COLLOID TITRATION OF WHEAT PROTEINS, DOUGH, AND FLOUR[1]

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ABSTRACT

Colloid titration, based on a stoichiometric combination between positive and negative ionic high-molecular-weight compounds, was applied to wheat flour, dough, and wheat proteins. Polyvinyl alcohol sulfate was used in the titer solution, and chitosan hydrochloride or methylated chitosan iodide was used as the standard positively charged polymer. Colloid titration curves, in which an equivalent charge per g. of protein was plotted against pH of medium, indicated that the positive net charge was increased in the following order at pH values lower than 4, crude gluten < defatted gluten < wheat flour < soluble protein. A marked increase of charge in gluten was observed when lipid was removed. The positive charge of dough dispersed in 3*M* urea at pH values lower than 4 decreased as mixing time increased. These findings suggest a possible interaction between polymer ions during mixing and dough development.

Some attempts to approach the specific properties of dough from the point of view of the electrical charge of the protein have interested the authors. Bungenberg de Jong and Klaar (1) and Hess (2) suggested that the rigidity of gluten is due to the force between two components having opposite charges. They indicated two components, gliadin *vs.* glutenin, wedge protein *vs.* adhering protein, as those which have opposite charges.

An acid-base titration method (3) and a dye-binding method (4) have been applied to the determination of the electric charge of wheat protein, originated by dissociation of basic and acidic amino acid residues. The data of Wu and Dimler (3) provided the most important information to the present authors.

As a simpler method than potentiometric titration, Terayama (5,6) proposed colloid titration, which involves titration with a negative or positive polymer, using a metachromasic dye such as toluidine blue, methylene blue, or brilliant cresyl blue as indicator of the end point. Metachromasy is the phenomenon of the color change of a dye combined with macromolecular ions. Toluidine blue, a basic dye, used as the indicator in the present work, combines with a minute excess of the negative polymer ion after the equivalent point is reached and changes the blue color to reddish purple. The principle of this method is based on a stoichiometric combination between positive and nega-

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tive ions, through which the equivalence of the charge can be estimated from that of a standard polymer. In this volumetric titration of polymer ions, normality of the titer solution is defined by the number of equivalents of the dissociable groups of the polymer in 1 liter of solution. This can be determined by colloid titration, using a known standard reagent, or by direct or indirect estimations of the electrolytic groups by chemical analysis.

This paper deals with the application of colloid titration to flour, dough, gluten, soluble proteins, and gluten freed from lipid, to obtain basic knowledge about dough formation and the physical properties of dough in terms of the electric charge of proteins.

Materials

The wheat flour used in these experiments was unbleached, improver-free, straight grade, commercially milled from Canadian hard red spring wheat, and supplied through the courtesy of Nissin Milling Co., Kobe, Japan. It contained 12.5% protein on 14.5% moisture basis.

The soluble fraction was extracted from flour with fourfold deionized water, using the Waring Blendor, and then clarified by centrifugation at 3,800 g. for 5 min. It was used in 3*M* urea final concentration.

Gluten was dispersed in 0.01*N* acetic acid. At first, about 20 g. of wet gluten, washed out from dough with tap water, was dispersed in 200 ml. of acetic acid in the blender. The dispersion was centrifuged twice at 1,100 g. for 5 min. each before and after heat-treatment at 95°C. for 3 min., to inactivate enzymes.

Flour and dough were dispersed in 3*M* urea at concentrations of about 2 and 4% respectively.

Sodium salt of polyvinyl sulfate (PVS), which was used as the standard negative colloid, was synthesized by sulfonation of polyvinyl alcohol (mean degree of polymerization: 435) in a pyridine bath with chlorosulfonic acid, according to the method of Terayama (5) and Takahashi *et al.* (7). Mean molecular weight of this negative colloid per sulfuric acid radical, calculated from the sulfur content determined by the procedure of Krober and Howell (8), was 356.2, which means 41% esterification of polyvinyl alcohol. This standard colloid can be used at a wide pH range, as it keeps a standard negativity through the dissociation of its sulfuric acid radical.

