Shear Stress Relaxation of Chemically Modified Gluten

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ABSTRACT

Chemically modified gluten was studied in shear stress relaxation. The gluten was treated with glutaraldehyde, potassium bromate, cysteine, and ascorbic acid. The resulting relaxation curves, shear stress versus log time, were analyzed in terms of the theory of cooperative flow. In this way, the experimental results gave information on the structure of gluten and how this structure is affected by chemical modifications. In gluten, two cooperative flow processes generally occur. The primary process, which in unmodified gluten has a relaxation time of approximately 1 sec and a cooperative coordination in flow of four, is proposed to occur in a partly oriented fibrillar structure. The secondary process, which has a relaxation time of approximately 500 sec and a coordination in flow of two, is proposed to occur in a lamellar superstructure. In unmodified gluten, both structures flow like a liquid at long times. The results of this work clarify how the applied chemical modifications affect the two flow structures in gluten.

The rheology of wheat flour dough in relation to the structure of gluten complex and to bread manufacture has received much attention. The rheological properties of dough are due primarily to the properties of gluten. Although the properties of dough have been studied considerably (Bloksma 1971, Hibberd and Parker 1975), few studies have been done on the rheological properties of gluten (Funt Bar-David and Lerchenthal 1975, Matsumoto 1979, Mita and Matsumoto 1981). Recently a new, simple, and accurate stress relaxation instrument suitable for materials like dough and gluten was developed (Bohlin et al 1980), and the experimental relaxation results were analyzed according to a cooperative flow theory (Bohlin 1980). The stress relaxation experiment, in which the decay of stress was observed at constant strain, is fundamentally simple and ideally suited for the construction of the model. The stress relaxation of dough and gluten was measured with this instrument (Bohlin and Carlson 1981), which showed that the observed relaxation curve for gluten consists of two processes. The first relaxation process, occurring at short times (1 to approx. 10 sec), is strongly cooperative with a fourfold coordination of flow units. The second relaxation process, occurring at longer times (10 to approx. 10^4 sec), can be interpreted as a weakly cooperative flow process with a twofold coordination of flow units.

Disulfide (SS) bonds and other intermolecular interactions are important factors in the rheology of gluten. This article describes an attempt to investigate the rheological properties of gluten through the measurements of shear stress relaxation of gluten modified with glutaraldehyde, potassium bromate ($KBrO_3$), cysteine, and ascorbic acid.

MATERIALS AND METHODS

Reagent

All chemicals used in this study were of reagent grade. Glutaraldehyde was purchased from Sigma Chemical Co., L-ascorbic acid and $KBrO_3$ from E. Merck, AG, and cysteine from BDH Chemicals Ltd. The amount of reagent used for modification of gluten was expressed in parts per million (ppm) based on flour weight.

Wheat Flour

The wheat flour was obtained from Swedish spring wheat Amy (from the 1975 crop). The wheat was milled to an extraction of 67% (w/w) in a Brabender Quadromat Senior experimental mill. Chemical analysis of Amy flour gave the following constants, calculated on a dry basis (w/w) as described in the AACC method (AACC 1976): protein 13.5% (N × 5.7), and ash less than 0.5%.

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Chemical Modification of Gluten

Gluten for glutaraldehyde treatment was obtained from the flour (10 g) by using a semiautomatic gluten washer (Glutomatic, Falling Number, Stockholm, Sweden). So that it would react with glutaraldehyde, the gluten prepared was soaked in water (20 ml) containing different amounts of glutaraldehyde (1,000–5,000 ppm) at room temperature. After standing for 22 hr, the glutaraldehyde-treated gluten was washed with a large quantity of water to remove excess glutaraldehyde. $KBrO_3$, cysteine, and ascorbic acid treatment was as follows: the flour sample (10 g) and 5.4 ml of water were mixed in the Glutomatic gluten washer. The reagent was dissolved in an aliquot of the added water. The dough made was then allowed to stand for 15 min so that it would react sufficiently with the reagent. The gluten, from which excess reagent and starch were well-removed, was prepared by washing with a large quantity of water (700–800 ml) in the Glutomatic washer. All gluten samples were prepared at 22 ± 1°C.

Stress Relaxation Measurements

In a shear stress relaxation experiment, the sample is deformed to a given shear strain, which is then kept constant throughout the measurement. In the following, shear stress at constant strain is denoted by τ, the initial stress by τ_o, and the relaxation time by $t_{1/2}$. A useful representation for the analysis of stress relaxation experiment is a graph of $d(\tau/\tau_o)/d\ln t$ versus τ/τ_o (rate-stress plot). This graph contains detailed information of the relaxation curve (τ/τ_o versus ln t), except for an arbitrary shift on the time axis.

