NOTE

Solubility of Flour and Gluten Protein in a Solvent of Acetic Acid, Urea, and Cetyltrimethylammonium Bromide, and Its Relationship to Dough Strength[1]

E. KUROWSKA,[2] and W. BUSHUK[3]

Cereal Chem. 65(2):156–158

ABSTRACT

This note presents a simple and accurate procedure for determining protein content of AUC (0.1*M* acetic acid, 3*M* urea, and 0.01*M* cetyltrimethylammonium bromide) extracts and residues of flour and gluten. AUC-soluble protein was determined directly by a modified Biuret assay. AUC-insoluble protein was first dissolved in sodium laurate solution and then analyzed by the Biuret assay. For good accuracy, it was necessary to use separate calibration curves for AUC-soluble flour protein, AUC-soluble gluten protein, and AUC-insoluble flour or gluten protein. Flours (and their glutens) that yield strong doughs contain a higher proportion of AUC-insoluble protein.

A very effective solvent for wheat gluten proteins comprising 0.1*M* acetic acid, 3*M* urea, and 0.01*M* cetyltrimethylammonium bromide in water (AUC) was introduced to cereal chemistry in 1966 by Meredith and Wren. Since that time it has been used widely in research on various aspects of wheat proteins (Huebner and Wall 1976, Bushuk and Wrigley 1971, Zawistowska et al 1985).

Determination of protein in AUC extracts and residues of flour and gluten is impossible by the standard Kjeldahl procedure because the solvent contains a high concentration of nitrogen. Meredith and Wren (1966) determined the protein in AUC-insoluble residues of flour by the biuret method after dialyzing exhaustively the starchy residue against toluene-saturated distilled water. Because of interference by the starch, they calibrated their method by adding known amounts of gliadin to the mixture containing the residue and then estimated the protein content of the residue by extrapolation. Bushuk and Wrigley (1971) determined protein content of the AUC-insoluble residue by first extracting the protein in the residue with 0.1*N* NaOH solution and then estimating the amount of protein in the extract with the biuret method. Unfortunately, the alkaline extraction does not dissolve all of the protein from the residue. Later, the biuret method was successfully modified to determine the protein content of Osborne fractions of flour proteins dissolved in AUC (Noll et al 1974), but the difficulties encountered in determining protein content of AUC-insoluble residue remained unsolved.

This note reports on the use of sodium (Na) laurate solution to

[1]Publication no. 117 of Food Science Department, University of Manitoba.
[2]Present address, Department of Biochemistry, University of Western Ontario, London, ON, Canada N6G 5C1.
[3]Grain Industry Research Group, Food Science Department, University of Manitoba, Winnipeg, MB, Canada R3T 2N2.

dissolve all of the AUC-insoluble protein of flour and gluten for analysis by the biuret method. The procedure was applied to determine solubility of flour and gluten proteins of wheat varieties of widely different dough strength.

MATERIALS AND METHODS

Materials

Seven wheat varieties of widely different dough strength were selected to test the method for determining protein content of AUC extracts and residues. Flour was milled on a Buhler experimental mill and gluten was washed out by the machine-washing method (AACC 1983). Dough strength was assessed with the standard farinograph test (AACC 1983).

Methods

Preparation of calibration curves. The calibration curve for the AUC-soluble protein was prepared by plotting the absorbance values from the modified biuret method (Noll et al 1974) against the protein in the reference sample as determined by the Kjeldahl method. For the Kjeldahl analysis, it was necessary to dissolve the protein of flour (or gluten) with solvents that did not contain any nitrogen. The solubilization was accomplished by the procedure summarized in Figure 1. Protein in residue C was extracted with phosphate-borate buffer containing sodium dodecyl sulfate and β-mercaptoethanol (SDS-ME) (Fullington et al 1980). Most of the starch from flour samples was removed as residue E. Subsamples of the combined, freeze-dried extracts were then used for protein analysis by the modified biuret method and nitrogen analysis by the Kjeldahl method.

The calibration curve for the AUC-insoluble protein was prepared similarly. In this case only supernatants B and D (Fig. 1) from flour were combined, dialyzed, and freeze-dried to obtain the protein for the calibration curve. Subsamples of the freeze-dried material were used to obtain both the absorbance and Kjeldahl nitrogen values for the calibration curve.

