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A STUDY OF THE RELATIONSHIP BETWEEN VISCOELASTIC PROPERTIES AND THE CHEMICAL NATURE OF WHEAT GLUTEN AND GLUTENIN[1]

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ABSTRACT

The effect of variations in the level of water, pH, salt, urea, lipids, soluble proteins, free amino groups, free carboxyl groups, and free sulfhydryl groups on the viscoelastic properties of crude gluten, purified gluten, and glutenin has been studied. Both stress-strain and stress-relaxation curves were obtained on the three materials with an Instron tensile-testing machine. Functions related to the stress-relaxation modulus and the distribution function of relaxation times were calculated from these curves, and the effects of the variables on them were determined with standard statistical methods. Among the conclusions reached in these studies are: the viscoelastic properties of crude gluten are primarily determined by the level of water present; complete acetylation of free amino groups destroys the cohesiveness of gluten; methylation of crude gluten decreases its strength relative to purified gluten and glutenin, possibly because of a modification of the starch in the crude gluten; the level of lipids had no effect on viscoelastic properties. Electron micrographs of stressed, chemically modified crude gluten support some of these conclusions.

A thorough knowledge of the viscoelastic properties of wheat gluten and their relationships to the chemical nature of gluten and flour is essential to the continued development of new food products and to improved utilization of surplus wheat production in the United States. Previous studies (1–8; 9, p. 19; 10; 11) have provided substantial information about the structure of dough, and have shown that gluten is the primary constituent governing the mechanical behavior of dough. The viscoelastic properties of gluten or of chemically modified gluten have received little attention. Although Hlynka (7) has emphasized the necessity of employing fundamental principles for studying doughs, most of the viscoelastic measurements have been con-

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ducted with devices more suited for commercial processing of dough or with experimental laboratory equipment. Fundamental knowledge about the relationships between the chemical nature of gluten and its viscoelastic properties could more readily be obtained if commercially available laboratory testing equipment were employed to measure viscoelastic properties.

The chemical and physical properties of wheat gluten can be determined by the level of many additives, by the level of certain naturally occurring compounds, and by the nature and amount of functional groups present in the gluten proteins. Changes in the levels of these may produce significant changes in the viscoelastic properties of the gluten. The moisture content will determine the amount of interstitial water between the protein platelets in the gluten mass. The imbibtional power depends upon the level of salts and acids present as well as the extent of cross-linking (9, pp. 143–144). The amount of salt has a marked effect on the level of protein dissociation and unfolding (12, p. 315). Urea may cause denaturation (12, p. 541). Lipids may act as a double layer between the gluten proteins; hence, an increase in the lipid level in gluten may cause the gluten layers to slip more easily, with the result that the gluten mass will be less elastic (13). Soluble proteins probably become intermingled in the gluten proteins during the preparation of gluten from dough (14) and should affect the viscoelastic properties of the gluten. The free amino groups present in lysine and arginine, and the carboxyl groups present in aspartic and glutamic acids, undoubtedly enter into hydrogen-bonding between the gluten proteins and between the gluten mass and the interstitial water. A continuous interchange mechanism is believed to take place in cysteine in which disulfide bonds open between proteins, and sulfhydryl groups combine to form new disulfide bonds (14).

Many techniques have been used for studying the viscoelastic properties of polymers. Ferry (15) has classified them as (a) transient, (b) dynamic, (c) high-stress, and (d) other time-dependent techniques. Choice of the technique for measuring viscoelastic properties is usually based upon the rigidity of the polymer and the range of time scale desired. Generally, transient techniques are used for studying the long end of the time scale. One transient technique, stress relaxation, has been widely used to study the behavior of doughs (2,3,5,6) with experimental laboratory equipment. These techniques do not require elaborate equipment. A time-dependent technique, the measurement of monotonic changes of stress with time, has been used (4,5) with commercial instruments, such as the extensimeter and the extensigraph.

Gluten and dough are often described by qualitative terms such as

"cohesiveness," "stickiness," "springiness," and "strength." If gluten is considered as a polymeric material with an equilibrium distribution of molecular configurations, it can be described as a material possessing both viscous and elastic, or viscoelastic, properties. In early studies of viscoelastic properties, dough was characterized in terms of a simple Maxwell model (1). However, Hlynka and co-workers (2,3) clearly showed that dough possesses complex viscoelastic properties that can only be represented by a generalized Maxwell model, an infinite number of Maxwell elements connected in parallel. Consequently, gluten cannot be described by a single relaxation time and stress-relaxation modulus, but by distribution functions of them. These functions are not simply related to viscous or elastic properties of gluten. However, the distribution function of relaxation times has proved useful for describing the fundamental viscoelastic properties of dough (3); both functions should be useful for describing gluten.

This study was designed to elucidate the effects of the levels of moisture, pH, salt, urea, lipids, soluble proteins, amino groups, carboxyl groups, and sulfhydryl groups on the viscoelastic properties of gluten and glutenin with a readily available commercial tensile-testing machine. Both stress-relaxation and stress-elongation curves were obtained. Functions related to the stress-relaxation modulus and the distribution function of relaxation times were calculated from these curves, and the effects of the variables on them were determined with standard statistical methods.