The stress relaxation measurements were made with a cone-plate instrument described in detail by Bohlin et al (1980). The radius of the cone plate used was 2.65 cm, and the cone angle was 0.053 radians, except for glutaraldehyde-treated gluten, in which a cone plate system with a radius of 1.23 cm and a cone angle of 0.169 radians was used. The deviation from constant shear strain due to the deflection of the transducer membrance varies with the shear rigidity of the sample but may be kept around 1%. All measurements were made at 22 ± 0.5°C, and freshly prepared samples were used. The gluten sample was kept on the plate for approximately 15 min for evaporation of excess water to ensure good adhesion to the surface of the cone and the plate. When the cone was lowered, the gluten filled the gap between the cone and the plate, and expelled materials were trimmed off with scissors. The sample at the edge of the cone-plate system was covered by silicone oil to prevent drying. All samples were then allowed to rest for 60 min. During this time, the cone axis was free to rotate so that some recovery motion might occur. The base plate was then rotated so that the lever on the cone axis came into contact with the pressure transducer. The shear strain applied was 0.406, except for the glutaraldehyde-treated gluten in which the strain was 0.38. The output signal of the transducer was converted to digital form with 12 bits of resolution and interfaced to a personal computer (ABC80, Luxor, Sweden). The relative stress $\tau(t)/\tau_o$ versus ln t was recorded on a digital plotter (Houston Instrument, Division of

Bausch & Lomb, TX). The flow rate $(d\tau/\tau_o)/d\ln t$ versus τ/τ_o was also analyzed by using the same computer system. Each part of the experiment was replicated at least three times and then averaged.

RESULTS AND DISCUSSION

Glutaraldehyde

Figure 1A shows the stress relaxation curves, and Fig. 1B shows the rate-stress curves ($\delta = d(\tau/\tau_o)/d\ln t$ verus τ/τ_o) for glutaraldehyde-treated gluten. The parameters are given in Table I. A large increase of the rigidity modulus (G_o) was found through glutaraldehyde treatment. At concentrations above 5,000 ppm of glutaraldehyde, the measurement was impossible because the gluten lump became too stiff. The relaxation time ($t_{1/2}$) of glutaraldehyde-treated gluten is considerably larger than that of the control gluten, and the inflection points t_1 and t_2 of the relaxation curve also varied, eg, from $t_1 \simeq 0.15$ sec, $t_2 \simeq 300$ sec for control gluten to $t_1 \simeq 0.4$ sec, $t_2 \simeq 5 \times 10^3$ sec for glutaraldehyde (1,000 ppm)-treated gluten. The relative stress at which the second process starts also changed from approximately 20% for control gluten to approximately 40% for glutaraldehyde-treated gluten. This was accompanied by an increase of the coordination number Z, indicating the increase in the number of cooperative flow units in the structure of gluten matrix. In addition, not only was the first process shifted from the origin to the right and the linear part intersected the stress axis at positive stress value, but the peak at the second process distinctly increased, compared with that of the control gluten.

Bifunctional aldehydes are often used as crosslinking reagent because of their high reactivity. It results in the formation of covalent intercrosslinking and intracrosslinking between ϵ-amino groups of lysine and terminal α-amino groups (Simmonds and Orth 1973), and between lysine and tyrosine residues (Ewart 1968). Accordingly, gluten is considered to be crosslinked chemically by glutaraldehyde, and the increased entanglement of gluten molecules significantly converts the stress relaxation behavior.