Separate calibration curves for flour and gluten were prepared for each wheat variety, because small but different amounts of starch present in the samples affect the biuret assay results (Meredith and Wren 1966).

Sample preparation. Preparation of AUC-soluble and insoluble fractions of flour is summarized in Figure 2. AUC extracts of flour were prepared according to Orth and Bushuk (1973). Supernatant 1F was clarified by centrifugation. Usually small amounts of AUC-insoluble protein remained in residue 1F' after clarification. This residue was combined with the residue 1F. Residue 2F was subjected to total extraction of protein with SDS-ME buffer, pH 8.9, and most of the starch was discarded as residue 3F.

Gluten protein was separated into AUC-soluble and insoluble fractions as in Figure 3. To ascertain the exact amount of protein in the sample, each sample of wet gluten was cut into halves; one half was used to determine protein content by the Kjeldahl method and the other for AUC fractionation.

Determination of protein in AUC extracts and residues. Protein content of AUC extracts of flour (supernatant 1F in Fig. 2) and gluten (supernatant 1G in Fig. 3) was determined by the modified biuret method (Noll et al 1974) using the calibration curves obtained as described above.

For determination of protein in the AUC residue, the protein was first dissolved in Na laurate according to Kobrehel and

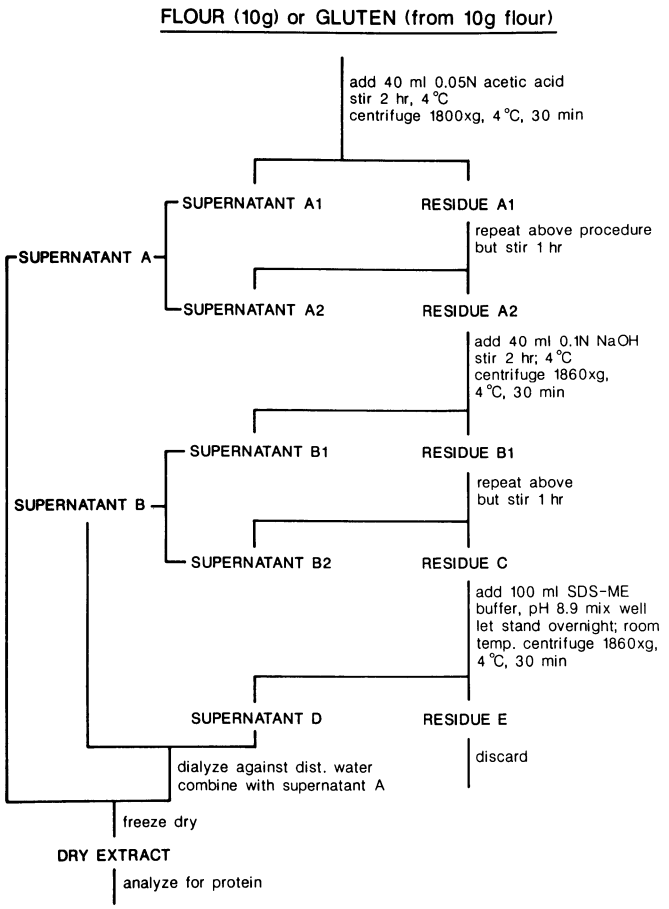
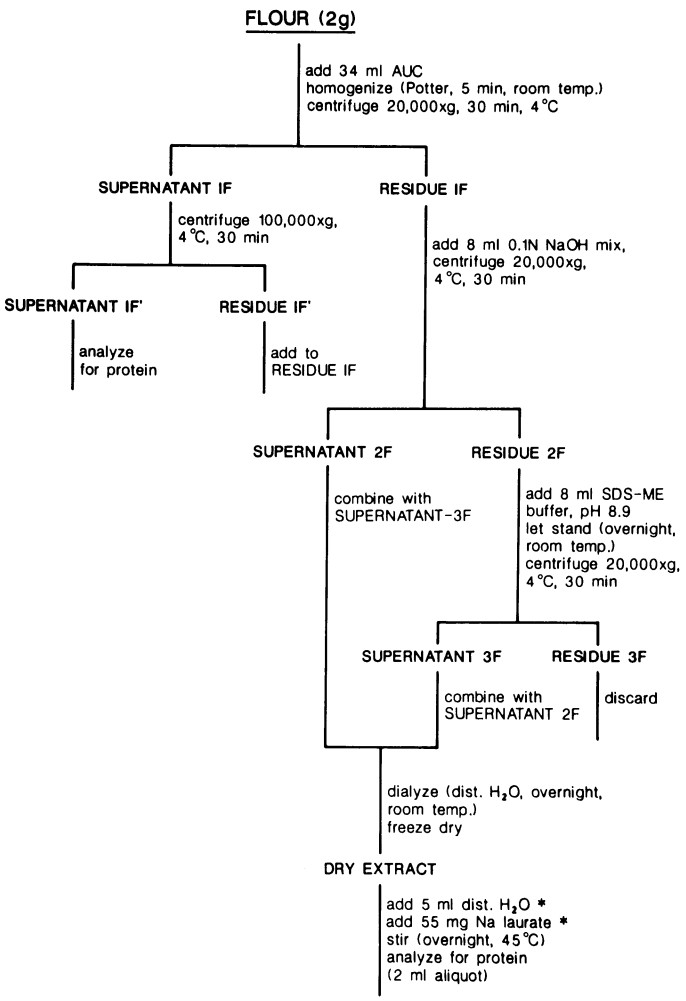


