant which had a high affinity to fat phase and retards the gelation of starch during the final stage of baking. In general, the effects of the acid on non-yeast leavened products are characterized by thicker, creamier batters, larger specific volumes of the finished products, finer grain and silkier texture with improved eating quality and shelf life. Feeding studies provide data as to the non-toxic nature and suitability for use in food products (11, 84, 85).

Thompson and Biddemeyer (83) first reported that calcium stearoyl-2 lactylate (CSL) increased the mixing tolerance of dough. Studies with farinograph and amylograph show that CSL affects the properties of both starch and gluten. Bechtel et al. (3) found that CSL altered the structure of wheat gluten. Thompson (83) suggested that this might be caused by a colloidal binding of the additive to the flour protein. Grosskreutz (34) suggested that CSL could orient on the outer perimeter of the gluten sheets in a manner similar to that proposed for the phospholipids or possibly crosslink between protein platelets in adjacent layers of the sheets. Also, CSL has marked inhibitory effect on transition temperature of dilute starch-water mixture, and hence retards the gelatinization and swelling of the starch (3).

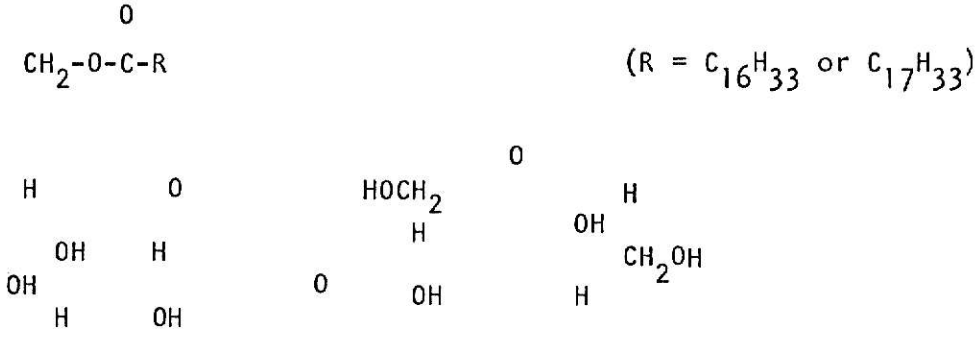
Sodium stearoyl-2 lactylate (SSL) is a newly available dough conditioner and emulsifier. It is produced by reacting stearic acid and lactic acid, and then neutralized to the sodium salt. The commercial product of SSL is mixture of sodium salts of a homologous series of stearoyl lactylic acid. The melting point range is 46^{o}-52^{o} C

(114.8°-125.6°F). It is dispersible in water and readily soluble in hot vegetable oil and lard. Average molecular structure is



In October, 1967, a food additive regulation was issued which provided for the safe use of SSL as a emulsifier, dough conditioner, or whipping agent (82, 91). Tsen has found that SSL can increase the mixing tolerance of dough and permit the production of uniform, high quality, baked products over a wide range of processing and ingredient variations. For example, SSL can increase the stability of dough containing 12 to 28 per cent soy flour. SSL can help the stored breads retain softness and spare or replace shortening normally required in white bread or bread containing 12 per cent soy flour (30, 66, 67, 82, 87, 89, 90, 91. 92, 93).

Sucrose monopalmitate is actually a mixture of mono-, di-, tri-palmitates and stearate esters. The chemical formula of the 6-monoester is shown below:



This nonionic surfactant plays a very important role in the baking industry, and acts as an emulsifier or reacts with starch or protein (57).

OBJECTIVES

The major objectives of this investigation were to elucidate the mechanisms of interaction between wheat flour proteins and added surfactants during dough mixing. The following parameters were evaluated:

1. Changes of glutenins and gliadins during dough mixing observed by scanning electron microscope, gel filtration, and disc electrophoresis.

2. The combined effects of dough mixing and surfactants on glutenins and gliadins observed and measured by scanning electron microscope and disc electrophoresis.

MATERIALS AND METHODS

Flours

Two varieties of hard red winter wheat (Eagle and Bison) and one of soft white wheat (Logan) were used. All wheats were milled on a Miag "multomat" mill. Proximate analyses of untreated, straight grade flours (Table 1) were performed as described by AACC Methods (14, 77).

TABLE 1 PROXIMATE ANALYSES OF FLOURS

	Flour Composition[a]		
	% Protein[b]	% Fat	% Ash
HRW			
Eagle	10.76	0.8	0.49
Bison	11.58	0.6	0.41
SW			
Logan	10.53	0.8	0.36

[a] 14% moisture basis

[b] 5.7 x N as a factor for protein conversion

Surfactants

Sodium stearoyl-2 lactylate (Emplex or SSL) was obtained from the C. J. Patterson Company, Kansas City, Missouri. Sucrose monopalmitate (SMP) was from Dai-Nippon Sugar Manufacturing Company, Ltd., Tokyo, Japan.

Unless otherwise stated, 0.5% of surfactants on flour weight was added to wheat flour.

Other Chemicals

All of the organic and inorganic solvents were of analytical reagent grade. Distilled water was used for mixing dough, gluten washing, and dialyzing samples.

Farinograph

The farinograph measures and records the resistance of a dough to mixing. It is used to evaluate the absorption of water by flour and to determine the stability and other characteristics of dough during mixing. Two basically different methods are in common use: Constant Dough Weight Procedure and Constant Flour Weight Procedure. The two procedures may not yield identical results.

These farinograms were obtained by the constant dough weight method as described by AACC (15), water absorptions adjusted to obtain a dough having a maximum consistency of 500 Brabender Units (B.U.). Whenever wheat flour was treated with a surfactant, the dry ingredients were blended for 5 minutes in a farinograph mixing bowl before adding distilled water and mixing.

Preparation of Dough

Dough samples were prepared from three wheat flours with or without 0.5% SSL or SMP and mixed for different periods. Doughs were mixed in a Farinograph mixed at 60 rpm and 30°C.

Fractionation of Samples

Dough was washed under a gentle stream of distilled water until a gluten ball was obtained, according to the procedure of Orth and Bushuk (Fig. 1). Overmixed dough produced the appearance of sticky, extensible characteristics--hard to wash the starch and water soluble protein away (60, 54). A gluten was dissolved in 200 ml of AUC

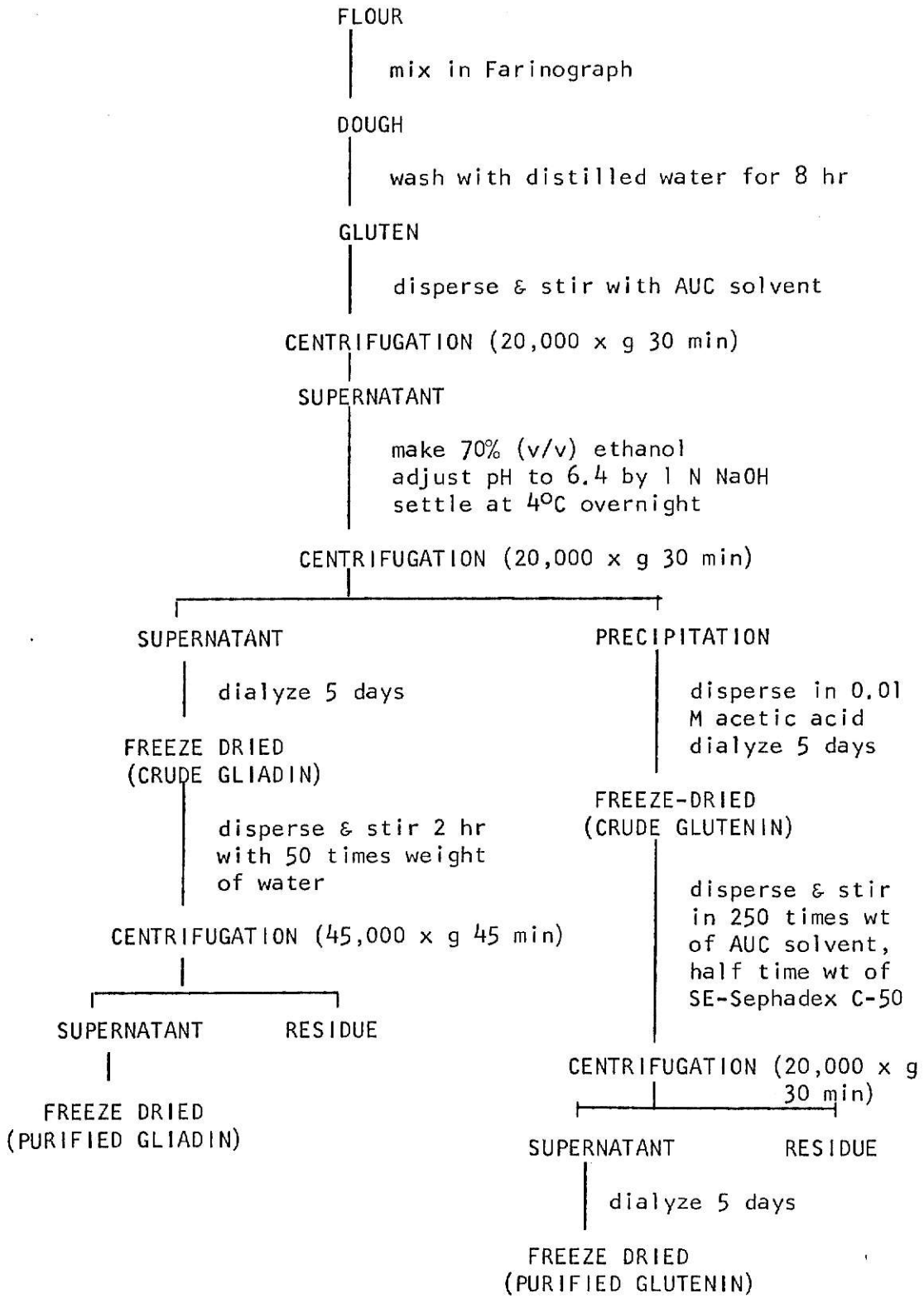


Fig. 1 Fractionation scheme of flours and doughs

solvent (0.1 M acetic acid, 3 M urea, and 0.01 M cetyltrimethylammonium bromide - CTAB) by overnight magnetic stirring. The solution was centrifuged at 20,000 x g, at 4°C 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 N NaOH. The resulting precipitate was allowed to settle overnight at 4°C and separated by centrifugation.

The precipitate (crude glutenin) was then dispersed in 0.01 M acetic acid, dialyzed against distilled water for 5 days, and freeze-dried. An aliquot of the supernatant from the centrifugation after the pH precipitation was also dialyzed 5 days and freeze-dried (crude gliadin).

The freeze-dried crude 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 x g at 4°C for 30 min. The supernatant was dialyzed against distilled water for 5 days and freeze-dried to yield purified glutenin (60). The freeze-dried crude gliadin was stirred for 2 hr, with 50 times its weight of distilled water and the dispersion centrifuged at 45,000 x g at 4°C for 45 min to remove glutenin. The clear supernatant was freeze-dried to give purified gliadin (24).

Scanning Electron Microscopy (SEM)

The outstanding characteristic of images from the scanning electron microscope is their remarkable three-dimensional quality,

as the transmission electron microscope produces only a two-dimensional image. It records not only the electrons passing through the specimen but the secondary electrons that are released from the sample by the electron beam impinging on it. The scanning electron microscope is useful over a magnification range from about 15 diameters to about 100,000 diameters, although the image begins to get fuzzy above 20,000 diameters. Most manufacturers guarantee a resolution of 200 angstrom (26).

Circular stubs were attached with double-side tape and put into small vials, then the dialyzed protein solution was poured into them, and the sample stubs were freeze dried and coated with 60% gold and 40% palladium. The mounted specimens were examined in a ETEC Autoscan scanning electron microscope at an accelerating potential of 20 KV and photographed on Polaroid 55 P/N type film (48, 61).

Gel Filtration

Gel filtration is a widely used method for fractionating wheat flour proteins (1, 86). In the process, protein molecules are separated according to size as they pass through a column of cross linked gel. Large molecules are unable to enter pores of gel particles, but smaller molecules can. An excellent linear correlation between the logarithm of molecular weight of a protein and the ratio of its elution volume-V_1, to the void volume-V_o of the column is found. The ratio of elution volume to void volume V_1/V_o is independent of protein concentration, column size, and ion exchange adsorption, but it is found to be temperature dependent for several proteins (61, 81, 98).

The gel filtration medium was cross-linked polyacrylamide gel, Bio-Gel P-150, 100-200 mesh (Bio Rad Laboratories, Richmond, California). The medium was prepared by suspending 169 g Bio-Gel in 800 ml buffer with gentle stirring. The buffer composition was 10 ml of 2 N acetic acid and 2 ml of 1 N sodium hydroxide and 3 M urea; pH was 3.8 diluted to one liter. The suspension was allowed to stand at room temperature for 24 hours after which the supernatant fluid was removed by suction; air bubbles entrapped in the swollen gel were removed by gentle stirring inside an evacuated desiccator. The chromatographic column (2.5 x 45 cm, Pharmacia, Uppsala, Sweden) was attached to a sturdy vibration-free support of an Isco Fraction Collector (Model 326). A gel reservoir (R25/26, Pharmacia), having the following dimension-length 20 cm, diameter (inner) 5.7 cm, volume 550 ml, was attached to the top of the column. With the column outlet closed, the entire volume of degassed gel slurry was poured into the column and reservoir. A glass rod was used to conduct the slurry along the inner wall of the column in order to prevent splashing and bubble formation. To assure uniform settling of the gel particles, the slurry was gently but briefly stirred with a long glass rod upon entry into the column. Particles were allowed to settle by gravity. Thereafter, the column outlet was opened and the supernatant solution allowed to drain at the rate of 11.0 ml/hr. Excess gel was removed by suction to permit insertion of a sample applicator above the bed. The final height of the gel was approximately 40 cm. The column was connected with a solvent reservoir via a Beckman Model 746 metering pump and was equilibrated for 24 hr by elution

at the rate of 11.0 ± 0.2 ml/hr. In order to preclude bubble formation within the system, eluting solvent was aspirated routinely in an evacuated desiccator before use.

Ten ml of solution containing the same amounts of protein (adjusted by UV spectrophotometer) was applied to the top of the column via the sample applicator. Immediately after all of the protein solution had penetrated into the column, 5 ml of buffer was placed in the sample applicator to wash residual protein into the column. Thereafter, the column was filled with eluting solvent and connected with reservoir via a Beckman metering pump operated at the rate 11.0 ± 0.2 ml/hr. Elution was monitored at 280 nm with an automatic UV analyzer.

Disc Electrophoresis

Polyacrylamide Gel. The electrophoresis procedure developed by Ornstein (59) and Davis (22) was used. A cationic gel system, pH 4.3, and with a 7.5% acrylamide concentration in the running gel was based on the work of Williams and Reisfeld (99). The composition of solution used for the gel preparation is shown in Table 2. The upper and lower buffer solutions were prepared as follows (if protein solvent changed to 3 M urea and 0.1 M acetic acid, every solution of disc electrophoresis also contained 3 M urea):

Glutenin or gliadin solution was prepared by dissolving lyophilized protein in 0.05 M acetic acid to give glutenin concentration of 0.6% and gliadin concentration of 0.35%. On model system, the protein solvents contained: (A) 0.1 M acetic acid, 3 M urea, and 0.5% (w/v) SSL;

TABLE 2 COMPOSITION OF SOLUTIONS FOR DISC ELECTROPHORESIS (99)

Chemicals		Upper gel, pH 6.7 per 100 ml	Volume ratio	Lower gel, pH 4.3 per 100 ml	Volume ratio
Solution 1			1		1
Acrylamide	g	10		30	
N, N-Methylene-bisacrylamide	g	0.8		0.8	
Solution 2			1		1
1 N Potassium hydroxide	ml	48		24	
Acetic acid (glacial)	ml	2.87		8.6	
N,N,N,N-tetramethyl-enediamine (Temed)	ml	0.1		0.24	
Solution 3			2		2
Potassium persulfate	mg	120		120	
Riboflavin	mg	2		2	

(B) 0.1 M acetic acid, 3 M urea, and 0.5% (w/v) SMP; (C) 0.05 M acetic acid and 0.5% (w/v) SSL; (D) 0.05 M acetic acid and 0.5% (w/v) SMP; (E) 0.5% (w/v) SSL; and (F) 0.5% (w/v) SMP. The protein solution's density was increased by adding sucrose to a concentration of 20%. Fifty μl of this final protein solution was layered on top of the upper gel by pipette. The buffer front was made visible by adding a few drops of 0.02% methyl green indicator solution. Electrophoretic separation was performed at a constant current of 4 ma/gel until the indicator marker reached a distance of 4 mm from the bottom of the gel column.

After electrophoresis, the gels were removed from the tubes and stained according to the procedure described by Fish et al. (31). The protein bands were fixed in a 20% sulfosalicylic acid solution for 1 hr and then stained in 0.25% solution of Coomassie Brilliant Blue R250 for 2 hr with gliadin, and for 3 hr with glutenin. Excess dye was removed by several changes of distilled water until the bands were clear. These gels were stored in test tubes containing 7% acetic acid.

Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis. Polyacrylamide gel electrophoresis (29, 50, 79, 96) in the presence of the anionic detergent sodium dodecyl sulfate (SDS) can be used to separate and estimate MW's of reduced protein and its subunits, and to detect differences in subunit composition of protein of different varieties. After disulfide bonds are cleaved, a large amount of SDS binds hydrophobically to the unfolded protein. Charged groups on the protein are masked by the large number of negative charges of SDS. The SDS-protein

complexes migrate through a porous polyacrylamide gel at a rate depending only on their molecular size. In contrast, ordinary gel electrophoresis separation depends on both protein size and charge.

Protein molecular weights are determined from the distance migrated from the origin in comparison to the mobilities of standard proteins of known molecular weight. The mobilities (distance of protein migration divided by distance of dye migration) are plotted against the known molecular weights on a semilogarithmic scale. The SDS gel electrophoresis can be used with confidence for a wide variety of proteins (7, 8, 88, 96).

The SDS-PAGE procedure was developed by Weber and Osborn (7).

Preparation of Protein Solution. The protein was incubated at 37°C for 6 hours in 0.01 M sodium phosphate buffer, pH 7.0, 1% in SDS, and 1% in beta-mercaptoethanol. The protein concentration was normally 4 mg/ml. The concentration of standard protein (ovalbumin and myoglobin) was 1 mg per ml.

Preparation of Gels. Gel buffer contained 7.8 g $NaH_2PO_4 \cdot H_2O$, 38.6 g of $Na_2HPO_4 \cdot 7H_2O$, 2 g of SDS per liter. For the 10% acrylamide solution, 22.2 g of acrylamide and 0.6 g of methylenebisacrylamide and 0.1% SDS were dissolved in water to give 100 ml of solution. Insoluble material was removed by filtration through Whatman No. 1 filter paper. The solution was kept at 4°C in a brown bottle.

For a typical run of 12 gels, 15 ml of gel buffer was deaerated and mixed with 13.5 ml of freshly prepared ammonium persulfate solution (15 mg per ml) and 0.045 ml of N,N,N,N-tetramethylethylenediamine were

added, and immediately poured into gel tubes. Before the hardening of the gel, a few drops of water were layered on top of the gel solution. After 10 to 20 min, an interface could be seen, indicating that the gel had solidified, then the water layer was sucked off.

Preparation of Samples. For each gel, 0.01 ml of dye solution (3 ml of 0.05% Bromphenol blue, 5 ml of mercaptoethanol, 5 ml of dialysis buffer and 20% sucrose) was applied. Then 0.05 ml of the protein solution was added. Gel buffer, diluted 1:3 with water, was carefully layered on top of each sample to fill the tubes. The two compartments of the electrophoresis apparatus were filled with gel buffer, diluted 1:3 with water. Electrophoresis was performed at a constant current of 8 ma per gel with positive electrode in the lower chamber. Under these conditions, the marker dye moved three-quarters through the gel in approximately 3 hr.

Staining and Destaining. The gels were placed in small tubes filled with staining solution for 2 hr. The staining solution was prepared by dissolving 1.25 g of Coomassie brilliant blue in a mixture of 454 ml of 50% methanol and 46 ml of glacial acetic acid. Its insoluble material was removed by filtration through Whatman No. 1 filter paper. Then the gels were rinsed with distilled water, and placed in destaining solution (75 ml of acetic acid, 50 ml of methanol, and 875 ml of water) for 2 days. The gels were stored in 7.0% acetic acid solution.

Experimental Variables and Designs

The experimental variables and the designs used for accomplishing the objectives of this study are summarized in Tables 3 and 4.