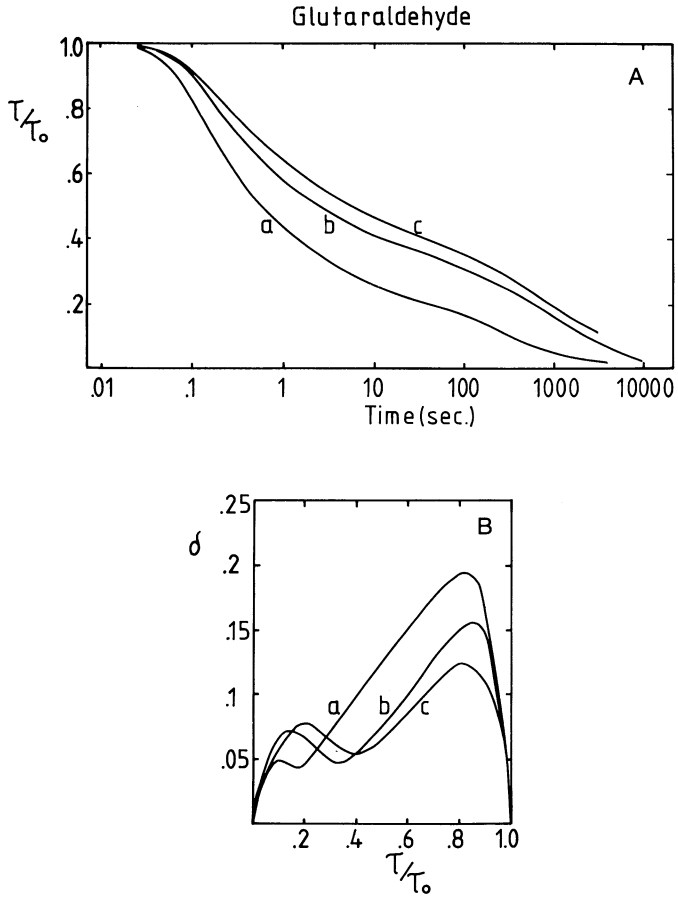

Fig. 1. Stress relaxation **(A)** and rate-stress curves **(B)** for glutaraldehyde-treated gluten at the shear strain 0.380. a, 0 ppm; b, 1,000 ppm; c, 2,000 ppm.

$KBrO_3$

Figure 2A shows the stress relaxation curves, and Fig. 2B shows the rate-stress curves for $KBrO_3$-treated gluten. The parameters are given in Table I. $KBrO_3$ has been widely used as oxidant in flour. It is also well-known as SS crosslinking reagent, and the level of 10 ~30 ppm of $KBrO_3$ has been used as a dough-improving component in the baking industry. When gluten was treated with 30 ppm of $KBrO_3$, a change of stress relaxation was found. This suggests that gluten is crosslinked even at the level of 30 ppm of $KBrO_3$. To clarify the contribution of $KBrO_3$ for gluten structure, large amounts of $KBrO_3$ were added during glutenmaking. With an increasing amount of $KBrO_3$, the rigidity modulus and relaxation time increased, becoming almost constant beyond 900 ppm of $KBrO_3$. The coordination number Z also increased with increasing amounts of $KBrO_3$ and indicates that the crosslinking by the use of $KBrO_3$ increases the cooperative flow units. When the amount of $KBrO_3$ increased more than 900 ppm, all parameters of stress relaxation became almost constant. This suggests that SH groups were almost completely oxidized to SS at the amounts above 900 ppm. This finding is in accordance with the results of Nagao et al (1981), who concluded that when the dough was mixed in the presence of 1,200 ppm of $KBrO_3$, no SH was detected.

Because a large amount of reagent on the flour basis is used in the chemical modification process of gluten, attention should be given to the salt effect that is caused by reagent remaining in gluten. Sodium chloride, which does not influence the crosslinking, was therefore added in the process of glutenmaking. The flow behavior of sodium chloride-treated gluten was almost the same as that of the control, even at levels as high as 8,500 ppm of sodium chloride.

TABLE I
Parameters of Stress Relaxation for Treated Gluten

Treatment (ppm)[a]	$t_{1/2}$[b] (sec)	Z[c]	q[d]	G[e] (Pa × 10^3)
Glutaraldehyde[f]				
0	0.66 ± 0.10	3.9 ± 0.1	0	1.10 ± 0.05
1,000	3.57 ± 0.85	4.4 ± 0.3	0.19 ± 0.02	4.43 ± 0.29
2,000	8.10 ± 2.52	4.7 ± 0.1	0.23 ± 0.01	8.41 ± 0.20
$KBrO_3$				
0	0.53 ± 0.05	4.0 ± 0.1	0	1.18 ± 0.05
30	0.67 ± 0.01	4.3 ± 0.1	0	1.52 ± 0.03
300	0.88 ± 0.06	4.5 ± 0.1	0	1.41 ± 0.10
600	1.07 ± 0.07	4.6 ± 0.1	0	1.57 ± 0.10
900	1.14 ± 0.01	4.8 ± 0.1	0	1.62 ± 0.07
1,500	1.15 ± 0.05	4.9 ± 0.1	0	1.63 ± 0.11
8,500	1.27 ± 0.13	5.0 ± 0.1	0	2.23 ± 0.03
Cysteine				
0	0.53 ± 0.05	4.0 ± 0.1	0	1.18 ± 0.05
50	0.46 ± 0.02	3.6 ± 0.1	0	1.19 ± 0.05
100	0.40 ± 0.03	3.4 ± 0.2	0	1.10 ± 0.08
200	0.34 ± 0.01	3.2 ± 0.1	0	1.10 ± 0.09
Ascorbic acid				
0	0.53 ± 0.05	4.0 ± 0.1	0	1.18 ± 0.05
1,500	0.96 ± 0.08	3.4 ± 0.1	0.14 ± 0.1	1.66 ± 0.04
3,000	1.34 ± 0.26	3.6 ± 0.2	0.15 ± 0.1	1.74 ± 0.10
5,000	3.34 ± 0.01	4.9 ± 0.1	0.13 ± 0.1	1.98 ± 0.28
Ascorbic acid in the presence of $KBrO_3$ (8,500)				
0	1.27 ± 0.13	5.0 ± 0.1	0	2.23 ± 0.03
1,500	1.57 ± 0.26	5.0 ± 0.1	0	2.25 ± 0.22
3,000	3.34 ± 0.01	6.0 ± 0.1	0	2.64 ± 0.06
5,000	4.68 ± 0.31	6.5 ± 0.2	0	3.56 ± 0.36