Fig. 1. Preparation of flour and gluten protein extracts for calibration curves for AUC (acetic acid, urea, cetyltrimethylammonium bromide)-soluble and insoluble proteins.



*For Glenlea only, 10 ml H_2O and 110 mg Na laurate

Fig. 2. Preparation of AUC (acetic acid, urea, cetyltrimethylammonium bromide)-soluble and insoluble protein fractions of flour.

Matignon (1980), and then protein in the solution was determined by the biuret method. Optimum conditions for sample preparation, determined by trials using a range of conditions, are indicated in Figures 2 and 3. The important points to take into account are the size of the starting flour sample (must be large enough to yield sufficient protein in the residue for detection by the biuret method), Na laurate concentration (should be high enough to dissolve all of the protein), and use of elevated temperature to improve solubilization (45° C).

RESULTS AND DISCUSSION

Standard Curves

The slopes, intercepts, and correlation coefficients of the regression lines for AUC-soluble flour, AUC-soluble gluten protein, and AUC-insoluble protein (flour) are given in Table I. The correlation coefficient for AUC-soluble protein is slightly lower for flour than for gluten. The probable reason for this is the higher amount of starch present in flour preparations, which might affect protein determination as noted by Meredith and Wren (1966).

Intervarietal Variation in Solubility of Flour and Gluten Protein

The methods for determining protein in AUC extracts and residues described above were used to determine the distribution of proteins between the two fractions for flours and glutens of seven wheat varieties of different dough strength (Table II).

For flours, the lowest amount of soluble protein was obtained for the variety Glenlea. For the other varieties, the proportion of soluble protein is similar except for the slightly higher values obtained for the two soft wheat varieties Fielder and Augusta. For glutens, the differences between strong and weak gluten varieties were accentuated. Glenlea, the variety with the strongest gluten of the group, had exceptionally low solubility (54%). This is probably related to the long dough-mixing requirements of this variety. All varieties, except Glenlea, were characterized by a lower percentage of AUC-insoluble protein in the gluten than in the flour. Presumably gluten washing increased protein solubility in AUC. The proteins of the strong gluten variety Glenlea appear to be particularly resistant to dissociation during gluten washing.

CONCLUSIONS

Methods for determining protein content of AUC extracts and residues of flour and gluten were described.

The methods were applied to determine the solubility in AUC of proteins of seven wheat varieties of different dough strength; both flour and gluten protein solubility was generally higher for weaker wheat varieties. Accordingly, solubility in AUC solvent may be a suitable technological test for distinguishing so-called strong varieties from weak varieties.

