Effect of Navy Bean Protein Flour and Navy Bean Globulin(s) on Composite Flour Rheology, Chemical Bonding, and Microstructure

NANCY L. LORIMER,[1,2] MARY E. ZABIK,[1] JANICE B. HARTE,[1] NANCY C. STACHIW,[1,3] and MARK A. UEBERSAX[1]

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ABSTRACT

Substitution of 5 and 10% dry-roasted, air-classified high-protein flour (fines) for bread flour in farinograph studies increased absorption and lengthened arrival and peak times. The 10% level of substitution decreased stability. The globular proteins, phaseolin and G2 lectin, were separated from the navy bean fines and added to wheat dough systems in the same amounts as those in which they occurred in the 5 and 10% high-protein flour substitutions. Wheat starch was used to make up the remaining weight. Phaseolins and the combination of phaseolins and G2 lectins also increased absorption and arrival time, while decreasing departure time and dough stability. Investigation of the effect of phaseolin and G2 lectins on the rheologically active thiol and disulfides did not produce sufficient evidence to conclude that these globular proteins disrupted the disulfide interchange. Results of low-temperature scanning electron microscopy support the rheological studies.

Replacement of wheat flour with high-protein, high-lysine ingredients such as legume flours, protein concentrates, and isolates has been given much attention in the last two decades (D'Appolonia 1977, 1978; Fleming and Sosulski 1977; Deshpande et al 1983; Silaula et al 1989). This practice improves the amino acid balance and increases the protein content of products baked from the blended flours.

Wheat flour is a unique substance. Its gluten-forming ability makes wheat flour the best-known material that, when blended with water, forms an elastic dough capable of gas retention. When composite flours were used to make bread, the nonglutenous additions resulted in decreased loaf volume (D'Appolonia 1977, 1978; Fleming and Sosulski 1977; Silaula et al 1989). In addition to low volume, legume flours tend to change the absorption, mixing tolerance, and other physical dough properties. The crumb, texture, and color are adversely affected. Adjustments in mixing time and water additions were found to somewhat improve breads using composite flour. Many of the deleterious effects of legume flour on breadmaking were minimized by the use of bromate and dough conditioners such as stearoyl-2-lactylate (SSL) (Silaula et al 1989).

In studies conducted by Silaula et al (1989), the use of oxidizing agents and SSL greatly improved the volume of breads from flours with substituted pinto and navy bean protein fractions. Oxidizing agents are believed to help control disulfide (SS) bond rupture and interchange. The importance of the sulfhydryl (SH)-SS interchange to bread quality and loaf volume is well documented (Hird et al 1968; Bloksma 1972, 1975; Ewart 1972).

Knorr and Betschart (1978) suggested that the weakening of the dough with the addition of non-gluten-forming proteins is a dilution effect. Competition for water between the bean seed and wheat protein or a disruption of the protein-starch complex by the foreign protein could also weaken the dough. Finally, the non-gluten-forming protein could disrupt the SS interchange.

The focus of this investigation was to determine how the high-protein flour fraction and globular proteins affected dough properties and to determine whether a link to the SS interchange could be established. The first objective was to determine the effect of the high-protein navy bean flour on farinograph parameters. The second was to extract the major globulin proteins from the high-protein bean flour fraction and investigate how the effect of these proteins on farinograph parameters compared to the effect of the high-protein flour fraction. This would help determine the role of these globulin proteins in product performance. The third objective was to distinguish rheologically important thiol and SS groups during dough development in wheat dough with substituted navy bean proteins for a clearer understanding of protein-protein interactions. Finally, it was important to view images of the doughs from each aspect of the research with the scanning electron microscope. We hoped that this tool could be used to explain findings from the physical dough testing.

MATERIALS AND METHODS

Commercial hard spring wheat flour (12.6% proteins, 14% moisture base, 14% moisture, 0.49% ash) was obtained from the Pillsbury Company, Minneapolis, MN. Aytex wheat starch (donated by Ogilvie Mills, Inc., Minnetonka, MN) contained 9.0% moisture and had maximum protein and ash of 0.4% each.

Navy beans (*Phaseolus vulgaris* L.) were dry roasted in a gravity-flow particle-to-particle heat exchanger to produce beans that reached an equilibrium exit temperature of 106°C at the Food Protein Research and Development Center, Texas A&M University, College Station, TX. These roasting conditions were developed to reduce the antinutritional factors of raw beans (Aguilera et al 1982a,b). After roasting, the beans were cracked and air aspiration was used to separate the hulls from the cotyledon. The dry-roasted bean cotyledon fraction was shipped to the Alpine American Corporation, Natuk, MA, to be pin milled and air classified (Aguilera et al 1982a) to produce high-protein and high-starch fractions. These flour fractions were shipped to Michigan State University and stored at 4°C until analyzed. The high-protein fines fraction was used in this study.

Isolation of Globulins from Flours of *Phaseolus vulgaris*

Globulin proteins were extracted from the air-classified flours using the modification of Sun and Hall (1975) for the procedure described by McLeester et al (1973). The phaseolin (G1) protein was precipitated by dilution of the clear supernatant with five volumes of distilled water.

Since globulins of *P. vulgaris* are soluble at acid pH, the largest portion of albumin proteins was precipitated under these same conditions. Thus, they were not extracted by the NaCl-ascorbate system and did not contaminate the globulin fraction. The globulins were separated from the minor acid-soluble albumins by lowering the salt concentration of the extract.

Five grams of high-protein navy bean flour was placed in a Sorvall Omni-Mixer 17150 (Ivan Sorvall Inc., Newtown, CT) with 50 ml of 0.5*M* NaCl, 0.25*M* ascorbate (1:1), and about 2 ml of Thomas antifoam spray (Arthur H. Thomas Co., Philadelphia, PA). Continuous mixing for 3 min at speed 5 (4,800 rpm) gave a homogeneous sample.

This suspension was centrifuged (4°C) at 28,000 × *g* in an IEC model B-2OA centrifuge (Damon/IEC Division; Needham

[1]Department of Food Science and Human Nutrition, Michigan State University, East Lansing, 48824.
[2]Current address: NutraSweet Company, 601 East Kensington Road, Mt. Prospect, IL 60056.
[3]Current address: Monsanto Company, 800 N. Lindbergh Blvd., St. Louis, MO 63167.