[a] The amount of reagent is expressed on a wheat flour basis.
[b] Relaxation time.
[c] Coordination number of first flow process.
[d] The value of the relative stress at which the linear part of the first flow process intersects the τ/τ_o-axis.
[e] Rigidity modulus.
[f] The prepared gluten is soaked in water with glutaraldehyde for 22 hr.

Furthermore, the peak at the second process decreased with increasing amounts of $KBrO_3$, and at the amounts above 900 ppm of $KBrO_3$ this peak almost disappeared, ie, the inflection point of relaxation, t_2, was not detected. Consequently, these results suggest that the second flow process depends on SS bridges and that this crosslinking gives fairly different flow properties compared to crosslinking by glutaraldehyde.

Cysteine

Figure 3A shows the stress relaxation curves, and Fig. 3B shows the rate-stress curves for cysteine-treated gluten. Table I gives the parameters. With increasing amounts of cysteine, the rigidity modulus and the relaxation time decreased. The coordination number Z also decreased with increasing amounts of cysteine. At concentrations above 300 ppm of cysteine, no coherent gluten lump was formed. An excess thiol reagent such as cysteine causes a reducing reaction that is opposite to the oxidative effect obtained by $KBrO_3$. In low concentrations, the thiol reagents cause a decrease in resistance to extension of gluten, and at high concentrations they assist in dispersing gluten (Hlynka 1949). We found that the gluten lump was not formed at concentrations above 300 ppm of cysteine.

Ascorbic Acid

Figure 4A shows the stress relaxation curves, and Fig. 4B shows the rate-stress curves for ascorbic acid-treated gluten. The parameters are given in Table I. At the lowest level of ascorbic acid (1,500 ppm), the effect is weak, but at higher concentrations of ascorbic acid the rigidity modulus and relaxation time increased considerably.

Ascorbic acid is expected to give a behavior characteristic of an oxidizing reagent in wheat flour dough (Kuninori and Matsumoto 1963). The effect of ascorbic acid, however, resembles glutaraldehyde rather than $KBrO_3$ in stress relaxation behavior.