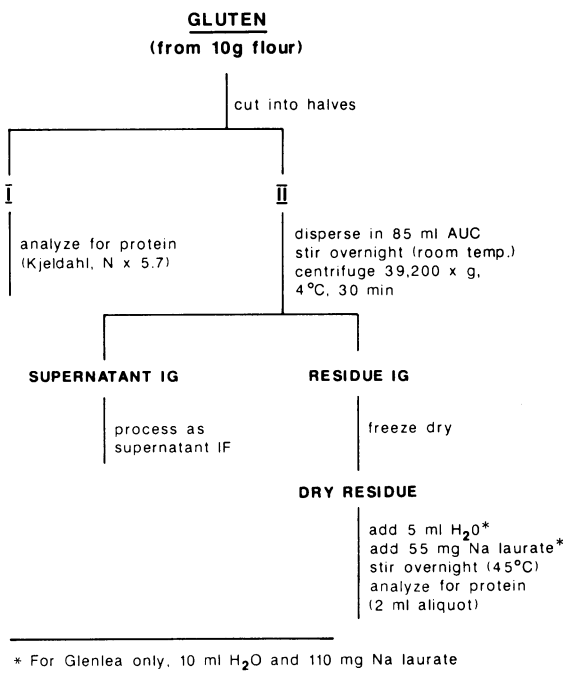


Fig. 3. Preparation of AUC (acetic acid, urea, cetyltrimethylammonium bromide)-soluble and insoluble protein fractions of gluten.

TABLE I
Correlation of Protein Contents Determined by the Biuret and Kjeldahl Methods

Protein	Solvent	Slope	Intercept	Correlation Coefficient[b]
AUC[a]-soluble (flour)	AUC	0.0199	0.020	0.987
AUC-soluble (gluten)	AUC	0.0184	0.021	0.994
AUC-insoluble (flour)	Na laurate	0.0238	0.006	0.997

[a]AUC = Solvent of acetic acid, urea, and cetyltrimethylammonium bromide.
[b]Biuret protein = Dependent variable; Kjeldahl protein = independent variable.

TABLE II
Protein Solubility of Different Wheat Varieties in AUC[a]

Cultivar	Protein (%)	Type of Gluten	Flour Soluble (%)	Flour Insoluble (%)	Gluten Soluble (%)	Gluten Insoluble (%)
Katepwa	12.5	strong	89.0	12.0	93.0	7.0
Glenlea	12.5	very strong	81.0	17.0	54.0	43.7
Marshall	12.4	strong	87.0	11.1	95.0	3.8
Norstar	12.4	medium	88.0	11.6	92.0	6.8
HY-320	9.6	medium	89.0	11.3	95.0	4.2
Fielder	10.2	weak	90.0	7.4	98.0	1.6
Augusta	8.2	weak	91.0	7.4	98.0	1.6

[a]AUC = Solvent of acetic acid, urea, and cetyltrimethylammonium bromide.

LITERATURE CITED

AMERICAN ASSOCIATION OF CEREAL CHEMISTS. 1983. Approved methods of the AACC. Methods 38-11 and and 54-21, approved April 1961. The Association: St. Paul, MN.

BUSHUK, W., and WRIGLEY, C. W. 1971. Glutenin in developing wheat grain. Cereal Chem. 48:448.

FULLINGTON, J. G., COLE, E. W., and KASARDA, D. D. 1980. Quantitative SDS-PAGE of total protein from different wheat varieties. J. Sci. Food Agric. 31:43.

HUEBNER, F. R., and WALL, J. S. 1976. Fractionation and quantitative differences of glutenin from wheat varieties varying in baking quality. Cereal Chem. 53:258.

KOBREHEL, K., and MATIGNON, B. 1980. Solubilization of proteins with soap in relation to the bread-making properties of wheat flours. Cereal Chem. 57:73.

MEREDITH, O. B., and WREN, J. J. 1966. Determination of molecular weight distribution in wheat flour proteins by extraction and gel filtration in dissociating medium. Cereal Chem. 43:163.

NOLL, J. S., SIMMONDS, D. H., and BUSHUK, W. 1974. A modified biuret reagent for determination of protein. Cereal Chem. 51:610.

ORTH, R. A., and BUSHUK, W. 1973. Studies of glutenin. I. Comparison of preparative methods. Cereal Chem. 50:106.

ZAWISTOWSKA, U., BEKES, F., and BUSHUK, W. 1985. Gluten proteins with high affinity to flour lipids. Cereal Chem. 62:284.

[Received August 6, 1987. Revision received December 21, 1987. Accepted December 29, 1987.]