

A Rapid Method for Quantitation of Insoluble Polymeric Proteins in Flour¹

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ABSTRACT

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The baking properties of several genotypes of U.S. hard wheats grown in state nurseries for the Wheat Quality Council (WQC) were analyzed by the Hard Winter Wheat Quality Laboratory. Flours (250 mg) from each individual line and location were extracted three times with 50% 1-propanol (1 mL) for 5 min each. Samples were vortexed continually during extraction. This method was effective in removing most monomeric proteins. Negligible detectable protein was found in the third extract. Significant amounts of polymeric glutenin were also extracted. Pellets were oven-dried (130°C) for 1 hr and analyzed for protein content using

nitrogen combustion analysis. Protein remaining in the pellet consisted mainly of polymeric protein. The amount of gliadin and soluble polymeric protein could also be measured by separating the supernatant by size-exclusion chromatography. Good correlations between dough strength parameters and amounts of pellet protein and the relative amount of pellet protein (pellet protein/flour protein) were found for all samples. This procedure was simple and rapid, with the potential of analyzing large numbers of samples per day with good reproducibility.

Gluten proteins are the major component of wheat flour and are responsible for the ability of flour to form a cohesive dough and thus make bread (Finney 1943). Because of this, many research programs have focused on correlating measurements of wheat protein fractions to bread and dough quality. As technology has progressed, many of these studies have focused on microscale testing that can be done quickly with minimal samples.

While it is known that gluten proteins control the properties of both dough and finished bread, the precise mechanism is still not completely understood. However, several relationships between various forms of gluten proteins, such as the high molecular weight glutenin subunits (HMW-GS) and breadmaking properties have been established (Payne et al 1979). Like the HMW-GS, the role of polymeric proteins is becoming better understood and the relationships between these proteins and dough and mixing strength are becoming well documented.

The largest glutenin polymers typically cannot be extracted from wheat unless reducing agents, acid-base hydrolysis, or sonication are used during extraction. A number of studies have found that the amount of polymeric protein left behind after removal of all other soluble proteins is directly related to quality parameters. Orth and Bushuk (1972) were among the first, finding that acetic acid insoluble glutenins were highly correlated with several breadmaking parameters. Later, Orth and O'Brien (1976) reported a residue test, based on Kjeldahl analysis, for quantifying the acetic acid insoluble glutenins. Other authors, using a wide variety of extraction procedures, have also reported the relationship between the insoluble glutenins and breadmaking properties (MacRitchie 1973, 1978; Huebner and Wall 1976; Field et al 1983; Huebner and Bietz 1985; Chakraborty and Khan 1988).

Recent studies have divided polymeric proteins into two fractions: extractable polymeric proteins and unextractable polymeric

proteins (Dachkevitch and Autran 1989, Gupta et al 1993). It was presumed that the different solubilities of these fractions were due to differences in molecular weight, with the largest being found in the unextractable fraction. In many cases, good correlation exists between the percentage of unextractable polymeric proteins in flours and the dough strength parameters. Thus it is not necessarily the total amount of polymeric proteins that is important, but rather the size distribution of these polymeric proteins (Gupta et al 1993).

The combination of sonication and size-exclusion chromatography (SEC) now provides cereal chemists with an indirect method of characterizing the molecular weight distributions of the polymeric proteins of wheat. However, Ciaffi et al (1996) recently reported that the amount of polymeric protein extracted during sonication may vary significantly in a cultivar-dependent manner, at least in conditions used in that study. In addition, the mechanics of using sonication to extract proteins (reproducibly positioning the probe, cleaning the probe between samples, etc.) were tedious and laborious, and when combined with use of SEC to measure protein levels, very time consuming. For these reasons, we have sought alternate and faster means of analyzing unextractable polymeric proteins of U.S. wheats for routine analysis.

Wieser et al (1994) and Fu and Sapirstein (1996a) demonstrated that 50% 1-propanol could effectively extract all monomeric proteins (albumins, globulins, and gliadins) from flour. Several other articles have also reported this, as well as the fact that aqueous alcohols can also extract soluble polymeric proteins (Kruger et al 1988, Marchylo et al 1989, Singh et al 1991, Zhen and Mares 1992, Fu and Sapirstein 1996a). Based on these earlier works, we tested three different alcohols at a wide range of concentrations, as well as acetic acid and SDS, for their ability to remove polymeric and monomeric proteins. The solvent able to remove all of the monomeric proteins and most of the smaller polymeric protein should leave behind a distribution of polymeric protein consisting of only the very largest polymers in the residue. Nitrogen (protein) in the residue could then be directly analyzed by combustion, thus eliminating the need to extract the insoluble protein and then analyze it. This eliminates the need for sonication and subsequent analysis by SEC and any problems associated with variable protein extractions during sonication, eliminating many potential sources of error. In addition, nitrogen combustion is an extremely rapid technique, requiring ≈ 3 min to analyze a sample.

