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# The Fractionation and Properties of Gluten Proteins

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# The Fractionation and Properties of Gluten Proteins

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ABSTRACT

Gluten protein may be separated into fractions of differing molecular weight distributions by a successive extraction procedure using urea solutions and dilute sodium hydroxide solution. Fractions containing mainly low molecular weight protein decrease dough strength and mixing stability but increase dough plasticity. Fractions rich in high molecular weight protein have the opposite effect. Doughs or glutens behave as uncross-linked systems in which entanglement coupling appears to play an important role.

Gluten is the constituent of wheat flour which is mainly responsible for the unique viscoelastic properties of flour doughs. Flours normally contain 7-17% by weight of gluten protein, 65-80% starch, while the remainder is made up of nongluten proteins, lipids, pentosans, and other minor components. Doughs usually contain between 35 and 40% by weight of water.

Two main classes of protein occur in flour, the gluten protein and the water-soluble or albumin and globulin class. The main characteristics of these two classes are summarized in Table 1.

TABLE 1. Comparison of Properties of Gluten and Water-Soluble Proteins

| | Gluten | Water-solubles |
|---|---|---|
| Amount in flour | 7-17% | 2% |
| Amino acid composition | Glutamic acid (mainly glutamine) 40%<br>Proline, 14%<br>Lysine, 10% | Glutamic acid, 15%<br>Proline, 7%<br>Lysine, 5% |
| Solubility in water | Low, increases with increasing temperature | High, decreases with increasing temperature |
| Effect on rheological properties of dough | Mainly responsible for viscoelasticity | Little effect |

Flours are usually classified in relation to the properties of the doughs they produce. This, in turn, has been traditionally evaluated in cereal laboratories by physical dough testing instruments. Two main types of instrument have been used. Recording dough mixers, such as the Farinograph or Swanson Mixograph [1], measure the mixing characteristics of the dough. They record the resistance to mixing as a function of time. The mixing curve consists of a rising part (dough development) followed by a more or less slow decrease in resistance, referred to as breakdown. Instruments such as the Extensograph or Chopin Alveograph [2] measure load-deformation responses of the dough. The loading pattern for these instruments approximates to deformation with a constant strain rate. The rheological properties of a dough depend mainly on the gluten protein, the starch which is present in the form of small granules acting as a rigid filler. In general, the higher the gluten protein content of a flour, the better the performance of the dough. The protein content of a flour depends to some extent on the wheat variety but mainly on the environmental conditions during growth of the wheat plant. It is possible, however, to have two flours of identical protein content but which perform quite differently. For example, one may be classed as a strong flour; this means that it will have a high mixing stability and high resistance to extension such as for the flours shown in Figs. 2c and 2d. The other may be a weak flour and have the Mixograph and Alveograph characteristics of Fig. 2b. The difference between flour strength at the same protein level is largely a varietal characteristic.

This poses the interesting question as to what causes these differences and how can they be explained at a fundamental and preferably a molecular level.

Gluten has been shown to be a protein complex with a molecular weight spectrum spanning a range between 30,000 and 2 or 3 million but with an amino acid composition not varying greatly with molecular weight [3]. No complete molecular weight distribution has so far been measured on a single flour gluten sample. It has proved difficult to investigate due to its relative insolubility, particularly the higher molecular weight portion. By using successive extractions with water, urea solutions, and finally sodium hydroxide solutions of about 0.1 M strength, it is possible to extract practically all the protein from a flour [4]. With this procedure, the results of Table 2 were obtained for the extraction of protein from a sample of flour from the Australian wheat variety Gluclub.

TABLE 2. Results for Extraction of Gluten Protein from Gluclub Flour

| Extracting solvent | | % of total protein extracted |
|---|---|---|
| Water | 1 | 16.0 |
| | 2 | 7.5 |
| 2 M urea | 1 | 27.0 |
| | 2 | 13.5 |
| | 3 | 6.0 |
| | 4 | 3.5 |
| | 5 | 4.5 |
| | 6 | 2.5 |
| | 7 | 4.5 |
| | 8 | 1.0 |
| 0.1 M NaOH | 1 | 8.5 |
| | 2 | 5.5 |

The first two water extractions remove practically all the albumin and globulin proteins. Figure 1 shows gel filtration profiles of some of the gluten protein extracts, which were measured by Dr. J. W. Lee of the CSIRO Wheat Research Unit. The proportion of low to high molecular weight protein is seen to progressively decrease with each extraction. Thus an effective fractionation is achieved by this procedure. This type of fractionation, which is well-known in high polymer chemistry, depends on the decrease in solubility of a polymer with increasing molecular weight [5].