Heights, MA) for 30 min. The supernatant was decanted and centrifuged for 30 min. This was repeated two additional times, and the pellet was discarded from each of the three runs. To the final clear supernatant, five volumes (about 200 ml) of deionized water was added. A white precipitate formed immediately and was pelleted by centrifugation for 30 min.

This pellet, designated phaseolin, was collected and frozen at −20°C before being freeze-dried. A small amount of supernatant was diluted with two volumes of distilled water to determine that no further precipitation would occur.

The supernatant was dialyzed for 18–20 hr at 4°C against deionized water, which was changed a minimum of four times. Silver nitrate (2%) was used as an indicator of chloride ions. The precipitate was sedimented as described above. This pellet was designated globulin fraction G2 (lectin) and frozen at −20°C before freeze-drying. Before being used, to remove any contamination (McPhie 1971), the dialysis tubing (Spectrum Medical Industries, Inc., Los Angeles, CA) was heated at 100°C for 20 min in a solution containing 1 g of $NaHCO_3$ and 0.1 g of sodium-ethylenediamine tetraacetic acid per 100 ml, then rinsed in distilled water. The process was repeated three times. The tubing was rinsed thoroughly in distilled water and stored at 4°C in 50% ethanol. Proteins were subsequently freeze-dried for 48 hr in a VirTis Unitrap II freeze dryer (VirTis Company, Inc., Gardiner, NY). Proteins were dried with a system pressure of $4–6 \times 10^{-2}$ Torr and a tray temperature approaching 50°C. Proteins were ground with mortar and pestle, and the fine powders were placed in tightly covered glass jars and stored at −20°C.

Chemical Analyses

Purified proteins, high-protein bean flour, wheat flour, and starch were analyzed for moisture using AACC Method 44-40 (1983). Purified proteins were analyzed for protein using the Folin-Ciocalteu assay (Lowry et al 1951), while the remaining materials were analyzed for nitrogen according to AOAC Method 14.026 (1984). Total SHs and SSs were determined according to Ellman (1959) and Cavallini et al (1966), respectively. Protein purity was assessed by discontinuous sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) according to the methods of Ornstein (1964) and Davis (1964).

Farinograph Studies

The effects of 5 and 10% substitutions of dry-roasted air-classified navy bean high-protein (fine) fraction on dough rheology was determined by AACC Method 54-21 (1983), using the constant flour weight techniques. Weight of the flour was adjusted according to AACC Table 82-23.

To study the effects of individual bean protein, the freeze-dried phaseolin and G2 proteins were added into the system in amounts equal to those determined to be present in the fines fraction when it was used at 5 and 10%. The proteins were added back individually and in combination with enough wheat starch to make up the remaining weight. Phaseolin made up 44% of the 45.22% protein in the high-protein navy bean fraction, whereas the G2 lectins were only 4.5% of the protein in the high-protein navy bean flour. Wheat starch was also substituted for 5 and 10% bread flour and was used as a second control to separate the effect of gluten dilution from the effect of the protein on the dough structure.

In the second farinograph study, estimates of rheologically active thiol and SS groups were obtained using a quantitative method of Jones et al (1974). Dithiothreitol (DTT) was the titrant used to determine the rheologically effective SS groups in the dough systems with protein substitution. Dough was mixed to full development as described by Jones et al (1974) to determine the content of SS important for mixing resistance. As mixing continued, DTT was added serially (10 to a cumulative 200 μmol) in 2.5- to 4-min intervals. Disulfides important to development were determined by addition of DTT (0–100 μmol) at time zero.

To distinguish rheologically important thiol groups, *N*-ethylmaleimide (NEMI, [0–60 μmol]) was added in 0.1 ml of ethanol to a saline solution containing 1 g of NaCl and a volume of water adjusted to produce a maximum resistance of 500 BU. This was used to titrate flours at time zero. The dough was mixed for 30 min, at which time resistance to mixing was measured.

Scanning Electron Microscopy

The primary objective of this phase of research was to determine whether structural differences could be discerned among a control wheat dough, doughs with high-protein bean flour substituted at the 10% level, and doughs with phaseolin and G2 proteins added at the levels present in the dough with the 10% high-protein substitution. Doughs were mixed in the 50-g bowl of the farinograph at high speed until peak time was reached.

Additionally, specimens from the rheologically active SH and SS studies were examined. Samples for these studies were taken from the farinograph at points that were determined to have the 1) maximum number of thiols involved in mixing tolerance, 2) maximum number of SSs involved in development, and 3) maximum number of SSs involved in resistance to mixing.

Low-Temperature Scanning Electron Microscopy

Doughs were mixed in the farinograph to the peak time. The instrument was then stopped and samples carefully excised with a surgical blade to avoid stretching the dough. Farinograph-prepared doughs were enveloped in plastic and, within a 5-min period, were transported to the Center for Electron Optics. Samples were prepared, and their microstructures were examined using the EMscope SP-2000 Sputter-Cryo system in conjunction with a JEOL JSM-35C scanning electron microscope. A small portion (less than 5 mm^2) of the fresh sample was mounted on a specially designed copper stub and was plunged into liquid nitrogen slush in the freezing chamber of the apparatus. During low-temperature scanning electron microscopy (SEM) preparation and viewing, the sample was held at a temperature of approximately −175°C under vacuum unless otherwise noted. The sample was moved to the preparation chamber equipped with the cold stage and was fractured before being transferred to the cold stage of the microscope. The specimen was then etched to remove the top layer of water, which otherwise would have obscured surface detail. This was accomplished by allowing the sample to warm to −65°C and holding it at that temperature for 3 min. Afterwards, the sample temperature was lowered to −120°C and the sample was retransferred to the preparation chamber, where it was cooled to −175°C and gold sputter coated for 4 min. The specimen was then reinserted onto the cold stage of the microscope for observation of the frozen specimen. Microscope settings for the cryo-stage work included a 15-kV beam, a condenser lens setting of 600, and a working distance of 39 mm.

Statistical Testing

The effects of high-protein navy bean flour and purified proteins on farinograph parameters were analyzed using the SPSS/PC Statistical Package for Social Sciences (SPSS 1986). The data derived from the experiments involving SS and SH groups were analyzed using the MSTAT (1985) program, version 3.01.