Chitosan hydrochloride and N-methylated chitosan iodide were used as the standard positive colloids. Chitin, extracted from lobster and crab shells after treatments with acid, alkali, permanganate, and bisulfite, was hydrolyzed with alkali under drastic conditions to yield chitosan. Chitosan hydrochloride was prepared from chitosan dis-

solved in dilute hydrochloric acid by repeated precipitation, with the addition of organic solvents such as acetone and ethanol. N-Methylated chitosan iodide (macramin) was synthesized from chitosan and methyl iodide in a sealed, brown-colored tube. These preparations have been described by Terayama (5).

Methods

In experiment 1, chitosan HCl and N-methylated chitosan iodide were titrated directly in a solution containing 5–3 $\times 10^{-6}$ equivalents in 10 ml. with the standard negative colloid solution, 0.001*N* sodium salt of PVS. In the other experiments, 1–2 ml. of solution or dispersion of wheat flour, dough, and wheat proteins containing 0.2–0.6% protein was mixed with 2 ml. of a standard positive colloid solution whose concentration was adjusted to give a positive charge up to 0.3–1.0 $\times 10^{-5}$ equivalents after compensation for the negative charge of protein. Urea was used to give the final concentration of 3*M*. After the pH of the mixture was adjusted with either HCl or NaOH solution of the various concentrations, deionized water was added to give a definite final volume of 6–10 ml. One drop of 0.5% toluidine blue solution was added as indicator, and then the mixture was titrated with the standard negative colloid solution containing 0.001*M* sodium salt of PVS until the color of indicator changed by metachromasy. The normality of the colloid solution was defined by the number of equivalents of the charged group per liter. Blank titration was carried out in the same way without sample. At each end point, pH was estimated with a glass-electrode pH meter. By the equation shown below, g. equivalence per unit g. of the sample was calculated from the difference between the titration value of sample and that of a blank test at the same pH value which was obtained from pH *vs.* titration value curve.

$$\frac{\text{equivalence}}{\text{g.}} = \frac{\text{normality of PVS} \times \text{difference in titer} \times 10^{-3}}{\text{amount of material (g.)}}$$

The change of the electrical charge of dough during mixing was determined as follows. Dough was prepared in a farinograph bowl with 50 g. of wheat flour, 1 g. of NaCl, and enough deionized water (24.8 ml.) to give 500 B.U. consistency at 30°C. Approximately 4 g. of dough was taken at various mixing times and submitted to colloid titration as described previously.

Results and Discussion

Colloid Titration of Standard Positive Colloid, Chitosan HCl, and "Macramin." The standard positive colloids used in the present ex-

periment were tested by direct titration with the standard negative colloid reagent. Results are shown in Fig. 1. Chitosan hydrochloride showed a positive charge in acidic solution; however, it lost charge in alkaline solution and precipitation resulted. The gram equivalent of chitosan HCl, determined by potentiometric titration carried out on the same solution with 0.5*M* potassium hydroxide, indicated reasonable agreement with that by colloid titration (Fig. 1). As the theoretical value for unit molecular weight is 197.5 (based on the unit chemical formula $C_6H_{12}O_4NCl$), the gram equivalent of 224 obtained in this colloid titration with the synthesized chitosan HCl is a little larger than theoretical. It could mean either the presence of unreacted acetyl amino groups or degradation of amino groups to some extent.

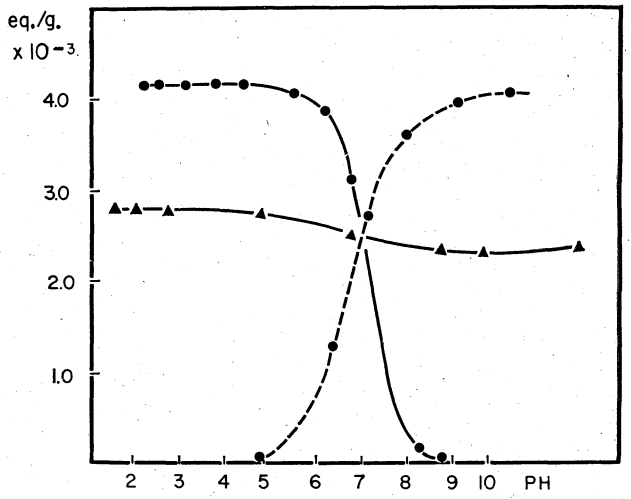

Fig. 1. Colloid titration curves of a standard positive colloid, chitosan HCl (solid circle) and N-methylated chitosan iodide (macramin) (solid triangles), with a standard negative colloid, polyvinyl sulfate, in comparison with potentiometric titration curve (dashed line, solid circles) of the former.

