The Effect of Gluten Protein Fractions on Dough Properties

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ABSTRACT

The protein component of the flours from four varieties of Australian wheats was fractionated by a successive extraction procedure using water, urea solutions, and 0.1M sodium hydroxide (NaOH) solution. The effects of the fractions on dough properties were measured by adding small amounts to a base flour and measuring changes in mixograph and alveograph patterns. Early urea extracts decreased mixing stability and gave weaker, more extensible doughs. Late extracts (urea and Na OH) increased mixing stability and gave stronger doughs. The gel-filtration profiles of the fractions showed that the proportion of high-molecular-weight protein increased with each successive extraction.

Considerable evidence exists on the importance of the protein component of flour in determining baking quality (1,2,3). Although quality is assessed by many tests, wheat varieties can be classified into strong and weak types, depending on the properties of their doughs.

The strong varieties are typified by high mixing stability, which includes long development time and slow breakdown, and alveograms of good height and area. The weak varieties show mixing instability and comparatively small alveograms. The search for a molecular basis to varietal differences has not met with great success, although protein molecular weight (MW) (4) and composition (5) have been implicated. The term "protein quality" is frequently invoked to explain these differences. This term implies that differences in properties of varieties reside in the

differences in composition of the protein, and in particular the gluten protein component.

During preliminary measurements in the present study, it was found that with the successive extraction procedures of Lee (6), the initial extractions of flour with 2M urea gave protein fractions which, when added to a base flour, imparted the properties of softness and extensibility as judged by the alveograph, together with reduction of mixing stability as measured by the mixograph. On the other hand, addition of the residue which remained after these early extractions produced the opposite effects, increased mixing stability and strength. This result suggested that the gluten portion of a flour could be fractionated by a successive extraction procedure, using 2M urea solution. The resulting fractions could then be characterized by their effects on addition to a base flour. Since not all of the protein could be easily extracted with 2M urea, final extractions were made with higher concentrations of urea and with 0.1M sodium hydroxide (NaOH) solution. With the same conditions for extraction as used here, earlier studies had shown almost complete removal of the protein with 2M urea from one sample of flour milled from the wheat variety Gabo (6). The reason for this inconsistency has not been satisfactorily explained, although differences undoubtedly exist in the ease with which protein can be extracted from different flour samples.

MATERIALS AND METHODS

Flour Samples and Protein Extraction

The base flour used for all physical tests was a commercial straight-run sample of 11.1% protein (14% moisture basis). Physical dough tests on this base flour showed it to have well-balanced dough characteristics and normal mixing behavior. Flour samples from which protein was extracted were experimentally milled to approximately 70% extraction from a range of wheat samples obtained from variety trials. The wheats used were Gluclub and Insignia, which are representative of soft and weak Australian varieties; and Gamut and Festiguay, which are hard and strong wheats. Protein was extracted by vigorously stirring 200 g. flour with 800 ml. of solvent in a Sorvall Omnimixer at 7,000 r.p.m. and centrifuging at 8,000 × g. Solvents used were water, urea of various molarities, and 0.1M NaOH. The sequences of solvent extractions are given with the results. All samples were exhaustively dialyzed against water and freeze-dried. Acetic acid-soluble gluten protein solutions which had been standardized by the Kjeldahl method were used for calibration of the absorbance graph. Protein concentration was measured on samples which had been filtered through 0.8 μm Millipore filters by determination of absorbance at 278 nm. A control experiment in which the two water extracts and the first two 2M urea extracts of Gamut flour were added back to the residual flour, after removal of urea and freeze-drying, showed that the mixograph and alveographs were identical to those of the original flour.

Physical Dough Testing

To evaluate the contribution of protein fractions to dough properties, the mixograph and Chopin alveograph were used in the following way. Controls were run by mixing 35 g. base flour with 20 ml. 2.5% sodium chloride solution in the mixograph for 10 min. at 25°C. Where protein was added, 2.2% of the flour was

replaced with freeze-dried extract which was thoroughly pre-mixed with the flour prior to the addition of liquor. Insufficient quantities of protein were available for the normal Chopin alveograph mixer. Doughs were therefore mixed to peak consistency in the mixograph, rounded by hand, rested 10 min., rolled and cut, and rested for a further 20 min. prior to stretching in the alveograph in the usual way.