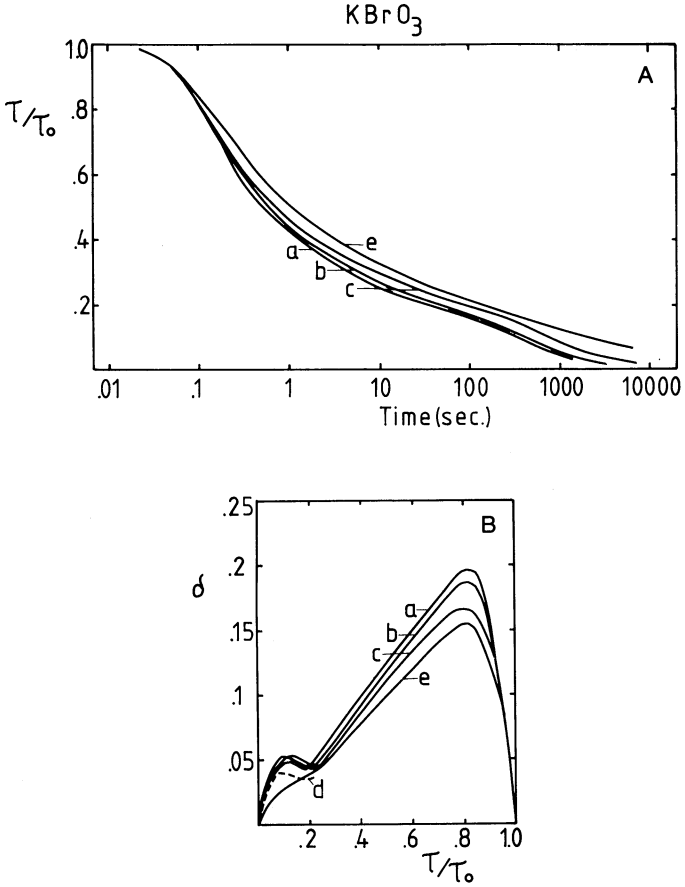

Fig. 2. Stress relaxation **(A)** and rate-stress curves **(B)** for $KBrO_3$-treated gluten at the shear strain 0.406. a, 0 ppm; b, 30 ppm; c, 300 ppm; d, 600 ppm; e, 900, 1,500, 8,500 ppm.

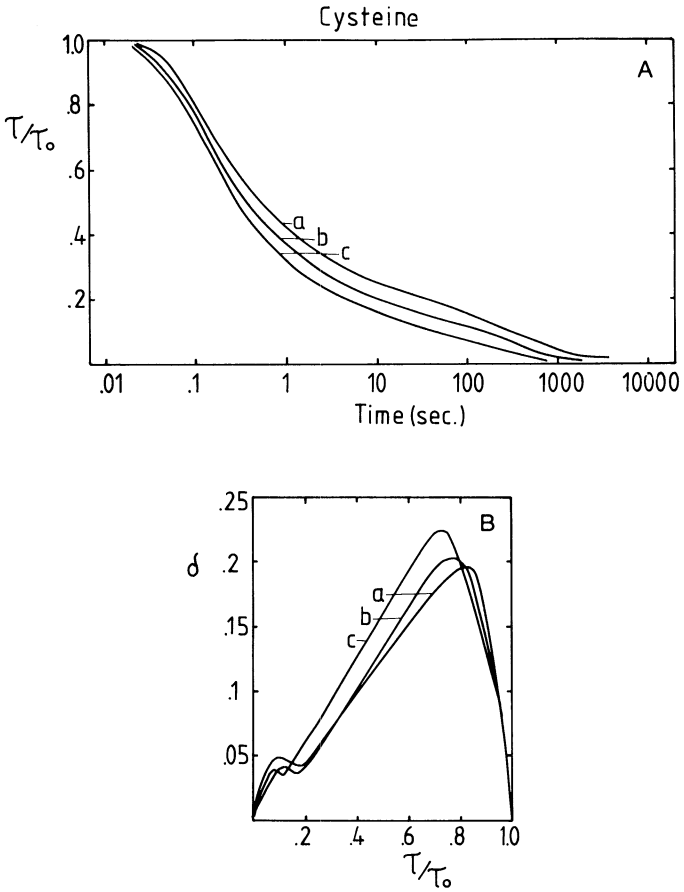

Fig. 3. Stress relaxation **(A)** and rate-stress curves **(B)** for cysteine-treated gluten at the shear strain 0.406. a, 0 ppm; b, 50 ppm; c, 100, 200 ppm.

