Dynamic Viscoelastic Properties of Concentrated Dispersions of Gluten and Gluten Methyl Ester: Contributions of Glutamine Side Chain

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ABSTRACT

Dynamic viscoelastic properties of concentrated dispersions of gluten and gluten methyl ester were investigated. The storage and loss moduli, G′ and G″, for dispersions of gluten and gluten methyl ester increased with increasing concentration. The values of G′ and G″ for gluten methyl ester dispersion appeared to increase steadily with increasing concentration compared to those of gluten. Tan δ for both dispersions decreased with increasing concentration. Significant decreases in tan δ were also observed when gluten methyl ester was tested at concentrations above 20%. The resulting viscoelastic spectra, log G′ versus logarithm of frequency, were analyzed in terms of the theory of cooperative flow. From a link between the cooperative flow analysis and the result of tan δ, gluten methyl ester dispersion forms loosely packed gel structure at higher concentrations, whereas gluten dispersion behaves as a viscoelastic liquid even at concentrations above 30%.

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 primarily due to the properties of gluten protein. The rheological methods, however, are usually empirical, and a few fundamental studies have been published on gluten rheology (Bernardin 1975, Cumming and Tung 1975, Funt Bar-David and Lerchenthal 1975). Considerably more studies have been conducted on dough rheology (Bloksma 1971, Hibberd and Parker 1975, Matsumoto 1979).

Recently, a new, simple, and accurate stress-relaxation instrument suitable for materials like soft gel was developed (Bohlin et al 1980). The shear-stress relaxations of gluten (Bohlin and Carlson 1981) and chemically modified gluten (Mita and Bohlin 1983) have been measured. The resulting relaxation curves have been analyzed in terms of the theory of cooperative flow (Bohlin 1980).

Glutamine comprises one third of the amino acids in gluten. To clarify the contribution of amide groups, amide groups are often replaced by ester groups (Beckwith et al 1963, Krull and Wall 1966). Measurements of the flow properties of aqueous dispersions of gluten and gluten methyl ester indicate that the hydrogen bonds resulting from glutamine side chains are an important factor in determining the rheological properties of gluten (Mita and Matsumoto 1981).

The theory of linear viscoelasticity should be applied only to linear viscoelastic behavior. A static experiment such as stationary-flow measurement is, therefore, fairly limited for the protein dispersion showing considerable non-Newtonian behavior. On the other hand, in dynamic testing, small deformations and short time spans are used, which will not alter the structure of a material and will satisfy requirements of linear viscoelastic theory.

The purpose of the work reported here was to estimate the contributions of glutamine side chain on the rheological properties of gluten through dynamic viscoelastic measurement of concentrated dispersions of gluten and gluten methyl ester. The present article also discusses an attempt to analyze the structures of their dispersions through the dynamic measurements.

MATERIALS AND METHODS

Reagents

All chemicals used in this study were of reagent grade. Anhydrous methanol containing 0.6N hydrogen chloride was prepared as follows. Anhydrous hydrogen chloride gas was dissolved with cooling in absolute methanol. The acid concentration was determined by titration with standard sodium hydroxide solution. Anhydrous methanol containing 0.6N hydrogen chloride was then prepared by diluting with appropriate methanol (Beckwith et al 1963).

Wheat Flour

Wheat flour used for extracting gluten was Kyokuba brand unbleached strong flour provided by Nissin Flour Mill Co., Japan.

Gluten

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

Gluten Methyl Ester

Gluten methyl ester was prepared from gluten by the procedure

of Beckwith et al (1963). A suspension of 5% gluten in anhydrous methanol containing 0.6*N* hydrogen chloride was incubated for 48 hr at 30° C. The reaction was then stopped by dialyzing against a large volume of water with frequent changes, and the nondialyzable product was lyophilized.

Chemical Analysis of Protein

Amide nitrogen content of protein was determined by incubating the protein with 2*N* hydrogen chloride for 3 hr at 100° C, distilling the ammonia from alkaline solution, and titrating the ammonia with standard acid as in the micro-Kjeldahl method (Chibnall et al 1958). Amino acid analysis was made by using a Hitachi KLA-5 amino acid analyzer according to the method of Spackman et al (1958). Tryptophan content was estimated with an unhydrolyzed protein sample by the spectrophotometric method of Edelhoch (1961).