RESULTS AND DISCUSSION

The dry-roasted air-classified high-protein navy bean flour (fines) contained 40.2% protein and 5.4% moisture. SDS-PAGE established the purity of the phaseolin and G2 lectin proteins to be 90% or greater. Furthermore, apparent molecular weights for the phaseolin polypeptides closely corresponded to those established for the "tender green" patterns as described by Brown et al (1981).

Farinograph Studies

The results of the farinograph study showing the effects of substituting 5 and 10% high-protein flour (fines) are presented in Table I. This table also presents the effects of substituting the two globulin proteins alone and in combination in amounts equal to the original phaseolin and/or G2 lectin content present in the fines. Wheat flour was also replaced with wheat starch

at the 5 and 10% levels to study the intrinsic effects of dilution of the gluten proteins as compared to the effects of bean proteins on flour proteins.

Absorption increased significantly ($P < 0.05$) when fines were substituted for bread flour. These results were in agreement with those of other researchers (D'Appolonia 1978; Sathe et al 1981; Deshpande et al 1983; Silaula et al 1989). Absorption trends were similar for systems with globular protein equivalents at both levels of substitution. Absorption increased significantly ($P < 0.05$) with the addition of phaseolin and the combination of phaseolin and the G2 lectin protein. However, addition of G2 alone resulted in decreased absorption that was not different ($P > 0.05$) from that of the dough system with wheat starch substituted. This was expected, as the G2 content required to achieve these levels of substitution was quite small. The high-protein fines fraction contained 45.22% protein. The G2 lectin proteins contributed approximately 4.5% of that total. Thus, to substitute G2 protein at levels found in the dough with substituted fines at the 5 and 10% level, using the 50-g bowl of the farinograph, only tenths of a gram were needed.

The substitution of fines significantly ($P < 0.05$) increased arrival time, indicating a delay in the rate of hydration of the composite flour. The addition of phaseolin as well as phaseolin plus G2 lectin in amounts equivalent to those when 10% fines was substituted for bread flour also significantly ($P < 0.05$) increased arrival time, whereas the substitution of phaseolin or phaseolin plus G2 lectin equivalent to 5% fines or of G2 lectin or starch at either level had no significant effect. At the 5% level of substitution, only starch substitution significantly reduced peak time. At the 10% level of substitution, navy bean portion fines delayed peak time significantly ($P < 0.05$), whereas the substitution of 10% starch or of G2-lectin equivalent to that in 10% fines shortened peak time. Departure time increased significantly ($P < 0.05$) for the 95:5 composite of wheat flour and fines. All other departure times were significantly lower (P 0.05) than that of bread flour.

Stability of the doughs decreased significantly as compared to stability of bread flour ($P < 0.05$) with the addition of 10% fines or of the phaseolins, both alone and when combined with the G2 proteins at levels equivalent to the 5 and 10% levels of high-protein bean flour substitution. The addition of G2 alone did not reduce the stability from that of the blend of wheat flour and starch. Thus, the effect on the stability for the wheat-G2 protein blend was due primarily to the dilution of the gluten with the starch. As previously discussed, very small quantities of the G2 protein were needed to equal the amounts present in the fines at either level of substitution.

Rheologically Active Thiols and Disulfides

In dough systems, only a portion of the thiol and SS groups participate in the reactions that contribute to the rheological properties of the dough (Jones et al 1974). The following studies were conducted to determine the chemical reactivity and thus the rheologically effective proportions of thiols and SSs in the dough. To obtain these proportions, it was necessary to estimate the total number of thiols and SSs present in each system. Estimations of total SS and SH groups were obtained photometrically using the methods of Ellman (1959) and Cavallini et al (1966), respectively. The values obtained for the total thiol and total SS content are listed in Table II along with protein content.

The farinograph study involved determination of rheologically active SH by adding NEMI, a SH blocking agent, to doughs

TABLE I
Farinograph Measures Performed on Doughs with High-Protein Navy Bean Flour and with Navy Bean Proteins Substituted at an Equivalent Protein Level[a]

Substitution Level	Fraction	Absorption[b] (%)	Arrival Time (min)	Peak Time (min)	Departure Time (min)	Stability (min)
...	Wheat flour	62.39 ± 0.1 c	2.0 ± 0 ab	5.0 ± 0 bc	10.0 ± 0 b	8.0 ± 0.0 a
5%	Fines	64.24 ± 0.1 e	3.5 ± 0.5 c	5.3 ± 0.3 b	11.8 ± 1.4 a	8.3 ± 1.9 a
	Phaseolin	63.20 ± 0.0 d	2.5 ± 0.5 b	5.0 ± 0.0 bc	8.5 ± 0.0 d	6.0 ± 0.0 c
	G2 lectin	61.89 ± 0.4 b	1.8 ± 0.8 a	4.5 ± 0.0 cd	9.0 ± 0.5 cd	7.2 ± 0.3 b
	Phaseolin + G2 lectin	63.34 ± 0.2 d	2.8 ± 0.3 b	5.0 ± 0.0 bc	8.3 ± 0.3 d	5.5 ± 0.5 cd
	Starch	61.64 ± 0.4 b	1.3 ± 0.3 a	4.3 ± 0.3 d	9.2 ± 0.6 c	7.8 ± 0.3 ab
10%	Fines	66.69 ± 0.0 f	4.5 ± 0.1 d	6.5 ± 0.0 a	9.2 ± 0.3 c	4.7 ± 0.3 de
	Phaseolin	64.51 ± 0.6 e	3.3 ± 0.3 c	4.7 ± 0.3 cd	7.3 ± 0.3 e	4.0 ± 0.0 ef
	G2 lectin	60.69 ± 0.8 a	1.2 ± 0.3 a	4.3 ± 0.3 d	8.0 ± 0.0 de	6.8 ± 0.3 b
	Phaseolin + G2 lectin	64.63 ± 0.2 e	3.7 ± 0.3 c	5.2 ± 0.3 b	7.3 ± 0.3 e	3.7 ± 0.3 f
	Starch	60.98 ± 0.4 a	1.3 ± 0.6 a	4.2 ± 0.3 d	8.2 ± 0.3 d	6.8 ± 0.8 b

[a] Mean value of three trials. Values with the same letter are not significantly different ($P < 0.05$) from each other (Duncan 1957).
[b] 14% mb.