N-Methylated chitosan iodide (designated as "macramin" by Terayama), synthesized in the present experiment, showed the titration curve indicated in Fig. 1. This compound appears to be an appropriate standard positive colloid reagent because of its definite electric charge over a wide pH range. This preparation was also used as standard positive colloid at higher pH where chitosan HCl was not available.

Colloid Titration of Wheat Flour, Soluble Fraction, Defatted Gluten, and Crude Gluten. The results of colloid titration of these samples at various pH values are shown in Fig. 2. The soluble fraction

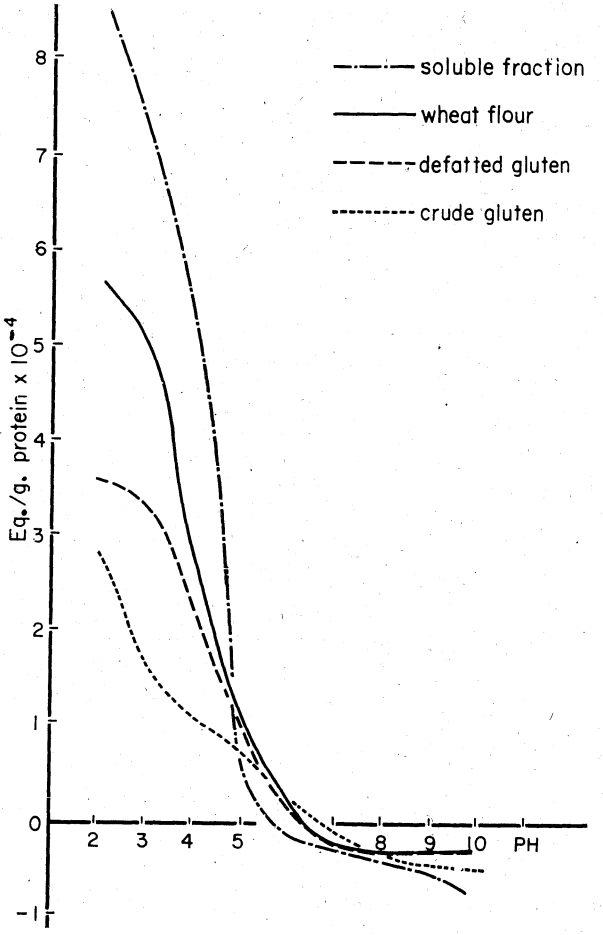

Fig. 2. Colloid titration curves of wheat flour, soluble fraction, defatted gluten, and crude gluten.

exhibited a higher electrical charge than that of gluten. This result is supported by the amino acid compositions (9,10) in which soluble protein contains more basic amino acids, such as arginine and lysine, than gluten; it also is supported by results obtained by Udy with a dye-binding capacity method (4). The electrophoretic study of gluten by Jones *et al.* (11) showed larger migration of soluble protein than of gluten in aluminum-lactate buffer, pH 3.1. This might partly support the result mentioned above, though the electrical charge is not the only factor determining the electrophoretic migration of protein.

A marked increase in positive net charge brought about by remov-

ing lipid from crude gluten may suggest a combination between gluten and lipid through their opposite electrical charges. The phospholipids, one of the major components of wheat lipids, include compounds with the phosphoric acid radical such as phosphatidic acid.

The polyvinyl sulfate combining capacity of defatted gluten obtained by colloid titration, 3.5×10^{-4} eq./g. at pH 2.0, was very close to the acid-binding capacity of about 4.0×10^{-4} eq./g. at the same pH reported by Wu and Dimler (3) with defatted gluten. These are in good agreement with values obtained by Udy (4) who showed $7.8–8.1 \times 10^{-4}$, $4.4–4.6 \times 10^{-4}$ equivalents per g. protein at pH 2.2, for the soluble fraction in 0.05% phosphate buffer, pH 7, and for that in 0.03*N* acetic acid respectively, using a dye-binding method. Though the effect of ionic strength on the titration curve was not examined, a reasonable agreement between the colloid titration value and others would suggest that this effect may be small. However, further experiments must be carried out on colloid titration with the addition of salts. Titration curves in strong alkaline solution were not determined, because of the difficulty in detecting the end point at the pH range 11–12. But the results in Fig. 2 apparently demonstrate a preponderance of basic groups in flour proteins, judging from the shapes of the curves, whereas all amino acid analyses of flour indicate that acidic groups predominate. The presence of carboxyl groups of glutamic acid in the amide form as shown by Cunningham *et al.* (12) may support this observation.