Preparation of Concentrated Dispersions of Gluten and Gluten Methyl Ester

Dilute acetic acid was used as the disperse medium, since gluten is insoluble in water, while gluten methyl ester is soluble in water. The desired amount of protein powder was weighed into a beaker and an appropriate volume of 0.1 *M* acetic acid was added with continuous mixing by a Homomixer (Tokushu Kikakogyo Co. Ltd.). After the protein had been sufficiently dispersed, contaminated air bubbles were removed by centrifugation at 2,000 × *g* for 20 min. Protein dispersions thus prepared were then stored for one day at 5° C and aliquots were withdrawn from each dispersion for the rheological measurements. The pHs of the dispersions of gluten and gluten methyl ester prepared were about 4.1 and 3.9, respectively. Accurate protein concentration in the dispersions was determined by measuring the weight of dry matter.

Measurement of Dynamic Viscoelasticity

The dynamic shear characteristics of the protein dispersions were examined with a Rheometer RM-1 (Shimazu Manufacturing Co. Ltd.) using the cone-plate geometry. This instrument is similar in principle to a Weissenberg's Rheogoniometer and permits measurements to be made either in steady strain (static) or with an imposed sinusoidal strain (dynamic), covering a wide range of shear rates or frequencies. In this instrument, oscillatory torque is sensed through the small movement of the cone supported by a torsion bar. Deflection of the cone is followed with an electrical detector and is registered on a recorder. Variable oscillatory motion can be imparted to the bottom plate. The radius of the cone used was 4.0 cm, and the cone angle was 0.070 rad. The dynamic measurements were made at the rotating angle of 1.753×10^{-2} rad and in the range of frequency from 5.82×10^{-2}–5.82 rad·s^{-1}. After the sample had been introduced on the lower plate and gap width had been set, the sample was allowed to relax and to equilibrate with the desired temperature for 30 min before the test began. The sample chamber was kept at constant humidity to prevent drying during measurement. The measurements were made at 25 ± 0.5° C. Each part of the experiment was made at least in duplicate. The results were reproducible to ±5%.

TABLE I
Amino Acid Compositions and Amide Contents of Gluten and Gluten Methyl Ester (mol/ 10^5 g of protein)

Amino Acid	Gluten	Gluten Methyl Ester
Alanine	25	27
Arginine	46	16
Aspartic acid	20	22
Half-cystine	3	4
Glutamic acid	304	300
Glycine	41	31
Histidine	11	15
Isoleucine	31	38
Leucine	56	63
Lysine	7	7
Methionine	9	10
Phenylalanine	35	31
Proline	170	152
Serine	44	42
Threonine	20	19
Tryptophan	11	15
Tyrosine	26	26
Valine	40	45
Glutamine and asparagine	309	66

Analysis of Dynamic Viscoelasticity

The storage and loss moduli, G′ and G″, respectively, can be calculated from observed phase shift and amplitude difference. In this instrument, G′ and G″ is given by

$$G' = \frac{-C_1\left(1-\frac{\cos\phi}{P}\right)}{\left(1-\frac{\cos\phi}{P}\right)+^2\left(\frac{\sin\phi}{P}\right)^2} \quad (1)$$

and

$$G'' = \frac{-C_1\left(\frac{\sin\phi}{P}\right)}{\left(1-\frac{\cos\phi}{P}\right)+^2\left(\frac{\sin\phi}{P}\right)^2} \quad (2)$$

where P is the angular amplitude ratio, ie, the ratio of the displacements of the cone and plate, ϕ is the phase angle lag between the cone and plate, and C_1 is the constant ($C_1 = 3\,\theta k/2R^3$, where R = radius of cone; θ = cone angle, and k = torsion bar constant) (Markovitz et al 1952). Furthermore, the loss tangent tan δ, a measure of the ratio of energy lost to energy stored in a cyclic deformation is given by