TABLE II
Chemical and Physical Mixing Properties of Doughs with Navy Bean Proteins Substituted for Wheat Flour

		Sulfhydryls			Disulfides						
			Involved in Mixing Tolerance			Involved in Development		Involved in Mixing Tolerance		Ratio of Disulfides to Sulfhydryls	
Type of Dough	Protein	Total (μmol/ 50 g)	(μmol/ 50 g)	(%)	Total (μmol/ 50 g)	(μmol/ 50 g)	(%)	(μmol/ 50 g)	(%)	Total	In Mixing
Bread flour	12.60	161.97	32	19.76	996.0	14	1.44	72	7.42	6.15	2.3
Wheat starch											
5%	11.97	153.87	27	17.55	946.2	12	1.30	81	8.79	6.15	3.0
10%	11.34	145.77	19	13.03	896.4	16	1.83	74	8.48	6.15	4.0
Phaseolin protein											
5%	12.48	154.70	30	19.39	956.6	15	1.61	104	11.16	6.18	3.5
10%	12.36	147.43	30	20.35	917.2	15	1.68	110	12.31	6.22	3.7
G2 + Lectin protein											
5%	12.01	154.56	28	18.12	947.9	16	1.73	82	8.88	6.13	2.9
10%	11.43	147.15	22	14.95	899.8	13	1.48	82	9.36	6.11	1.6

containing 5 or 10% protein equivalents, as previously detailed. Figure 1 shows the effect of NEMI on the loss of resistance to mixing of bread flour and bread flour with substitution of phaseolin equivalent to that in 10% fines. The loss of resistance to mixing shows two distinct linear areas. When these linear regions are extended as shown by the dashed lines, the amount of NEMI consumed in this region is considered to be the amount of SHs important to mixing tolerance. Details related to these evaluations are given by Jones et al (1974). In the control dough, 32 μmol/50 g were consumed. Amounts were similar for the systems with protein substituted. For the doughs with phaseolin added, the maximum amount of thiol involved in mixing tolerance was 30 μmol/50 g at both the 5 and 10% levels. Values reported for the doughs with G2 protein approximated those of the starch-replaced doughs (Table II).

To determine the number of SSs involved in mixing, dithiothreitol (DTT) was added serially to the doughs after they had reached full development. The resistance to mixing after addition of DTT to the control and to doughs with 10% phaseolin substituted, respectively, is shown in Figure 2. The first linear region of the curve, which shows a steep decline in resistance as DTT was added, represents the SS bonds important to mixing. This region is extended by a dashed line until it crosses the dashed line extension of the region that is essentially flat and thus not sensitive to DTT addition. The control system revealed the range of SS to be 72 μmol/50 g. The phaseolin-treated dough showed a definite increase in the number of SSs important to mixing properties; these rose to 104 μmol/50 g at 5% substitution and to 110 μmol/50 g at 10%. For the dough system containing G2 protein, 82 μmol/50 g were involved in resistance to mixing.

DTT was also added at time zero to determine the amount of SSs involved in dough development. However, the number of SSs involved in development remained relatively unchanged for any treatment. The range was 12–16 μmol/50 g. Mean values for each treatment are found in Table II.

The amounts of SS and SH involved in mixing were converted to a ratio (Table II). The ratio varied from 1.6 for the system containing G2 equivalent to a 10% substitution to 3.7 for the system containing phaseolin equivalent to a 10% substitution. The ratio for the control was 2.3.

The proportion of thiol groups involved in rheological properties was higher than the proportion of SSs. The percentage of total SH groups involved in mixing was 19.76 for the control, whereas the percentage of SS bonds found important to mixing was 7.42. Phaseolin substitution had the greatest impact on resistance to mixing. When expressed as a percentage of total SS, the dough containing phaseolin equivalent to 10% high-protein flour had a value of 12.31. The total percentage of thiol involved in mixing, 20.35, was close to that of the control. Results indicated that the ratio of mixing SS to mixing SH was not affected by the phaseolin or G2 lectin proteins. However, phaseolins appeared to influence the number of SSs involved in mixing. The evidence

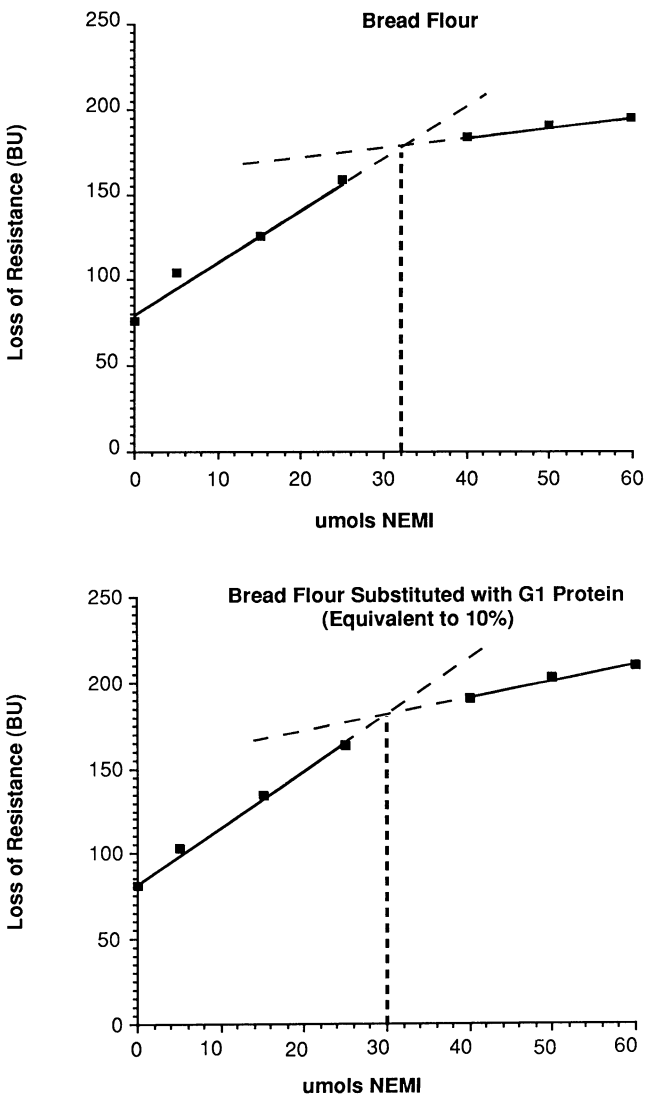

Fig. 1. Determination of sulfhydryls involved in mixing tolerance by treatment with *N*-ethylmaleimide (NEMI).