In this article, we present an extraction scheme and analysis developed for use by the Hard Winter Wheat Quality Laboratory at the Grain Marketing and Production Research Center in Manhattan, KS. This procedure is based on multiple short (5 min) extractions with 50% 1-propanol followed by protein determination using combustion in a nitrogen analyzer. This procedure is simple and rapid, with the potential to analyze large numbers of samples per day with good reproducibility.

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MATERIAL AND METHODS

Flour Samples

Flour samples were from the 1996 Wheat Quality Council (WQC) hard red winter wheat samples ($n = 28$). Flours were milled on a Brabender Quadrumat Senior experimental mill.

Extraction Procedure

Flours (250 mg) were mixed with 1 mL of solvent and the mixture was dispersed mechanically with a spatula. Samples were placed in a vortex stirrer (Vortex Genie2, Scientific Industries, Bohemia, NY) equipped with a 30-place vial holder and vortexed continually for 5 min at an instrument setting of four. Samples were then centrifuged with an Eppendorf 5415C at $8,160 \times g$ for 5 min (2 min can also be used with no change in the data and the procedure is shortened) and the supernatant discarded. This extraction procedure was done a total of three times. When testing effects of different solvents, the first supernatant was kept for analysis by SEC. After extraction, pellets were either prepared for nitrogen analysis or sonicated for SEC analysis.

Pellets to be analyzed by nitrogen combustion were mixed with ≈ 1 mL of acetone to remove residual solvent and facilitate drying. Samples were centrifuged and the acetone discarded. Pellets were broken with a spatula to allow moisture to escape during drying and placed in an oven at 130°C for 1 hr. Dried pellets were analyzed with a LECO FP-428 nitrogen determinator (St. Joseph, MI). Nitrogen values were converted to protein by multiplying by a conversion factor of 5.7.

To extract pellet protein for SEC, the sonication procedure of Gupta et al (1993) was used. Solvent (1 mL) was added and the pellet dispersed with a spatula and vortexed for 5 min. The mixture was then sonicated for 15 sec at 10W (22.5 kHz) with a sonic dismembrator (Fisher Scientific, Pittsburgh, PA).

SEC Analysis

Proteins were analyzed on a Hewlett-Packard 1090A HPLC system using a Waters ProteinPak 300SW SEC column. Separation

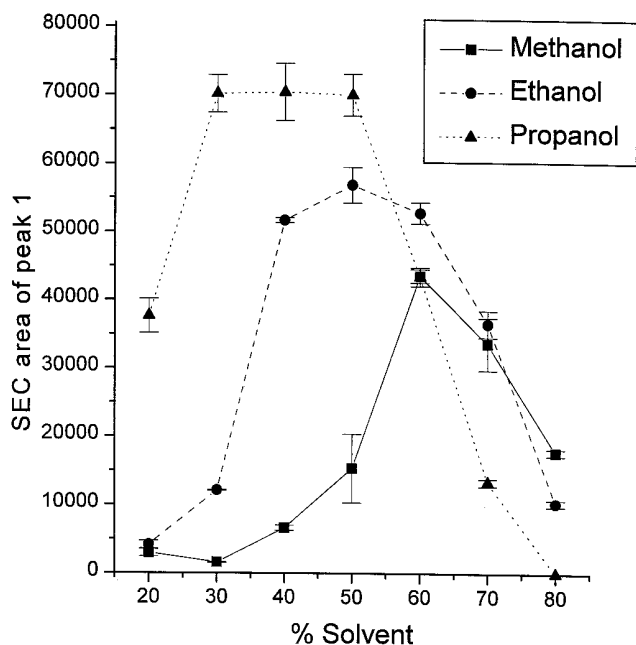


Fig. 1. Area of peak 1 from size-exclusion chromatography (SEC) separation of soluble polymeric glutenin extracted with three different alcohols at concentrations of 20–80%. Error bars indicate standard deviations ($n = 2$). SEC analysis was performed with a Waters ProteinPak 300SW column as utilized by Batey et al (1991).

tion conditions were as described by Batey et al (1991). Column temperature was maintained at 40°C . All samples were filtered through $0.45\text{-}\mu\text{m}$ filters before analysis.