In order to evaluate the contributions of different gluten fractions

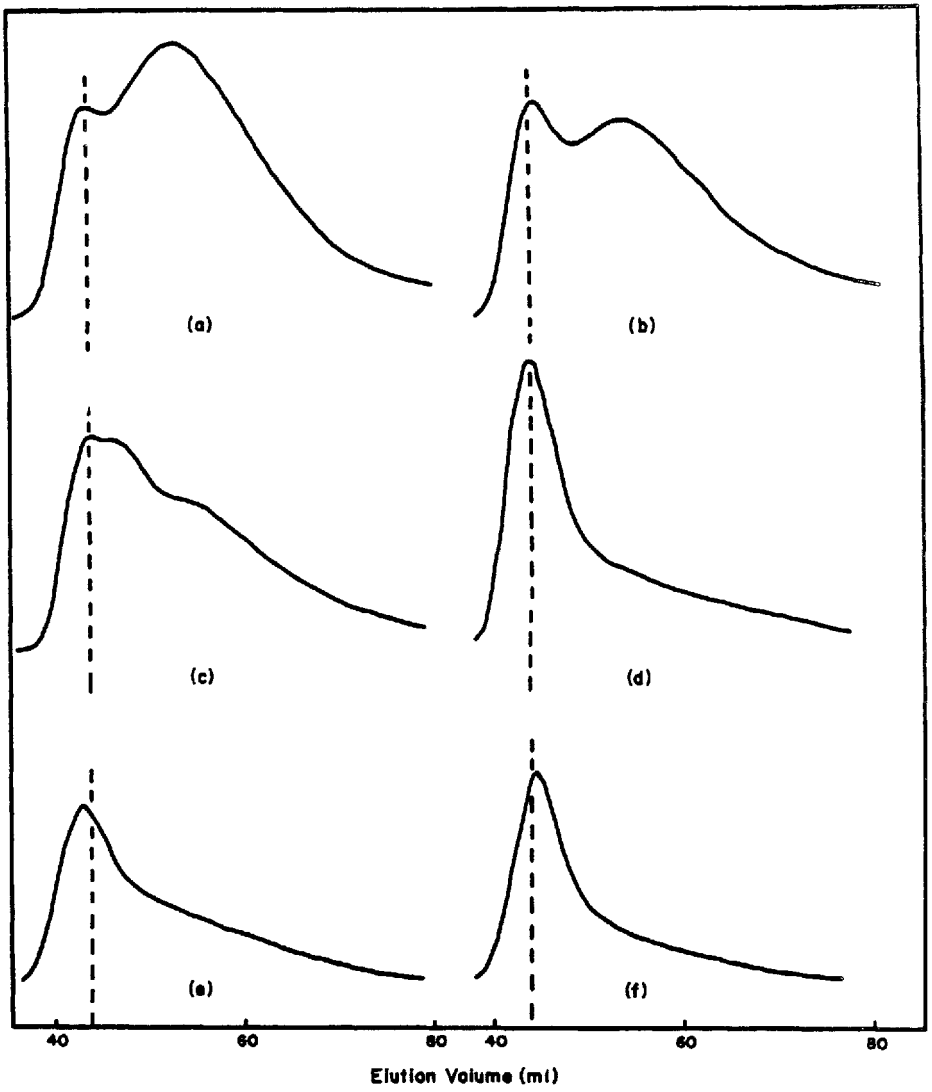


FIG 1. Gel filtration profiles of gluten protein fractions from Gluclub flour. (a) 1st 2 M urea. (b) 3rd 2 M urea. (c) 5th 2 M urea. (d) 7th 2 M urea. (e) 8th 2 M urea. (f) 1st 0.1 M NaOH.