Functional Relationships and Curve Characterization

Stress-Strain to Stress-Relaxation Modulus. If a generalized Maxwell model receives a constant rate of strain at zero time, the stress-strain curve is defined (16) by the equation

$$\sigma(\epsilon) = R \int_{-\infty}^{\infty} \tau H(\tau) \, [1-\exp(-\epsilon/R\tau)] \, d\ln\tau \qquad (1)$$

where $\sigma(\epsilon)$ = stress at strain ϵ
τ = relaxation time
R = rate of strain
$H(\tau)$ = distribution function of relaxation times, defined such that $H(\tau)\, d\ln\tau$ is the contribution to the instantaneous tensile modulus by those elastic mechanisms whose relaxation times lie between $\ln\tau$ and $\ln\tau + d\ln\tau$.

If t is substituted for ϵ/R in equation 1, and the resulting equation is differentiated, then

$$\frac{d(\sigma[\epsilon]/R)}{dt} = \int_{-\infty}^{\infty} H(\tau)\exp(-t/\tau)d\ln\tau \equiv E(t) \quad (2)$$

where E(t) is the stress relaxation modulus. For computational purposes, equation 2 can be written as

$$E(t) = \left(\frac{\sigma[\epsilon]/R}{t}\right)\left(\frac{d\ln[\sigma(\epsilon)/R]}{d\ln t}\right). \quad (3)$$

Thus, the stress relaxation modulus, E(t), for time t can be obtained from a plot of $\ln(\sigma[\epsilon]/R)$ *vs.* ln t. Stress-relaxation moduli have been calculated for polyisobutylene (17) from stress-strain curves obtained with a tensile-testing machine.

Stress-Relaxation Moduli to $H(\tau)$. Once E(t) has been determined either directly from stress-relaxation studies or indirectly from stress-strain data, the Alfrey approximation (16,18,19) may be used for estimating $H(\tau)$. The appropriate equation is

$$H_1(\tau) = \left[\frac{dE(t)}{d\ln t}\right]_{t=\tau} = -E(t)\left[\frac{d\ln E(t)}{d\ln t}\right]_{t=\tau} \quad (4)$$

where $H_1(\tau) \cong H(\tau)$.

To use this approximation, experimental stress-relaxation curves are normally replotted as $\sigma(\epsilon)/\epsilon$ or $\ln\sigma(\epsilon)/\epsilon$ versus ln t; in the latter case, the negative of the slope of this curve at $t = \tau$ approximates $H_1(\tau)$. Hlynka and co-workers (2,3) successfully used the Alfrey approximation to describe the viscoelastic behavior of dough after various treatments.

Two differentiations of the stress-strain curve but only one of the stress-relaxation curves are required to evaluate $H_1(\tau)$; hence, the stress-relaxation curve is preferable to the stress-strain curve. However, a finite time is required for application of the strain in stress-relaxation studies, so that relaxation times shorter than the extension time cannot be measured. Furthermore, measurements at short extension times may be adversely affected by vibrations caused by sudden stops. Finally, precise measurement of the cessation of extension may be difficult. As a result, it is not possible to predict which method will give more reliable values of $H_1(\tau)$.

Total Modulus of Elasticity and Total Tensile Viscosity. Because $H(\tau)$ is defined so that $H(\tau)d\ln\tau$ is the contribution to the instantaneous modulus by those elastic mechanisms whose relaxation times lie between $\ln\tau$ and $\ln\tau + d\ln\tau$, the integral of $H(\tau)d\ln\tau$ over all relaxation times will give the total modulus of elasticity E. Also, because τ is the ratio of viscous to elastic components, the integral of $\tau H(\tau)d\ln\tau$

over all relaxation times will give the total "tensile" viscosity η. The corresponding equations are

$$E_1 = \int_{-\infty}^{\infty} H_1(\tau) d\ln\tau = \int_{0}^{\infty} \frac{H_1\tau}{\tau} d\tau \tag{5}$$

and

$$\eta_1 = \int_{-\infty}^{\infty} \tau H_1(\tau) d\ln\tau = \int_{0}^{\infty} H_1(\tau) d\tau. \tag{6}$$

Finite limits will usually be required to prevent the integral from diverging. Thus, E_1 and η_1 can only be interpreted as the apparent modulus and viscosity. If short relaxation times have not been studied, E will be underestimated; similarly if long relaxation times have not been studied, η will be underestimated.

Characterization of Experimental Curves. In this study, the functional forms of the experimental curves were approximated by fitting least-squares orthogonal polynomials to six values obtained from experimental stress-elongation and stress-relaxation curves. These approximations were of the form

$$\ln S_S = a_0 + a_1(A_1[\ln t]) + a_2 (A_2[\ln t]) + \ldots \tag{7}$$

for the stress-strain data, and

$$\ln S_R = \beta_0 + \beta_1 (B_1[\ln t]) + \beta_2 (B_2[\ln t]) + \ldots \tag{8}$$

for the stress-relaxation data, where S is the measured stress, and $A_i(\ln t)$ and $B_i(\ln t)$ are i^{th} degree polynomials in $\ln t$ which are uniquely determined by the choice of time points, a_i and β_i and their respective coefficients.

In the stress-strain studies, measured stress was used for computations instead of true stress and time was used instead of true strain, and in the stress-relaxation studies measured stress was also used for computations instead of true stress. Measured stress *vs.* time gave straight-line log-log plots, while true stress, calculated on actual cross-section with a value of 0.5 for Poisson's ratio, gave log-log plots that were concave upward. Conversion of elongation to time rather than to true strain simplified the computations without affecting the validity of the conclusions.