Experimental approaches:

A. Scanning Electron Microscope

B. Gel Filtration

C. Polyacrylamide Gel Electrophoresis (PAGE) - 3 M Urea and 0.1 M Acetic Acid

D. PAGE - 0.05 M Acetic Acid

E. PAGE - Model System

F. SDS-PAGE

TABLE 3 EFFECT OF MIXING TIME

Flour	Protein Fractionation from Dough	Dough Mixed min	Experimental Approaches A	B	C	D	E	F
Eagle	Glutenin	0	+		+	+		+
		2			+	+		+
		5	+		+	+		
		21 (28)[a]	+		+	+		+
		46 (56)[b]	+		+	+		+
	Gliadin	0	+	+	+	+		+
		2			+	+		+
		5	+		+	+		
		21 (28)[a]		+	+	+		+
		46 (56)[b]	+	+	+	+		+
Bison	Glutenin	0	+		+	+	+	+
		2	+		+			
		5	+			+	+	+
		21	+		+			
		46	+		+	+	+	+
		92	+		+	+	+	+
	Gliadin	0	+	+	+	+	+	+
		2	+		+			
		5		+		+	+	+
		21	+	+	+			
		46		+	+	+	+	+
		92	+		+	+	+	+
Logan	Glutenin	0	+		+	+	+	+
		1	+		+	+	+	+
		4	+		+	+		
		10	+		+	+	+	+
	Gliadin	0	+	+	+	+	+	+
		1	+	+	+	+	+	+
		4			+	+		
		10	+	+	+	+	+	+

[a]The departure time of SSL treated dough

[b]The double departure time of SSC treated dough

TABLE 4 COMBINED EFFECT OF MIXING TIME AND SURFACTANTS (SSL OR SMP)

Flour	Protein Fractionation from Dough	Dough Mixed min	Experimental Approaches A	B	C	D	E	F
Eagle	Glutenin	2			+	+		+
		5	+		+	+		
		21 (28)[a]	+		+	+		+
		46 (56)[b]	+		+	+		+
	Gliadin	2		+	+	+		+
		5			+	+		
		21 (28)[a]		+	+	+		+
		46 (56)[b]		+	+	+		+
Bison	Glutenin	2	+		+			
		5				+	+	+
		21	+		+			
		46			+	+	+	+
		92	+		+	+	+	+
	Gliadin	2	+		+			
		5		+		+	+	+
		21	+	+	+			
		46		+	+	+	+	+
		92	+	+	+	+	+	+
Logan	Glutenin	1	+		+	+	+	+
		4	+		+	+		
		10	+		+	+	+	+
	Gliadin	1		+	+	+	+	+
		4			+	+		
		10		+	+	+	+	+

[a]The departure time of SSC treated dough

[b]The double departure time of SSC treated dough

RESULTS AND DISCUSSION

Farinograph

Farinograms were obtained from wheat flour and wheat flour containing surfactant (0.5% level). The farinograph characteristics of certain doughs are presented in Table 5.

TABLE 5 FARINOGRAPH CHARACTERISTICS OF DOUGHS PREPARED FROM WHEAT FLOURS WITH OR WITHOUT SURFACTANTS

Flour	Surfactants	Water Absorption %	Arrival Time min	Peak Time min	Departure Time min	Dough Stability min	M.T.I. B.U.
Eagle							
	Control	67.6	4.5	10.0	21.0	16.5	22
	SSL	66.9	7.5	15.0	28.0	20.5	6
	SMP	68.0	4.5	10.0	21.0	16.5	12
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

Water Absorption. Water absorptions of Eagle, Bison, and Logan flours were 67.6, 58.2, and 56.4, per cent, respectively. Addition of surfactant to the wheat flour induced only minor changes in water absorption.

Arrival Time. The arrival time is an indication of how quickly the flour and water mix to form a homogeneous mass which provides resistance to mixing. The quality and quantity of gluten can affect the mixing properties of dough. In general, soft wheat flour has a shorter arrival time than hard wheat flour.

The arrival time of control dough was 4.5 min for Eagle flour, 2.0 min for Bison flour, and 0.5 min for Logan flour. Surfactants added to dough resulted in only a little change in arrival time except SSL added to Eagle dough had a long arrival time.

Peak Time. The farinograph peak time was found to be 10.0 min, 8.0 min, and 1.0 min for the Eagle, Bison, and Logan flours, respectively. Addition of SSL increases all of the dough development time in all cases. SMP had little effect except on Logan flour (Table 5).

Departure Time. The departure time is an indication of the strength of the flour proteins and how well they can withstand mixing action. The data (Table 5) indicates that the SSL, added to Eagle and Bison flours, have a longer departure time than the controls.

Dough Stability. The dough stability is determined by the difference between the arrival time and departure time. It is also a good criterion for protein strength of a dough. As shown in the Table, the addition of SSL greatly increases Eagle and Bison (HRW) dough stability that is

indicative of strengthening of gluten structure during mixing, whereas the addition of SMP hardly shows any effect, as compared to that of the controls.

Mixing Tolerance Index. The mixing tolerance index (M.T.I.) value of dough containing SSL and SMP decreases with all the wheat flours tested when compared with the control flours, but one exception is SMP with Logan wheat flour which has almost the same M.T.I. value as the control. SSL is more effective than SMP in changing mixing tolerance.

Summary. The results obtained from the farinograph study strongly indicate that the addition of surfactants alters the mixing characteristics of dough. The alteration is probably due to interaction or complexing of the surfactants with gluten and other flour constituents. The different patterns in dough stability obtained with SSL and SMP suggest that a mechanism for improving dough rheology may be different between SMP (nonionic surfactant) and SSL (ionic surfactant).

Scanning Electron Microscopy

Figure 2-a depicts the Eagle gluten obtained from 2 min mixed dough washed under a gentle stream of distilled water. Gluten is a complex coherent mass without a fibrillar structure. It contains starch granules which adhere to the gluten matrix (5). Figures 2-b, 2-c, and 2-d are SEM micrographs of Eagle glutenins in a stretched sheet form, showing the initial stages in the transformation to a fibrillar structure. Many small holes are visible in the sheet in the area where the sheet appears to be under stress. The sheet has rolled back at the ruptured edges forming thick fibers and thin fibrous strands (2).

Effects of Mixing on Wheat Glutenins. Figures 3, 4, and 5 show the effect of mixing on Eagle, Bison, or Logan glutenin structure individually. The following points can be derived from these figures:

The glutenins isolated from the flour have a structure quite different from the glutenins isolated from mixed doughs. They are comprised of large protein aggregates (Fig. 3-a).

Initial mixing ruptures the sheet structure of glutenin (Fig. 3-a) and then associates into large amounts of fibrous structure (Fig. 3-b). Shearing action of the mixing process dissociates and orients glutenins for the formation of the protein network (Fig. 3-c). The glutenins reach a maximum in viscous and elastic properties between the two stages of fibrous and network structure (T24). With prolonged mixing, the protein network gradually breaks down (breaks sheetlike structure and cuts short fibrous structure (Fig. 3-d). The broken pieces of network structure cause adhesions between them (Figs. 3-e and 3-f).

From Fig. 4, it can be seen that Bison glutenins have different structure compared with Eagle glutenins. At the initial mixing stage, Bison glutenin changes more rapidly than Eagle glutenin.

The structural change of Logan glutenin (Fig. 5) is different from that of Eagle or Bison glutenin (Figs. 3 and 4). The differences are largely due to variation in glutenin quality between soft wheat and hard wheat flours. The molecular size of Logan glutenins may be small, as shown in Disc Electrophoresis in Results and Discussion.

Combined Effects of Mixing and Surfactants on Wheat Glutenins. Figures 6, 7, and 8 show the combined effects of mixing and SSL (Figs. 6-a,b,c; 7-a,b,c; and 8-a,b.c) or SMP (Figs. 6-d,e,f; 7-d,e,f; and 8-d,e,f) on wheat (Eagle, Bison, and Logan) glutenins.

Surfactants, SSL and SMP, appear to assist glutenins to develop a sheetlike structure (Figs. 6-a and 6-d) and to maintain such a structure longer with mixing (Figs. 6-b and 6-e). In other words, surfactants can protect the sheetlike structure from breaking.

The action of SSL and SMP on Logan glutenins (Fig. 8) is hard to ascertain, largely because the change of glutenin structure takes place so rapidly during mixing.

Glutenin-Surfactant Interactions in Model System. Surfactants, SSL and SMP, appear to associate and to maintain the structure of glutenins. But they do not produce clear changes, especially with Bison glutenins, probably because the surfactants added at 0.5% level (based on flour weight) are too little to affect the glutenin structure. Surfactant could associate not only with protein but also with carbohydrate and lipid in dough system. Additional work was therefore undertaken to characterize the interactions of Bison glutenins and surfactant SSL (Fig. 9) or SMP (Fig. 10) in a model system.

The model system consisted of 0.5% surfactants (based on protein weight) and Bison glutenins dispersed in 0.1 M acetic acid and 3 M urea solvent for overnight in a refrigerator at $4^{o}C$. The protein solution was dialyzed, freeze-dried, and examined with scanning electron microscope. The results can be summarized as follows:

Original fibrous and sheetlike structure of Bison glutenins (Fig. 4) disappear and form many associated glutenin structures with surfactants, (Figs. 9 and 10). The small glutenin-surfactant complex (Figs. 9-a and 10-a) readily undergoes molecular orientation and forms a large sheetlike

glutenin-surfactant complex (Figs. 9-b,c,d,e; and 10-b,c,d,e). But with prolonged mixing, this complex is gradually broken down (Figs. 9-f and 1 -f).

Between the surfactants, the SSL seems more effective than SMP in interacting with glutenins because SSL appears to bind more glutenins than SMP as reflected by the size of complex structure.

Effects of Mixing on Wheat Gliadins. Figures 11 and 12 show the effects of mixing on Eagle, Logan (Fig. 11), and Bison (Fig. 12) gliadins. The following points can be derived from these figures:

All the gliadin particles are smaller than glutenins. Gliadins isolated from flours (Figs. 11-a,d; and 12-a) seem to have a relatively amorphous structure.

Prolonged mixing decreases the amount of amorphous protein structure and increases the narrow sheetlike structure (Figs. 11-b and 12-b) or the concentration of round particles (Fig. 11-e). The overmixed gliadins are comprised of sheet- and rod-like structures (Figs. 11-c,f; and 12-c,d).

All gliadins, shown in SEM micrographs, contain a large amount of starch, but largely because the gliadins are not completely purified.

Combined Effects of Mixing and Surfactants on Wheat Gliadins. Figure 13 shows combined effects of mixing and surfactant SSL (Fig. 13-a, b,c) or SMP (Fig. 13-d,e,f) on Bison gliadins. The effects can be summarized as follows:

All surfactant treated gliadins have a similar pattern of change when compared to control gliadins isolated from doughs mixed for different mixing times.

The surfactant treated gliadins contain more sheet-like and fibrous structure than do control gliadins (Fig. 12) during the initial mixing time. The overmixed gliadins show a pattern between sheet-like and amorphous structure (Fig. 13-c,f).

Surfactant SSL and SMP show different effects on Bison gliadins. SSL alters the gliadin structure more than the SMP.

Gel Filtration

Gel filtration is a widely used method for fractionating wheat glutenins and gliadins, for estimating the molecular weight of each fraction, and for confirming the association and dissociation of wheat proteins during mixing with or without surfactants. The gel filtration of glutenins will not be dealt with here because the molecular size of glutenins is too large to enter pores of Bio-Gel P-150.

Effects of Mixing on Wheat Gliadins. Figure 14 shows the gel filtration of gliadins isolated from Eagle, Bison, and Logan flours and doughs mixed for indicated periods. Extended mixing dissociates and breaks the protein aggregates. As a result, the concentration of first fraction is reduced and the concentration of the second fraction increased.

All of the Eagle, Bison, and Logan gliadins show the rate of association and dissociation vary among different flours.

Combined Effects of Mixing and Surfactants on Wheat Gliadins. Figures 15 and 16 show the gel filtration of gliadins isolated from SSL (Fig. 15) or SMP (Fig. 16) treated doughs mixed for indicated periods.

Surfactant SSL decreases the rate of protein dissociation (Fig. 15). Surfactant SMP seems less effective than SSL because SMP does not produce a clear-cut change of gliadins.

Comparison of Electrophoretic Patterns of First Gel Fractions of Glutenins and Gliadins. Although gel filtration is used to estimate the molecular weight of glutenin and gliadin fractions, the first effluent peak is very heterogeneous with different molecular weights. This experiment was conducted to find out the components differences of the first gel fraction of glutenins or gliadins isolated from mixed Logan dough. There is no electrophoretic difference between glutenins isolated from doughs mixed for 1 min and 10 min, largely because the molecular size of glutenins is too large to get into the electrophoretic gel.

Figure 17 shows the disc electrophoretic patterns of the first gel fraction of glutenins and gliadins isolated from doughs. This fraction shows different electrophoretic patterns. The results can be summarized as follows:

The reduction of first fraction in gel filtration and the increase in the second fraction, in another part of this section, indicates that the mixing dissociates gliadins into smaller size. With disc electrophoresis, even the first fraction shows some reduction on its size and increases the concentration of the third electrophoretic component (c).

THIS BOOK CONTAINS NUMEROUS PAGES THAT WERE BOUND WITHOUT PAGE NUMBERS.

THIS IS AS RECEIVED FROM CUSTOMER.

Fig. 2 SEM micrographs of gluten and glutenin isolated from Eagle flour-water dough mixed for 2 min.

EAGLE

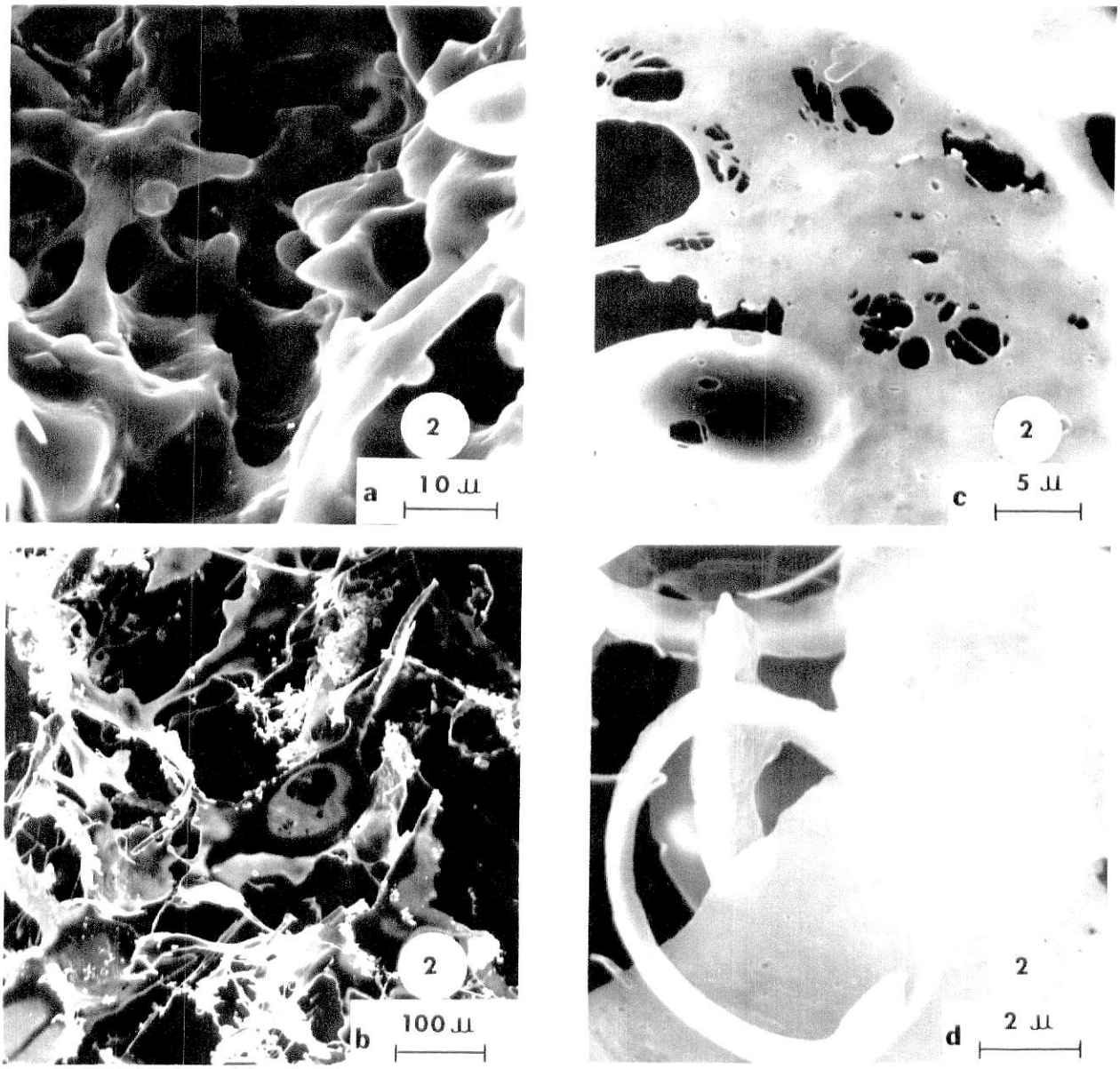


Fig 3 SEM micrographs of glutenins isolated from Eagle flour and flour-water doughs mixed for indicated periods.

EAGLE GLUTENIN

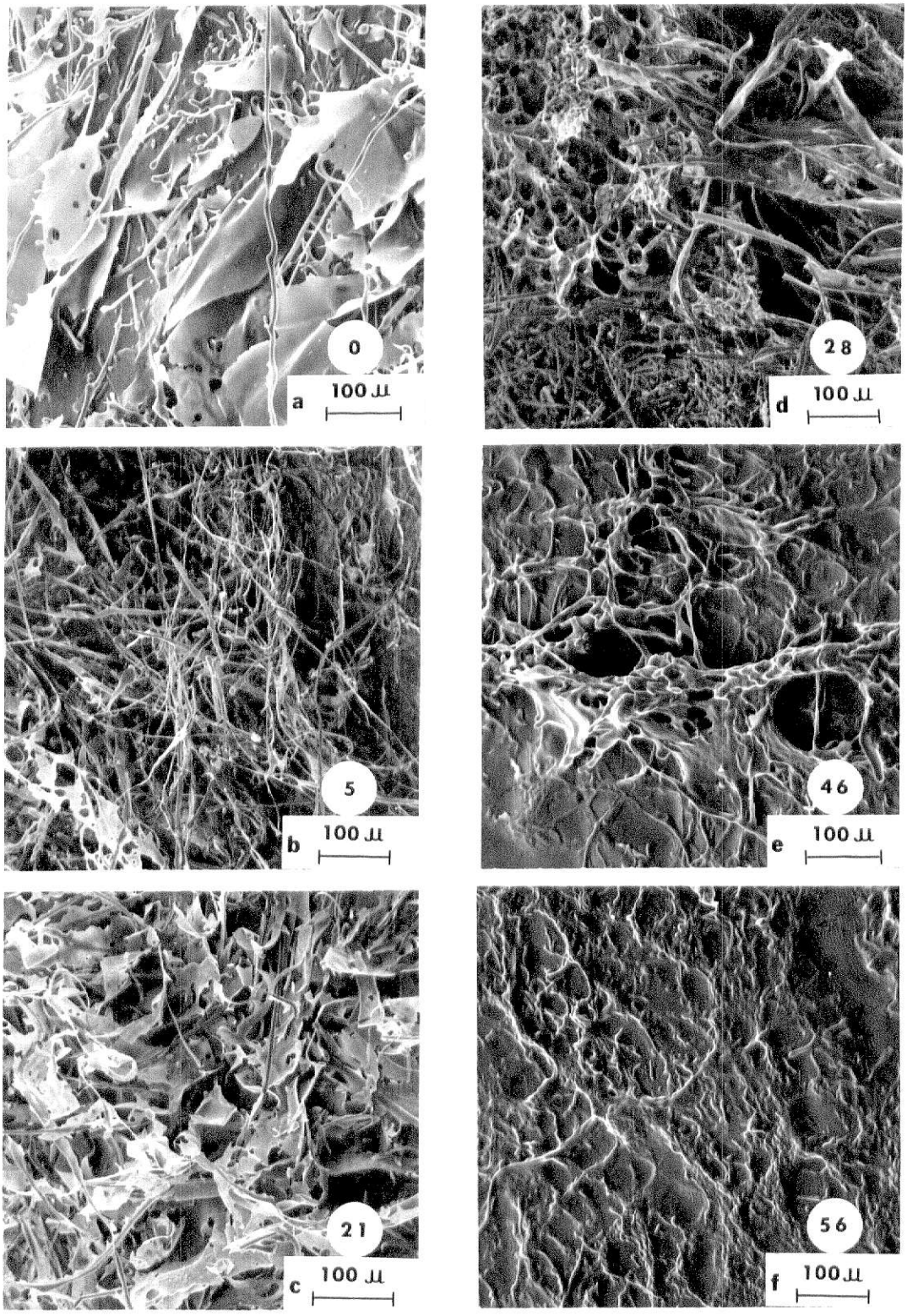


Fig. 4 SEM micrographs of glutenins isolated from Bison flour and flour-water doughs mixed for indicated periods.

BISON GLUTENIN

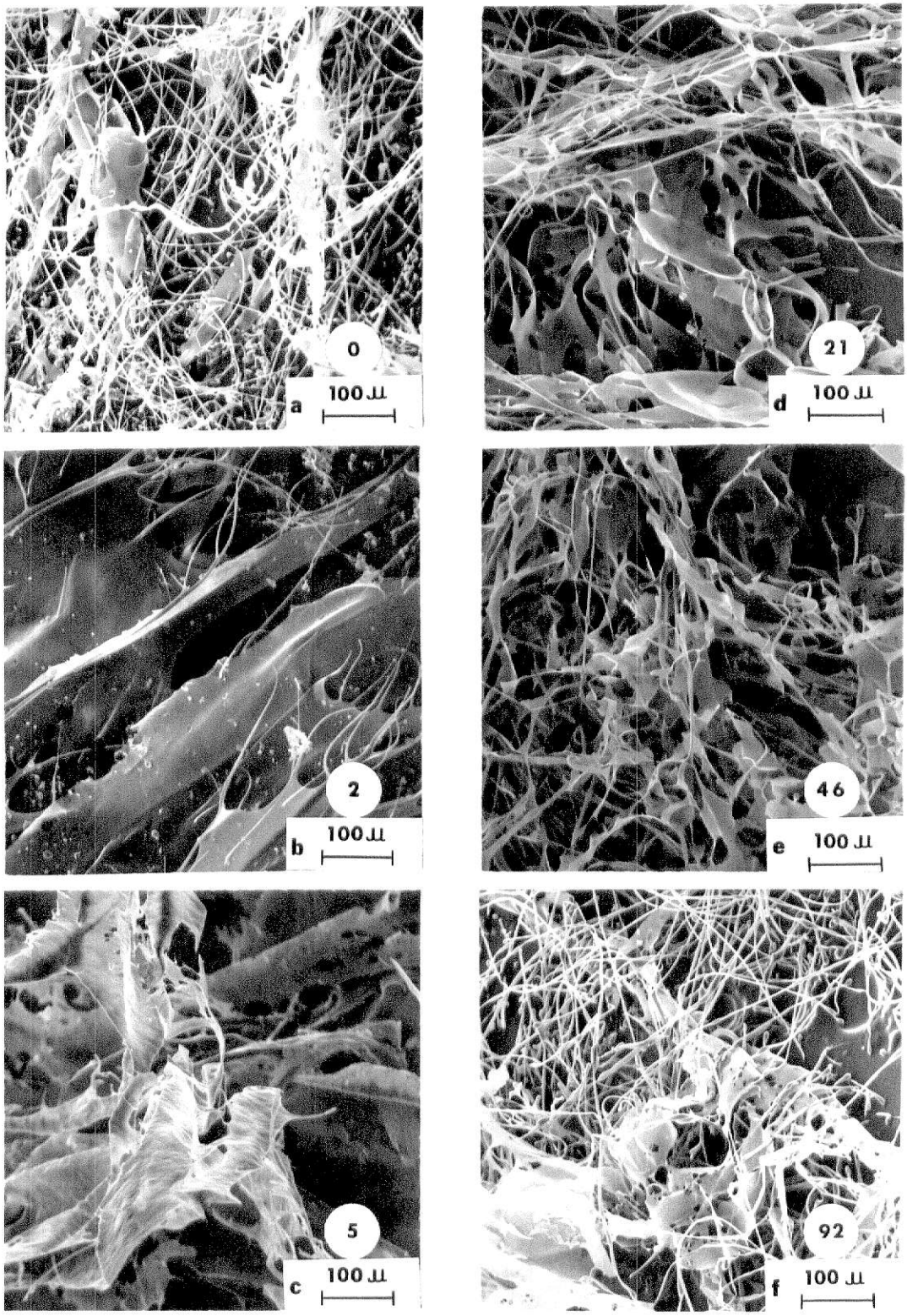


Fig. 5 SEM micrographs of glutenins isolated from Logan flour and flour-water doughs mixed for indicated periods.

