# Flow Properties of Aqueous Gluten and Gluten Methyl Ester Dispersions

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## ABSTRACT

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Flow properties of dispersions of aqueous gluten and gluten methyl ester were investigated. Gluten dispersions exhibited Newtonian flow behavior at concentrations below 4%, but non-Newtonian flow behavior became more pronounced with increasing concentration above 6%. At concentrations above 14%, a hysteresis loop was observed. Gluten methyl ester, in which side-chain amide groups of gluten were partly converted to ester groups, was prepared by a treatment with methanol containing hydrogen chloride.

The flow properties of gluten methyl ester dispersion differed considerably from those of gluten dispersion. At concentrations above 4%, the apparent viscosity of gluten methyl ester dispersion became significantly lower than that of gluten, although the apparent viscosities of gluten and of gluten methyl ester were almost equal at concentrations below 4%. Hydrogen bonds resulting from glutamine side-chains seem to be an important factor in determining the flow properties of gluten.

The rheological properties of wheat flour dough, one of the principal intermediate stages in the transformation of wheat through flour to bread, are primarily due to the properties of gluten. However, few studies have been conducted (Bernardin 1975, Funt Bar-David and Lerchenthal 1975, Matsuo 1978, Rinde et al 1970) on the rheological properties of gluten, although many have been conducted on those of dough (Bloksma 1971).

Nearly one quarter of the total nitrogen of gluten is amide nitrogen, and one third of the amino acids in gluten is glutamine. Because of the large amount of glutamine, hydrogen bond interactions between glutamine side-chains play an important role in promoting association between gluten molecules (Krull et al 1965). Beckwith et al (1963) have investigated the changes by converting the glutamine residues of gluten into $\gamma$-methyl glutamate esters and have shown that the products considerably increased solubility in water. Therefore the hydrogen bonds resulting from glutamine side-chains are an important factor determining the rheological properties of gluten.

This article reports investigation of the contributions of glutamine side-chain on the rheological properties of gluten through measurements of flow properties of gluten methyl ester.

## MATERIALS AND METHODS

### Wheat Flour
Wheat flour used for separating gluten was a "milling" brand of unbleached baking flour provided by Nissin Flour Mill Co., Japan.

### Preparation of Gluten
Gluten was prepared from defatted wheat flour by the method of Jones et al (1959). Starch and other nongluten materials were removed from wheat flour, previously defatted with n-butanol, by handkneading in 0.1% sodium chloride. The crude gluten thus separated was then dispersed in 0.01 $M$ acetic acid and centrifuged at 22,000 × $g$ for 1 hr. The supernatant was heated to 90° C for 10 min to inactivate the proteinase and then lyophilized.

### Preparation of Gluten Methyl Ester
Gluten methyl ester was prepared from the gluten by the procedure of Beckwith et al (1963). A suspension of gluten (5%) in anhydrous methanol containing 0.6 $N$ hydrogen chloride was incubated for 48 hr at 30° C. The protein suspension was agitated vigorously at first to prevent coagulating. The reaction was stopped by dialysis against large volumes of water. The nondialyzable product was lyophilized.

### Chemical Analyses
Amide nitrogen content was determined by incubating the proteins with 2$N$ hydrogen chloride for 1–8 hr at 100° C, distilling the free ammonia from alkaline solution, titrating the ammonia

with standard acid as in the Kjeldahl procedure, and then extrapolating the amount of liberated ammonia to zero time by the procedure of Chibnall et al (1958). Ester content was determined by Zeisel's method (Arima 1934).

### Flow Properties of Protein Dispersions
Protein dispersions containing 2–16% by weight of dry matter were prepared in a Homomixer (Tokushu Kikakogyo Co.). Solutions were adjusted with 0.1 $M$ acetic acid to pH 4.5. Dispersed air bubbles were removed by centrifugation at 2,000 × $g$ for 10 min. Protein dispersions thus prepared were then stored for one day at 5° C. The shear stress-shear rate characteristics of the protein dispersion were examined at 25° C with a Rheometer RM-1 (Shimazu Seisakusho Co.) using coaxial cylinder attachments. This instrument is generally similar to a Weissenberg's Rheogoniometer, in which rotational and vibrational stresses are combined. In a flow test, the outer cylinder rotates and the torsion (ie, tangential stress) imparted to the inner cylinder is measured. The shear rate range covered was 2.3–1,076 sec$^{-1}$.