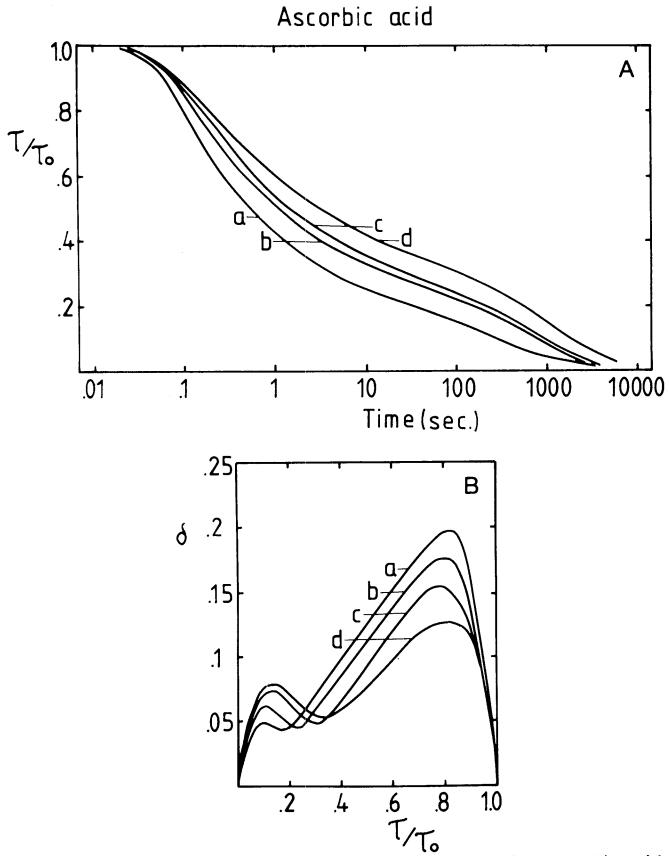

Fig. 4. Stress relaxation **(A)** and rate-stress curves **(B)** for ascorbic acid-treated gluten at the shear strain 0.406. a, 0 ppm; b, 1,500 ppm; c, 3,000 ppm; d, 5,000 ppm.

Thus, through treatment with ascorbic acid, the rate-stress plots at the first flow process are shifted to the right, and the peak at the second flow process became considerably larger than that of the control.

To obtain further information on the effect of ascorbic acid, the stress relaxation of ascorbic acid in the presence of a constant amount of $KBrO_3$ (8,500 ppm) was measured. At this concentration of $KBrO_3$ the SH groups should be almost completely crosslinked to SS bonds. Figure 5A shows the stress relaxation curves, and Fig. 5B shows the rate-stress curves for ascorbic acid containing this amount of $KBrO_3$. The corresponding parameters are given in Table I. When $KBrO_3$ was used with ascorbic acid, a stronger effect was found compared to $KBrO_3$ or ascorbic acid. The shift observed in ascorbic acid disappeared, and the peak at second process also decreased significantly. This suggests that the reactive site of ascorbic acid differs, at least partly, from that of $KBrO_3$, although the reaction mechanism of ascorbic acid is still obscure.

Gluten Structure—Cooperative Flow Analysis

The analysis of the stress relaxation experiment based on the theory of cooperative flow was described in detail by Bohlin (1980). The formal theory applies only to a flow process with a simple cooperative flow unit. Such flow is commonly found in experiments on solid metals and polymers. In a recent work on dough and gluten (Bohlin and Carlson 1981), stress relaxation results showed the existence of two cooperative flow units in dough as well as in gluten. The results of the present work confirm this for gluten and show how the relative contribution of the two flow processes can be changed considerably by chemical modifications of the gluten. The implications on the structure of gluten that are obtainable from a cooperative flow analysis of the results of the present work are discussed.

The spatial arrangement of particles constituting a flowing substance, whether the particles be atoms, molecules, or molecular aggregates, generally occurs in three dimensions. On a higher level of organization, the aggregates may arrange in two dimensions, like fibrils, or in one dimension, like lamellar aggregates. The coordination in space of aggregates closely packed in three, two, and one dimension(s) is 12, six, and two, respectively. The shear flow coordination number, however, will generally differ from the spatial coordination: in shear flow significant rearrangements occur in two dimensions only, ie, in the planes perpendicular to the stress planes. Thus, we can understand why cooperative flow analysis of stress relaxation in shear (or uniaxial tension) never seems to give a flow coordination number greater than six.[2]

The primary flow process in control gluten has a flow coordination of four. We believe that this corresponds to the rearrangement in nonclose-packed oriented fibrillar structures (Bernardin 1975). Such a coordination may, however, also be obtained in a nonclose-packed particle structure.

The secondary flow process in gluten has a flow coordination of two. This should correspond to rearrangements in a lamellar structure. A schematic representation of a cross section of the fibrillar structure is shown in Fig. 6A. The theoretical relaxation plots are shown to the right; δ is the relaxation rate $(-d(\tau/\tau_o)/d\ln t)$. Assuming that strong cooperation exists only between the nearest neighbors, the coordination number Z of this structure is four. The slope of the linear part of the rate-stress plot is, hence, $1/Z = 1/4$. Furthermore, the structure is assumed to relax back to a stress-free state of equilibrium. The nature of the interplay between the

[2]L. Bohlin. 1982. Unpublished data.