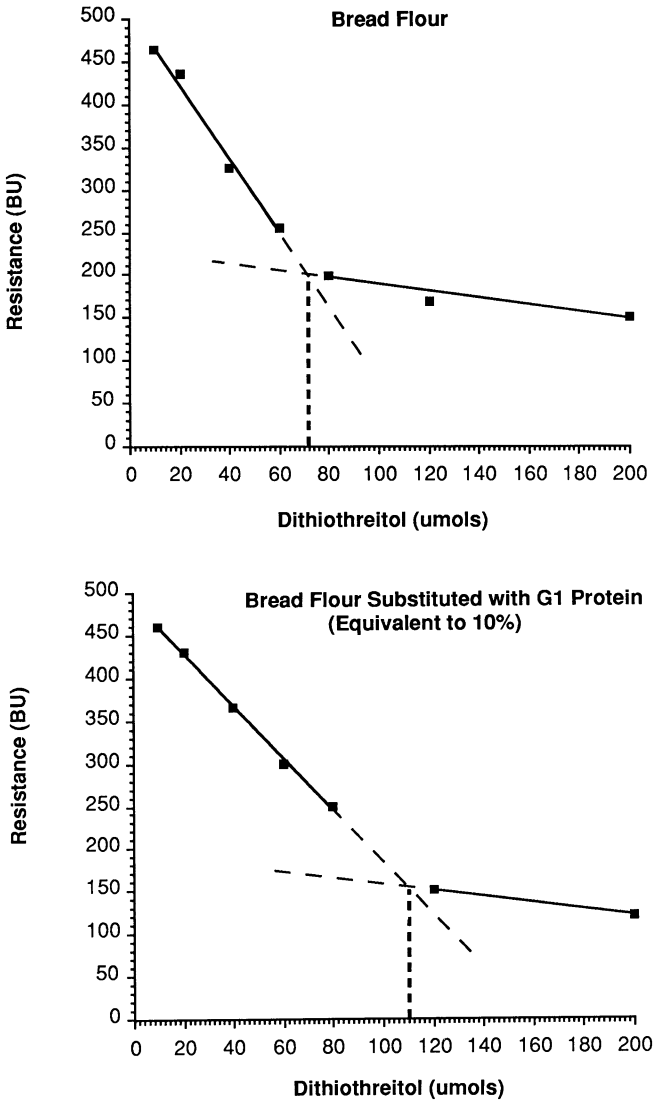

Fig. 2. Effect of dithiothreitol on resistance to mixing.

is insufficient to conclude that the globulin proteins of *Phaseolus vulgaris* disrupted the SS interchange.

It was evident from the farinograph data that the phaseolin proteins do affect dough properties, stability in particular. The amino acid profile of this protein as determined by Slightom et al (1983) may provide some insight into these phenomena. Phaseolin contains a high number of glutamine residues capable of contributing amide groups that could potentially impede hydrogen bonding between glycolipid and gliadin, glutenin, or the starch. Another important aspect of the amino acid picture is the presence of eight nonpolar residues. Of these, leucine is reportedly present in the largest amount, with valine, glycine, isoleucine, phenylalanine, and alanine occurring slightly less frequently. Methionine and tryptophan are the least available amino acids in this group. Nonpolar amino acids participate in hydrophobic interactions. These have the ability to interact with flour lipids. Removal of lipid from wheat flour leads to a decrease in the glutenin-gliadin ratio and thus interferes with gluten formation (Chung 1986). If hydrophobic interactions between the many bean proteins and the wheat flour lipids were tying up the wheat flour lipids, this could contribute to the decrease in stability attributed to doughs with substituted phaseolin proteins.

Hydrophobic and hydrogen bonds are essential to bread-baking performance. The phaseolin proteins have the potential to interfere with either or both types of bonding. The occurrence of either possibility would alter the farinograph mixing profile, as dough strength and stability would be reduced.

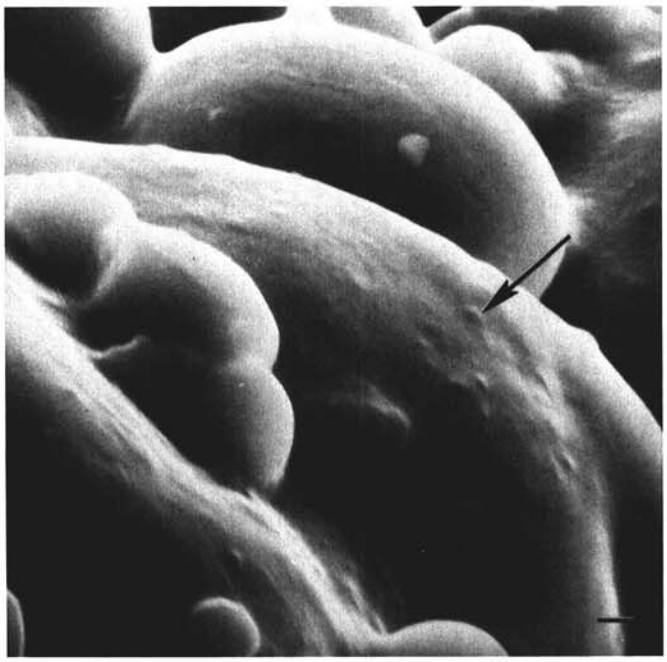

Fig. 3. Scanning electron micrograph of dough prepared by low-temperature methods from a wheat flour-phaseolin protein blend. Bar = 1 μm; arrow = blistered surface.