A statistical analysis was performed on each set of coefficients to determine if and how they were influenced by the experimental variables. In the range of times studied, the log-log plots were nearly linear, so that the four constants a_0, a_1, β_0, β_1 contain all of the information about the curves. Therefore, discussion is limited to these constants.

Examination of equations 7 and 8 shows that large values of α_0 and β_0 correspond to large stress-to-strain ratios for the average time of measurement. Also as α_1 approaches 1 and β_1 approaches 0, the relaxation time increases for the average time of measurement. $H_1(\tau)$, E(t), and η were not calculated, because conclusions can be obtained simply by examining the four constants.

Materials and Methods

Preparation of Materials. All of the gluten and glutenin used in these studies was prepared from an unbleached, straight-grade flour obtained from a certified source of Triumph variety wheat. This flour contained an average of 13.55% moisture as measured by AACC Method 44-15 (21), 11.49% protein (AACC Method 46-10) (21), and 0.368% ash.

The crude gluten was prepared (20) by mixing a dough containing 5 parts by weight of flour and 3 parts by weight of 0.1% aqueous sodium chloride solution, allowing the dough to develop, washing the dough three times with 58 parts of 0.1% aqueous sodium chloride solution, preparing a suspension of gluten in water containing 8% by weight of protein with a Waring Blendor, and freeze-drying the suspension in a Repp Model 42 freeze-dryer. The gluten suspension was not heated above 150°F. during the initial stages of freeze-drying and not above 130°F. during the final stages. The crude gluten was distinctly whiter than all sources of commercial vital gluten examined. The improved color is attributed to the relatively low temperature to which the crude gluten was subjected during the final stages of freeze-drying.

The purified gluten was prepared in the same manner as the crude gluten, except that a suspension containing 5% by weight of protein in 0.01*N* acetic acid was freeze-dried after clarification at 20,000 *g* in a Sharples Super centrifuge. Part of the purified gluten was heat-treated after clarification to destroy enzymatic activity: the acetic-acid dispersion of the crude gluten was heated rapidly to boiling and cooled rapidly to room temperature. The purified gluten was whiter than the freeze-dried crude gluten. It could not be reconstituted as readily as the latter because it was not readily wetted with water.

Glutenin was prepared by a precipitation method (20) and by an extraction method (9, pp. 143–144). In the precipitation method, freeze-dried purified gluten was mixed with 0.01*N* acetic acid to form a dispersion containing 0.25% protein, wet glutenin was precipitated by adding 0.1*N* sodium hydroxide dropwise with stirring until the pH of the slurry was 5.80, the wet glutenin was washed twice with

water and was mixed in a Waring Blendor with water to form a dispersion containing 2% by weight protein, and the dispersion was freeze-dried as described previously. Part of this glutenin was prepared from heat-treated freeze-dried purified gluten. Both the unheated and the heat-treated gluten were difficult to reconstitute. In the extraction method, freeze-dried crude gluten was extracted in a Waring Blendor with 70% ethyl alcohol at a gluten concentration of 10 g. per 100 ml., and the white gelatinous glutenin residue was vacuum-dried at 35°C. to remove as much water as possible. This glutenin is identified as "classic" glutenin in this paper.

The chemical properties of the glutens and glutenins are summarized in Table I. Protein was determined by AACC Method 46-10 (21); moisture was determined by AACC Method 44-15 (21), except that the sample was dried for 3 hr. Lipids and lipid-free crude gluten were prepared from wheat flour by a modification of the method of Mecham and Mohammad (22). Lipids were obtained by extracting reconstituted

TABLE I
CHEMICAL PROPERTIES OF GLUTENS AND GLUTENINS

MATERIAL	PROTEIN	MOISTURE	LIPIDS	SOLUBLE PROTEINS
	%, dry basis	*%*	*%*	*%*
Crude gluten	74.4	1.00	5.35	3
Purified gluten	90.0	2.87	0	0
Heat-treated purified gluten	89.3	3.40	..	..
Glutenin	88.5	3.08	0	0
Heat-treated glutenin	87.9	2.60	..	..
Classic glutenin	...	10.5	..	..

freeze-dried crude gluten in a Waring Blendor with n-butyl alcohol which had previously been saturated with dionized water. The alcohol-water extract was evaporated in a Buchler flash evaporator at 35°C., additional water was added, and evaporation was continued until the odor of n-butyl alcohol in the solvent had disappeared. This aqueous solution was freeze-dried to yield an oily, yellow liquid. Lipid-free gluten was prepared by extracting the gluten or glutenin twice in a Waring Blendor with n-butyl alcohol which had previously been saturated with deionized water. The extracted wet material was dispersed in deionized water in the blender, the dispersion was evaporated in the flash evaporator as before to remove n-butyl alcohol, and the aqueous dispersion was freeze-dried.

Soluble proteins were obtained by washing flour according to the procedure used for preparing crude gluten, except that the wash liquid

was permitted to stand overnight at 40°F. to allow starch to settle. The liquid was clarified by centrifugation at 20,000 *g* and dialyzed for 96 hr. in cellophane tubes against deionized water. The tan, chloride-free liquid in the dialysis tubes was freeze-dried to yield the soluble protein.