After each sample had been introduced into the viscometer, it was allowed to rest for 30 min before beginning the test so that it achieved thermal equilibrium and returned to its original state of dispersion. Additional measurements of dispersions with concentrations lower than 1% were made in an Ostwald-type viscometer. Each part of the experiment was made in duplicate at least.

Flow properties can be generally expressed by following a power law equation.

$$\sigma = k\dot{\gamma}^n \qquad (1)$$

or

$$\log \sigma = n \log \dot{\gamma} + \log k,$$

in which $\sigma$ is the shear stress (dyne/cm$^2$), $\dot{\gamma}$ is the shear rate (sec$^{-1}$), n is the flow behavior index, and k is the consistency coefficient in the power law relation (Holdsworth 1971). For a Newtonian fluid, n = 1; any other values indicate a deviation from Newtonian flow. The apparent viscosity was also calculated by the single-bob method of Krieger and Maron (1954).

### Effect of Shearing Time
The time dependence of viscosity was determined at the desired shear rate by recording the shear stress against time. When the equilibrium of the shear rate was reached, the shear stress at time t = 0 was recorded.

### Effect of Temperature
The viscosity of a liquid decreases with increasing temperature. Temperature-dependent measurements were made on the 0.5 and 12% dispersions, typical Newtonian and non-Newtonian fluids, respectively.

In considering the effect of temperature on viscosity ($\eta$) of a Newtonian fluid, Andrade (1934) proposed the following equation:

$$\eta = A \exp (Ev/RT) \qquad (2)$$

or

$$\ln \eta = Ev/RT + \ln A,$$

in which Ev is the activation energy for viscous flow, A is a constant, R is the gas constant, and T is the absolute temperature. For a non-Newtonian fluid, however, the Andrade equation is not directly appropriate. The shear rate is used as a parameter, and the activation energy for flow, $Ev(\dot{\gamma})$, is identified by the following equation:
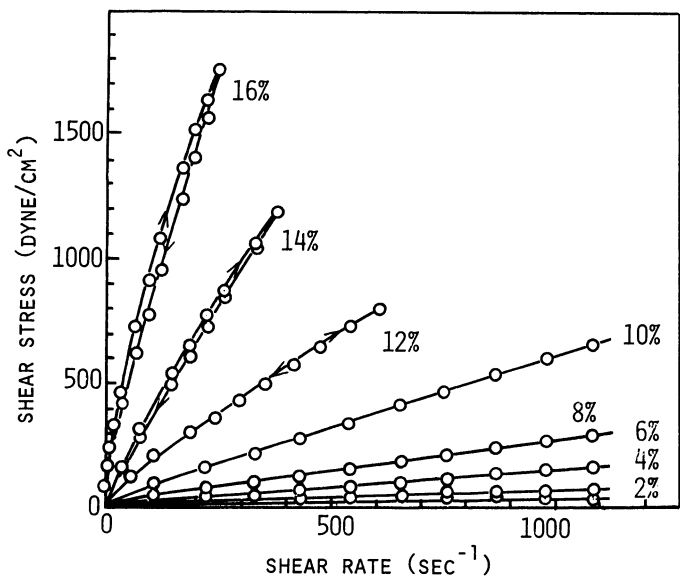