LOGAN GLUTENIN

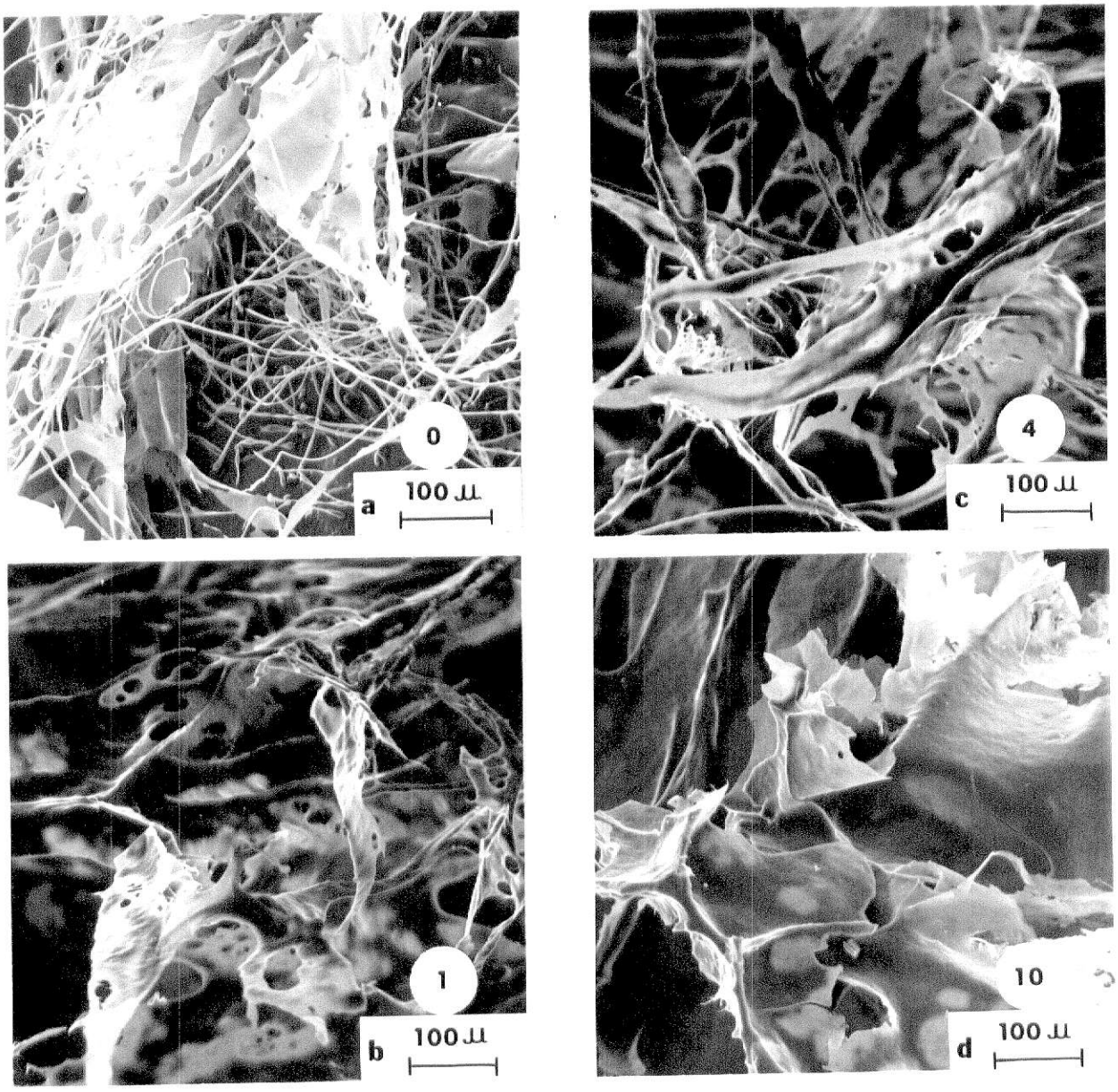


Fig. 6 SEM micrographs of glutenins isolated from SSL (a,b,c) or SMP (d,e,f) treated Eagle flour-water doughs mixed for indicated periods.

EAGLE GLUTENIN

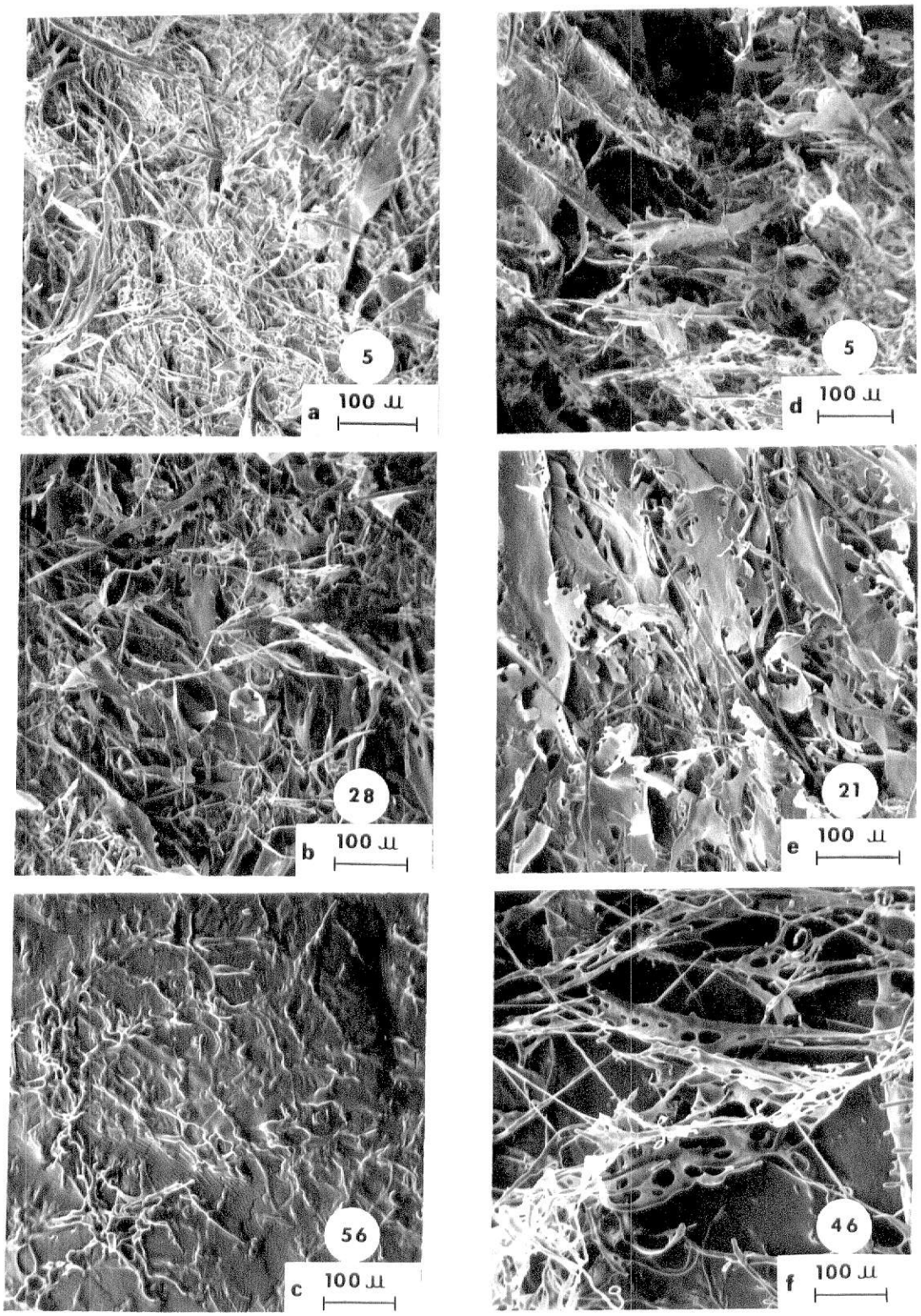


Fig. 7 SEM micrographs of glutenins isolated from SSL (a,b,c) or SMP (d,e,f) treated Bison flour-water doughs mixed for indicated periods.

BISON GLUTENIN

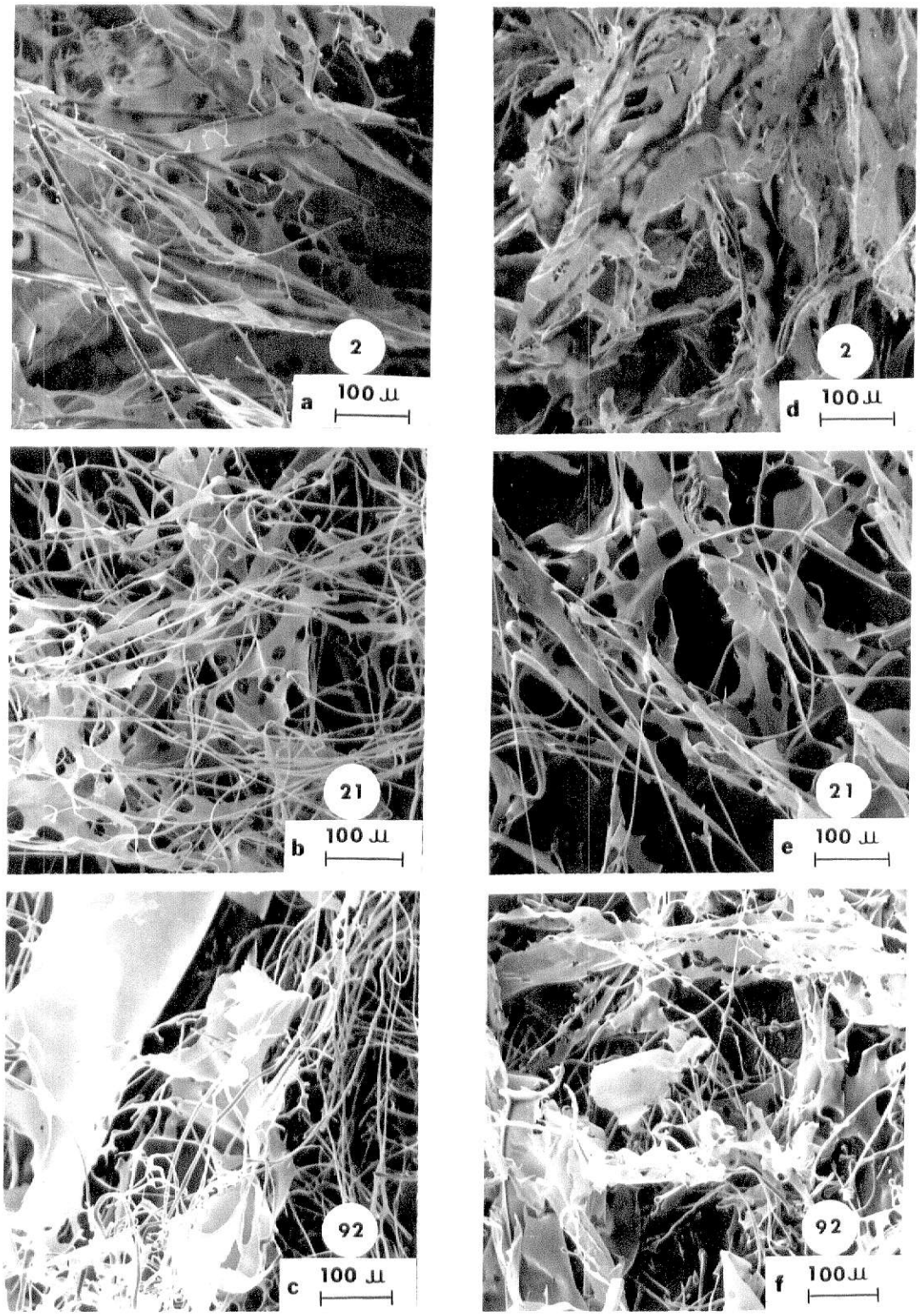


Fig. 8 SEM micrographs of glutenins isolated from SSL (a,b,c) or SMP (d,e,f) treated Logan flour-water doughs mixed for indicated periods.

LOGAN GLUTENIN

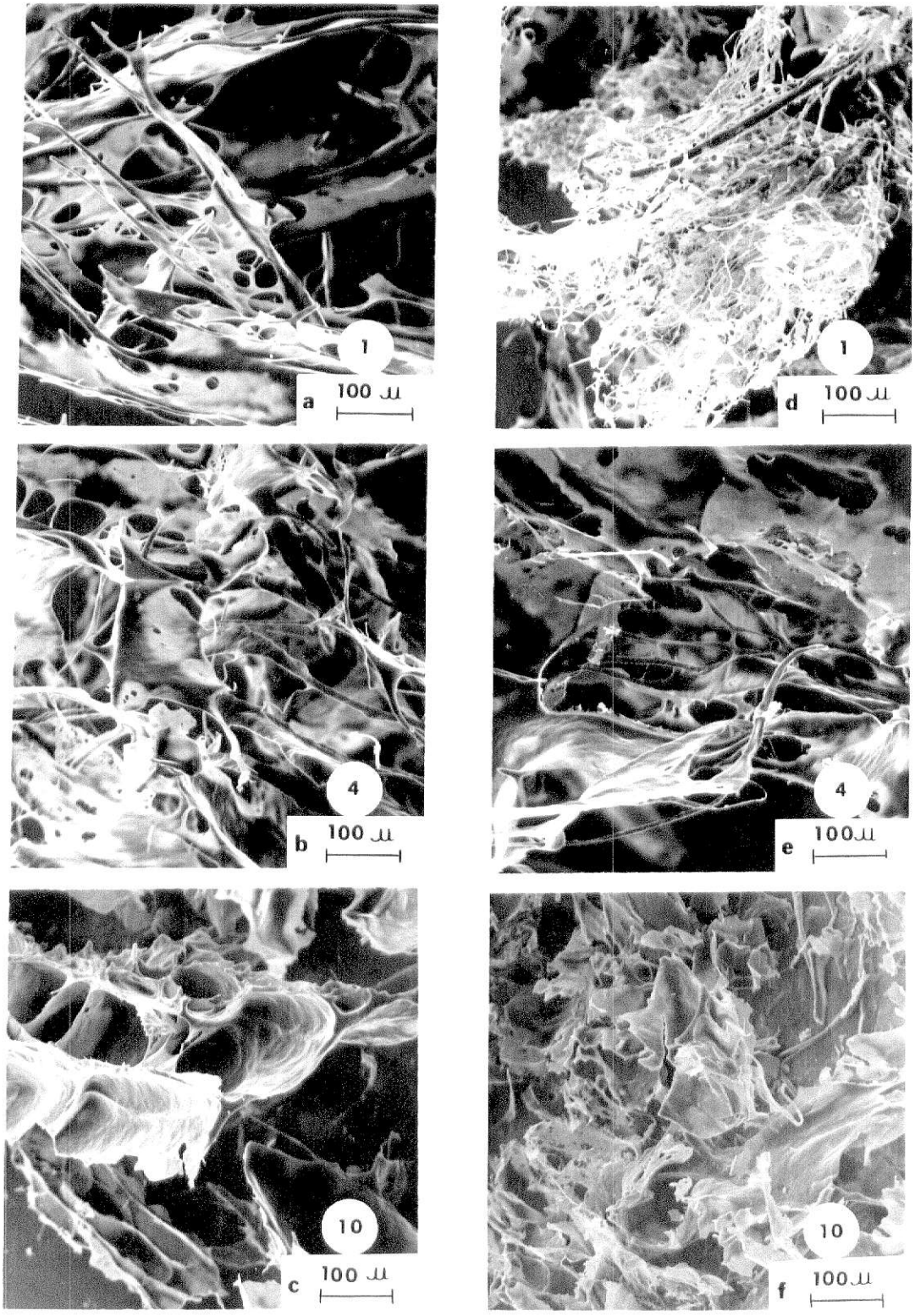


Fig. 9 SEM micrographs of glutenins in model system represents interactions between SSL and glutenins isolated from Bison flour and flour-water doughs mixed for indicated periods.

BISON GLUTENIN

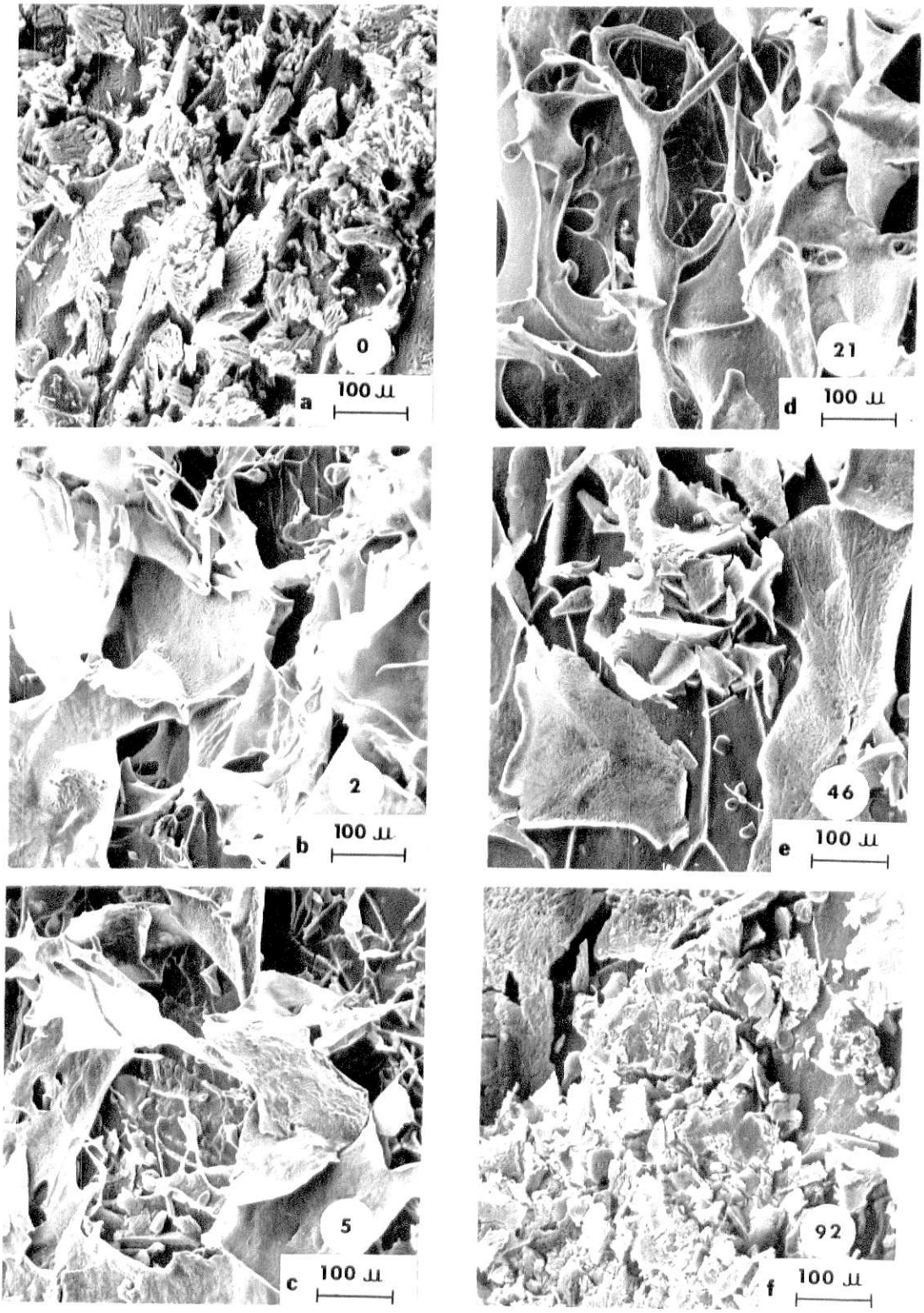


Fig. 10 SEM micrographs of glutenins in model system represents interactions between SMP and glutenins isolated from Bison flour and flour-water doughs mixed for indicated periods.