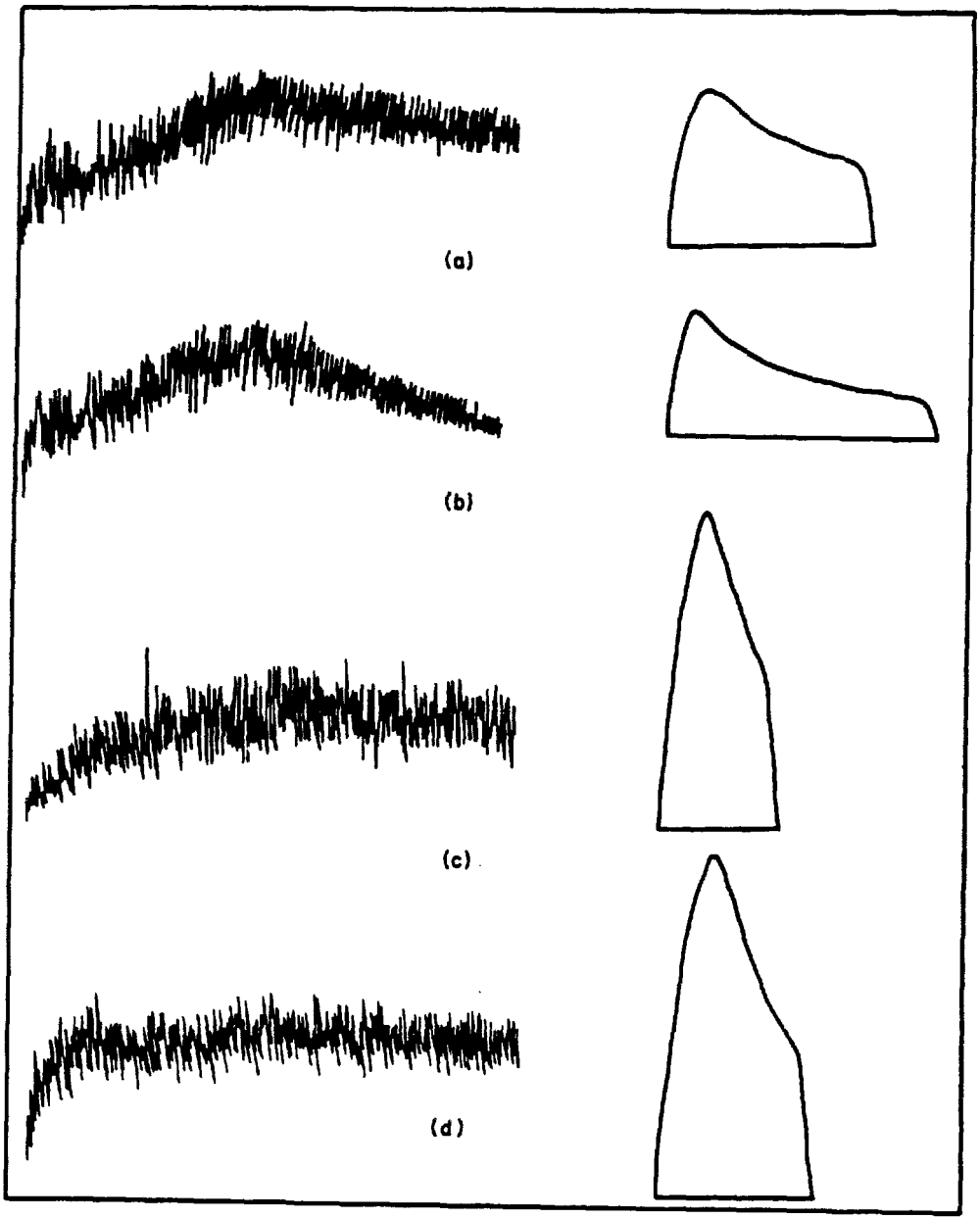


FIG. 2. Effect of additions of gluten protein fractions on Mixographs (left) and Alveographs (right) of a base flour. (a) Base flour . (b) Base flour + 2% 1st 2 M urea extract. (c) Base flour + 2% 7th 2 M urea extract. (d) Base flour + 2% 1st 0.1 M NaOH extract.

to dough properties, small amounts of the fractions (representing about 2% of the total flour weight) were added to a base flour and their effects on Mixograms and Alveograms measured. Some results are shown in Fig. 2. The early extracts which contained largely low molecular weight protein caused an increased rate of breakdown during mixing together with a decrease in dough strength and increased plasticity as measured by the Alveograph. The later extracts had the opposite effects. The results suggest that the rheological properties of doughs are very sensitive to changes in the molecular weight distribution of the gluten protein.

The instruments used in conventional dough testing, although very useful for comparisons between doughs, are not suitable for yielding fundamental information on dough structure. This is because, first, the loading pattern is very restricted and second, the load-deformation curves cannot easily be translated into stress-strain relationships due to the complicated change of geometry of the sample during deformation. Recent studies [6] using apparatus for measuring creep and creep recovery of dough rings have shown that there is no permanent cross-linked network in doughs. In an uncross-linked polymer there is generally a critical value of the molecular weight above which the molecular weight dependence of the rheological properties changes fundamentally due to the contribution of chain entanglements [7]. This value is not known for gluten protein in a wheat flour dough, but results suggest that it could be a value which divides the protein into roughly two equal portions. Stress relaxation or creep measurements on well-characterized gluten fractions should throw light on this aspect.

At present several results point to the importance of entanglement coupling in determining the properties of a gluten. The characteristic mixing curve of a dough could be explained on the basis of the formation and breakdown of an entanglement network. Stronger flours would have a greater proportion of high molecular weight protein, therefore the concentration of entanglement points would be greater and the temporary network slower to be broken down. When doughs are mixed well past their peak development and then allowed to relax, they tend to recover their former properties, consistent with the re-formation of entanglements on removal of stress. A dough made from starch and high molecular weight gluten protein shows the characteristic mixing curve of a normal dough except that the development time is greatly extended and the breakdown rate considerably decreased. On the other hand, starch plus low molecular weight gluten protein does not exhibit the properties of a normal flour dough. It behaves as a cream with little elastic strength and no breakdown in the mixing curve.

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