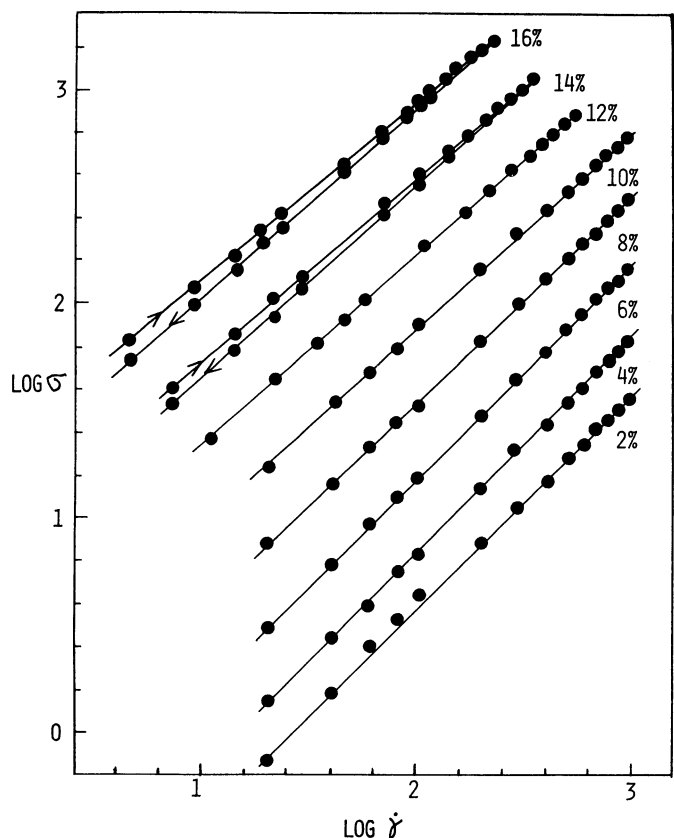Fig. 1. Flow curves at various concentrations of gluten (percent based on dry weight).



Fig. 2. Shear stress ($\sigma$) as a function of shear rate ($\dot{\gamma}$) on a logarithmic scale for gluten dispersions at various concentrations.

$$\ln\sigma(\dot{\gamma}) = Ev(\dot{\gamma})/RT + \ln C, \qquad (3)$$

in which C is a constant.

## RESULTS

### Flow Properties of Gluten Dispersion

The slope of flow curves of gluten dispersions increased with increasing concentration (Fig. 1). At concentrations above 14%, a hysteresis loop was observed. Figure 2 shows double logarithmic shear stress-shear rate plots for gluten dispersion. Hermansson (1975) and Lefebvre and Sherman (1977) have indicated that the flow properties of protein dispersions are characterized by the power equation at various concentration levels. Within the shear rate range 100–1,000 $sec^{-1}$, the excellent linearity of the plots indicates that the flow properties of gluten dispersions conform to the power equation. Table I shows the power law constants. At concentrations below 4%, the dispersions were Newtonian (n = 1), and k had the dimensions of viscosity. At concentrations above 6%, non-Newtonian behavior became more pronounced and k increased enormously with increasing concentration. In addition, k followed a semilogarithmic relation to concentration (Fig. 3), and n decreased more or less linearly with increasing concentration.

### Effect of Shearing Time

Concentrated gluten dispersions (12–16%) were subjected to constant shear rate for up to 15 min. The shear stress of the dispersions decreased with shearing time for all shear rates tested (Fig. 4), and the decrease was more rapid at higher shear rates and at higher concentrations.

**TABLE I**
**Effect of Concentration on the Power Law Constants**
**of Gluten Dispersions at pH 4.5 (25°C)**

| Gluten Concentration (%) | n[a] | k[b] |
|---|---|---|
| 2 | 1.00 | 0.04 |
| 4 | 1.00 | 0.07 |
| 6 | 0.98 | 0.18 |
| 8 | 0.95 | 0.45 |
| 10 | 0.90 | 1.4 |
| 12 | 0.87 | 3.4 |
| 14 | 0.86 | 8.3 |
| 16 | 0.81 | 21 |