BISON GLUTENIN

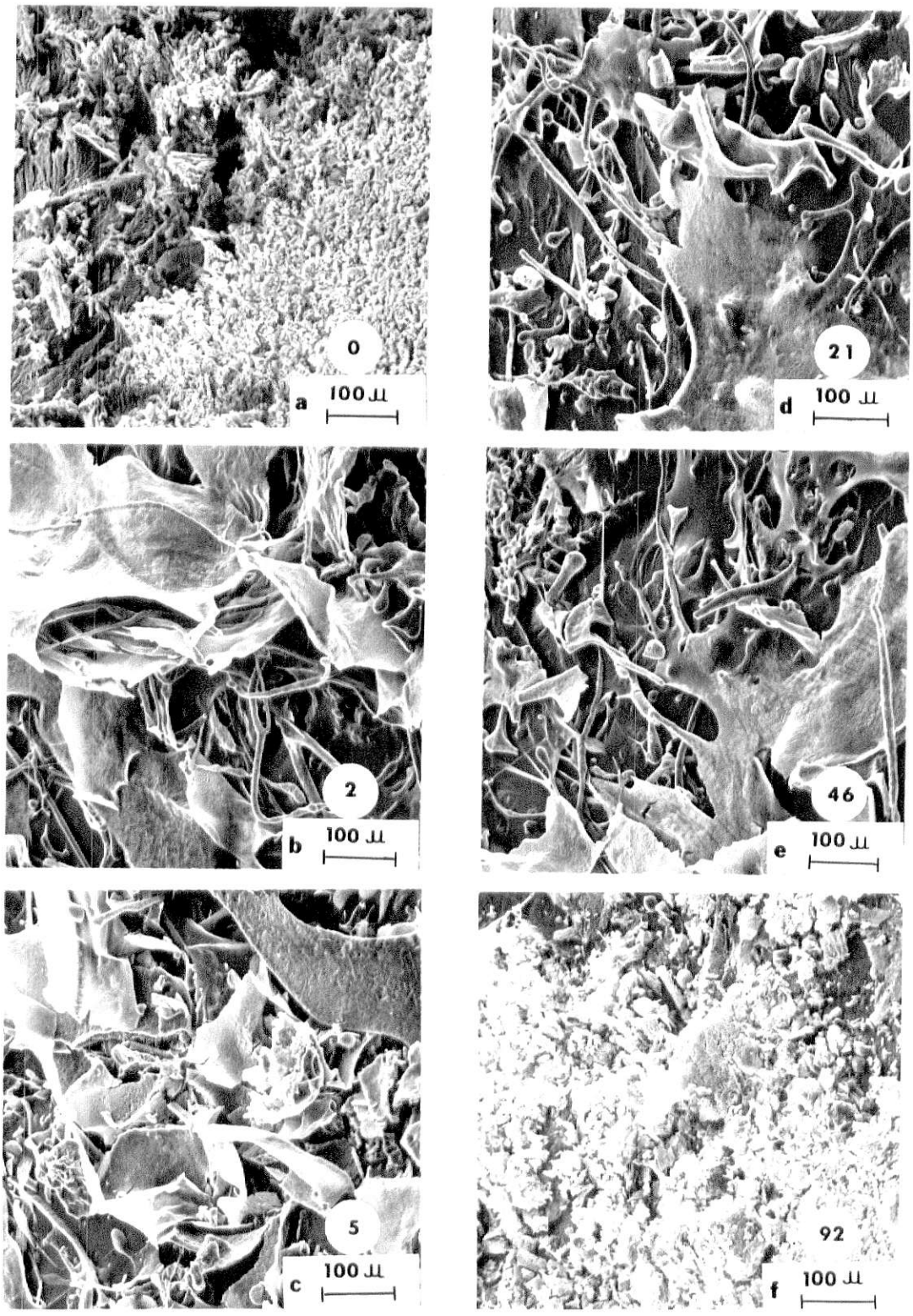


Fig. 11 SEM micrographs of gliadins isolated from Eagle and Logan flour and flour-water doughs mixed for indicated periods.

EAGLE GLIADIN

LOGAN GLIADIN

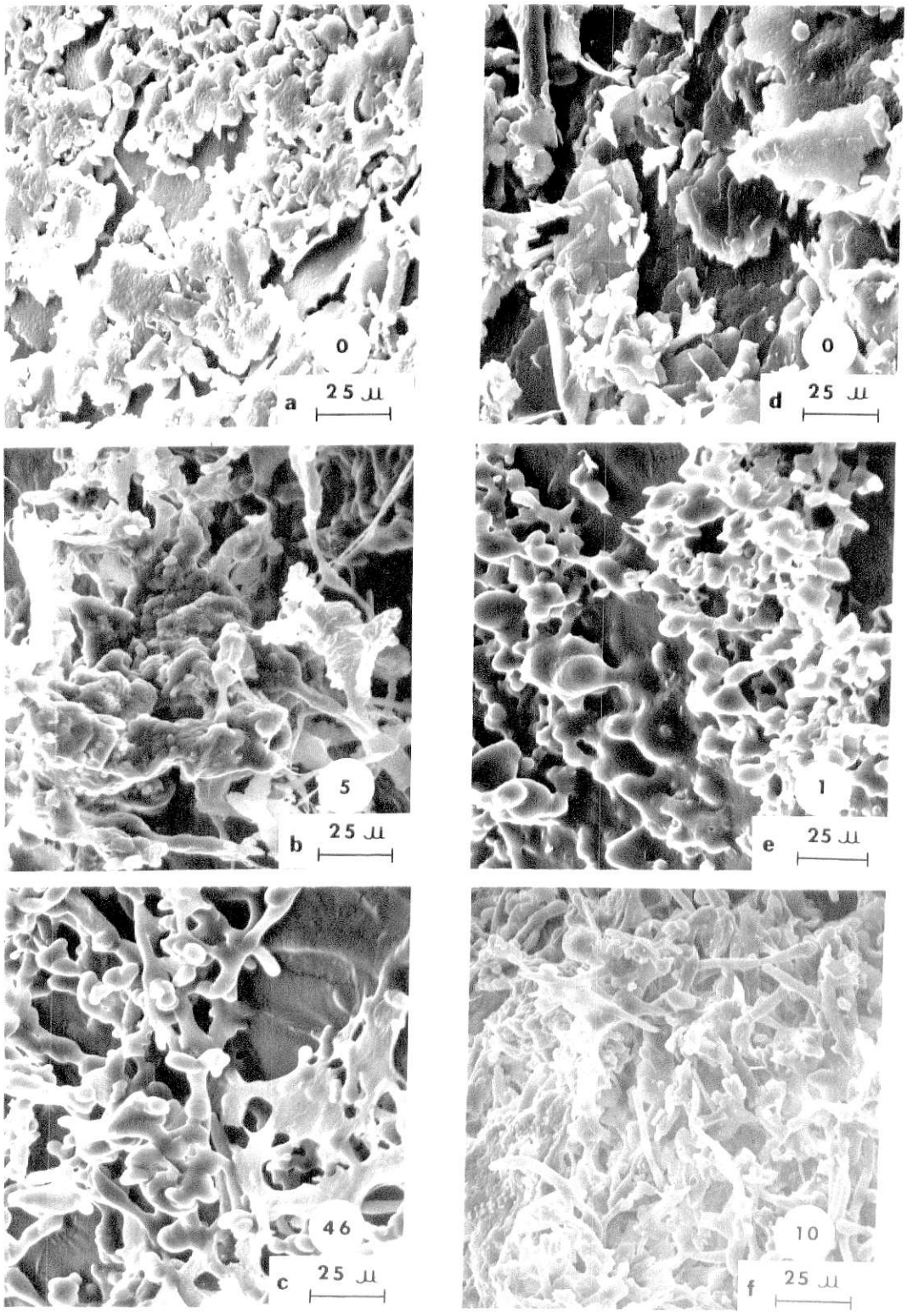


Fig. 12. SEM micrographs of gliadins isolated from Bison flour and flour-water doughs mixed for indicated periods.

BISON GLIADIN

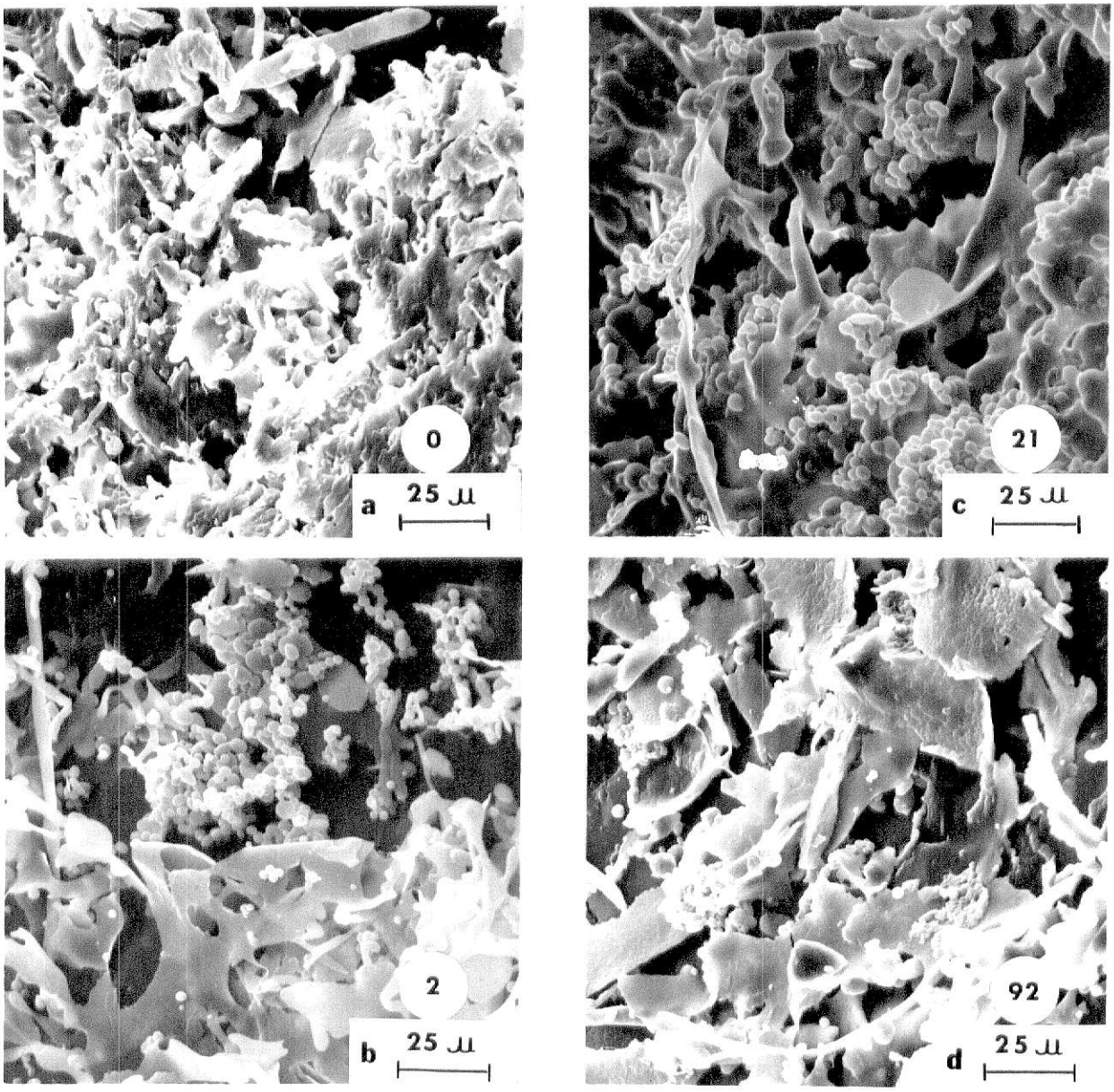


Fig. 13 SEM micrographs of gliadins isolated from SSL (a,b,c) or SMP (d,e,f) treated Bison flour-water doughs mixed for indicated periods.

BISON GLIADIN

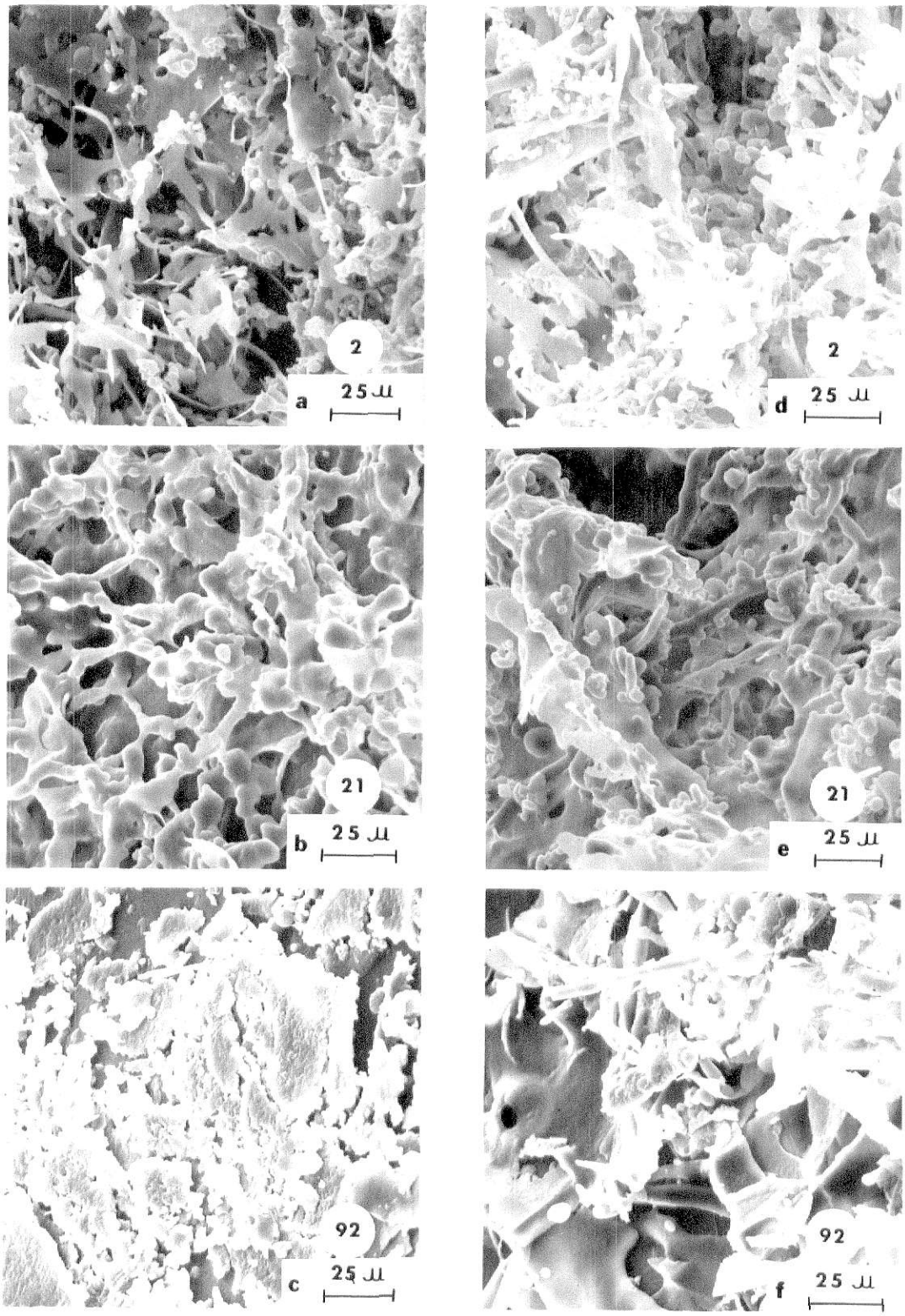


Fig. 14. Gel filtration of Eagle, Bison, and Logan gliadins isolated from flour and flour-water doughs mixed for indicated periods, on a column (45 x 2.5 cm) of Bio-Gel P-150 (100-200 mesh) using 0.1 M acetic acid and 3 M urea as protein solvent and eluant at a flow rate of 11 ml/hr.

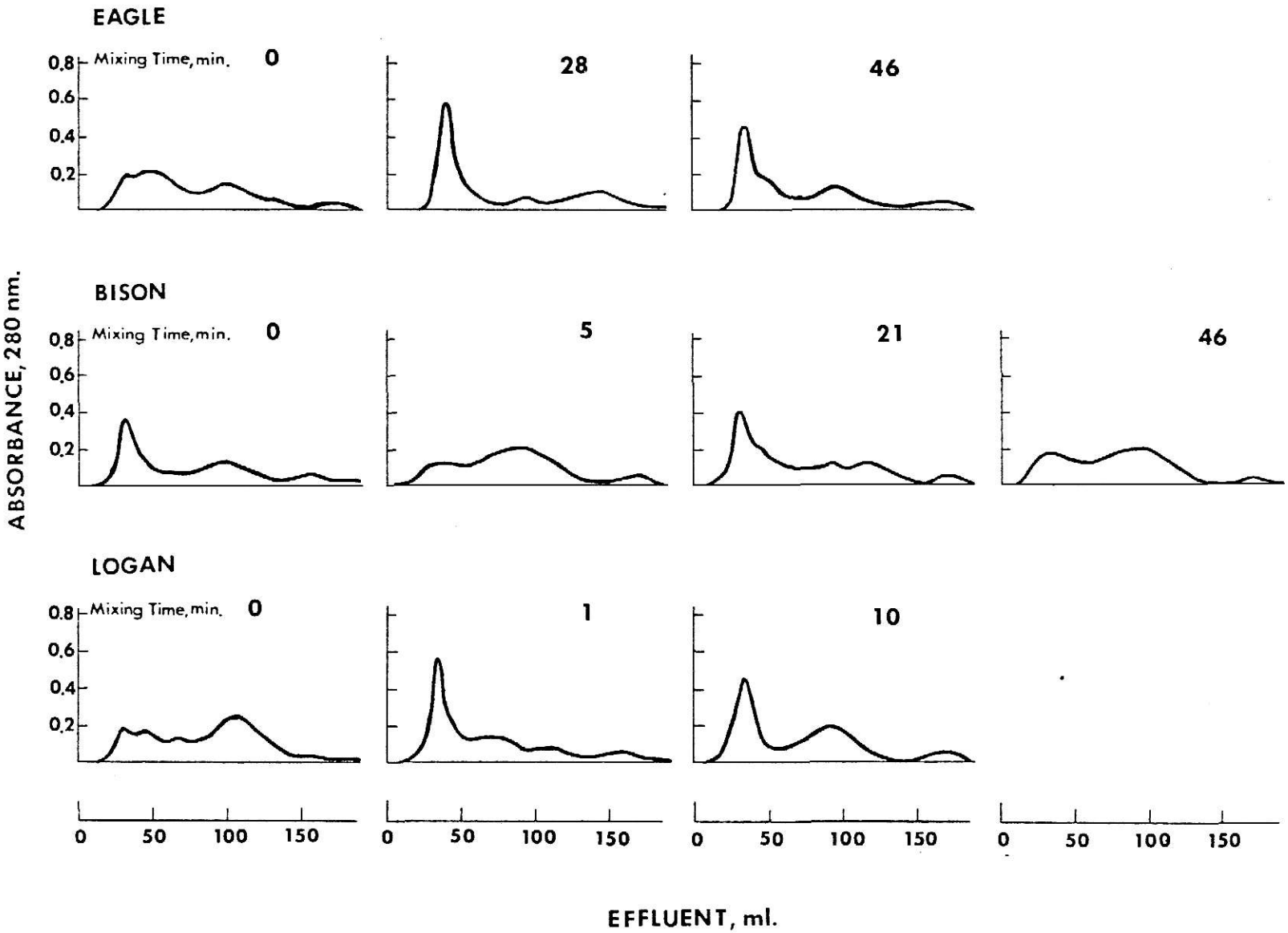

EAGLE
Mixing Time, min.
0
28
46
BISON
Mixing Time, min.
0
5
21
46
LOGAN
Mixing Time, min.
0
1
10
0.8
0.6
0.4
0.2
0
50
100
150
ABSORBANCE, 280 nm.
EFFLUENT, ml.

Fig. 15 Gel filtration of Eagle, Bison, and Logan gliadins isolated from SSL treated doughs mixed for indicated periods, on a column (45 x 2.5 cm) of Bio-Gel P-150 (100-200 mesh) using 0.1 M acetic acid and 3 M urea as protein solvent and eluant at a flow rate of 11 ml/hr.