Glutens and glutenin containing acetylated amino groups were prepared by treatment with acetic anhydride (12, p. 547). Freeze-dried gluten or glutenin was dispersed in saturated sodium acetate solution at 2°C., and the required amount of reagent-grade acetic anhydride was added in 2.5-ml. increments at 10-min. intervals. This dispersion was dialyzed in cellophane tubes at 20°C. against deionized water for 72 hr., after which the solution in the tubes was freeze-dried. Properties of the acetylated glutens and glutenins are shown in Table II. Assay of the free amino groups was carried out by AACC Method 46-31 (21).

TABLE II

EXPERIMENTAL CONDITIONS FOR PREPARATION OF CHEMICALLY MODIFIED CRUDE GLUTEN, PURIFIED GLUTEN, AND GLUTENIN

MATERIAL	MODIFICATION	AMOUNT	VARIABLE (UNITS)	AMOUNT	MODIFICATION ACHIEVED
		g.			%
Crude gluten	Acetylation of amino groups	50	Acetic acid (ml.)	17	44
Crude gluten		50		28	100
Purified gluten		30		12	100
Glutenin		30		12	100
Crude gluten	Methylation of carboxyl groups	50	Time of reaction (hr.)	1.5	37
Crude gluten		50		7	56
Purified gluten		30		1.5	24
Glutenin		30		1.5	32
Crude gluten	Blocking of -SH groups	50	NEMI solution (ml.)	19	52
Crude gluten		50		28	100
Purified gluten		30		10	52
Purified gluten		30		10	52

Glutens and glutenin containing methylated free carboxyl groups were prepared by a modification of the method of Fraenkel-Conrat and Olcott (23). Freeze-dried gluten or glutenin was treated with 500 ml. of anhydrous reagent-grade methanol and 5 ml. of concentrated, reagent-grade hydrochloric acid in a stoppered flask for a specified number of hours. The solution was poured into 500 ml. of deionized water and dialyzed in cellophane tubes for 24 hr. at 20°C., after which the solution in the tubes was freeze-dried. Properties of the esterified

glutens and glutenin are shown in Table II. Assay of the methyl groups was carried out by AOAC Method 38.029-9 (24).

Glutens and glutenin containing sulfhydryl groups blocked with N-ethylmaleimide were prepared by a modification of the method of Mecham *et al.* (25). Freeze-dried crude gluten, purified gluten, or glutenin was dispersed in air-free 0.01*N* acetic acid solution at 2°C., 0.001*N* N-ethylmaleimide (Mann Chemical Co.) was added over a 1-hr. interval while the dispersion was maintained between 2° and 6°C., and the dispersion was dialyzed for 48 hr. in cellophane tubes against air-free deionized water. The solution in the dialysis tubes was freeze-dried by the method described previously, except that the drum cage of the freeze-dryer was filled with nitrogen just prior to start-up. Properties of the sulfhydryl-blocked glutens and glutenin are presented in Table II. An amperometric method (26) was used to determine the free sulfhydryl groups in the modified glutens and glutenin.

Preparation of Test Specimens. Freeze-dried gluten and glutenin and derivatives were reconstituted by adding 20 or 30 g. of gluten or glutenin and the required amounts of salt, urea, distilled water, and hydrochloric acid or sodium hydroxide solution, in that order, to a 2-mil polyethylene bag about 5 in. wide and 5 in. long. The bag was heat-sealed, and the contents were hand-kneaded until visual inspection showed that the reconstituted gluten was homogeneous and free from bubbles.

To prepare the test specimens, or coupons, from these materials, the bags were pressed under a heavy load for 48 hr. at 40°F. between aluminum plates separated by 0.635-cm. spacers. These slabs were quick-frozen at 0°F. under compression in a commercial home freezer. Coupons with nominal dimensions of 5 by 1 by 0.635 cm. were cut from these slabs with a band saw equipped with a serrated-edge blade. This edge resembled the cutting edge on a household bread knife. These coupons were stored in sealed polyethylene bags at 0°F. until they were tested. Immediately prior to their use in the testing program, individual coupons were rapidly brought to 23°C. with an infrared lamp.

Operation of Instron. In the stress-strain studies on crude gluten and chemically modified crude gluten and in some studies on purified gluten and glutenin, the coupons were mounted and drawn between two clamps constructed from the cork stoppers shown in Fig. 1. Fine sandpaper was cemented to the faces of the cork clamp in contact with the coupon. Some of the coupons of purified gluten and glutenin swelled when they were thawed; apparently, the 48-hr. relaxation period at 40°F. was not sufficient for these materials. These coupons

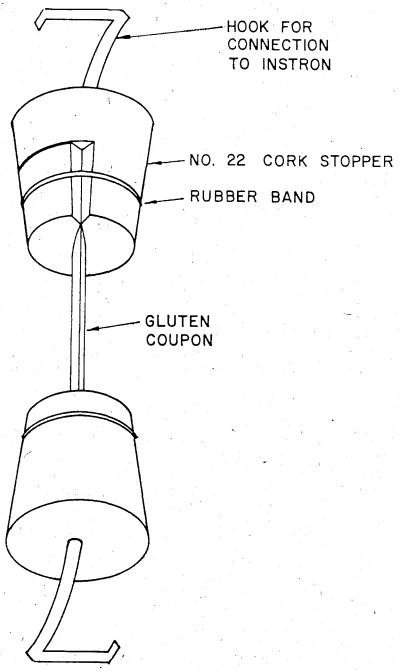

Fig. 1. Cork clamps for gluten testing.