[a] n = Flow behavior index.
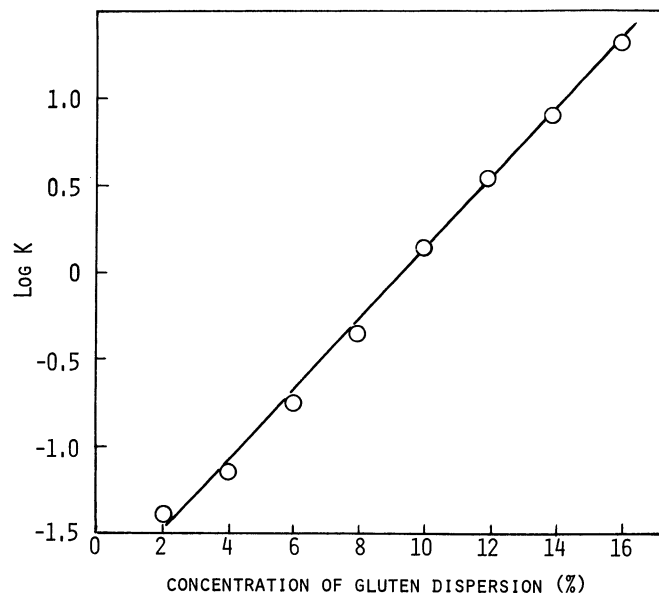[b] k = Consistency index.



Fig. 3. Plots of log k against concentration of gluten dispersion.

## Amide-Ester Conversion of Gluten

Gluten ($10^5$ g) containing 308 mole of amide was converted to gluten methyl ester ($10^5$ g) containing 107 mole of amide and 236 mole of ester. Thus, approximately 65% of the amide of gluten was removed by esterification. The larger ester content may be due to the direct esterification of free carboxyl groups of gluten. Beckwith et al (1963) have reported that, in spite of the significant changes in solubility and intrinsic viscosity, little decrease was found in the size of the peptide chain in gluten methyl ester (because N-terminal amino nitrogen increased only slightly) and that the amide-ester interchange apparently did not cause any significant change in the structure of gluten, as measured by optical rotatory dispersion.

## Flow Properties of Gluten Methyl Ester Dispersion

At concentrations below 4%, the dispersions were almost Newtonian and of low viscosity (Fig. 5). At concentrations above 8%, they became slightly non-Newtonian and the viscosity increased significantly with increasing concentration.

The flow properties of gluten methyl ester dispersion differed considerably from that of gluten dispersion. At protein concentrations above 4%, the apparent viscosity of gluten methyl
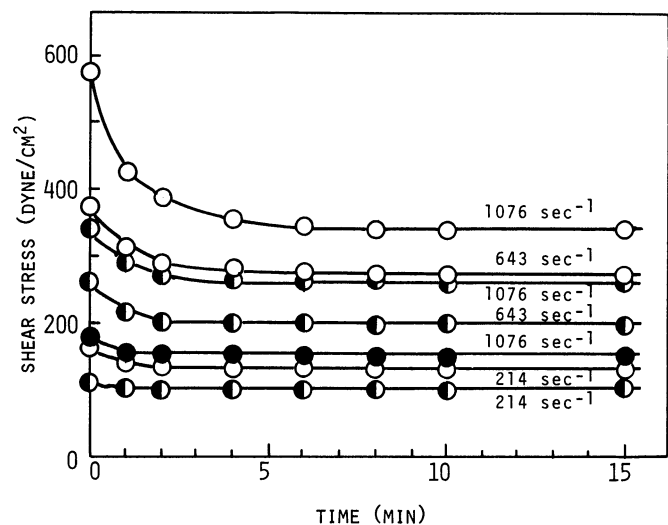
ester dispersion became significantly lower than that of gluten dispersion (Fig. 6), although the apparent viscosity of gluten and gluten methyl ester was equal at concentrations lower than 4%.