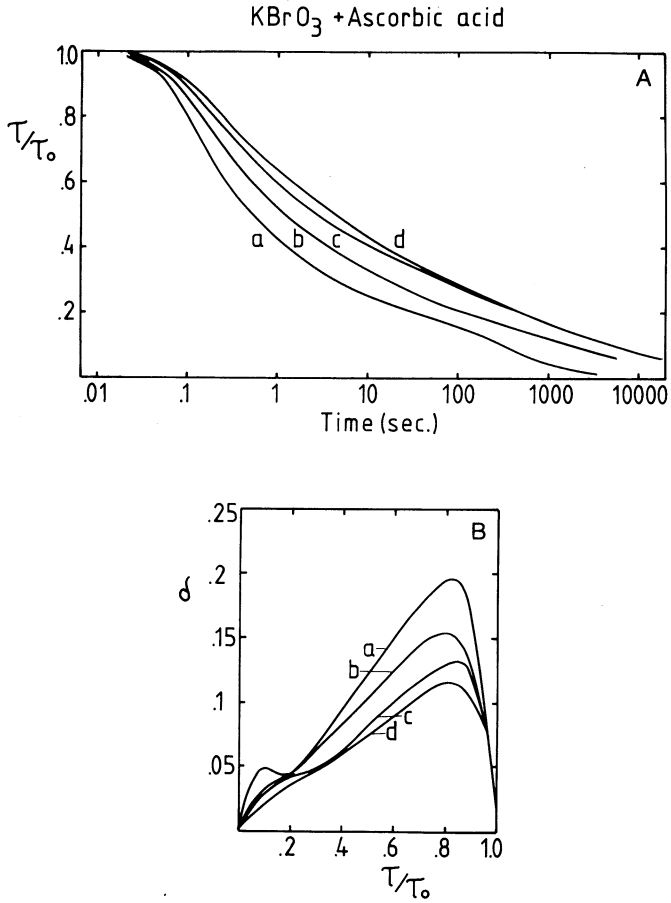

Fig. 5. Stress relaxation **(A)** and rate-stress curves **(B)** for ascorbic acid in the presence of constant amounts of $KBrO_3$ (8,500 ppm). a, 0 ppm; b, 1,500 ppm; c, 3,000 ppm; d, 5,000 ppm.

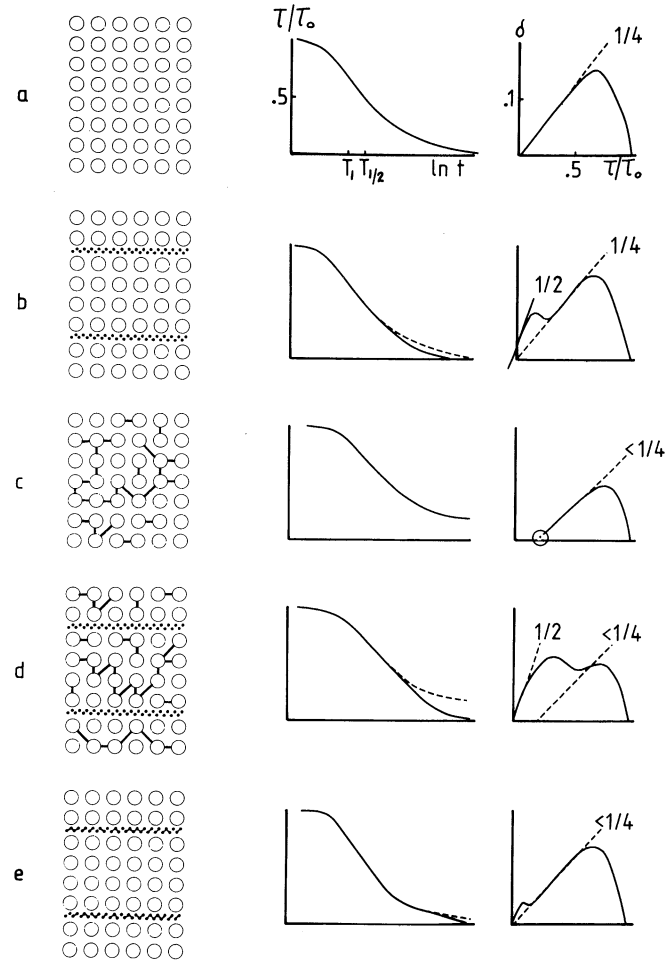

Fig. 6. Schematic structure of flow units. **a,** Cross-section of nonclose-packed fibrils; **b,** same as **a,** but with interface between homogeneous domains, creating a lamellar superstructure; **c,** same as **a,** but with crosslinks between the fibrils; **d,** same as **b,** but with crosslinks between the fibrils; **e,** same as **b,** but with crosslinks in the interface structure.

primary and the secondary flow in gluten implies that the lamellar structure is a superstructure in the fibrillar system. Figure 6B shows how the lamellar domains are bounded by interfaces. The theoretical relaxation plots are shown at the right. The lamellar peak is drawn to show the existence of a less strongly cooperative process, where the tangential line intersects the τ/τ_0 axis on the negative side.