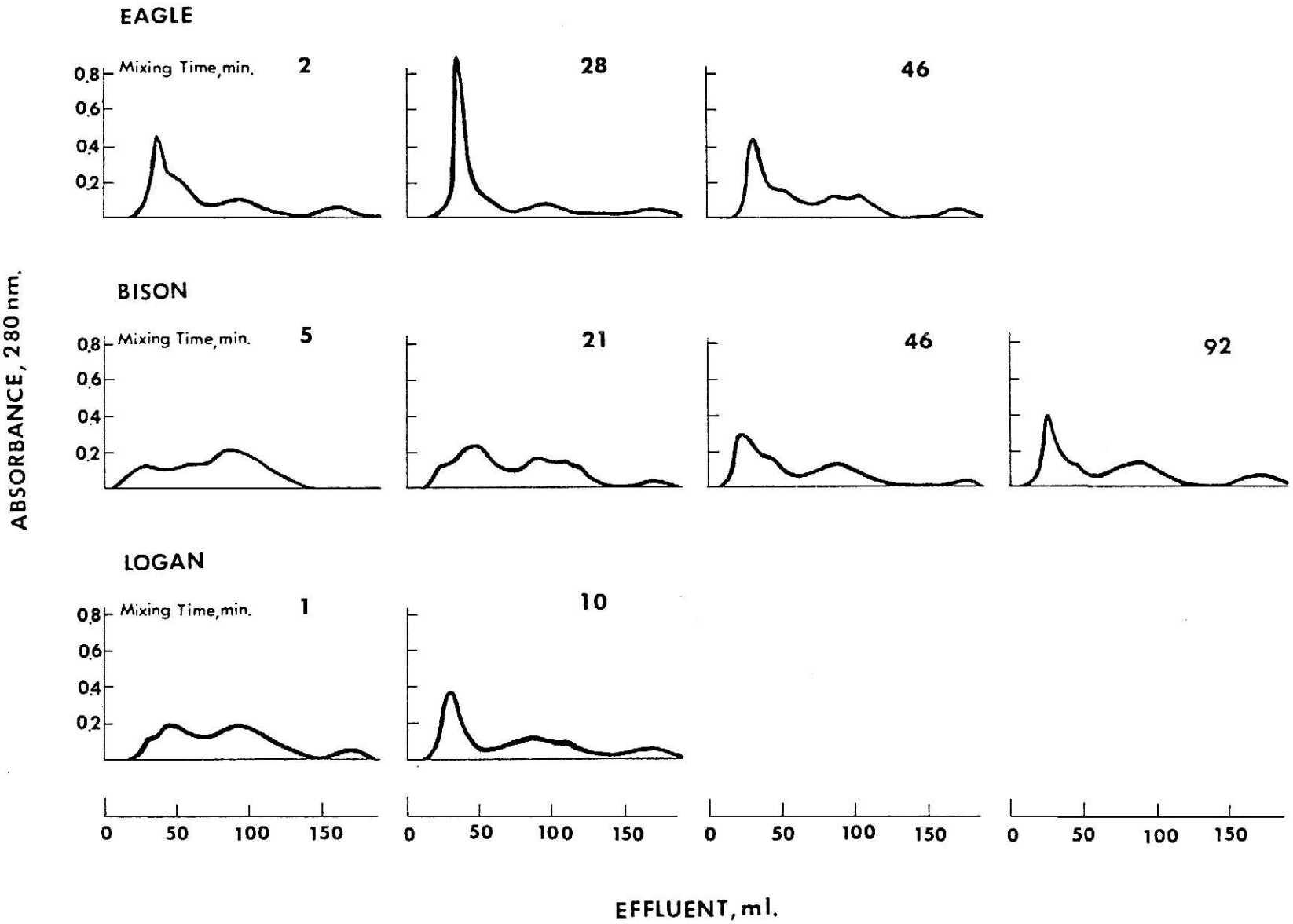

EAGLE
Mixing Time, min.
2
28
46
BISON
Mixing Time, min.
5
21
46
92
LOGAN
Mixing Time, min.
1
10
0.8
0.6
0.4
0.2
0
50
100
150
ABSORBANCE, 280 nm.
EFFLUENT, ml.

Fig. 16 Gel filtration of Eagle, Bison, and Logan gliadins isolated from SMP treated doughs mixed for indicated periods, on a column (45 x 2.5 cm) of Bio-Gel P-150 (100-200 mesh) using 0.1 M acetic acid and 3 M urea as protein solvent and eluant at a flow rate of 11 ml/hr.

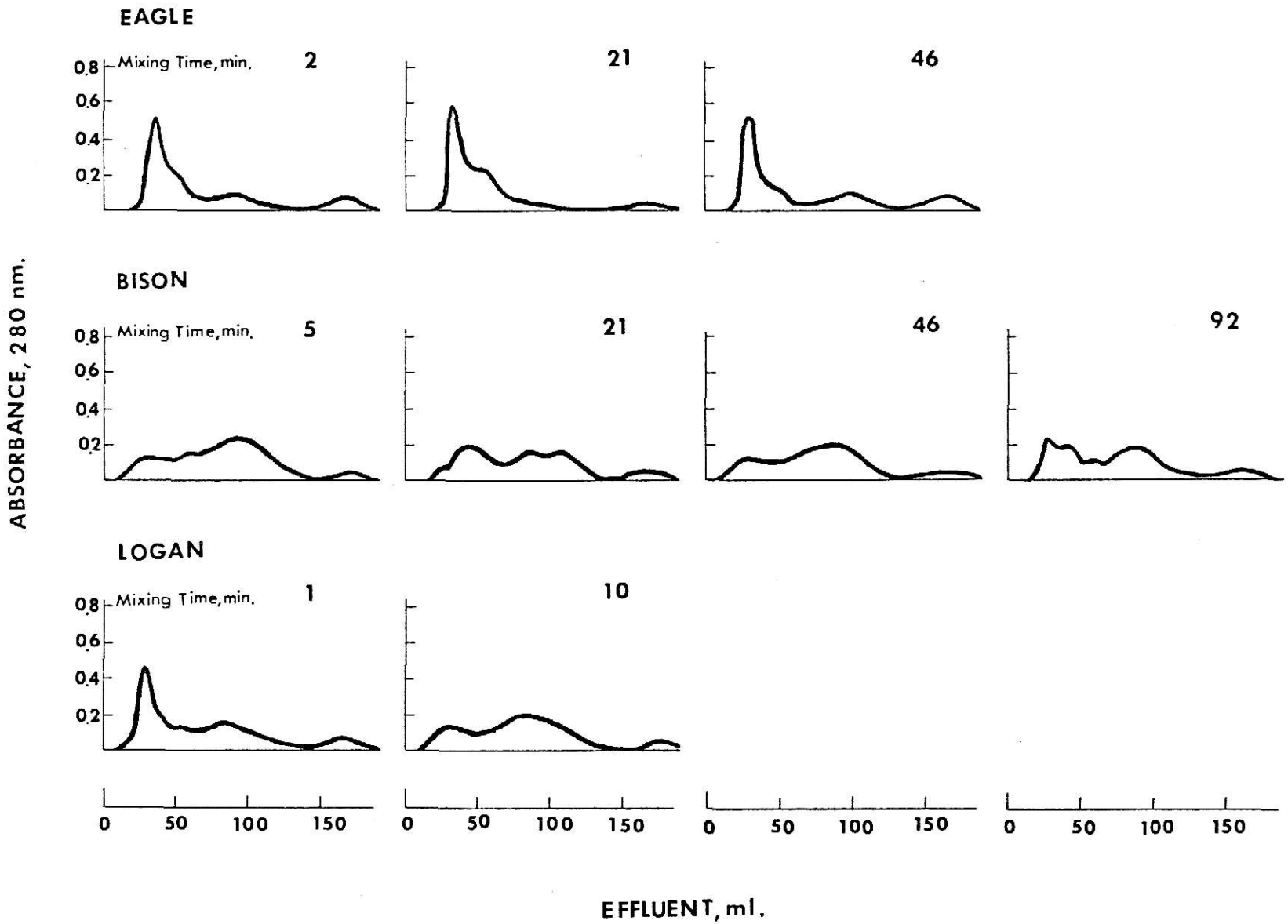
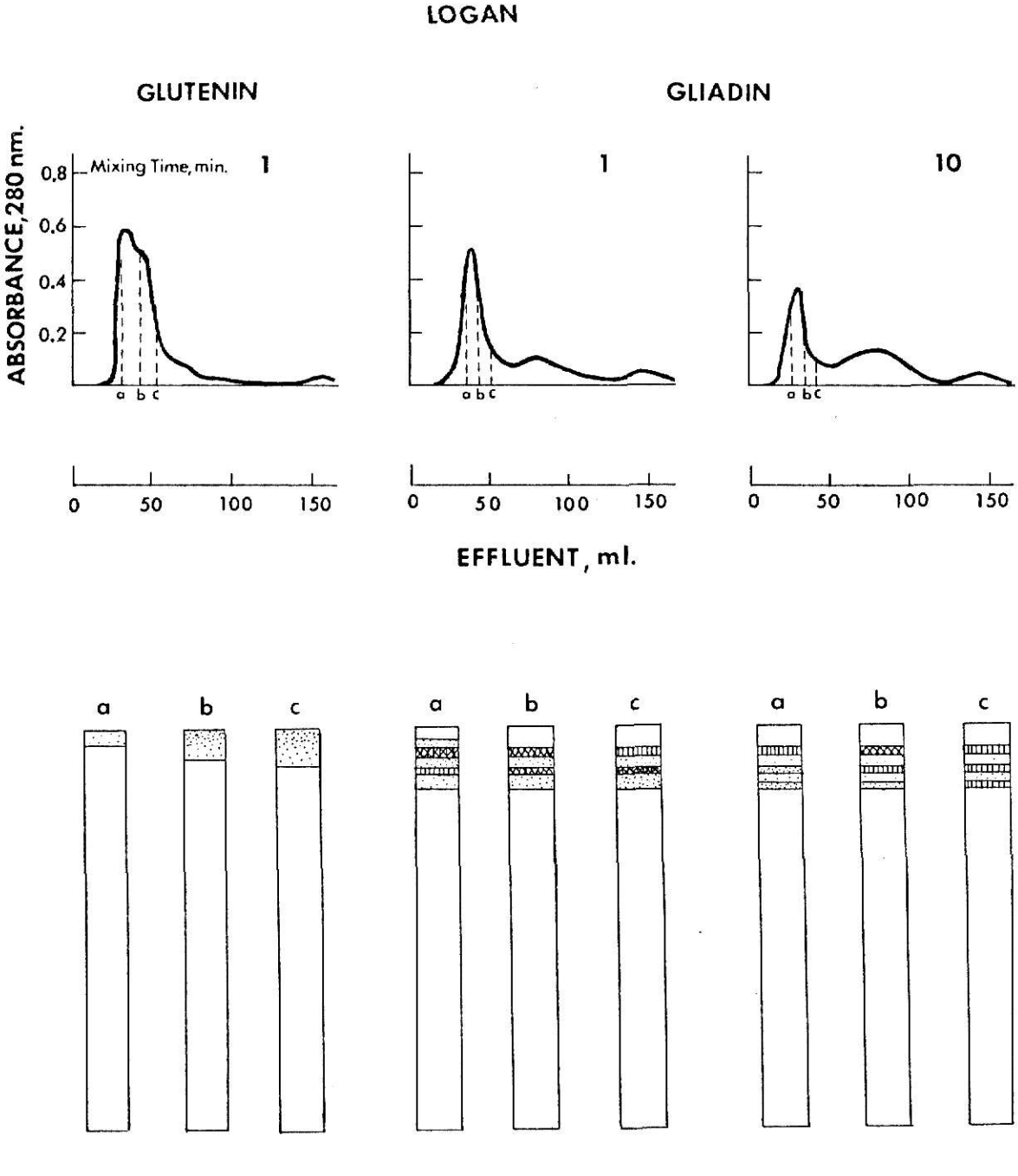

EAGLE
Mixing Time, min.
2
21
46
BISON
Mixing Time, min.
5
21
46
92
LOGAN
Mixing Time, min.
1
10
0.8
0.6
0.4
0.2
0
50
100
150
ABSORBANCE, 280 nm.
EFFLUENT, ml.

Fig. 17 A comparison of electrophoretic patterns on first gel fraction of glutenins and gliadins isolated from Logan flour-water doughs mixed for indicated periods, on a column (45 x 2.5 cm) of Bio-Gel P-150 (100-200 mesh) using 0.1 M acetic acid and 3 M urea as protein solvent and eluant at a flow rate of 11 ml/hr.


LOGAN
GLUTENIN
GLIADIN
ABSORBANCE,280 nm.
Mixing Time, min.
1
1
10
0.8
0.6
0.4
0.2
a b c
0
50
100
150
EFFLUENT, ml.

Disc Electrophoresis

Polyacrylamide Gel. Glutenins and gliadins can be separated into many components by disc electrophoresis. This experiment was made to characterize the component changes of glutenins and gliadins isolated from doughs mixed for various periods and treated with or without surfactants by using polyacrylamide gel electrophoresis (PAGE).

Three solvent systems were used to dissolve the glutenins and gliedins. (A) 0.1 M acetic acid and 3 M urea can dissolve 93% of glutenins and gliadins (60); (B) 0.05 M acetic acid is widely used and has less denaturing effect on protein structure; (C) distilled water is used to examine the electrophoretic patterns of the nature of glutenins and gliadins which can get into the gel.

The slow moving components of disc electrophoresis of glutenins and gliadins likely indicate their high molecular weight; and some glutenins because of their large molecular size cannot get into the electrophoretic gel.

Effects of Mixing on Wheat Proteins. Figures 18 and 19 show the electrophoretic patterns representing the effects of mixing on glutenins and gliadins. The solvents used for electrophoretic studies are 0.1 M acetic acid + 3 M urea (Fig. 18) and 0.05 M acetic acid (Fig. 19). The results can be summarized as follows:

Gliadins isolated from different wheat flours (Eagle, Bison, and Logan) show different electrophoretic patterns, varying in the number, migration and concentration of their components.

Mixing changes the electrophoretic pattern drastically, indicating that gliadins first undergo a rapid process of association, then dissociation. With initial dough mixing, gliadins undergo molecular association and decrease the concentration of fast moving components and increase the concentration of slow moving components. Extended mixing can increase the concentration of fast moving components and decrease the concentration of slow moving components, probably because prolonged mixing dissociates the large protein aggregates.

The gliadins' electrophoretic pattern with the acetic acid and urea (Fig. 18) as protein solvents is different from that with the acetic acid along (Fig. 19), because urea is a high polar solvent which helps acetic acid to dissociate the gliadins. In spite of the difference, the rapid change in association and dissociation of gliadins is evident.

Some glutenins, because of their large molecular size, can not get into the electrophoretic gel. This is clearly reflected by the finding that more glutenins are present in the electrophoretic gel with the urea and acetic acid than with acetic acid alone.

Glutenins isolated from different wheat flours (Eagle, Bison, and Logan) show different electrophoretic patterns, varying in the number, migration and concentration of their components.

The changes of association and dissociation of glutenins are similar to those of gliadins. With initial dough mixing, glutenins undergo molecular association; as a result, the concentration of slow moving components is reduced. Extended mixing dissociates the glutenin aggregates and increases the concentration of all the components.

Combined Effects of Mixing and Surfactants on Wheat Proteins. Figures 20, 21, 22, and 23 show the electrophoretic patterns of glutenins and gliadins isolated from surfactant SSL (Figs. 20 and 22) and SMP (Figs. 21 and 23) treated doughs. The solvents used for these studies are 0.1 M acetic acid + 3 M urea (Figs. 20 and 21), and 0.05 M acetic acid (Figs. 22 and 23). The results can be summarized as follows:

The concentration of fast moving components of SSL-treated gliadins is less than control gliadins (Figs. 18 and 19) largely because surfactant SSL decreases the rate of protein dissociation and maintains gliadin components longer with mixing. Some of fast moving components of SSL treated gliadins can be associated into narrow and dark bands.

SMP does not produce clear changes on gliadin components. It seems that SMP is less effective than SSL.

Like gliadins, glutenins isolated from surfactant treated dough show a similar effect. SSL decreases the rate of dissociation and SMP is less effective than SSL.

Interactions of Wheat Proteins and Surfactants in Model System. Figures 24, 25, 26, 27, and 28 show the effects of SSL (Figs. 24, 25, and 26) and SMP (Figs. 27 and 28) on the disc electrophoretic patterns of glutenins and gliadins isolated from Bison and Logan flours and doughs. The model system was composed of 0.5% surfactants (based on protein weight) and wheat protein (glutenins or gliadins) which, isolated from dough mixed for various periods, dispersed the sulfactant and protein solvent. The protein solution was placed in a refrigerator at $4^{o}C$ overnight before electrophoresis.

The solvents used for these studies are (A) 0.1 M acetic acid + 3 M urea (Fig. 24); (B) 0.05 M acetic acid (Figs. 25 and 27); and (C) distilled water (Figs. 26 and 28). The results can be summarized as follows:

The effects of SSL *on glutenins and gliadins* (Figs. 24, 25, and 26) are perhaps best demonstrated by the model system. The action of SSL to associate glutenins and gliadins is evident on account of new bands formation, slow migration, and less protein components getting into the electrophoretic gel. For example, in acetic acid or distilled water solvent system, surfactant SSL associates with slow moving components of gliadins and does not get into the gel.

Less interaction between wheat protein (glutenins and gliadins) and surfactant SSL was observed when the protein was isolated from overmixed doughs. As a result, more protein components got into the gel. This suggests that surfactants do not associate the dissociated protein components efficiently.

Surfactant SSL seems more effective in interaction with glutenins than with gliadins.

The data obtained from the model system with acetic acid or distilled water confirm those from the model system with acetic acid + urea as the solvent, but the action of SSL to associate glutenin and gliadin components is more apparent when acetic acid or distilled water is used as the solvents in the model system, as shown by the fact that less gliadin and glutenin components can get into the gel.

Although there are minor variations between the effects of SSL and SMP, to a large extent the effects of SMP are similar to those of SSL, but are less effective.

Figure 29 shows the disc electropherograms of Bison glutenins in presence of lipid, starch, or surfactant (SSL or SMP), showing whether lipid and starch have any effect on the association of SSL and SMP on glutenin components.

For this experiment, total lipid extracted from Bison flour and wheat starch were used. Three model systems were used: (A) Bison glutenin (0.05 ml of 0.6% glutenin solution), starch, and/or lipid; (B) A in presence of 0.5% SSL (base on protein weight); (C) A in presence of 0.5% (base on protein weight). The solvent used for this study was 0.05 M acetic acid. The proportion of glutenin, starch, and lipid is 10:70:2. Protein solution was placed in a refrigerator at $4^{o}C$ overnight before electrophoresis. The results can be summarized as follows:

The slow migration and less concentration of starch treated glutenins getting into gel indicate that starch produces an associated action with glutenins. Lipid appears to homogenize the slow moving components and increase the concentration of glutenin components (Fig. 29 A).

Less concentration of SSL treated glutenins getting into the gel confirmed all previous work of protein association with SSL. SSL modifies the effects of starch as shown by the fact of maintaining the migration of glutenin components. This can be suggested that SSL involves the complex formation with starch and glutenins (Fig. 29-B).

Fig. 18 A comparison of electrophoretic patterns representing the effects of mixing on glutenins and gliadins isolated from flours and flour-water doughs of A, Eagle; B, Bison; C, Logan. The solvent used for electrophoretic study was 0.1 M acetic acid and 3 M urea.

EAGLE

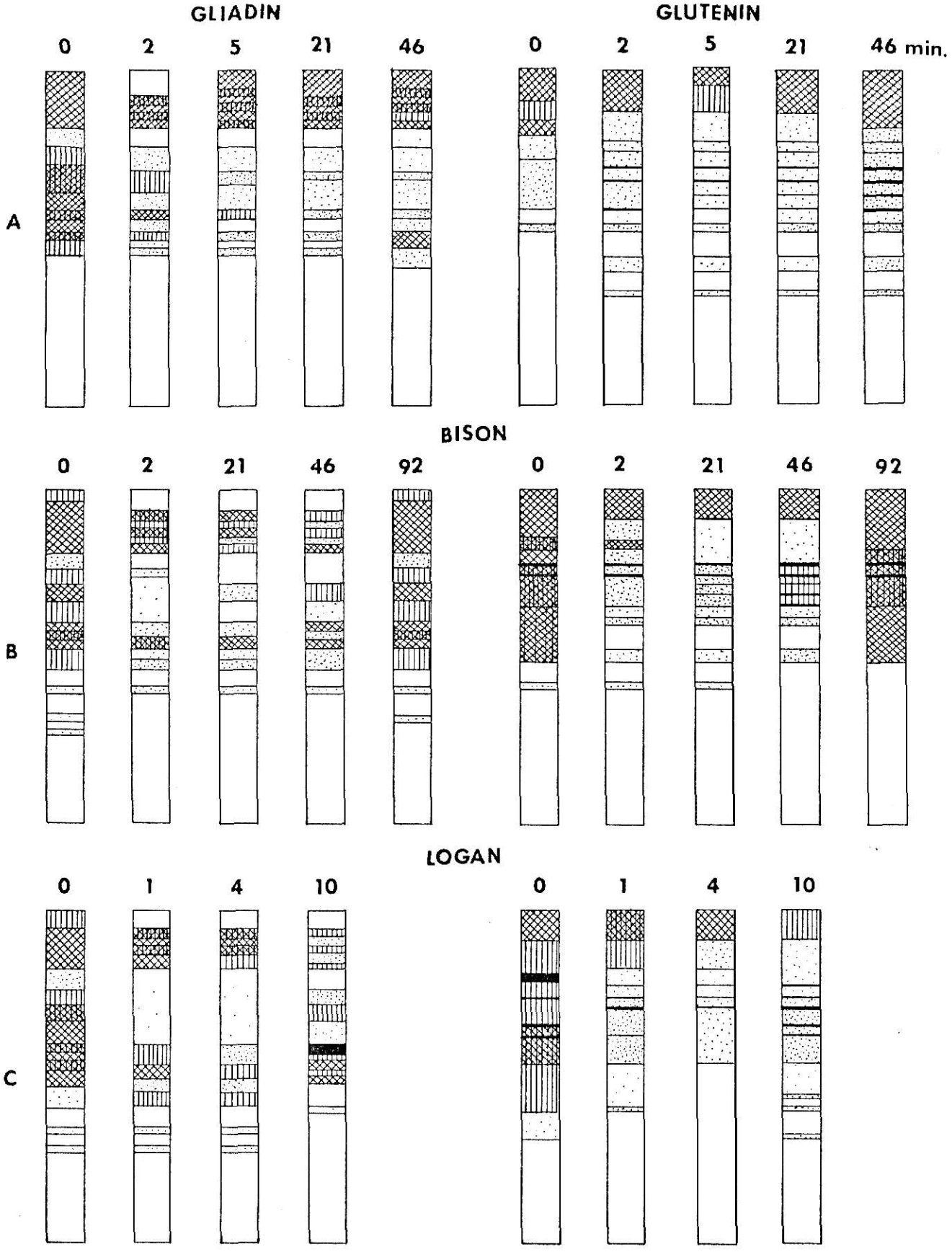

GLIADIN
GLUTENIN
0
2
5
21
46
0
2
5
21
46 min.
A
BISON
0
2
21
46
92
0
2
21
46
92
B
LOGAN
0
1
4
10
0
1
4
10
C

Fig. 19 A comparison of electrophoretic patterns representing the effects of mixing on glutenins and gliadins isolated from flours and flour-water doughs of A, Eagle; B, Bison; C, Logan. The solvent used for electrophoretic study was 0.05 M acetic acid.