were mounted and drawn between standard Instron clamps. Width and thickness of all coupons were measured immediately before the tests. Testing conditions were: testing machine, Instron Model TT-C; load cell, B; range, 100 g.; cell deflection factor, 0.0007 mil/g.; sensitivity, normal; cross-head travel, 2 cm./min.; chart drive, 10 cm./min.; gage length, 3 cm.; T = 23°C. All coupons were pulled to 110% extension. A few coupons appeared to slip from the grips; all such tests were repeated. A typical stress-elongation curve is shown in Fig. 2. Stresses for the program were read from the stress-elongation curves at 20, 40, 50, 60, 80, and 100% elongation.

In the stress-relaxation studies the coupons were also mounted and drawn between the cork clamps previously described and shown in Fig. 1. Experiments were conducted with the apparatus shown in Fig. 3. When the cord was cut, the 100-g. brass weight fell to the Instron cross-head in less than 0.5 sec. and produced a 300% elongation of the coupon. Testing conditions were: testing machine, Instron Model TT-C; load cell, B; range, 100 g.; cell deflection faction, 0.0007 mil/g.; sensitivity, normal; chart drive, 50 cm./min.; gage length, 3 cm.; T = 23°C. A typical stress-relaxation curve is shown in Fig. 4.

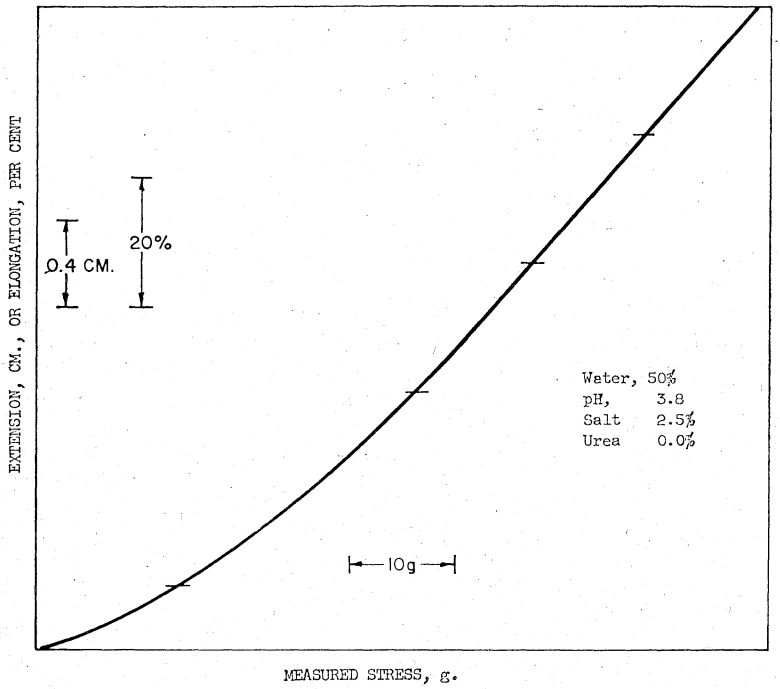

Fig. 2. Typical stress-strain elongation curve. Test 83 on crude gluten.

Stresses were read from the stress-relaxation curves at 1, 2, 3, 4, 5, and 6 sec. for use in the program.

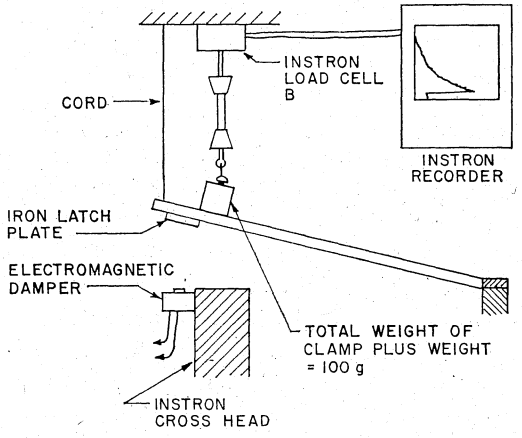

Fig. 3. Stress-relaxation apparatus.

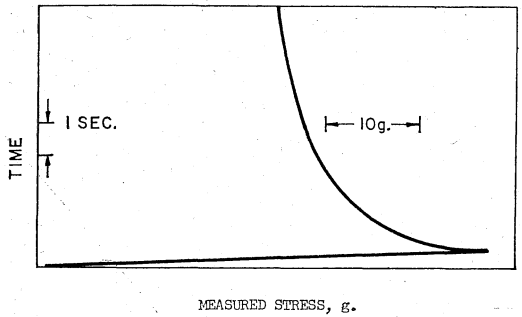

Fig. 4. Typical stress-relaxation curve. Test 83 on crude gluten (see Fig. 2).

Electron Microscopy. Stretched gluten coupons were replicated for electron microscope examination by methods developed for wheat proteins (27). This type of surface examination reveals the sheetlike structure of gluten and the response of these sheets to the applied tensile stress.