Quality Data

Mixograph and baking tests were performed by the Hard Winter Wheat Quality laboratory personnel as part of the 1996 WQC evaluation program. Procedures are described in detail in the WQC report (1996). Tolerance data was taken from the 1996 WQC report and was the average of tolerance scores obtained from several different laboratories.

Statistical Analysis

Statistical analyses were performed with programs from either SAS Institute (Cary, NC) or MicroCal Origin (Northampton, MA).

RESULTS AND DISCUSSION

Protein Extraction

Three different alcohols (methanol, ethanol, 1-propanol) were tested for their effectiveness in removing both monomeric and soluble polymeric protein. The cultivar TAM 105 was used for all solvent tests. All solvents tested extracted some levels of polymeric material (peak 1) as well as various amounts of other monomeric protein (referred to as peak 2). For each solvent, the amount of soluble polymeric material extracted exhibited roughly bell-shaped curves. 1-Propanol was most effective at extracting soluble polymeric protein (Fig. 1) and reached a maximum at 30–50% (v/v), with nearly constant amounts across that entire range (Fig. 1). Ethanol reached a maximum extraction at 50%, while methanol reached a maximum at 60%. At their maxima, both methanol and ethanol extracted substantially less polymeric protein than did 1-propanol (Fig. 1). Since little soluble polymeric protein was extracted at 70% 1-propanol (Fig. 1), this data cor-

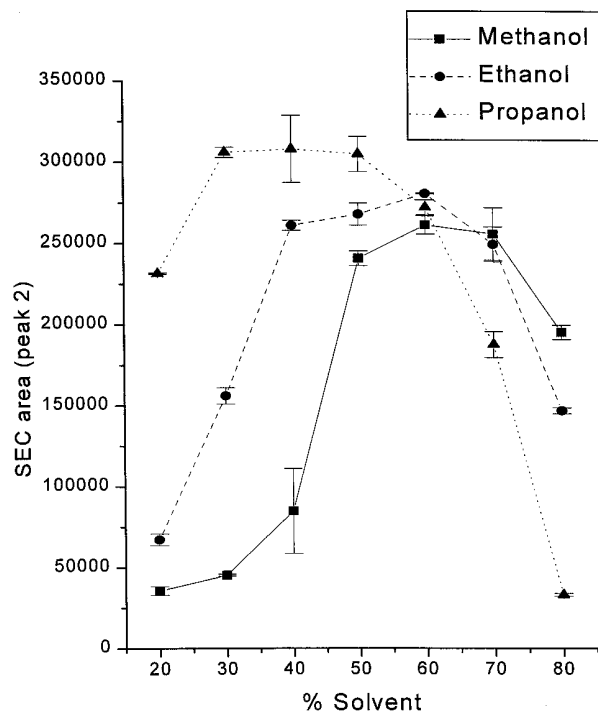


Fig. 2. Area of peak 2 (all nonpeak 1 material) from size-exclusion chromatography (SEC) separation of soluble polymeric glutenin extracted with three different alcohols at concentrations of 20–80%. Error bars indicate standard deviations ($n = 2$). SEC analysis was performed with a Waters ProteinPak 300SW column as utilized by Batey et al (1991).

roborates that of Fu and Sapirstein (1996a), who reported that polymeric protein could be precipitated from 50% 1-propanol extracts by increasing the amount of propanol in the sample to 70%.

Each alcohol was also investigated for its ability to solubilize monomeric proteins (peak 2). Again, 1-propanol was most effective in this regard, again reaching a maximum at 30–50% (Fig. 2). Both methanol and ethanol showed maximum effectiveness at solubilizing monomeric proteins at 60% (Fig. 2). This data, in general, follows that of Wieser et al (1994). An interesting observation of this data is that 50% methanol extracts nearly as much monomeric protein as does 50% 1-propanol, but extracts very little soluble polymeric protein (Fig. 2). Thus, 50% methanol may be the best solvent for extracting monomeric proteins (such as gliadins) with little contamination by soluble glutenin.