Scanning Electron Micrograph Studies

Examination of low-temperature SEM micrographs of the wheat flour-fine blend indicates that the gluten is not as smooth and thick as that of the bread flour control (Lorimer et al, *unpublished data*). Areas of thick and thin gluten were both present. Ingredients in the wheat flour-phaseolin blend were evenly dispersed (Fig. 3). The gluten sheet was continuous but slightly blistered. Doughs with phaseolin substituted were significantly different from the control for every farinograph parameter examined. Arrival time was delayed; peak and departure times were shortened; and stability decreased. Had micrographs been taken of samples removed from the farinograph bowl at departure time, the loss of stability would likely have been more evident.

The G2 lectin proteins incorporated into the dough system were characterized by smaller starch granules, which formed a ring around the larger granules (Fig. 4A). This phenomenon was also noted in systems with substituted bean flour. The cut surface images of the dough with substituted G2 lectin showed evidence of disruption at the protein-starch interface (Fig. 4B). The starch granules were not clearly cleaved from the gluten. Many areas exhibited fingerlike projections spanning the distance between the parting edges.

When phaseolins and lectins were used in combination in the wheat blend dough, the gluten sheet was thick and continuous, covering the starch grains without interruption. Thin spots and breaks in the gluten were minimal (Fig. 5A). The fractured surface showed large starch granules that were connected to the thick gluten filaments by thinner branches of the gluten strands (Fig. 5B). These wisps surrounded the smaller starch grains and appeared attached to the larger starch granules. Farinograph data and micrographs both provide evidence of little difference in doughs produced from wheat flour and phaseolin blends, or wheat flour plus the combination of phaseolin and G2 protein.

Microscopy studies corresponding to the rheologically active thiol and SS investigations were performed since no other literature existed for SEM. Nemi, at 32 μmol/50 g of flour, was added to a control dough and mixed for 30 min. After 30 min, the doughs were broken down, making them tenacious and extremely

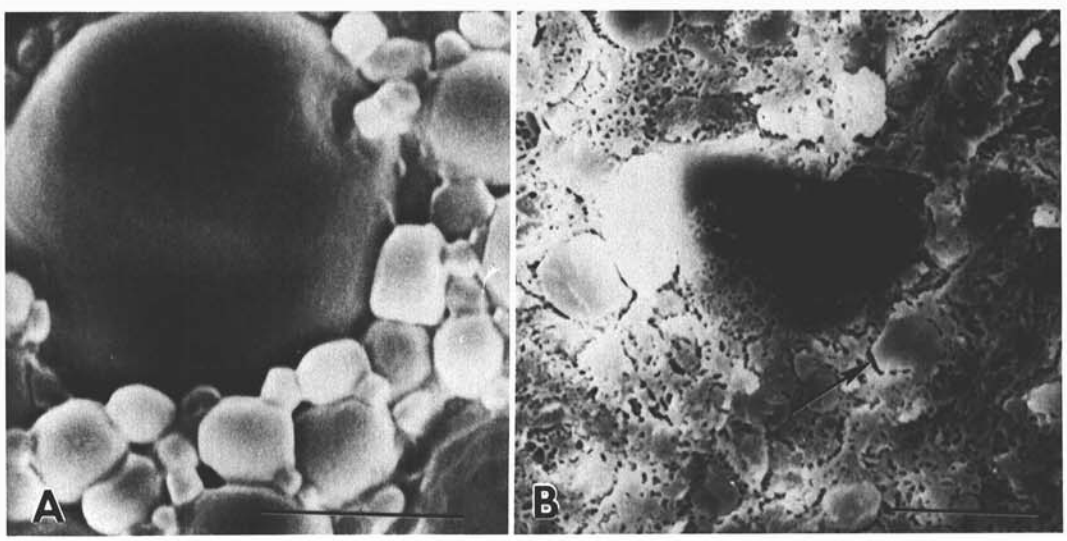

Fig. 4. Scanning electron micrographs of dough prepared by low-temperature methods from a wheat flour-G2 protein blend. **A,** Surface image interface; **B,** fractured surface. Bars = 10 μm. Arrow = protein-starch interface.

sticky. This resulted in an alteration of the system as shown in Figure 6A. The gluten network appeared to have broken down. The gluten sheet was thin and translucent. Some regions may have completely lacked a gluten covering. There were gaps between starch granules without a connecting gluten bridge.

The specimen with wheat starch substituted was severely altered (Fig. 6B). The gluten coat was studded with tiny orbs, and in some places these spheres apparently tended to aggregate. These may have been globular proteins that had precipitated due to a shift in pH caused by the addition of NEMI. The gluten film was thick and velvety. Small cracks at the bases of the starch granules gave evidence of disruption of the protein-starch interface. The system with substituted wheat and phaseolin was similar to the wheat-starch dough. The gluten also was thick and patchy. The surface was dotted with the identical orbicular matter that occurred on the surface of the dough with substituted starch.

To estimate the number of SSs involved in mixing, DTT was added to the doughs, which caused their rapid deterioration. Doughs became thick and sticky and had the appearance of whipped cream. The micrograph of the control dough system (Fig. 7) resembled the images of the wheat-starch and wheat-phaseolin specimens in the NEMI series of micrographs. The surface was spotted and rough. Large spaces appeared between starch granules; some were held together by thick fingerlike projections (see arrow in Fig. 7A). As could be expected in a ruptured dough, the gluten did not drape evenly over all of the starch grains. The fractured surface showed a dense gluten network (Fig. 7B). Many starch granules were completely separated from the network or remained loosely attached (arrow, Fig. 7B).

The effect of DTT was less physically disruptive to the dough containing substitute wheat flour and starch (Fig. 8). The gluten sheet was slightly rough and open in some areas. Thick and thin regions were also detectable. No irregular markings or patterns were apparent on the gluten surface. The fractured image (Fig. 8B) showed starch granules embedded in the gluten matrix (arrow).