EAGLE

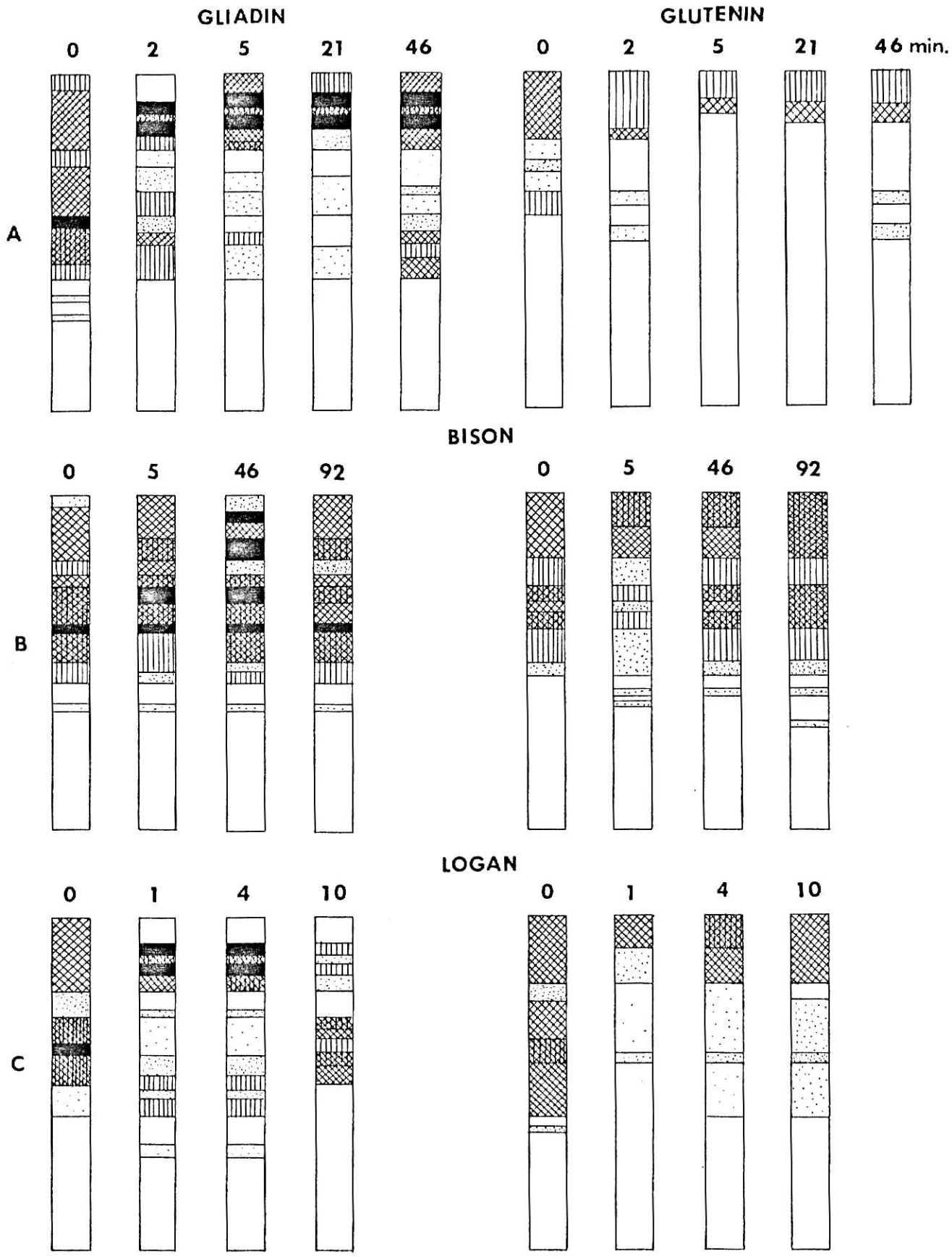

GLIADIN
GLUTENIN
0
2
5
21
46
0
2
5
21
46 min.
A
BISON
0
5
46
92
0
5
46
92
B
LOGAN
0
1
4
10
0
1
4
10
C

Fig. 20 A comparison of electrophoretic patterns representing the effects of mixing on glutenins and gliadins isolated from SSL treated flour-water doughs of A, Eagle; B, Bison; C, Logan. The solvent used for electrophoretic study was 0.1 M acetic acid and 3 M urea.

EAGLE

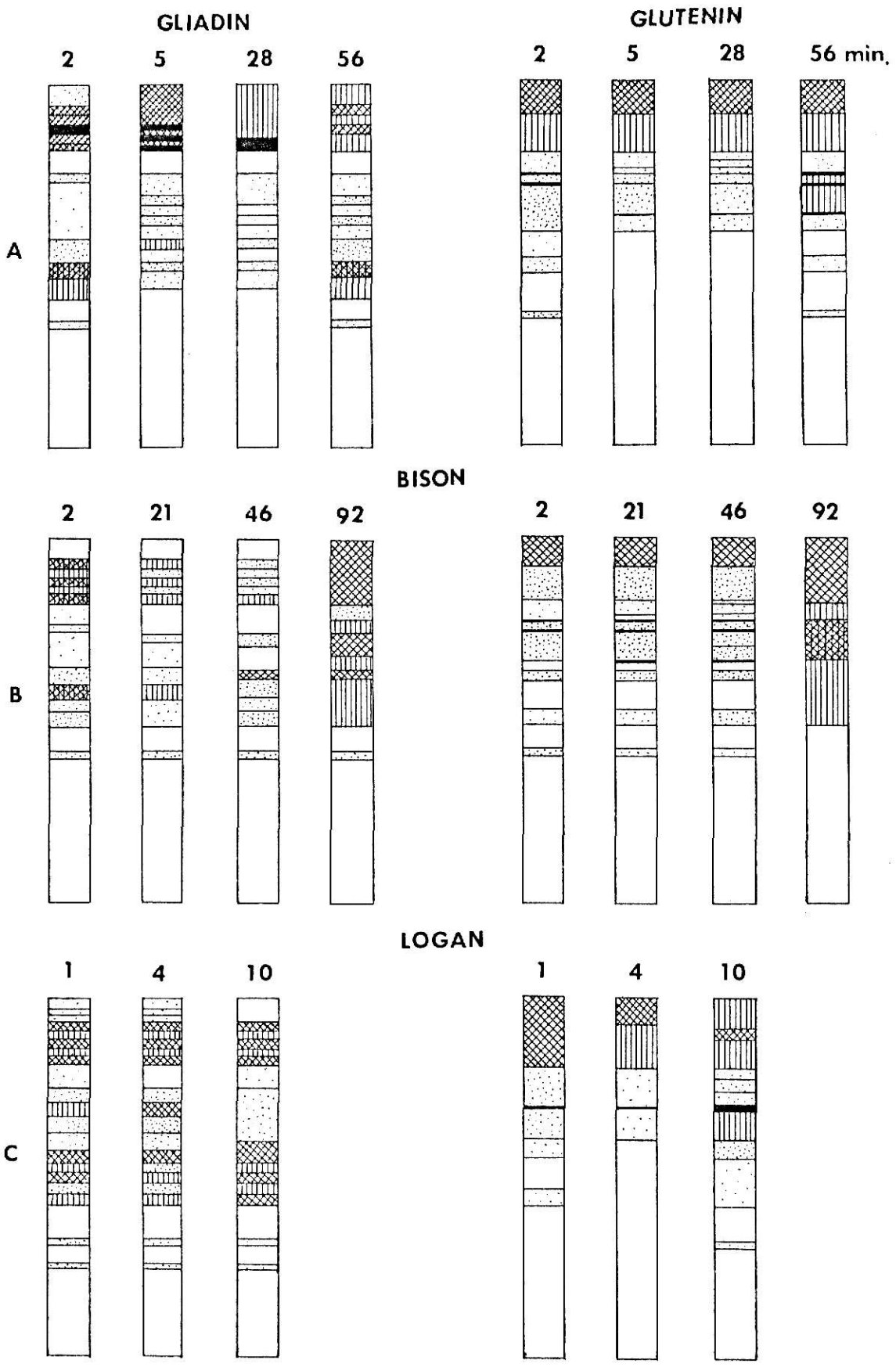
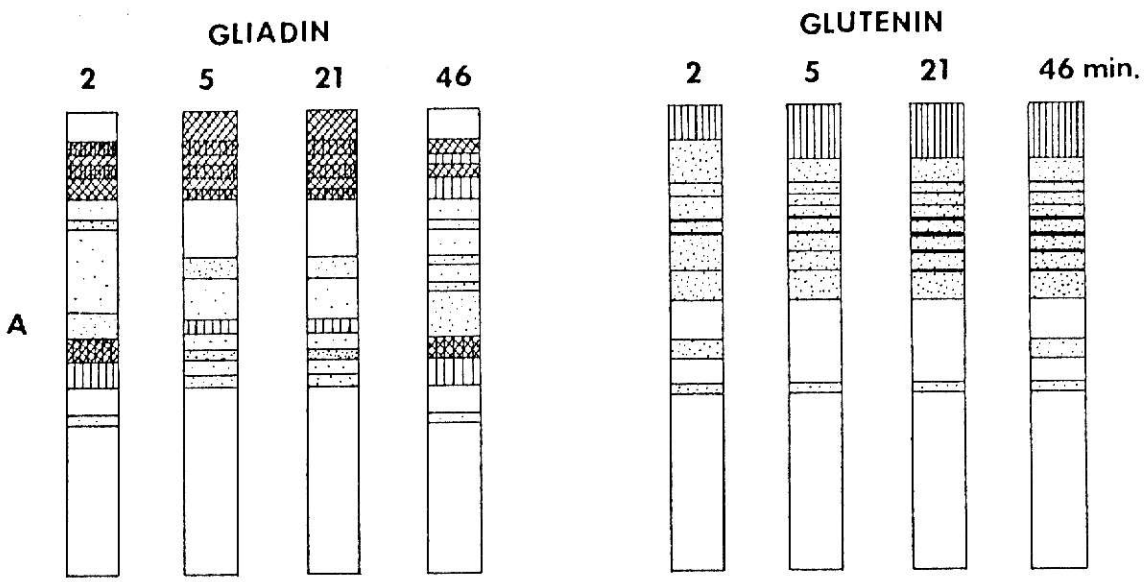
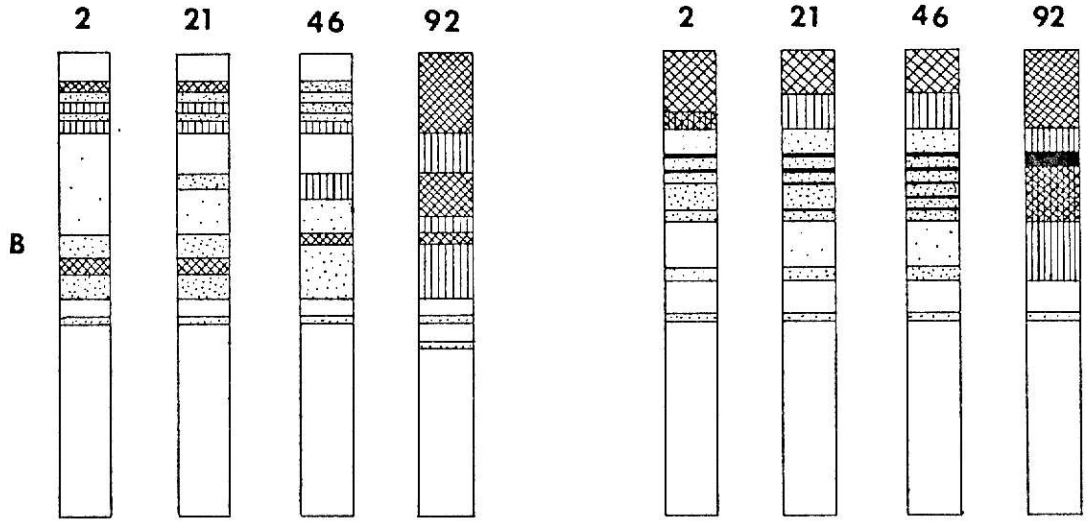

GLIADIN
GLUTENIN
2
5
28
56
2
5
28
56 min.
A
BISON
2
21
46
92
2
21
46
92
B
LOGAN
1
4
10
1
4
10
C

Fig. 21 A comparison of electrophoretic patterns representing the effects of mixing on glutenins and gliadins isolated from SMP treated flour-water doughs of A, Eagle; B, Bison; C, Logan. The solvent used for electrophoretic study was 0.1 M acetic acid and 3 M urea.

EAGLE



BISON



LOGAN

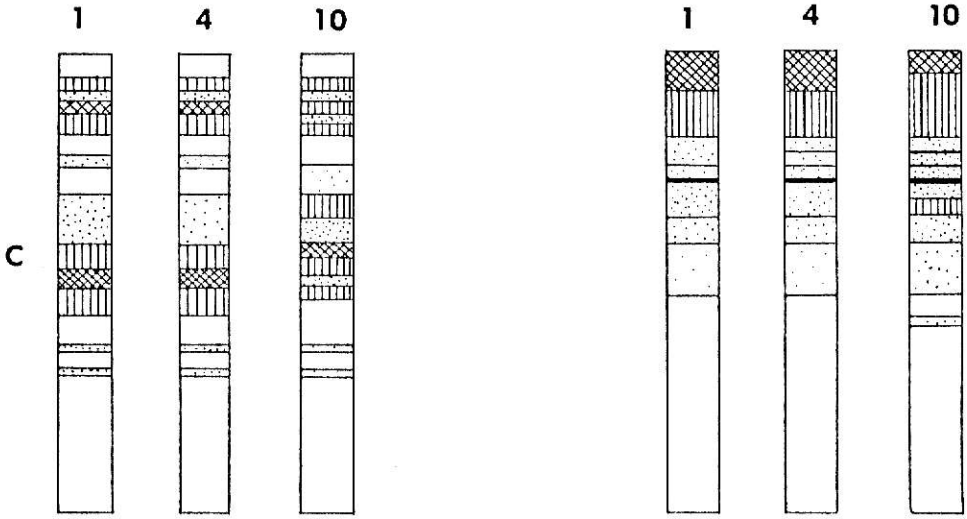


Fig. 22 A comparison of electrophoretic patterns representing the effects of mixing on glutenins and gliadins isolated from SSL treated flour-water doughs of A, Eagle; B, Bison; C, Logan. The solvent used for electrophoretic study was 0.05 M acetic acid.

EAGLE

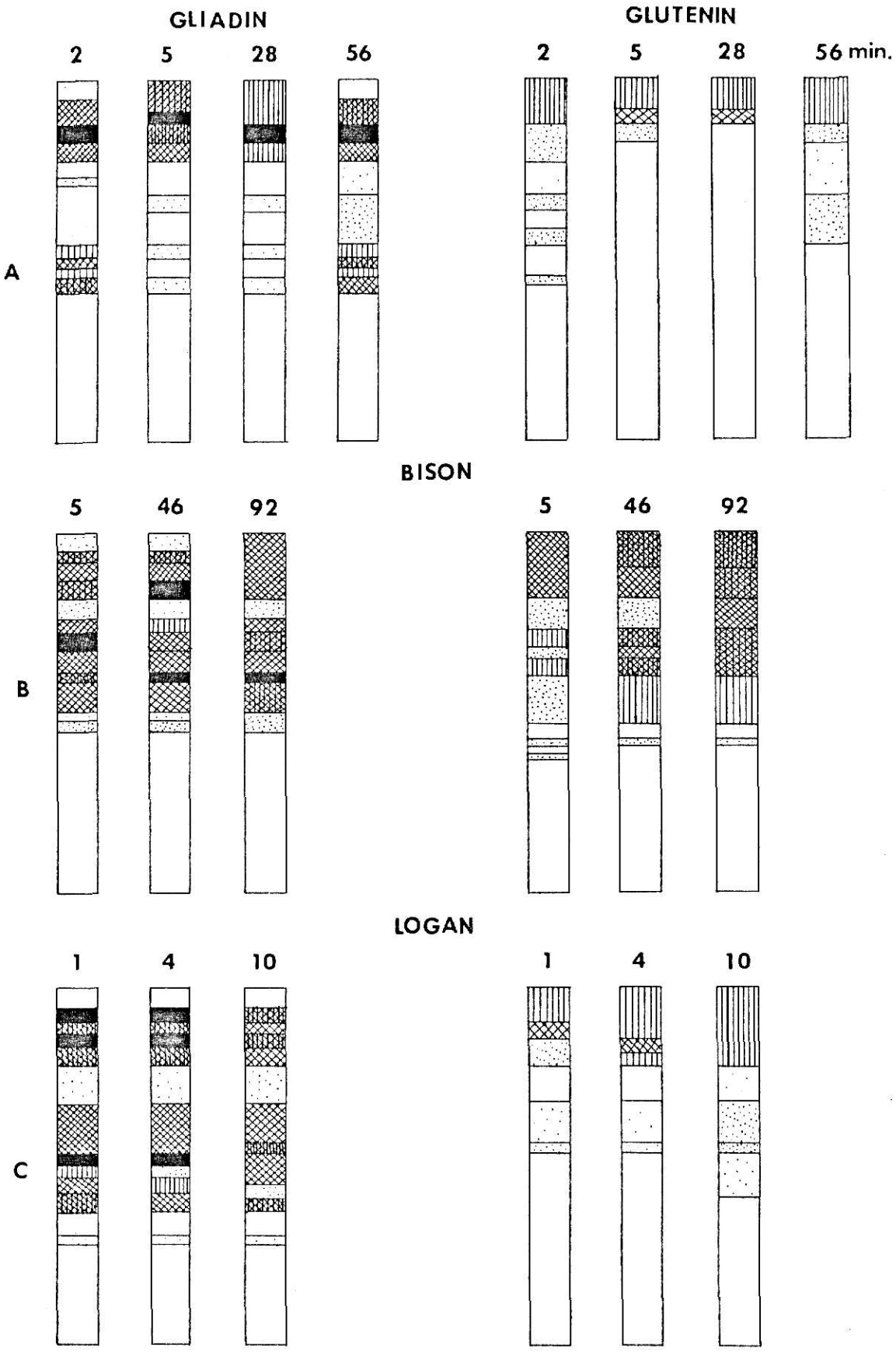

GLIADIN
2
5
28
56
GLUTENIN
2
5
28
56 min.
A
BISON
5
46
92
5
46
92
B
LOGAN
1
4
10
1
4
10
C

Fig. 23 A comparison of electrophoretic patterns representing the effects of mixing on glutenins and gliadins isolated from SMP treated flour-water doughs of A, Eagle; B, Bison; C, Logan. The solvent used for electrophoretic study was 0.05 M acetic acid.

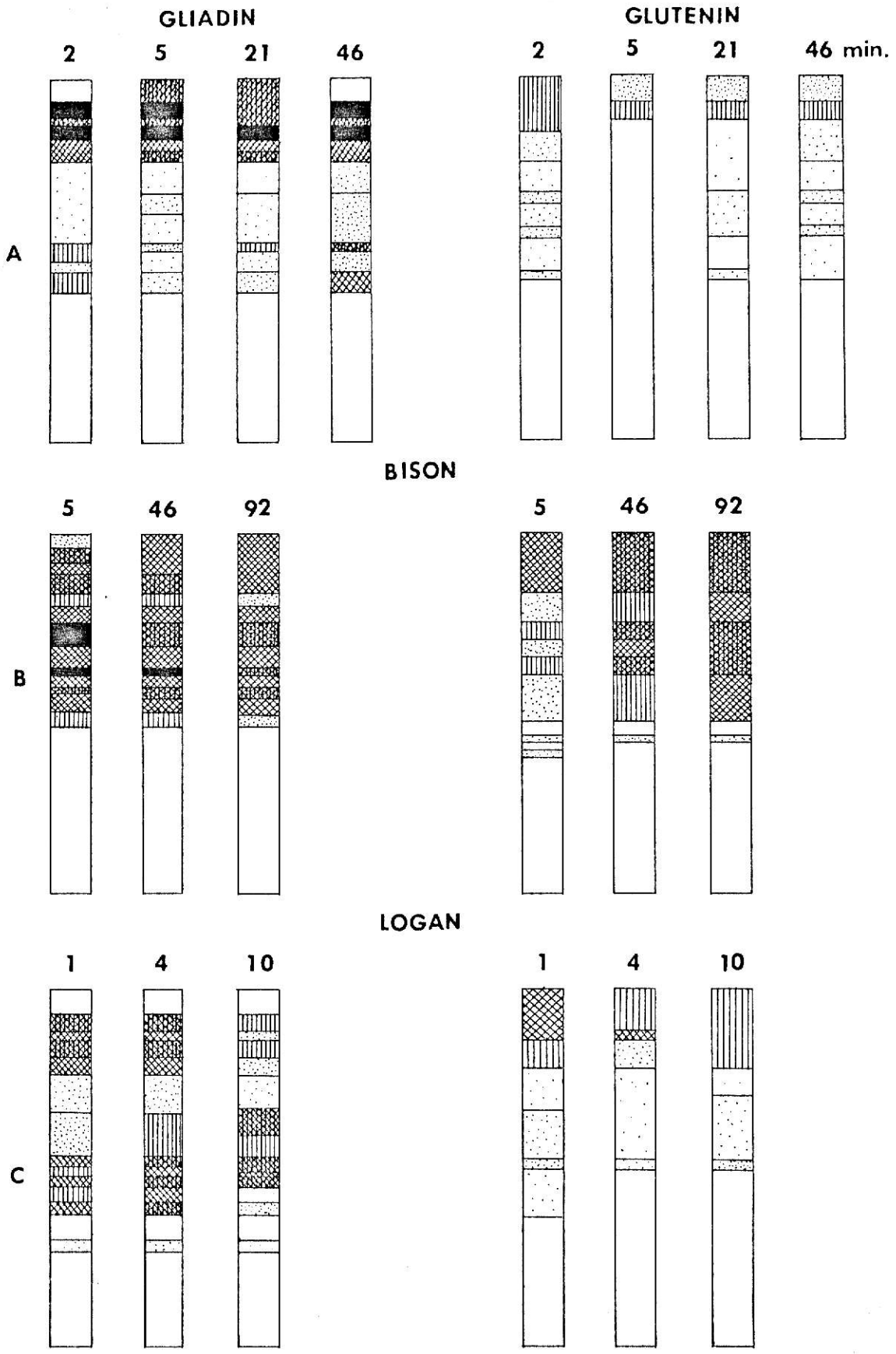

GLIADIN
2
5
21
46
GLUTENIN
2
5
21
46 min.
A
BISON
5
46
92
5
46
92
B
LOGAN
1
4
10
1
4
10
C

Fig. 24 Effects of SSL (A) and SMP (B) on the disc electrophoretic patterns of glutenins and gliadins isolated from Bison flour and flour-water doughs mixed for indicated periods. The solvent used for this study was 0.1 M acetic acid and 3 M urea.