Experimental Results

Program A. Three programs were carried out in both the stress-strain and stress-relaxation studies. In program A, a 2×4^3 factorial experiment was conducted on crude gluten at four levels of water, pH, and salt, and two levels of urea. The variables studied and their levels were:

Water		*Salt*	*Urea*
%	*pH*	%	%
45.0	3.8	0.0	0.0
50.0	6.0	1.0	2.5
52.5	6.7	2.5	..
55.0	7.8	5.0	..

The four parameters, α_0, α_1, β_0, β_1, for each test in program A were calculated, a third-order orthogonal polynomial surface was fitted to each set of constants, and the residual error for 98 degrees of freedom was used to test the significance of the observed effects on the experimental variables. In addition to the individual analyses of the parameters, two multivariate joint analyses were performed, the first with α_0 and β_0, and the second with all four parameters.

The standard deviations of α_0 and β_0 for the third-order model were 1.35 and 1.29, or 35 and 29%, respectively, for duplicate tests. The 95% confidence limits of α_0, α_1, β_0, and β_1 were 0.23, 0.13, 0.22, and 0.031, respectively, for a single run. These results indicate that

reasonable precision in the measurements was obtained. Nevertheless, few of the regression coefficients were significant, although the four variables were selected because of their suspected importance in determining the viscoelastic properties of gluten. The values of F and χ^2 for salt, pH, and water, especially those for a_0 for pH-water, showed large excursions. A fifth-order model and a complex hypothesis would be required to explain these excursions if they are real.

Table III lists the regression coefficients that were significant in one

TABLE III

SIGNIFICANT TERMS IN REGRESSION COEFFICIENTS

TERM [a]	COEFFICIENT [b]					a_0,β_0 a_1,β_1	EFFECT ORDER
	a_0	a_1	β_0	$-\beta_1$	a_0,β_0		
S_C	..	..	b	..	..	b	3
W_L	d	..	d	..	d	d	1
S_LW_Q	a	..	c	..	b	a	3
W_QP_L	..	..	b	..	..	..	3
S_LP_Q	b	a	..	..	a	a	3
W_LP_Q	c	..	..	..	b	b	3
$S_LP_LU_L$	..	..	b	..	..	..	3

[a] S, salt; W, water; U, urea; P, pH; L, linear term; Q, quadratic term; C, cubic term.
[b] a, Approaching significance; b, significant; c, highly significant; d, very highly significant.

or more tests. The constant β_1 was not significantly affected by any variable, while a_1 was significantly affected by only one variable. All the significant coefficients showed third-order effects except the coefficient for the linear effect of water. The absence of other significant first- or second-order coefficients suggests that the third-order effects may have been caused by variables not studied. Because a_0 and β_0 were highly significantly affected by water while a_1 and β_1 were not, either the latter coefficients are not influenced by the experimental variables or they show the effect outside the time intervals studied.

Program B. Program B was conducted on crude gluten, purified gluten, and glutenin at four levels of water and two levels of salt and pH, and on heat-treated and untreated purified gluten and glutenin. The variables studied and their levels were:

Water	*pH*	*Salt*
%		%
45.0	6.7	0.0
50.0	7.8	2.5
52.5		
55.0		

The four parameters a_0, a_1, β_0, $-\beta_1$ for each test are shown in Table IV. Imbalance was introduced into the program because one

TABLE IV
CURVE PARAMETERS FOR PROGRAM B

	WATER	pH	SALT	α_0	α_1	β_0	$-\beta_1$
	%		%				
Crude gluten	45	6.7	0	1.43	0.67	1.95	0.146
	45	6.7	2.5	1.59	0.58	2.16	0.179
	50	6.7	0	1.27	0.63	1.68	0.161
	50	6.7	2.5	1.37	0.58	1.75	0.170
	45	7.8	0	1.49	0.58	1.82	0.166
	45	7.8	2.5	1.37	0.70	1.88	0.182
	50	7.8	0	1.33	0.64	1.81	0.205
	50	7.8	2.5	1.27	0.59	1.75	0.195
Purified gluten	45	6.7	0	1.50	0.71	1.90	0.187
	45	6.7	2.5[a]	1.53	0.80	2.12	0.292
	50	6.7	0[a]	1.06	0.95	1.77	0.274
	50	6.7	2.5	1.29	0.89	1.94	0.213
	45	7.8	0[a]	1.47	0.71	2.14	0.350
	45	7.8	2.5	1.74	0.67	2.27	0.257
	50	7.8	0	1.05	0.71	1.73	0.149
	50	7.8	2.5[a]	1.16	0.98	1.93	0.217
Glutenin	45	6.7	0[a]	[b]	[b]	[b]	[b]
	45	6.7	2.5	2.22	0.90	[c]	[c]
	50	6.7	0	1.74	0.82	2.52	0.124
	50	6.7	2.5[a]	2.15	0.79	2.63	0.119
	45	7.8	0	2.34	0.86	2.65	0.140
	45	7.8	2.5[a]	2.26	0.93	[c]	[c]
	50	7.8	0[a]	2.22	0.88	2.55	0.138
	50	7.8	2.5	1.99	0.81	2.57	0.137

[a] Heat-treated.
[b] Insufficient sample.
[c] Broke in tension

sample of glutenin was insufficient for testing and two others broke in the stress-relaxation test. This imbalance and the small size of the experiment limited the analysis of the data. Four observations are:

1. Values of α_0 and β_0 for glutenin were significantly higher than those for purified gluten, whereas α_0 and β_0 for purified gluten are not significantly different from those for crude gluten.