The dough with substituted wheat flour and phaseolin protein and treated with DTT looked very different from the control and starch-supplemented doughs (Fig. 9). Farinograph data suggested that phaseolin protein affected the mixing tolerance of the dough. The dough surface took on a mosaic pattern when treated with DTT (Fig. 9A, small arrow). The gluten veil was torn in a few places, as indicated by the large arrow on Figure 9A, and it thinned out at the base of many of the starch grains. Figure 9B shows the cleaved surface of the wheat flour-phaseolin composite dough. Many of the starch granules were no longer associated with the gluten. Craterlike pockets suggested that starch granules, which were no longer firmly positioned in the network,

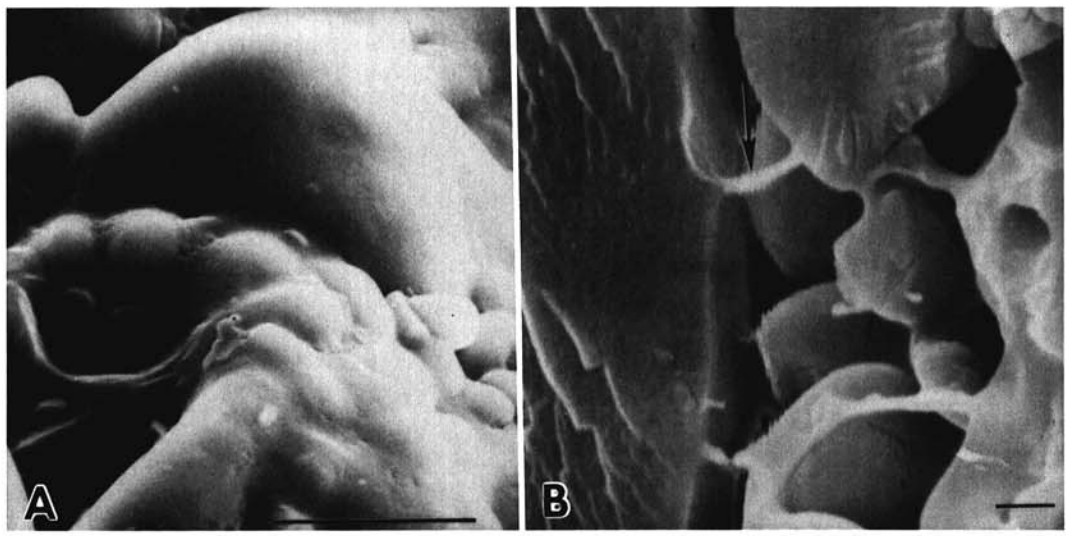

Fig. 5. Scanning electron micrographs of dough prepared by low-temperature methods from a blend of wheat flour, phaseolin, and G2 protein. **A,** Surface image (bar = 10 μm); **B,** fractured surface (bar = 1 μm, arrow = protein filaments).

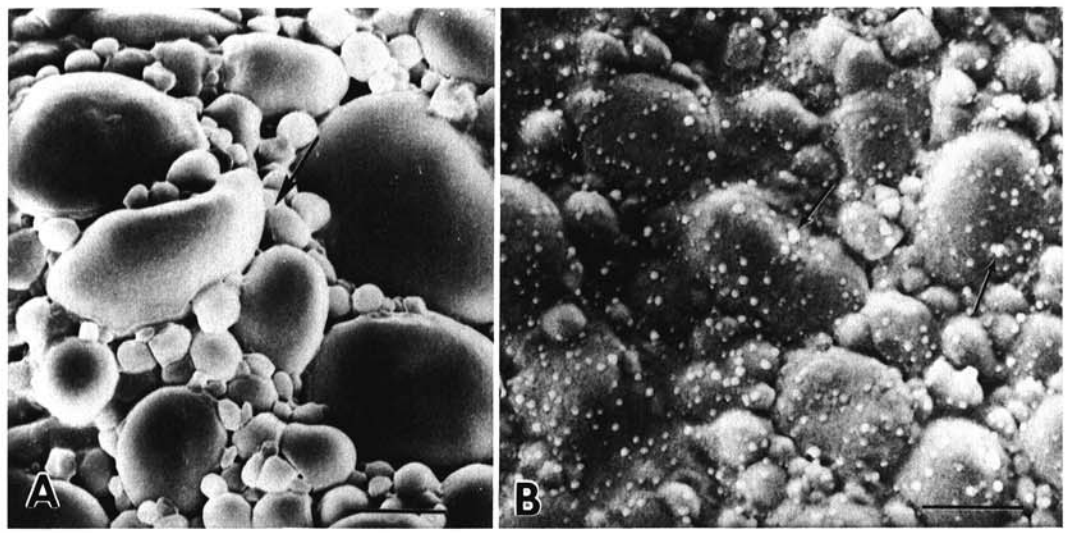

Fig. 6. Scanning electron micrographs prepared by low-temperature methods, illustrating the effect of *N*-ethylmaleimide on loss of resistance. **A,** Wheat flour control (bar = 10 μm, arrow = broken-down gluten network); **B,** wheat flour-starch blend (bar = 10 μm, arrow = tiny orbs).

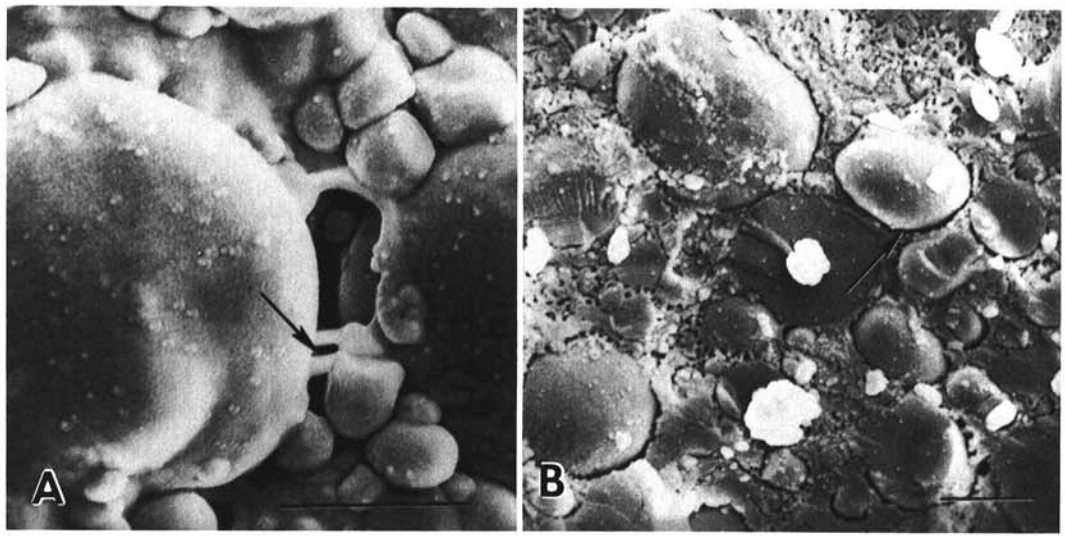

Fig. 7. Scanning electron micrographs prepared by low-temperature methods, illustrating the effect of dithiothreitol on resistance to mixing in a wheat flour dough. **A,** Surface (bar = 10 μm, arrow = thick projections); **B,** fractured (bar = 10 μm, arrow = separated starch granules).