Gel Filtration

Columns (2.5 X 40 cm.), with upward flow and jacketed at 25°C., of Bio-Gel P-150 polyacrylamide beads (Bio-Rad Laboratories, Richmond, Calif.) were prepared in 0.01M sodium acetate buffer, pH 4.0, containing 2M urea. Protein (10 mg. in 1.0 ml. buffer) was loaded and the flow rate maintained at 1 ml. per min. The column effluent was monitored by the Lowry (7) method on a Technicon Auto-Analyzer.

RESULTS AND DISCUSSION

The yields of protein following extractions of flour samples with water, 2M and 4M urea, and 0.1M NaOH are given in histogram form in Fig. 1. Not all the protein was extracted from the two hard wheats examined, but the total yield from the two soft wheats was close to 100%.

The effect of "early" and "late" urea extracts on mixograph and alveograph patterns is shown in Fig. 2, together with the gel-filtration profiles of the protein present in these extracts. Addition of the first 2M urea extracts from all wheat varieties reduced mixing stability and tolerance and markedly increased alveograph extensibility. The gel-filtration profiles showed that these "early" urea extracts contained the bulk of the low-MW protein which was eluted from the Bio-Gel

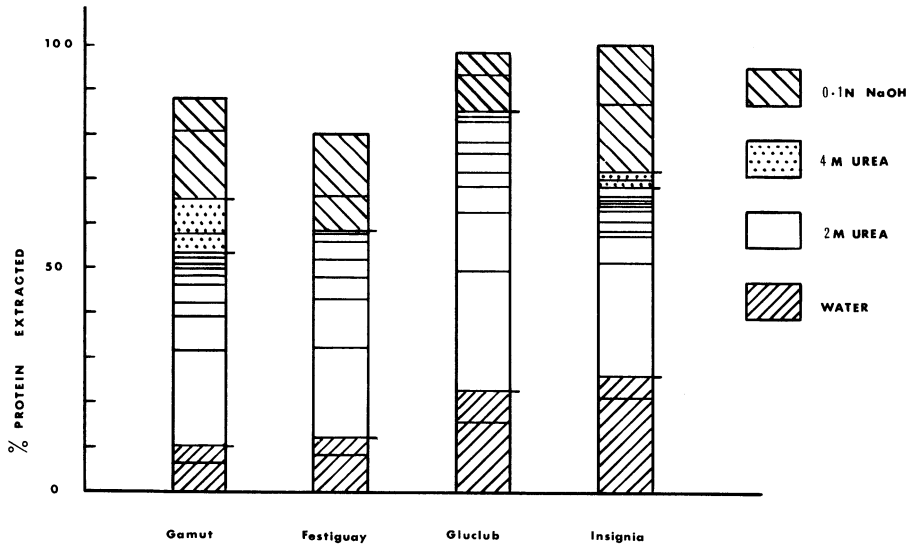

Fig. 1. Extraction of protein from flour by different solvents. The quantity of protein removed by successive extractions is represented by the distance between the horizontal lines on each bar-graph.

columns at a volume greater than the void volume. The later urea and the Na OH extracts all increased mixing stability and increased alveograph height. These extracts contained almost exclusively high-MW protein which on gel filtration was eluted at or near the void volume. It was not possible to make quantitative measurements from the gel-filtration profiles of the proportion of high- and low-MW protein, but the trend to higher MW as the number of urea extractions increased was, nevertheless, clear, as is illustrated in Fig. 2.