The colloid titration curve obtained with wheat flour (Fig. 2) was found between soluble protein and gluten. The equivalent per g. protein in flour was higher than the expected value based on the ratio of gluten content to that of soluble protein in wheat flour. Two possible causes are suggested for this phenomenon: 1) the presence of charged components other than protein in the soluble fraction; 2) a decrease in the charge in gluten during its extraction and dispersion.

Colloid titration was also carried out with wheat starch which was separated from a flour suspension by centrifugation. The equivalent per g. of starch was found to be lower than 10^{-6} eq./g. Thus the effect of starch can be neglected; however, the charge of other polysaccharides should be considered carefully.

Colloid Titration of Doughs at Various Mixing Times. The equivalence per g. protein, that is, the PVS combining capacity of dough, in strongly acidic solution decreased rapidly as mixing time increased, and decreased to a value similar to that of crude gluten after 6 min. of mixing, as shown in Fig. 3. This decrease might be brought about by interaction between the positive charge of wheat protein and the latent

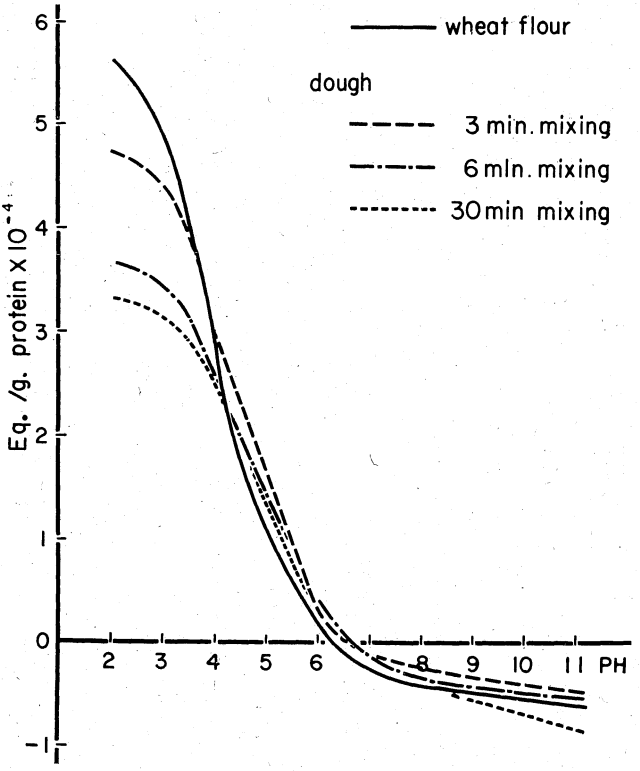

Fig. 3. Colloid titration curves of doughs mixed for various times.

negative charge of some components which may come in close contact during mechanical mixing. The reaction of lipid to protein may be involved in this phenomenon.

The essential electrostatic force between wedge protein and adhering protein in the formation of dough reported by Hess (2) may be related to this decrease of charge. This may be brought about by gel-forming wheat protein produced during mixing as reported by Meredith (13), or because gluten is different from wheat proteins originally present in wheat flour, as mentioned by Mecham (14).

General Discussion

Sullivan (15) reported that anionic surface-active compounds had a marked effect on the properties of gluten, as shown by farinograph curves, whereas little influence was observed with nonionic and cationic compounds. Glabe *et al.* (16,17) found that carrageenan, a mixture of salts of polysaccharide sulfates and hydroxylated lecithin, improved

bread quality by reaction with wheat proteins. Zentner (18) has shown that cysteic acid groups of the proteins contribute to dough strength. Recently the effect of dextran sulfate on mixing properties of dough was described by Mullen and Smith (19,20). The results with these negatively dissociated substances suggest their interaction with wheat protein which has a positive net charge on the acid side. Interaction between polysaccharides and wheat proteins, as reviewed by Gilles (21) and Udy (22), could be explained by a similar principle, though Cawley (23) showed all charged polysaccharides were ineffective except dextran sulfate.

These speculations should, however, be confirmed by experimental results. The colloid titration established by Terayama and applied by the present authors presumably gives an effective method by which to explore this field.

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