$$\tan\delta = G''/G'. \quad (3)$$

RESULTS AND DISCUSSION

Chemical Composition of Gluten and Gluten Methyl Ester

Table I shows amino acid compositions and amide contents of gluten and gluten methyl ester. Gluten (10^5g) containing 309 mol of amide (glutamine and asparagine) was converted to gluten methyl ester (10^5g) containing 66 mol of amide; ie, approximately 79% of the amide of gluten was removed by esterification. Esterification of the limited number of free carboxyl groups also occurred in the early stages of the reaction. Beckwith et al (1963) have reported that, despite significant changes in the physical properties, such as solubility or viscosity, the size of the peptide chain in gluten methyl ester decreased little (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. However, small differences were found in the amino acid compositions between gluten and gluten methyl ester, particularly in proline content. This might indicate that small amounts of peptide cleavage were accompanied by the amide-ester interchange.

Storage and Loss Modulus for Dispersions of Gluten and Gluten Methyl Ester

Figure 1 shows the changes of the storage and loss moduli, G′ and G″ as a function of frequency ω for gluten dispersions at various concentrations. The values of G′ and G″ increased with increasing concentration and increased in proportion to ω. The values of G′ were smaller than those of G″ for all concentrations and for all frequencies studied, showing the relatively predominant viscous character of gluten dispersion. Figure 2 shows the changes of G′ and G″ as a function of ω for dispersions of gluten methyl ester at various concentrations. The values of G′ and G″ significantly increased with increasing concentration. In addition, at concentrations above 20% the values of G′ became predominant compared to those of G″, showing considerable increase of elastic character. Figure 3 shows the plots of G′ and G″ against protein

concentration for dispersions of gluten and gluten methyl ester at constant frequency ($\omega = 0.59$ rad·s^{-1}). At concentrations above 15% the values of G′ and G″ for gluten methyl ester dispersion were slightly lower than those of gluten dispersion, while at concentrations above 17% the values of G′ and G″ for gluten methyl ester dispersion became significantly higher than those of gluten.

Mita and Matsumoto (1981) reported that at concentrations above 4% (up to 12%) the apparent viscosity of gluten methyl ester dispersion was significantly lower than that of gluten, while the apparent viscosity of gluten and gluten methyl ester was almost equal at concentrations lower than 4%. Additionally, the difference in the values of activation energy for flow (Ev) of dilute gluten and gluten methyl ester dispersions was not significant. At higher concentrations, however, the value of Ev of gluten dispersions was considerably higher than that of gluten methyl ester for all shear rates applied. 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). Then, the difference in the flow characteristics between the dispersions of gluten and gluten methyl ester may be mainly attributed to hydrogen bond interactions between glutamine side chains, becoming pronounced with increasing concentration, although differences other than glutamine side chain content might contribute to the observed differences in viscoelastic properties of gluten and gluten methyl ester. This assumption is not directly applicable for significantly concentrated dispersion, however, because at concentrations above 17% the values of G′ and G″ for gluten methyl ester dispersion were considerably higher than those of gluten. Such a conflict will be resolved by the phenomenon whereby gluten methyl ester dispersion forms a gel-like structure at concentrations above 20%.

Furthermore, to characterize the relationships between G′ and G″, the loss tangent, tan δ, was determined for the dispersions of gluten and gluten methyl ester. Figure 4 shows the frequency dependence on tan δ obtained by the dispersions of gluten and gluten methyl ester. In gluten dispersion, the small inflection points observed for low concentrations at low frequencies might be the result of entanglement coupling (Ferry 1970) in which extended linear fragments interact in a specific frequency range. To obtain the definite information on the entanglement coupling, further studies should be made through measurement in an extended range of frequency. Tan δ for both dispersions decreased with increasing concentration. Significant decreases in tan δ were also observed when gluten methyl ester dispersion was tested at concentrations above 20%. If tan $\delta \ll 1$, the material will behave as a solid (ie, deformations within the linear range will be essentially elastic or recoverable), whereas if tan $\delta \gg 1$, material will behave as a liquid; that is, the energy used to deform the material will be dissipated viscously (Ferry 1970, Evans and Haisman 1979). As shown in Fig. 4, at concentrations above 20%, tan δ of gluten