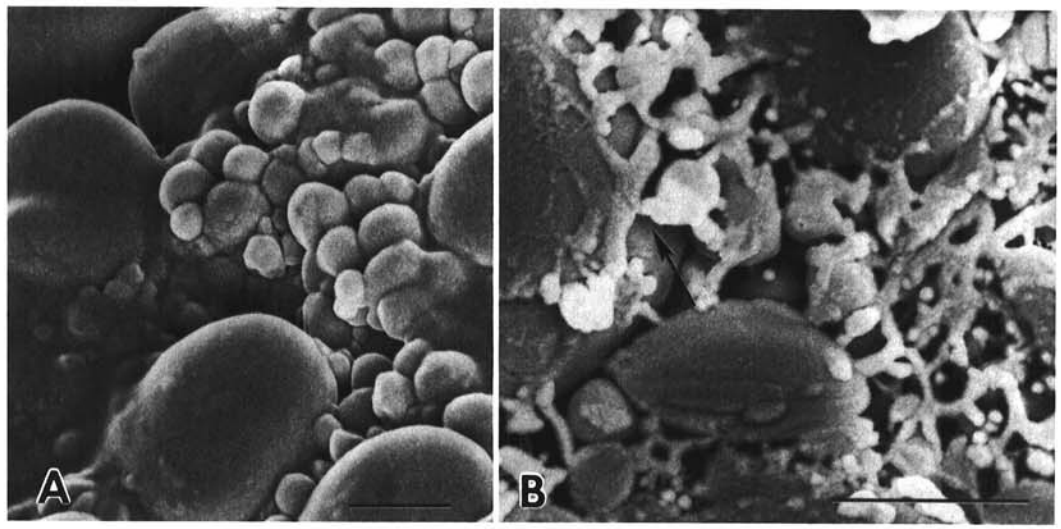

Fig. 8. Scanning electron micrographs prepared by low-temperature methods, illustrating the effect of dithiothreitol on resistance to mixing in a flour-starch blend. **A,** Surface (bar = 10 μm); **B,** fractured (bar = 10 μm, arrow = starch granules embedded in gluten matrix).

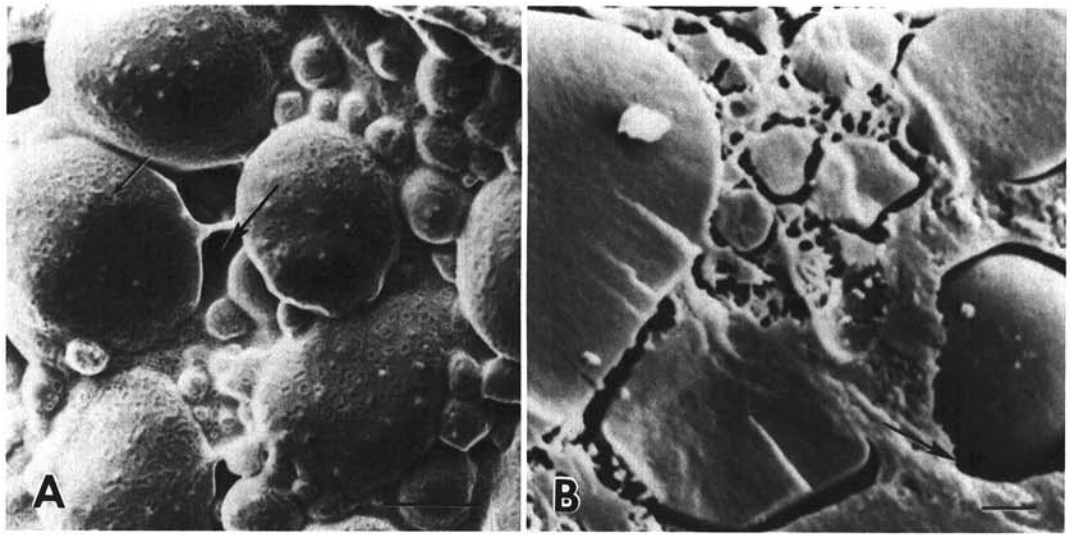

Fig. 9. Scanning electron micrographs prepared by low-temperature methods to illustrate the effect of dithiothreitol on resistance to mixing in a wheat flour-phaseolin blend. **A,** Surface (bar = 10 μm, arrow = mosaic pattern); **B,** fractured (bar = 1 μm).

may have been dislodged in the fracture process. Many small starch granules were still attached to each other by spindly filaments. The major portion of the gluten network between the starch granules had collapsed and appeared solid.

High-protein navy bean flour and the major storage protein, phaseolin, had a negative effect on dough stability, causing a faster rate and greater magnitude of breakdown than other variables. The ratio of mixing SS to mixing SH was not affected by phaseolin or lectin protein. Phaseolin did influence the number of SSs in mixing. However, as noted earlier, evidence is insufficient to conclude that the globulin proteins of *Phaseolus vulgaris* disrupted the SS interchange.

In the doughs with protein substituted, ingredients were well distributed. The gluten sheet was continuous and smooth. The farinograph demonstrated little evidence of the negative impact of the phaseolin protein. Since the specimens were taken from the farinograph bowl at peak time, this may indicate that the subsequent breakdown was mechanically induced or that the reaction rate was slow.

Micrographs of the reactive SH and SS groups showed some interesting results. As expected, doughs were broken down by the chemical treatment. Curiously, the doughs with substituted wheat starch or and those with substituted phaseolin and NEMI treatment were flecked with small spheres. The effect of DTT on mixing resulted in a mosaiclike pattern on the surface of the flour-phaseolin dough.

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