2. Fundamental differences were observed for both α_1 and β_1, in contrast to the results obtained in program A. Significantly larger values of α_1 and β_1 were obtained for glutenin than for crude gluten. The values of β_1 for purified gluten were significantly higher than those for crude gluten, while the values of α_1 for purified gluten and crude gluten were not significantly different.

3. Variations in the water level produced similar changes in all four parameters for all three types of gluten and glutenin.

4. Heat-treatment of the purified gluten and glutenin caused no significant changes for all four parameters.

Program C. Program C was conducted on crude gluten which had been chemically modified at three levels, and on purified gluten and

glutenin modified at two levels, both including the base level, by acetylation of amino groups, methylation of carboxyl groups, and blocking of sulfhydryl groups with N-ethylmaleimide. Levels of chemical modification are shown in Table II. The program also included measurements on crude gluten containing three levels of lipids (0, 5.35, and 18.0%) and soluble proteins (3.0, 6.7, and 17.9%), and on purified gluten and glutenin containing two levels of lipids (0 and 15.0%) and soluble proteins (0 and 17%). In all of the lipid and soluble protein experiments, one of the levels was the base level.

The four parameters a_o, a_1, β_o, and $-\beta_1$ for each test are shown in Table V. Again, breakage of coupons or failure of samples to recon-

TABLE V

CURVE PARAMETERS FOR PROGRAM C

		None[a]	Acetylation	Methylation	Blocking	Total Lipids	Total Soluble Proteins
Crude gluten	%		44	36	52	0	6.7
	a_o	1.32	1.85	[b]	1.58	1.94	[b]
	a_1	0.54	0.86	[b]	0.96	0.95	[b]
	β_o	1.83	2.51	[b]	1.89	2.40	1.68
	$-\beta_1$	0.153	0.144	[b]	0.158	0.158	0.178
	%		100	54	100	18.3	18.0
	a_o		[c]	[b]	1.67	1.97	1.38
	a_1		[c]	[b]	0.81	0.87	0.60
	β_o		[c]	[b]	2.01	2.33	1.78
	$-\beta_1$		[c]	[b]	0.160	0.148	0.228
Purified gluten	%		100	24	52	15.0	17.0
	a_o	1.36	[c]	1.87	[c]	1.63	[b]
	a_1	0.62	[c]	0.83	[c]	0.69	[b]
	β_o	1.71	[c]	2.30	[c]	1.98	1.61
	$-\beta_1$	0.155	[c]	0.131	[c]	0.207	0.221
Glutenin	%		100	32	52	15.0	17.0
	a_o	2.26	[c]	2.24	2.21	1.99	[d]
	a_1	0.64	[c]	0.92	0.91	0.90	[d]
	β_o	2.71	[c]	2.67	2.50	2.62	1.97
	$-\beta_1$	0.128	[c]	0.093	0.270	0.121	0.169

[a] Crude gluten contained 5.35% lipids and 3% soluble proteins; purified gluten and glutenin contained none.
[b] Broke in tension.
[c] Did not reconstitute.
[d] Sample insufficient for testing.

stitute limited the analysis of the data. Five observations are:

1. As in program B, glutenin exhibited the highest a_o and β_o, while a_o and β_o for crude and purified gluten were not significantly different.

2. Complete acetylation destroyed the ability of gluten and glutenin to reconstitute, although crude gluten acetylated at the 44% level did reconstitute.

3. Two coupons of methylated crude gluten broke during the stress-

strain tests; two broke during the stress-relaxation tests.

4. When purified gluten was sulfhydryl-blocked at the 52% level, it did not reconstitute, although crude gluten and glutenin, blocked at the same and higher levels, did reconstitute.

5. The effect of adding soluble proteins and adding or removing lipids was not consistent.

Classic Glutenin. In contrast to the studies on purified glutenin, the classic glutenin could not be reconstituted to produce a cohesive sample with the experimental techniques used in this study. Evidently the two different methods employed for preparing the glutenins produced materials of different composition. Very probably the purified glutenin also contained enough gliadin to bind the glutenin proteins in a cohesive structure.

Electron Microscopy. Electron micrographs of the surfaces of un-

Fig. 5. Shadowed carbon replica of the surface of stretched unmodified crude gluten.

modified and modified crude gluten are shown in Figs. 5 to 8. All samples contained 50% water and no other components. The coupons of crude, methylated, and acetylated crude gluten were quick-frozen at 100% elongation prior to preparation of the specimens, whereas the coupon of sulfhydryl-blocked crude gluten was quick-frozen at only 10% elongation because other coupons of this sample broke at greater elongations.

Figures 5 and 6 show the surfaces of crude and methylated gluten, respectively. Both glutens exhibit the usual sheet and filimentary structure; the methylated gluten has ruptured more extensively, however, indicating that the elementary bond strength has been lowered. Figure 7 depicts the surface of acetylated gluten. In this sample, the normal sheets of stretched filaments are not seen. The numerous black

Fig. 6. Shadowed carbon replica of the surface of stretched 36% methylated crude gluten.