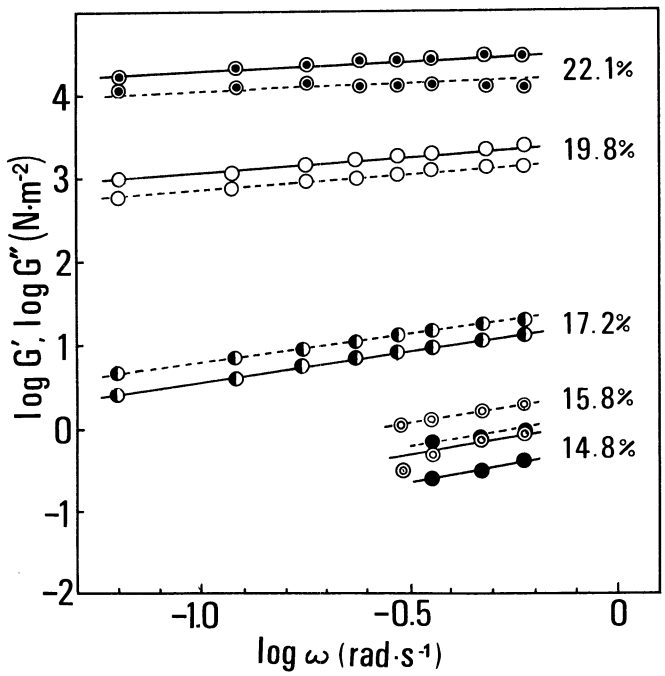

Fig. 2. G′ and G″ as a function of ω on a logarithmic scale for dispersions of gluten methyl ester at various concentrations. —— = G′; - - - - = G″.

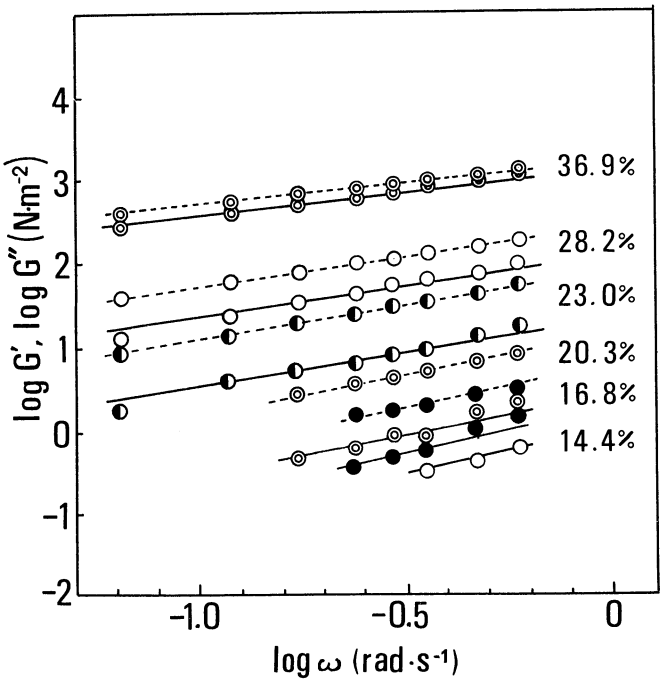

Fig. 1. Storage and loss moduli, G′ and G″, as a function of frequency ω on a logarithmic scale for gluten dispersions at various concentrations (percent based on dry weight). —— = G′; - - - - = G″.