Chemically modified gluten may give relaxation plots, where the primary process is of the type shown in Fig. 6C. In this case, the linear part extrapolates to a point on the positive τ/τ_0 axis, indicating an apparent residual stress $q\tau_0$ at long times. The general explanation of such behavior is that the system was in a state of nonequilibrium before the relaxation experiment. The q is a measure of the deviation from equilibrium in the nonstrained state relative to the initial deviation from equilibrium in the strain state. This may be explained by the structure depicted in Fig. 6C, which shows the fibrillar structure of the same type with crosslinks between the fibrils. These crosslinks symbolize partial flow arrest and may represent, eg, chemical crosslinking or aggregation. The expected effects on the relaxation experiment include an increased shear modulus, an increased relaxation time, an increased cooperative coordination number, and the appearance of nonequilibrium effects as respresented by the finite q-value. When crosslinks are introduced in the fibrillar structure, flow by rearrangements in the lamellar structure become increasingly important. This is shown in Fig. 6D. A quite opposite effect on the relaxation plot is obtained when crosslinks, which are effective in the interface regions, are introduced. In this case, the lamellar peak gradually disappears at increasing degree of crosslinking (Fig. 6E). The relaxation curve of a composite structure with no flow at interfaces differs, however, from the flow of the simple fibrillar structure because in this case the fibrillar structure is constrained by the elastic interfaces. The relaxation time and the coordination number increase slightly. If crosslinks are applied simultaneously to the fibrillar structure and the interface, the effects on the lamellar peak tend to cancel.

Gluten chemically modified by glutaraldehyde is of the type shown in Fig. 6D, ie, the fibrillar structure is crosslinked. For potassium bromate the behavior seen in Fig. 6E is obtained, ie, crosslinks preferably appear to arrest the flow in the lamellar structure. Ascorbic acid has an effect on gluten relaxation that is similar to that of glutaraldehyde. The mechanism of ascorbic acid is not clear to us, however, and further investigation should be made.

LITERATURE CITED

AMERICAN ASSOCIATION OF CEREAL CHEMISTS. 1976. Approved Methods of the AACC. The Association, St. Paul, MN.

BERNARDIN, J. E. 1975. The rheology of concentrated gliadin solutions. Cereal Chem. 52(3,II):136r.

BLOKSMA, A. H. 1971. Rheology and chemistry of dough. Page 523 in: Wheat Chemistry and Technology. Y. Pomeranz, ed. Am. Assoc. Cereal Chem., St. Paul, MN.

BOHLIN, L. 1980. A theory of flow as a cooperative phenomenon. J. Colloid Interface Sci. 74:423.

BOHLIN, L., CARLSON, T. L.-G., and BÄCKSTRÖM, G. 1980. Cone-plate instrument for stress relaxation measurements. J. Colloid Interface Sci. 73:61.

BOHLIN, L., and CARLSON, T. L.-G. 1981. Shear stress relaxation of wheat flour dough and gluten. Colloid and Surfaces 2:59.

EWART, J. A. D. 1968. Action of glutaraldehyde, nitrous acid, or chlorine on wheat proteins. J. Sci. Food Agric. 19:370.

FUNT BAR-DAVID, C. B., and LERCHENTHAL, Ch. H. 1975. Rheological and thermodynamic properties of gluten gel. Cereal Chem. 52:154r.

HIBBERD, G. E., and PARKER, N. S. 1975. Measurement on the fundamental rheological properties of wheat flour doughs. Cereal Chem. 52:lr.

HLYNKA, I. 1949. Effect of bisulfite, acetaldehyde, and similar reagents on the physical properties of dough and gluten. Cereal Chem. 20:307.

KUNINORI, T., and MATSUMOTO, H. 1963. L-Ascorbic acid oxidizing system in dough and dough improvement. Cereal Chem. 40:647.
MATSUMOTO, S. 1979. Rheological properties of synthetic flour dough. Page 291 in: Food Texture and Rheology. P. Sherman, ed. Academic Press, New York.

MITA, T., and MATSUMOTO, H. 1981. Flow properties of aqueous gluten and gluten methyl ester dispersions. Cereal Chem. 58:57.

NAGAO, S., ENDO, S., and TANAKA, K. 1981. Scanning electron microscopy studies of wheat flour fractions from doughs mixed with oxidants at high temperature. J. Sci. Food Agric. 32:235.

SIMMONDS, H., and ORTH, R. A. 1973. Structure and composition of cereal proteins as related to their potential industrial utilization. Page 51 in: Industrial Uses of Cereals. Y. Pomeranz, ed. Am. Assoc. Cereal Chem., St. Paul, MN.

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