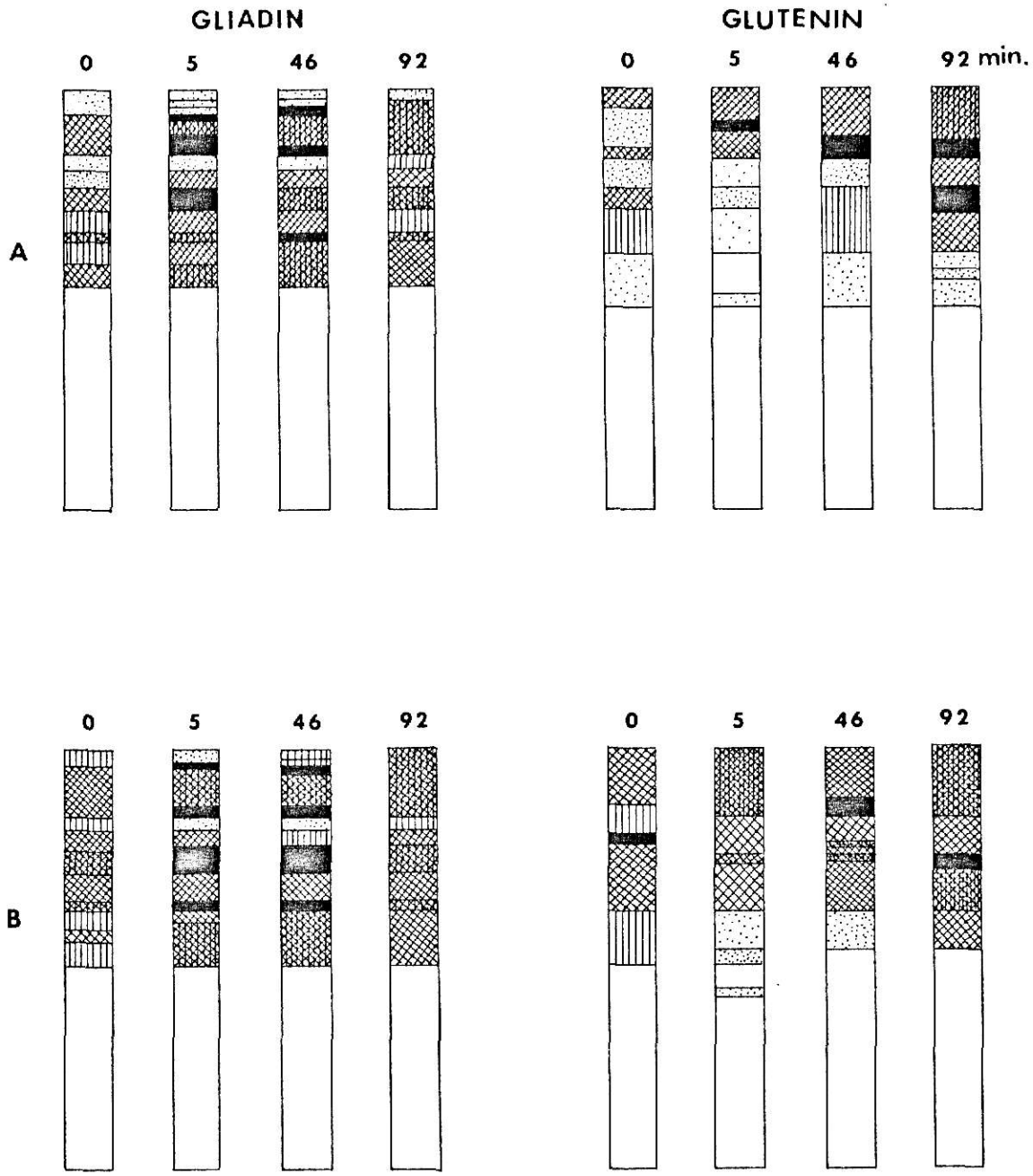
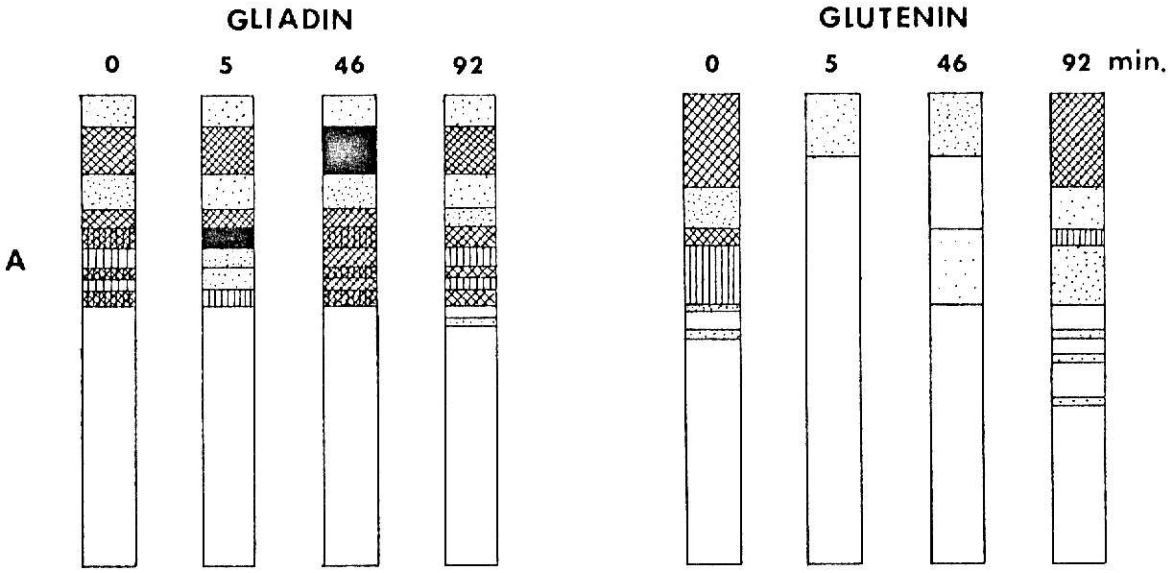

GLIADIN
GLUTENIN
0
5
46
92
92 min.
A
B

Fig. 25. Effects of SSL on the disc electrophoretic patterns of glutenins and gliadins isolated from Bison, Logan flours and flour-water doughs mixed for indicated periods. The solvent used for this study was 0.05 M acetic acid.

BISON



LOGAN

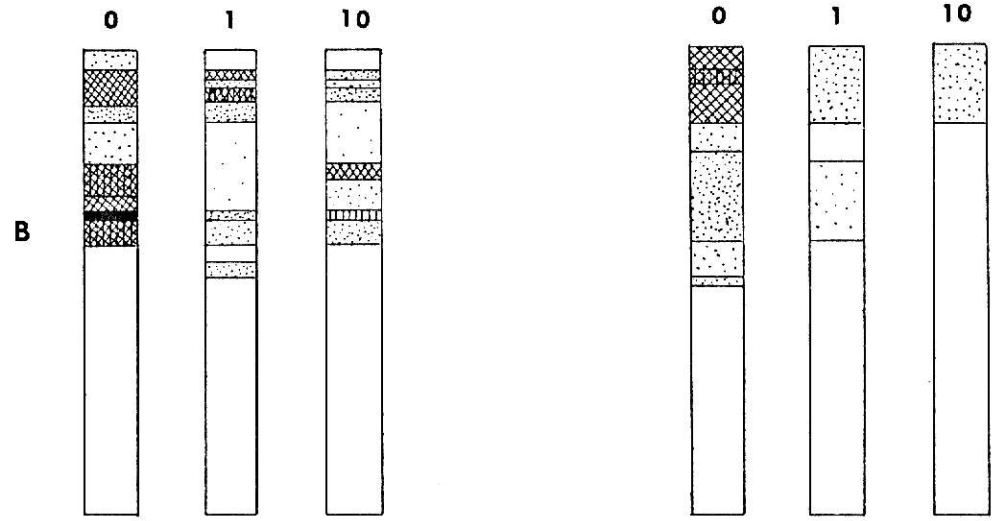
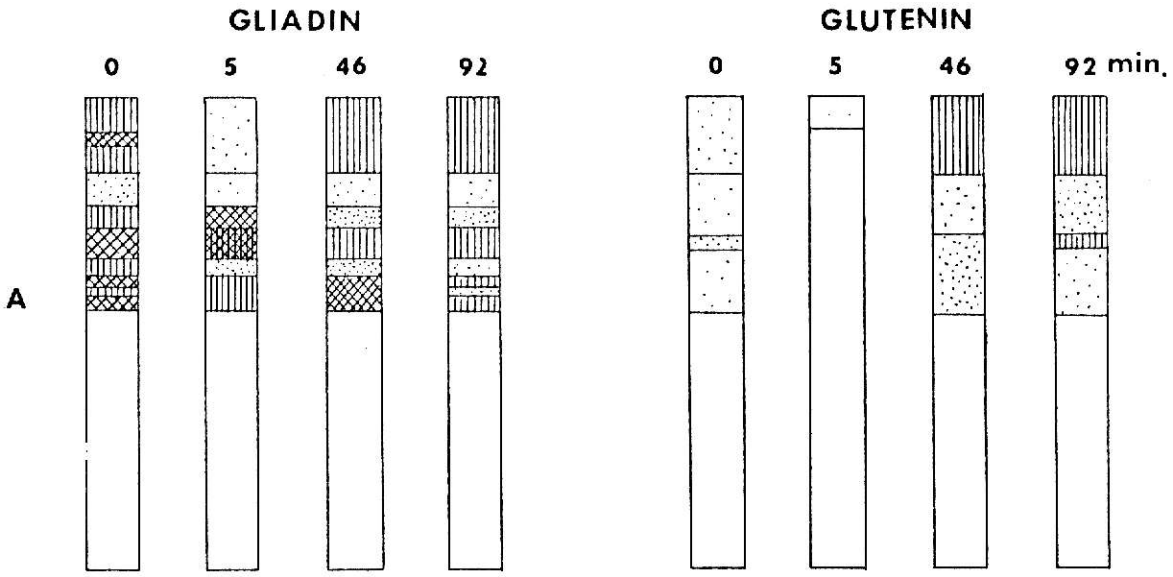


Fig. 26. Effects of SSL on the disc electrophoretic patterns of glutenins and gliadins isolated from Bison, Logan flours and flour-water doughs mixed for indicated periods. The solvent used for this study was distilled water.

BISON



LOGAN

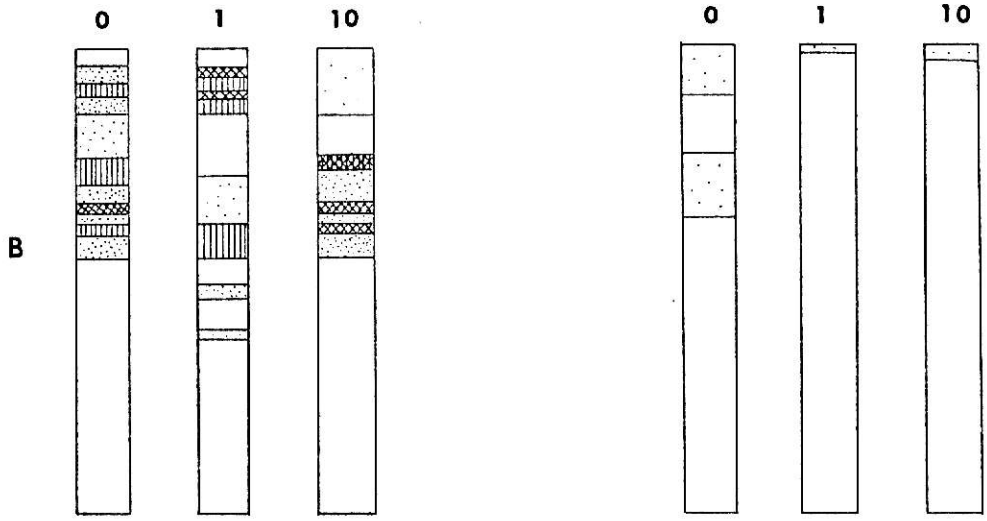


Fig. 27 Effects of SMP on the disc electrophoretic patterns of glutenins and gliadins isolated from Bison, Logan flours and flour-water doughs mixed for indicated periods. The solvent used for this study was 0.05 M acetic acid.

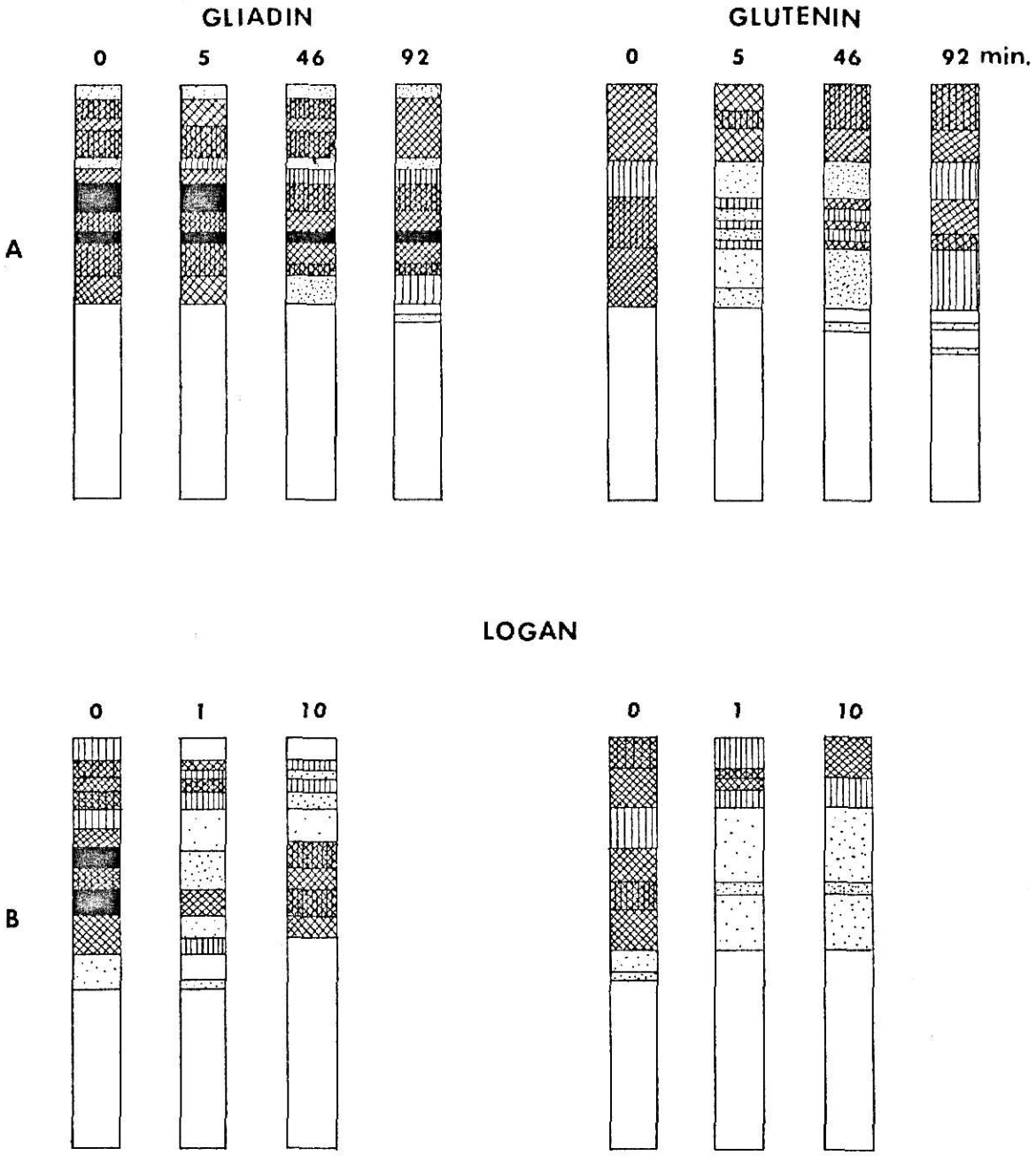

GLIADIN
0
5
46
92
GLUTENIN
0
5
46
92 min.
A
LOGAN
0
1
10
0
1
10
B

Fig. 28. Effects of SMP on the disc electrophoretic patterns of glutenins and gliadins isolated from Bison, Logan flours and flour-water doughs mixed for indicated periods. The solvent used for this study was distilled water.

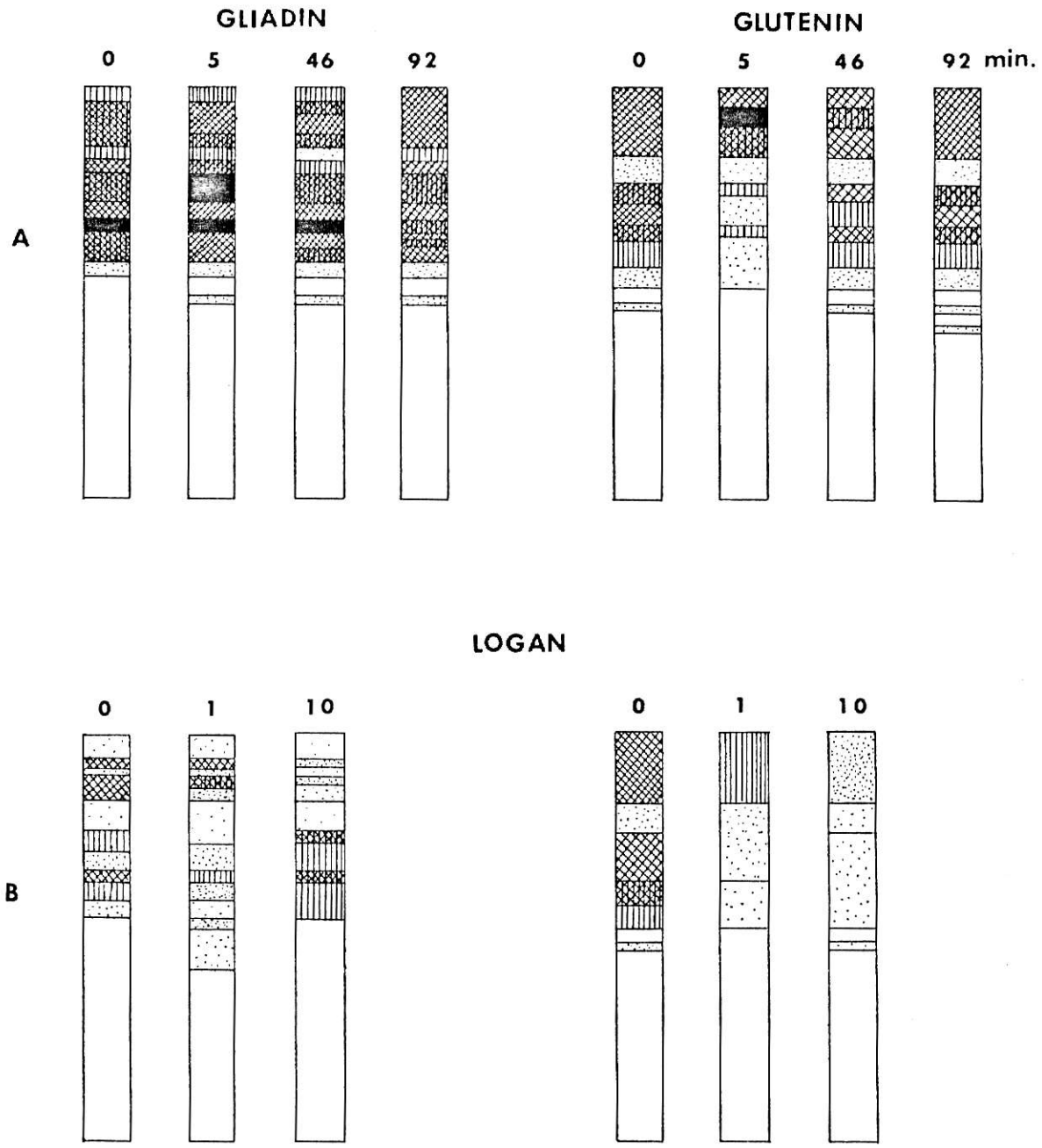
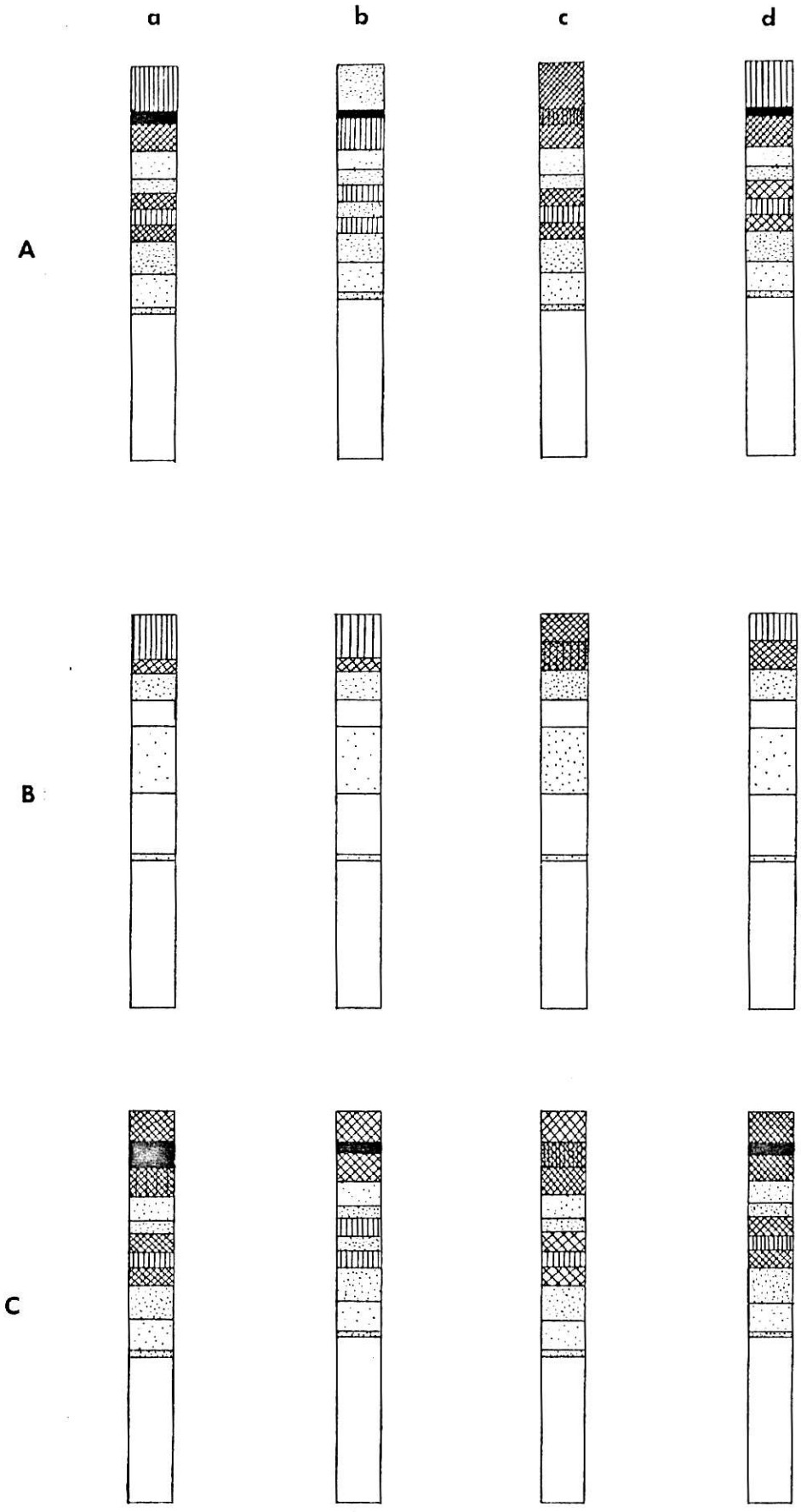