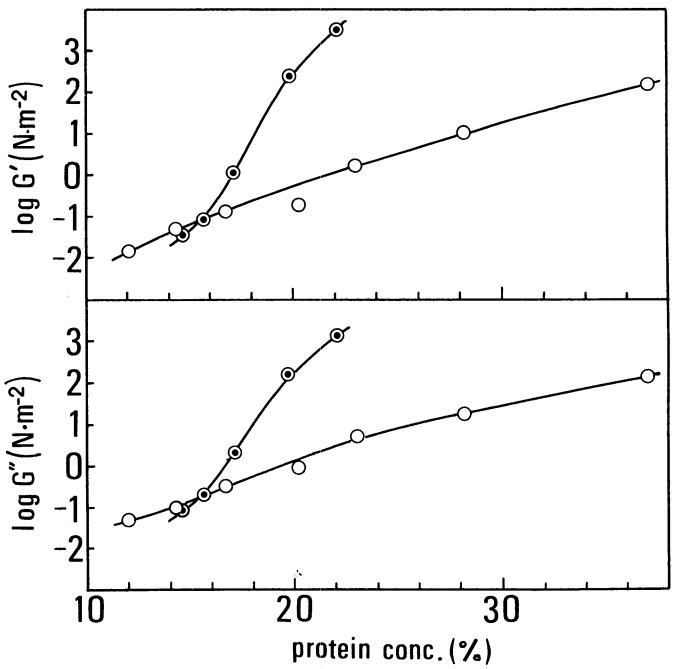

Fig. 3. Plots of G′ and G″ against protein concentration for dispersions of gluten and gluten methyl ester at constant frequency ($\omega = 0.59$ rad·s^{-1}). O = gluten; ⊙ = gluten methyl ester.

methyl ester dispersion became less than one, suggesting the increase in solidlike behavior, whereas tan δ of gluten dispersion was larger than one even at concentrations above 30%, showing liquid behavior. In addition, at concentrations above 20%, significant spinnability in gluten dispersion was observed. In contrast to the case of gluten dispersion, no spinnability in gluten methyl ester dispersion was observed. They may indicate that the concentrated gluten dispersion behaves like a liquid and that the concentrated gluten methyl ester dispersion behaves like a solid.

Structure of Concentrated Dispersions of Gluten and Gluten Methyl Ester

The analysis of the stress-relaxation experiment based on the theory of cooperative flow has been described in detail by Bohlin (1980). This theory may provide a link between the rheology and the microstructure of a flowing substance. Based on the methods of nonequilibrium statistical mechanics a kinetic equation for stress relaxation is derived. In this theory the flow is largely determined by the coordination number z of cooperative flow units in the structure. For stress relaxation, the rate equation describing the relaxation process before the stationary state is reached, is given by

$$ds/dt = -(1/\tau)\cdot s(s+\alpha)^z, \quad (4)$$

where s is the relative stress, τ is the relaxation time, and α is a measure of the strength of the cooperativity. A dynamic experiment at a frequency ω is qualitatively equivalent to a transient experiment at the time $t = 1/\omega$ (Ferry-1970). It follows, therefore, that the characteristic dependence of a dynamic experiment on the cooperative coordination number z should be of the form

$$G'(\omega) \sim \omega^{1/z} \quad (5)$$

in the range of frequencies corresponding to times $\tau \lesssim t <$ (stationary state) (Bohlin 1980).

The dynamic flow of the well-established liquid crystalline lamellar and close-packed rod hexagonal phases of the hexanol-hexadecyltrimethylammonium bromide-water system has the frequency dependence $\omega^{1/2}$ and $\omega^{1/6}$, respectively (Bohlin and Fontell 1978, Bohlin 1979). The coordination in flow in these structures is, hence, equal to the coordination in the colloidal structure. 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 is, 12, six, and two, respectively. Furthermore, the dynamic data on wheat flour dough have indicated a structure having approximately a fourfold coordination ($z \simeq 4$) of flow units in the frequency range 0.08–20.9 rad·s^{-1} (Bohlin and Carlson 1980). They have also analyzed the creep data on wheat flour dough by Hibberd and Parker (1979) according to the flow theory. From these creep data, a fourfold coordination flow process can be identified.

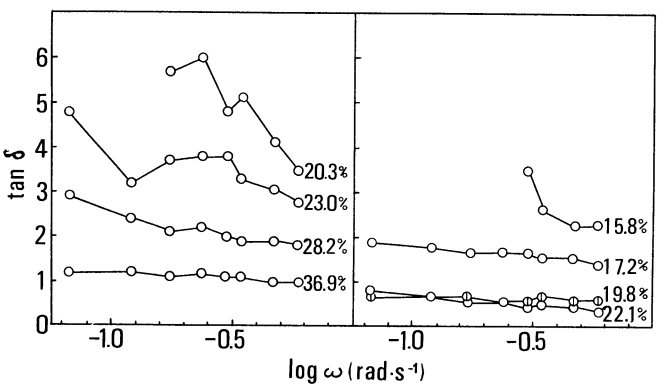

Fig. 4. Plots of loss tangent, tan δ, against log ω for dispersions of gluten (**left**) and gluten methyl ester (**right**) at various concentrations.