## Effect of Temperature

In the temperature range $20-50°$ C, Ev for dilute (0.5%) gluten was 3.98 Kcal/mol and for gluten methyl ester dispersions, 4.12 Kcal/mol. (That of water was 3.83 Kcal/mol.) The difference in the two values of Ev was not significant. However, at high concentration, the viscosity dependence on temperature was more prominent for gluten dispersions than for those of gluten methyl ester. Figure 7 shows the effect of temperature upon the apparent viscosity of gluten and gluten methyl ester dispersions at a constant



**Fig. 6.** Plots of apparent viscosity against concentration at $\dot{\gamma} = 110$ sec$^{-1}$ (left) and $\dot{\gamma} = 1,076$ sec$^{-1}$ (right). $-\circ-$ = gluten, $-\bullet-$ = gluten methyl ester.



**Fig. 4.** Changes in shear stress with shearing time at constant shear rates for gluten dispersions. Concentrations: $-\circ-$ = 16%, $-\mathbf{o}-$ = 14%, $-\bullet-$ = 12%.
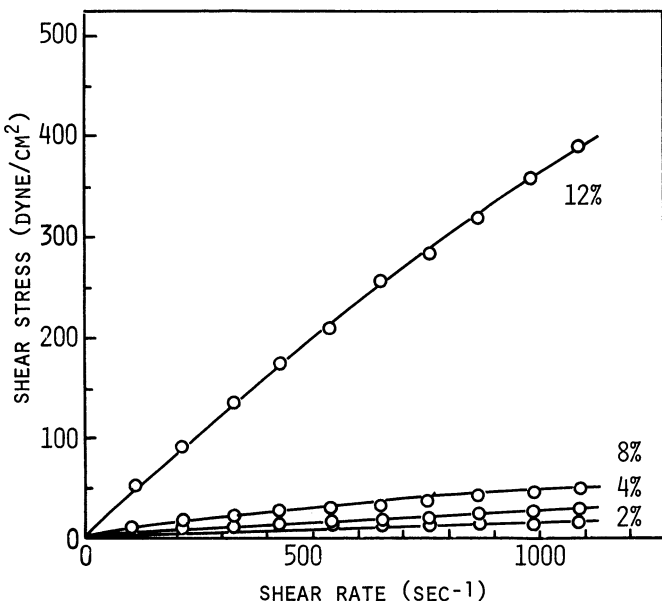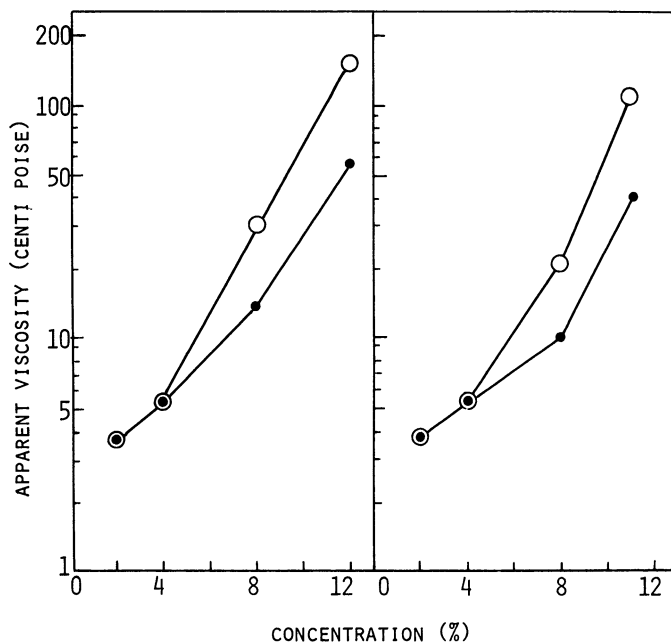