GLIADIN
GLUTENIN
0
5
46
92
92 min.
A
LOGAN
0
1
10
B

Fig. 29. Effects of starch and lipid on the disc electrophoretic patterns of A, control; B, SSL treated; C, SMP treated glutenins isolated from 21 min mixed Bison dough. The proportion of glutenin, starch, and lipid is 10:70:2 and a, glutenins; b, glutenins and starch; c, glutenins and lipid; d, glutenins, starch, and lipid. The solvent used for this study was 0.05 M acetic acid.


a
b
c
d
A
B
C

SMP is less effective in interacting with glutenins than SSL because the electropherograms of glutenins isolated from SMP treated doughs indicate that more glutenin components get into the gel. SMP does not appear to modify the effects of starch on glutenins.

Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis. Polyacrylamide gel electrophoresis in the presence of the anionic detergent sodium dodecyl sulfate (SDS) and reducing agent 2-mercaptoethanol (2-ME) can be used to separate and estimate MW's of wheat proteins, and to detect differences in the subunit composition of glutenins and gliadins. This experiment was made to examine the MW's and changes of glutenin and gliadin subunits of different wheat flours and doughs mixed for various periods and treated with or without surfactants.

Tables 6 and 9 show the distinguishable components of glutenins and gliadins isolated from Eagle, Bison, and Logan flours and doughs. Tables 7, 8, 10, and 11 show the distinguishable components of glutenins and gliadins isolated from SSL treated (Tables 7 and 10) and SMP (Tables 8 and 11) doughs. The results can be summarized as follows:

The SDS-electrophoretic components of glutenins from the different varieties (Eagle, Bison, and Logan) of wheat are similar in general form, but no two are identical (e.g., components of 75,500, 71,450, and 42,300). HRW wheat (Eagle and Bison) has more higher molecular weight components than SW wheat (Logan, Table 6).

The change of color with coomassie brilliant blue indicates that the dissociation and association of protein components take place during the mixing.

The data of SSL treated glutenins (Table 7) indicate that SSL can assist the glutenin components to associate with one another into higher molecular weight components (e.g., 75,500), thus reducing the number of components. Table 8 indicates the action of SMP on the association of protein components, as shown by the reduction in the number of protein bands. However, SMP is less effective than SSL in exerting such an action.

Table 9 shows that the high molecular weight fractions (76,700, 75,500, and 73,600) show more clearly the variation among three flours (Eagle, Bison, and Logan), and soft-hard wheat differences are greater than hard-hard wheat.

The reduction in the number of components indicates that SSL and SMP can assist the gliadin components to associate with one another as shown in Table 10 and 11.

GENERAL CONCLUSIONS

The possible explanations for the results of scanning electron microscope, gel filtration, and electrophoresis are as follows:

(A) Due to the interaction of protein with starch and/or lipid during dough mixing.

(B) Resulting from enzyme attack during dough mixing.

(C) By protein denaturation during dough mixing.

(D) By protein association and dissociation during dough mixing.

There are so many factors involved and each of them need a lot of time to solve. In this study, the focus was placed on the changes of protein themselves by mixing.

TABLE 6 DISTINGUISHABLE COMPONENTS OF GLUTENIN ISOLATED FROM WHEAT (EAGLE, BISON, AND LOGAN) FLOURS AND FLOUR-WATER DOUGHS MIXED FOR THE INDICATED PERIODS

Molecular Weight of Component	Dough Mixing Time, min										
	Eagle				Bison				Logan		
	0[a]	2	21	46	0	5	46	92	0	1	10
76,700											
75,500	+	+	+	+		+	+				
73,600	+[c]	+	+	+[c]	+	+[b]	+[b]	+	+[c]	+	+
71,450	+	+	+	+[c]	+[c]	+[c]	+[c]	+[c]	+[c]	+	+
68,700						+[b]	+[b]				
66,400	+[b]	+[b]	+[b]	+[b]	+[b]	+[b]	+[b]	+[b]	+[b]	+[b]	+[b]
64,580		+	+	+	+	+	+	+			
63,700	+	+	+	+		+	+		+[c]	+	+
61,200		+	+	+	+	+	+	+			
58,350	+[c]	+	+	+	+[c]	+[c]	+[c]	+[c]	+[c]	+	+
53,200	+	+	+	+	+			+	+[c]	+[c]	+[c]
51,800		+[c]	+[c]	+[c]	+	+[c]	+[c]	+	+[c]	+[c]	+[c]
51,800-48,300	+	+	+	+	+	+	+	+	+	+	+
46,300-43,000	+	+	+[b]	+	+	+	+	+	+	+[b]	+
42,300		+	+		+	+	+	+		+	
40,000		+									
37,800-34,000	+	+	+	+	+	+	+	+	+	+	+
33,600	+	+	+	+	+	+	+	+	+	+[c]	+[c]
32,700-30,900	+	+	+	+	+	+	+	+	+[c]	+	+
29,300-27,500	+	+	+	+	+	+	+	+	+	+	+
24,700-22,900	+	+	+	+	+	+	+	+	+	+	+
20,500-18,700	+	+	+	+	+	+	+	+	+	+	+
16,500-12,500	+	+	+	+	+	+	+	+	+	+	+
Total Bands	16	21	20	19	18	20	20	18	16	17	17

[a] Control glutenin isolated from Eagle, Bison, and Logan flour.

[b] Very dark blue band; + alone represented light blue band.

[c] Purple band.

TABLE 7 DISTINGUISHABLE COMPONENTS OF GLUTENIN ISOLATED FROM SSL TREATED WHEAT (EAGLE, BISON, AND LOGAN) FLOUR-WATER DOUGHS MIXED FOR THE INDICATED PERIODS

Molecular Weight of Component	Dough Mixing Time, min										
	Eagle				Bison				Logan		
	0[a]	2	21	46	0	5	46	92	0	1	10
76,700											
75,500	+	+	+	+		+	+	+			+
73,600	+[c]	+	+[c]	+[c]			+[c]		+[c]		+[c]
71,450	+[c]	+[c]	+[c]	+[c]	+[c]	+[c]	+[cb]	+[c]	+[c]	+[c]	+[c]
68,700						+[b]	+[c]				
66,400	+[b]	+[b]	+[b]	+[b]	+[b]	+[b]	+[b]	+[b]	+[b]	+[b]	+[b]
64,580					+	+[c]	+[c]	+			
63,700	+	+[c]	+	+					+[c]	+	+
61,200					+	+	+				
58,350	+[c]	+[c]	+	+	+[c]	+[c]	+[c]	+[c]	+[c]	+	+[c]
53,200	+	+	+	+	+			+	+[c]	+[c]	+[c]
51,800		+			+	+[c]	+[c]	+	+[c]	+[c]	+[c]
51,800-48,300	+	+[cb]	+[cb]	+	+	+	+	+	+	+	+
46,300-43,000	+	+	+	+	+	+	+	+	+	+[b]	+
42,300											
40,000											
37,800-34,000	+	+	+	+	+	+	+	+	+	+	+
33,600	+	+	+	+	+	+[c]	+[c]	+	+	+	+[c]
32,700-30,900	+	+	+	+	+	+	+	+	+[c]	+	+
29,300-27,500	+	+	+	+	+	+	+	+	+	+	+
24,700-22,900	+	+	+	+	+	+	+	+	+	+	+
20,500-18,700	+	+	+	+	+	+	+	+	+	+	+
16,500-12,500	+	+	+	+	+	+	+	+	+	+	+
Total Bands	16	17	16	16	18	19	19	18	16	16	17

[a]Control glutenin isolated from Eagle, Bison, and Logan flour.

[b]Very dark blue band; + alone represented light blue band.

[c]Purple band.

TABLE 8 DISTINGUISHABLE COMPONENTS OF GLUTENIN ISOLATED FROM SMP TREATED WHEAT (EAGLE, BISON, AND LOGAN) FLOUR-WATER DOUGHS MIXED FOR THE INDICATED PERIODS

Molecular Weight of Component	Dough Mixing Time, min										
	Eagle				Bison				Logan		
	0[a]	2	21	46	0	5	46	92	0	1	10
76,700											
75,500	+	+	+	+		+					
73,600	+[c]	+[c]	+	+[c]	+	+	+	+	+[c]	+[c]	+[c]
71,450	+	+[c]	+[c]	+	+[c]	+[c]	+[c]	+[c]	+[c]	+[c]	+[c]
68,700						+	+				
66,400	+[b]	+[b]	+[b]	+[bc]	+[b]	+[b]	+[b]	+[b]	+[b]	+[b]	+[b]
64,580					+	+	+	+			
63,700	+	+	+	+		+	+		+[c]	+	+
61,200					+	+	+	+			
58,350	+[c]	+	+[b]	+	+[c]	+[c]	+[c]	+[c]	+[c]	+	+
53,200	+	+	+	+	+			+	+[c]	+[c]	+
51,800					+	+[c]	+[c]	+	+[c]	+[c]	+[c]
51,800-48,300	+	+[c]	+	+	+	+	+	+	+	+	+
46,300-43,000	+	+	+	+	+	+	+	+	+	+	+
42,300					+	+	+	+			
40,000											
37,800-34,000	+	+	+	+	+	+	+	+	+	+	+
33,600	+	+	+	+	+	+[c]	+[c]	+	+	+	+
32,700-30,900	+	+	+	+	+	+	+	+	+[c]	+	+[c]
29,300-27,500	+	+	+	+	+	+	+	+	+	+	+
24,700-22,900	+	+	+	+	+	+	+	+	+	+	+
20,500-18,700	+	+	+	+	+	+	+	+	+	+	+
16,500-12,500	+	+	+	+	+	+	+	+	+	+	+
Total Bands	16	16	16	16	18	20	19	18	16	16	16

[a]Control glutenin isolated from Eagle, Bison, and Logan flour.

[b]Very dark blue band; + alone represented light blue band.

[c]Purple band.

TABLE 9 DISTINGUISHABLE COMPONENTS OF GLIADIN ISOLATED FROM WHEAT (EAGLE, BISON, AND LOGAN) FLOURS AND FLOUR-WATER DOUGHS MIXED FOR THE INDICATED PERIODS

Molecular Weight of Component	Dough Mixing Time, min										
	Eagle				Bison				Logan		
	0[a]	2	21	46	0	5	46	92	0	1	10
76,700							+[c]				
75,500			+				+[c]	+[c]			
73,600			+	+		+[c]	+[c]	+[c]			
71,450			+	+							
68,700	+[c]	+	+[c]		+[c]	+[c]	+[cb]	+[c]	+[c]	+[c]	
66,400	+	+	+[c]	+	+[c]	+[c]	+[cb]	+[c]	+[c]	+[c]	+[c]
64,580	+							+[c]			
63,700	+[c]	+[c]	+[c]		+[c]	+[c]	+[c]	+[c]	+[c]	+[c]	+[cb]
61,200											
58,350	+[c]	+[c]	+[cb]	+	+[c]	+[c]	+[c]	+[c]		+[c]	
53,200	+		+								
51,800	+	+	+	+	+	+	+	+	+	+[cb]	+
51,800-48,300			+	+	+	+	+	+	+	+	+
46,300-43,000	+	+		+	+	+	+	+	+	+	+
42,300	+	+	+	+	+	+[c]	+	+	+		+[c]
40,000			+	+						+	
37,800-34,000	+	+	+	+	+	+	+	+	+	+[cb]	
33,600				+							
32,700-30,900										+[c]	
29,300-27,500	+	+		+	+	+[b]	+	+	+		+
24,700-22,900											
20,500-18,700	+[b]			+	+[b]	+[b]	+[b]	+[b]	+[b]		
16,500-12,500	+[b]	+[b]	+	+	+[b]	+[b]	+[b]	+[b]	+[b]	+	+[b]
Total Bands	13	10	14	14	12	13	15	15	11	11	8

[a]Control gliadin isolated from Eagle, Bison, and Logan flour.

[b] Very dark blue band; + alone represented light blue band.

[c] Purple band.

TABLE 10 DISTINGUISHABLE COMPONENTS OF GLIADIN ISOLATED FROM SSL TREATED WHEAT (EAGLE, BISON, AND LOGAN) FLOUR-WATER DOUGHS MIXED FOR THE INDICATED PERIODS

Molecular Weight of Component	Dough Mixing Time, min										
	Eagle				Bison				Logan		
	0[a]	2	21	46	0	5	46	92	0	1	10
76,700											
75,500											
73,600							+[c]				
71,450		+	+	+		+[c]	+[c]				
68,700	+[c]	+	+	+	+[c]	+[cb]	+[c]	+[c]	+[c]	+[c]	
66,400	+[c]				+[c]	+[c]	+[c]	+[c]	+[c]	+[cb]	+[c]
64,580	+[c]	+	+	+				+[c]			
63,700	+[c]				+[c]	+[c]	+[c]	+[c]	+[c]	+[cb]	+[cb]
61,200			+								
58,350	+[c]				+[c]	+[c]	+[c]	+[c]		+[c]	
53,200	+	+	+	+				+[c]		+[c]	
51,800	+	+	+	+	+	+	+	+	+	+	+
51,800-48,300		+	+	+	+	+	+	+	+		
46,300-43,000	+				+	+	+	+	+	+	+
42,300	+	+	+	+	+	+	+	+	+	+	+
40,000		+		+							+
37,800-34,000	+	+	+	+	+	+	+	+	+	+	+
33,600		+	+	+							
32,700-30,900											
29,300-27,500	+				+	+	+	+	+	+	+
24,700-22,900											
20,500-18,700	+[b]	+			+[b]		+[b]	+[b]	+[b]	+[b]	+[b]
16,500-12,500	+[b]	+	+	+	+[b]	+[b]	+[b]	+[b]	+[b]	+[b]	+[b]
Total Bands	13	12	11	11	12	12	14	14	11	11	10

[a]Control gliadin isolated from Eagle, Bison, and Logan flour.

[b]Very dark blue band; + alone represented light blue band.

[c]Purple band.

TABLE 11 DISTINGUISHABLE COMPONENTS OF GLIADIN ISOLATED FROM SMP TREATED WHEAT (EAGLE, BISON, AND LOGAN) FLOUR-WATER DOUGHS MIXED FOR THE INDICATED PERIODS

Molecular Weight of Component	Dough Mixing Time, min										
	Eagle				Bison				Logan		
	0[a]	2	21	46	0	5	46	92	0	1	10
76,700											
75,500											
73,600											
71,450						+[c]	+[c]	+[c]			
68,700	+[c]	+	+[c]	+	+[c]	+[c]	+[c]	+[c]	+[c]		+[c]
66,400	+[c]				+[c]	+[c]	+[c]	+[c]	+[c]		+[c]
64,580	+[c]	+	+[c]	+				+[c]			
63,700	+[c]		+[c]	+	+[c]	+[c]	+[c]	+[c]	+[c]	+[c]	+[c]
61,200											
58,350	+[c]		+[c]	+	+[c]	+[c]	+[c]	+[c]		+[c]	
53,200	+	+	+[c]	+				+			
51,800	+	+	+	+	+	+	+	+	+	+	
51,800-48,300		+	+		+	+	+	+	+	+	+
46,300-43,000	+			+	+	+	+	+	+	+	+
42,300	+	+	+	+	+	+[c]	+[c]	+	+		
40,000		+	+	+						+[c]	+
37,800-34,000	+	+	+		+	+	+	+	+		
33,600		+	+								
32,700-30,900										+[c]	+
29,300-27,500	+				+	+[b]	+	+	+		+
24,700-22,900											
20,500-18,700	+[b]				+[b]		+	+[b]	+[b]		
16,500-12,500	+[b]	+	+	+	+[b]	+[b]	+	+[b]	+[b]	+	+[b]
Total Bands	13	10	12	10	12	12	13	15	11	8	9

[a]Control gliadin isolated from Eagle, Bison, and Logan flour.

[b]Very dark blue band; + alone represented light blue band.

[c]Purple band.

The addition of SSL (ionic surfactant) increases the HRW (Eagle and Bison) flour-water dough stability, indicating that SSL can strengthen gluten structure; whereas the addition of SMP hardly shows any effects, compared to that of control.

SEM data show that glutenin appears to associate and dissociate itself with mixing. Surfactants can delay these changes in dough system. Glutenin seems to bind with surfactant (especially with SSL), and form large glutenin-surfactant complexes.

Prolonged mixing can dissociate gliadin, as shown by the reduction of the first gel-fraction's concentration with the gel filtration. Surfactants seem to reduce the dissociation rate. The glutenin and gliadin seem to associate itself with initial dough mixing and reduce the concentration of fast moving components of disc electrophoresis.

The concentration of the fast moving protein-bands of glutenin and gliadin increases with prolonged mixing of dough samples from which the protein has been isolated. This observation also supports that mixing can dissociate glutenin and gliadin in dough. SSL seems to bind some glutenin or gliadin components to form protein-SSL complexes on account of the reduction of protein-components which enter the gel. SMP is less effective in exerting the binding action. The improving mechanism of SSL appears different from that of SMP.

The SDS-electrophoretic components of glutenins and gliadins from different varieties of wheat have been similar in general form, but no two have been identical. Surfactants can assist the glutenin and gliadin components to associate with one another; as a result, the number of electrophoretic components are reduced.

The present study by using scanning electron microscope, gel filtration, and disc electrophoresis shows that the association and dissociation of glutenins and gliadins take place during dough mixing. SSL (ionic surfactant) displays a strong complex and association ability with glutenins and gliadins. The interaction between SSL and the proteins is dependent on mixing. SMP (nonionic surfactant) is less effective than SSL. The mechanisms of their action are probably different.

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EFFECTS OF SURFACTANTS ON GLUTENIN AND GLIADIN DURING DOUGH MIXING AND IN MODEL SYSTEM

by

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Dough mixing is a critical step in the breadmaking process. It has become more important with the introduction of the continuous breadmaking processes in which dough development is achieved primarily by high-speed mixing. Surfactants (dough conditioners) have been widely used in the baking industry. Their function is being emphasized because they can improve the baking performance of wheat flour fortified with a high level of protein-high additives such as soy flour and nonfat dry milk.

The objective of this investigation was to study the interaction between wheat proteins and surfactants during dough mixing in order to elucidate the improving mechanism of surfactants.

For this study, two hard red winter wheat flours (Eagle and Bison) and one soft winter wheat flour (Logan), together with two surfactants sodium stearoyl-2 lactylate (ionic surfactant) and sucrose monopalmitate (nonionic surfactant), were used. Dough samples were mixed for various periods. Glutenin and gliadin were isolated from dough samples and characterized by scanning electron microscopy, gel filtration, and electrophoresis. Results are summarized below:

SEM data show that glutenin or gliadin appears to associate and dissociate itself with mixing. Surfactants can delay these changes.

Prolonged mixing can dissociate gliadin, as shown by the reduction of the first gel-fraction's concentration with the gel filtration. Surfactants seem to reduce the dissociation rate.

The concentration of the fast moving protein-bands of glutenin and gliadin increases with prolonged mixing of dough samples from which the proteins have been isolated. This observation also supports that mixing can dissociate glutenin and gliadin in dough. SSL seems to bind some glutenin- gliadin-components to form new protein-SSL complexes on account of the formation of new protein bands and the reduction of protein-components which enter the gel. SMP is less effective in exerting the binding action. The improving mechanism of SSL appears different from that of SMP.