Dispersions of gluten and gluten methyl ester are complex systems compared to the hexanol-hexadecyltrimethylammonium bromide-water model system. Since our analysis is based on the model, it necessarily involves an element of speculation. As shown in Figs. 1 and 2, G′ was found to be almost proportional to frequency ω for all concentrations studied. The coordination number was then calculated from the reciprocal of the slope of the straight line obtained by the plots of log G′ as a function of log ω. The coordination number of gluten dispersion increased more or less with increasing concentration, but even at concentrations above 30% the cooperative flow analysis did not give a flow coordination number exceeding two, ie, z = 1.1, 1.1, 1.3, 1.3, and 1.8 at 14.4, 16.8, 23.9, 28.2, and 36.9%, respectively. On the other hand, the coordination number of the dispersion of gluten methyl ester increased distinctly with increasing concentration, and at concentrations above 20% the cooperative flow analysis gave a flow coordination number about four, ie, z = 1.1, 1.2, 1.3, 2.5, and 4.1 at 14.8, 15.8, 17.2, 19.8, and 22.1%, respectively. Even at considerably higher concentrations the flow process in gluten dispersion has a flow coordination of two. This should correspond to the rearrangement in a lamellar structure. At concentrations above 20%, however, the flow process in gluten methyl ester dispersion has a flow coordination of four. This may correspond to the rearrangment in a nonclose-packed particle structure. Accordingly, from a link between the cooperative flow analysis and the result of tan δ, gluten methyl ester dispersion forms a loosely packed gel structure at higher concentrations, whereas gluten dispersion behaves as viscoelastic liquid even at concentrations above 30%.

The insolubility of the gluten proteins is due to hydrogen bonds between protein molecules. The relatively low bond energy (about 20 kJ/mol) is compensated for by their abundance, which is due to the high amide content of gluten proteins (Holme and Briggs 1959). The amide group is both an acceptor and donor of hydrogen bond capable of interacting with other amide groups and with groups that are only acceptor or only donor. The high proline content hinders helix formation; this, in turn, favors the formation of intermolecular rather than intramolecular hydrogen bond (Bloksma 1971). On the other hand, methyl esters have considerably smaller dipole moments than do amide esters, and their tendency to engage in hydrogen bonding is correspondingly less. 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. Actually, gluten methyl ester was soluble in water even at neutrality, whereas the untreated gluten was insoluble in water above pH 6. They may be caused by the cleavage of peptide chain other than the amide-ester conversion, since peptide cleavage should increase solubility in water by lowering the molecular weight and increasing the charge per unit weight of protein. However, no difference in solubility between gluten and gluten methyl ester was observed in the range of pH 3–4 (ie, by using 0.1% protein solution the solubility was estimated in terms of optical density [turbidity] at 370 nm). They suggest that small viscoelastic changes in the esterified gluten result in the effect of the peptide cleavage, since the peptide cleavage should increase solubility even in the pH range observed.

Hydrogen bonding and hydrophobic attraction are important to the onset of gelation (Stainsby 1977, Schmidt 1981). In the acidic condition studied (pH 4.1 for gluten, pH 3.9 for gluten methyl ester), the electrical repulsive force due to an excess positive charge upon protonation of free carboxyl groups in gluten may be relatively stronger than the attractive force due to the hydrogen bond, so that gluten dispersion will not be easily aggregated. On the other hand, the hydrophobic nature of gluten molecules on conversion of amides to the less polar ester group must be increasing. Eagland et al (1974) have proposed that the gelation is caused by the van der Waals-London attractive force between the hydrated shells of a partial folded molecule. Accordingly, in gluten methyl ester dispersion at higher concentrations, the attractive

force from hydrophobic interaction would become significantly stronger than the repulsive force, and gelation formation may be a consequence of this. The mechanism of the gel formation, however, is still not clear to us, and further investigation should be done.

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