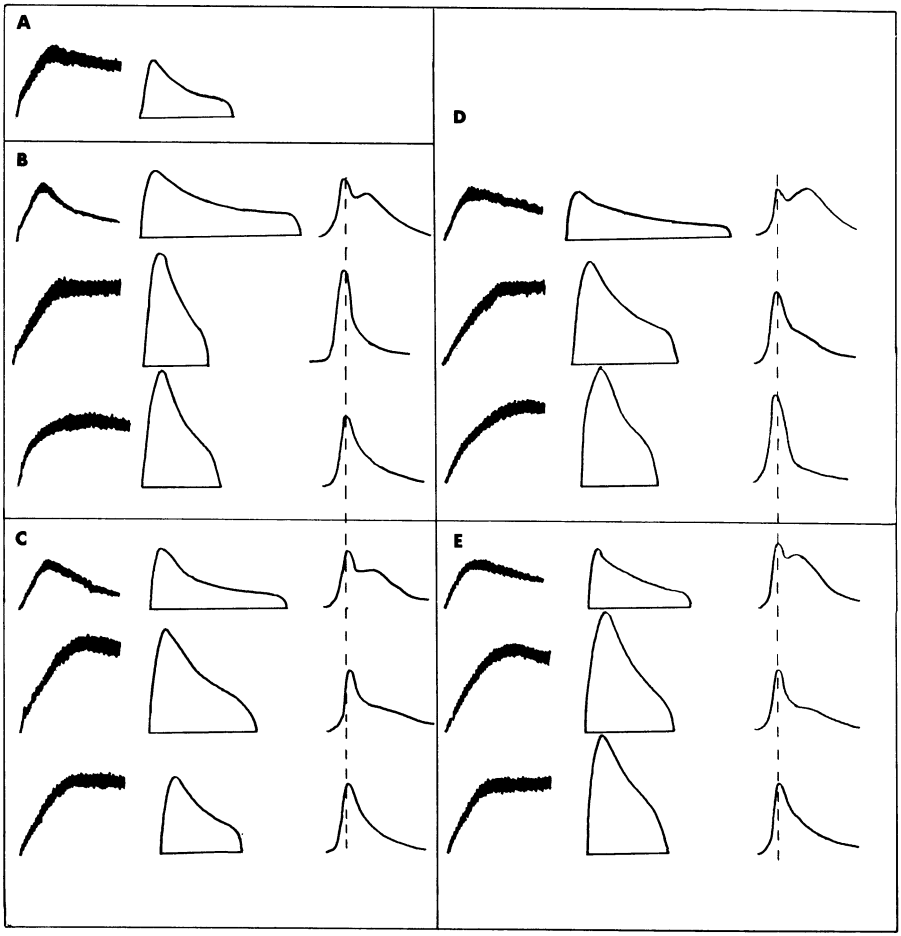

Fig. 2. Mixograph, alveograph, and gel-filtration patterns for protein extracted from different flours. A) Base flour, B) protein from Festiguay flour, C) protein from Gluclub flour, D) protein from Gamut flour, and E) protein from Insignia flour.

For each variety: 1st row = 1st 2M urea extract, 2nd row = combined 6th and 7th 2M urea extracts, and 3rd row = 1st 0.1M NaOH extract.

Mixograph and alveograph curves show the effect of the addition of 2.2% (flour basis) protein. The vertical broken line on the gel-filtration profiles indicates the void volume of the Bio-Gel P-150 column.

GENERAL DISCUSSION

It can be seen from the results that the addition of a relatively small proportion of an "early" extract to a balanced flour of 11.1% protein content produced what would be classed as a weak flour. Addition of the "late" extract increased the apparent strength of the base flour. The effects of these fractions on the rheological properties of doughs appear to be largely determined by the MW distribution of the proteins, although factors such as the SS:SH ratio (8,9) may be involved. Evidence based on gel-filtration studies (4,10) indicates that the "early" and "late" urea extracts correspond closely to gliadin and glutenin, respectively. However, a complete separation into high- and low-MW protein is probably never achieved in the type of extraction procedure used in this work or in the classic separation of gliadin and gluten. This may explain failure to correlate the baking quality of wheat varieties with the gliadin:glutenin ratio.

If we assume that gluten is composed of two main classes of protein, corresponding to "early" and "late" extracts, we see that addition of a small amount of one of them can affect dough properties far more than would be expected from addition of the same amount of unfractionated gluten. Flours milled from different wheat varieties of similar protein content normally differ in quality to a relatively small degree when compared to the effects resulting from the addition of urea extracts reported in this paper. The procedures described in this study were not sufficiently precise to detect a difference between the two strong and the two weak varieties. Apparently we must look for quite small variations in MW or protein composition to account for the commonly observed differences in quality between varieties.

While protein content is probably the most significant single parameter influencing baking quality, the results presented in this paper establish that dough properties are markedly influenced by the MW distribution in this protein. To establish, on a molecular basis, the reason for varietal differences in gluten quality, very precise methods would seem to be required. Such experiments would involve complete extraction of the protein from flour and reliable *quantitative* methods for measuring the MW distribution.

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