Fig. 7. Shadowed carbon replica of the surface of stretched 44% acetylated crude gluten.

splotches are relaxed filaments that have already broken. Such behavior indicates a serious disruption of normal bonding. Figure 8 shows the surface of sulfhydryl-blocked gluten and illustrates a structure which is difficult to interpret. It is totally unlike the other three gluten surfaces. Inasmuch as this sample could not sustain even small loads without rupturing, the formation of stretched sheets, as in Fig. 5, was not possible. The cellular arrangement observed must result from relaxation of ruptured surface layers.

The Test Program. The Instron tensile-testing machine appears to be useful for determining the viscoelastic properties of gluten. However, several precautions should be taken so as to decrease the experimental error. A uniform thawing procedure and testing temperature must be established. In addition, a better method for reconstituting

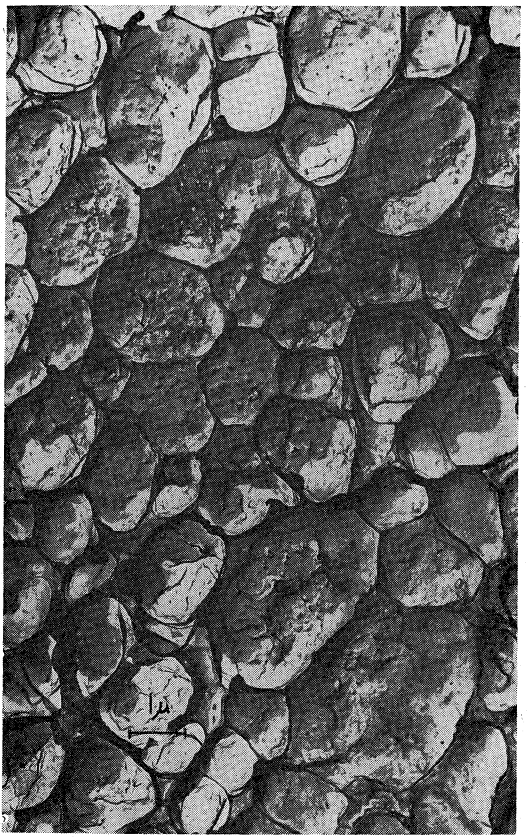

Fig. 8. Shadowed carbon replica of the surface of stretched 52% sulfhydryl-blocked crude gluten.

the gluten and glutenin is needed. Although the samples were kneaded until they appeared homogeneous, mixing of some samples may have been incomplete so that the subsequent measurements largely reflected the properties of the weakest portion of the coupon. The coupon dimensions and the rate of extension were selected arbitrarily; a larger cross-section probably would have increased the precision. If the error associated with the method could be reduced, then the distribution function of relaxation times could be calculated. However, the stress-strain curve should also be measured over a longer time. Furthermore, Hlynka (3) showed that a considerable portion of the total elastic modulus is present at times smaller than 1 sec. Unfortunately, the two methods used here are not capable of resolving the curves at such small times.

Conclusions

1. The viscoelastic properties of crude gluten in the time interval of 1 to 96 sec. are primarily determined by the amount of water present. As the water content increases, the stress-strain modulus decreases, while rate of stress decay does not change. The viscoelastic properties of crude gluten were not significantly changed by salt, pH, and urea at the levels studied.

2. Glutenin is the important viscoelastic component of gluten, as it has a higher elastic modulus and a slower rate of stress decay than crude gluten. However, the ratio of water to protein is lower for glutenin than for crude gluten (40 *vs.* 50%), so some increase in the modulus should be expected. Extrapolation of the water curve for crude gluten indicates that a_o and β_o should be increased by approximately 0.5 $\log_{10}$ units by the effect of water alone.

3. The viscoelastic properties of purified gluten are more similar to those of crude gluten than to those of glutenin.

4. Purified glutenin and classic glutenin have fundamentally different viscoelastic properties.

5. Complete acetylation of free amino groups destroys the cohesiveness of gluten. Because acetylation should greatly reduce the ability of the gluten proteins to enter into hydrogen bonding, the latter must play a significant role in the reconstitution of gluten and in determining its cohesive properties.

6. Methylation of free carboxyl groups apparently greatly reduces the strength of crude gluten relative to purified gluten and glutenin. However, crude gluten contains much more starch than either purified gluten or glutenin. Consequently, this effect could have been produced by modification of the starch during the methylation step. Because crude gluten and purified gluten exhibited similar viscoelastic properties, starch probably does not affect these properties of the former. Nevertheless, the methylation reaction could have produced moieties in the starch which would adversely affect viscoelastic properties.

7. Blocking of free sulfhydryl groups may have modified a basic property of purified gluten, as evidenced by the fact that this sample did not reconstitute. However, similar samples of crude gluten and glutenin did reconstitute, and the parameters for the modified materials were not significantly different from the unmodified material. This anomaly cannot be explained at present.

8. The level of lipids and soluble proteins showed no consistent effect on viscoelastic properties of all three materials.

9. Heat-treatment of the purified gluten had no effect on viscoelastic properties.

10. Either of the two techniques for measuring viscoelastic properties is useful for a fundamental study of macro time phenomena. However, the viscoelastic properties of gluten very probably are related to the entire time spectrum, so that additional studies with dynamic methods are probably required.

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