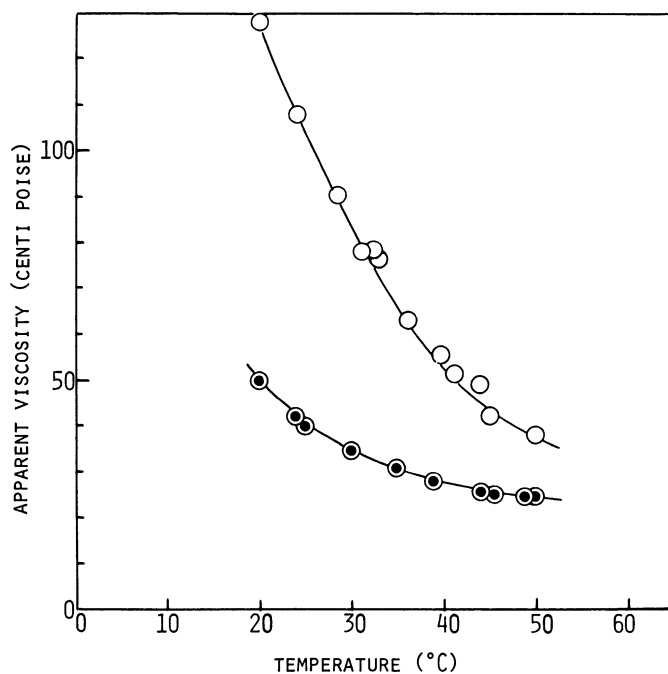**Fig. 5.** Flow curves at various concentrations of gluten methyl ester (percent based on dry weight).



**Fig. 7.** Effect of temperature upon apparent viscosity of gluten and gluten methyl ester dispersions (12%) at a constant shear rate (1,076 sec$^{-1}$). $-\circ-$ = gluten, $-\bullet-$ = gluten methyl ester.

shear rate. Figure 8 shows the plots of $\ln\sigma$ ($\dot\gamma = 1{,}076$ sec$^{-1}$) against the reciprocal of the absolute temperature. $Ev(\dot\gamma = 1{,}076$ sec$^{-1})$ was calculated from the slope of the straight line in Fig. 8. These results were further confirmed by the measurements of $Ev(\dot\gamma)$ as a function of shear rate $(\dot\gamma)$, shown in Fig. 9.

## DISCUSSION

As in the case of other proteins such as whey protein concentrate (Hermansson 1975), $\beta$-lactoglobulin (Pradipasena and Rha 1977), single-cell protein concentrate (Huang and Rha 1971), and sunflower protein (Lefebvre and Sherman 1977), the effect of shear rate on the viscosity of gluten dispersions was dependent on
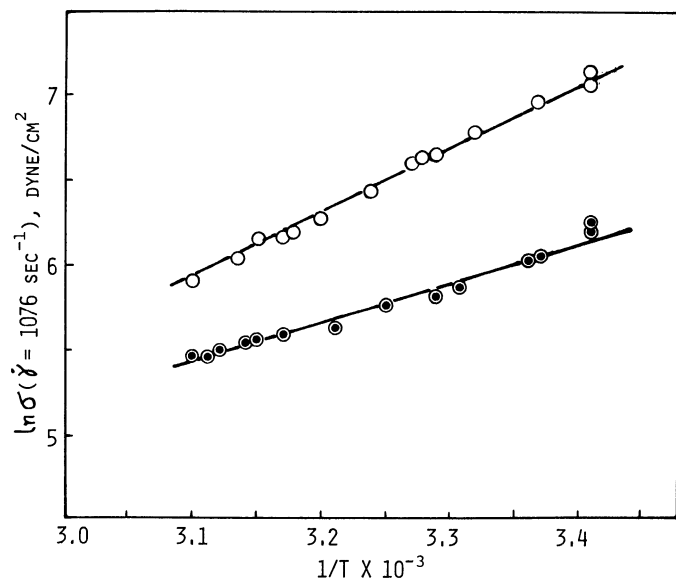


**Fig. 8.** Plots of $\ln\sigma$ ($\dot\gamma = 1{,}076$ sec$^{-1}$) against reciprocal of absolute temperature (T). $-o-$ = gluten, $-\bullet-$ = gluten methyl ester.
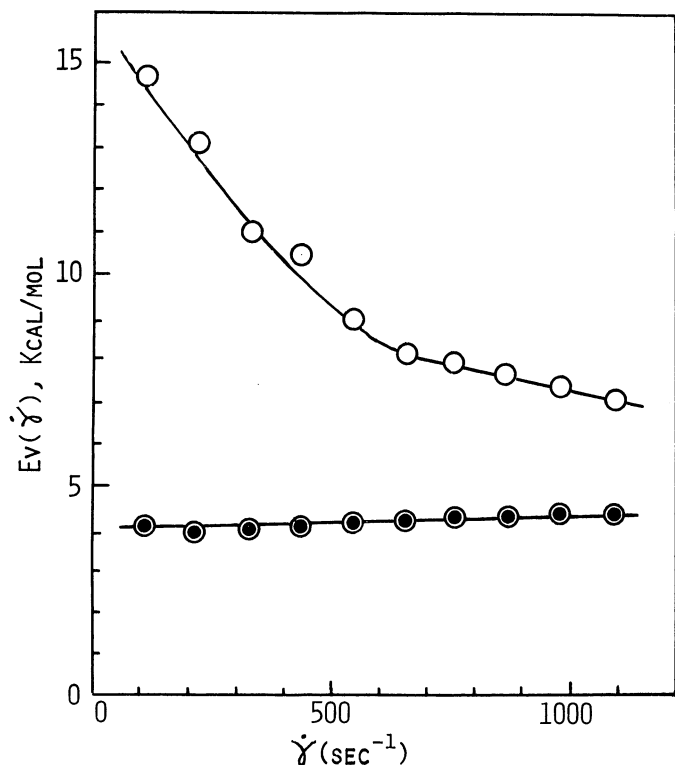


**Fig. 9.** Change of $Ev(\dot\gamma)$ as a function of $\dot\gamma$ within temperature range 20–50°C. $-o-$ = gluten, $-\bullet-$ = gluten methyl ester.

their concentrations. Non-Newtonian behavior became more pronounced with increasing concentration, and the hysteresis loop was observed at high concentration. Such flow characteristics of gluten dispersion indicate the presence of protein-protein interactions, leading to the formation of a protein network at high concentration. Thixotropic behavior of gluten dispersion may be explained by the disaggregation of molecules from shearing, which occurs at a higher rate than does the formation of aggregates.

Beckwith et al (1963) have reported that the amide groups of gluten contribute significantly to the intermolecular associations of the protein through hydrogen bonding. In order to demonstrate the contribution of these groups to flow properties of gluten, they were replaced by ester groups and the properties of gluten methyl ester dispersions were investigated. Esters have considerably smaller dipole moments than do amides, and their tendency to engage in hydrogen bonding is correspondingly less (Krull and Wall 1966). The conversion of side-chain amides to ester groups, therefore, should reduce intermolecular hydrogen bonding in gluten and significantly alter properties influenced by such bonding. The flow properties of gluten dispersions do differ considerably from those of gluten methyl ester (Fig. 6). At high concentrations the apparent viscosity of a gluten dispersion is significantly higher than that of a gluten methyl ester dispersion.

The presence of permanent dipoles in molecules, such as those with amide side-chains, causes a restriction of external molecular rotation through the directional interaction of the dipole of neighboring molecules. Hydrogen bonding therefore contributes to an abnormal increase in the value of Ev (Bondi 1956). The difference in the values of Ev of dilute gluten and gluten methyl ester dispersions was not significant. However, at higher concentrations the value of $Ev(\dot\gamma)$ of gluten dispersions was considerably higher than that of gluten methyl ester for all shear rates applied. As the rate of shear increased, $Ev(\dot\gamma)$ of gluten dispersion decreased, whereas that of gluten remained almost unchanged (Fig. 9). A lower $Ev(\dot\gamma)$ value of gluten dispersion at higher shear rates may be caused by the breaking of intermolecular hydrogen bonding by the shear force.

Accordingly, the difference in the flow properties of gluten and gluten methyl ester dispersions may be attributed to hydrogen bond interactions between gluten molecules becoming more pronounced with increasing concentration. Hydrogen bond interactions between glutamine side-chains may occur more rapidly in a dough system because the water content of dough is much lower than that of the gluten dispersions investigated.

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