Because 30–50% 1-propanol extracted the most soluble polymeric protein, 50% 1-propanol was chosen for further testing. Initially, single extractions with 50% 1-propanol were used to remove monomeric proteins and soluble polymeric protein. However, we found the reproducibility of a single extraction to be very poor (5–15% relative standard deviation [RSD] *unpublished data*). To maintain a high level of reproducibility, a protocol based on multiple extractions was therefore developed. However, multiple extractions significantly lengthen the time of the procedure. A compromise was reached by using multiple (three) short (5-min) extractions. Although many published extraction schemes call for extensive extraction time periods or very high solvent-to-flour ratios, we have found that the majority of protein can be extracted in as little as 5 min and at a flour-to-solvent ratio of 1:4. Figure 3 shows SEC patterns obtained from analysis of three consecutive 5-min extracts (using 50% 1-propanol) of the cultivar TAM 105. The majority of soluble protein ($\approx 95\%$) is removed with the first 5-min extraction (Fig. 3). By the third extract, little soluble protein is left ($<1\%$) (Fig. 3).

The sonication technique of Gupta et al (1993) was used to solubilize proteins remaining in the pellet for characterization by SEC. Analysis of the sonicated sample by SEC showed essentially only polymeric protein (peak 1), demonstrating that the multiple 50% 1-propanol extracts were removing essentially all monomeric

proteins (Fig. 4). Similar results were reported by Fu and Sapirstein (1996).

Gupta et al (1993) also demonstrated that protein remaining after a brief SDS extract consisted mainly of polymeric protein. Acetic acid was used in a similar method by Orth and O'Brien (1976) to remove proteins for a residue test. Thus, solutions of SDS (2%) and acetic acid (200 mM) were also tested and compared to the results obtained using 50% 1-propanol. All solvents produced similar results, however SDS extracted the most soluble polymeric material: $\approx 16\%$ more than 50% 1-propanol and $\approx 17\%$ more than acetic acid. We have also found that some polymeric protein remains soluble after the three 50% 1-propanol extractions. Even though SDS extracted the most soluble polymeric proteins, we selected 50% 1-propanol. When using SDS buffer or acetic acid in the multiple extraction scheme, there were difficulties in drying the pellets for nitrogen combustion. When either was used, the residue contained two layers, with the top layer containing a large amount of light yellow sticky material that swelled considerably and retained significant amounts of moisture after extraction. These samples tended to explode during oven drying as moisture trapped in this layer escaped. These layers also separated during drying, making it difficult to reproducibly remove the material for nitrogen analysis. To try and overcome the swelling problem, we utilized a procedure consisting of two 50% 1-propanol extracts followed by an extraction with 2% SDS. While this did result in less swelling, some problems were still encountered when using both solvents.

We were able to obtain useable results with this procedure, though the variance of the measurements was almost twice as high with some samples (data not shown). When using this dual solvent system, $\approx 15\text{--}20\%$ more polymeric protein appeared to be extracted. However, correlations to various quality parameters were virtually the same, and in some cases, lower than when using only 50% 1-propanol. Thus, no real benefit was gained by trying to incorporate SDS into the procedure, and the variance of the measurements was increased. This does point out the fact that 50% 1-propanol insoluble polymeric proteins probably do not have the same molecular weight distribution as SDS-insoluble poly-

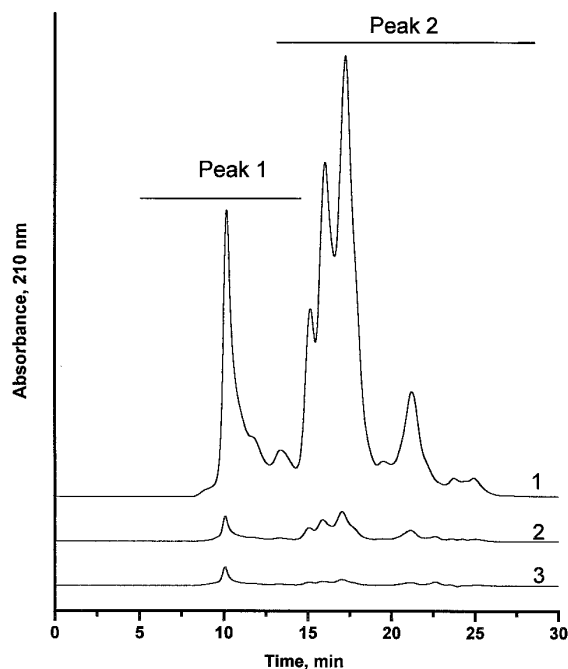


Fig. 3. Size-exclusion chromatography (SEC) patterns of three multiple 50% 1-propanol extracts of TAM 105. Extractions one, two, and three are labeled in sequence. SEC analysis was performed with a Waters ProteinPak 300SW column as utilized by Batey et al (1991).

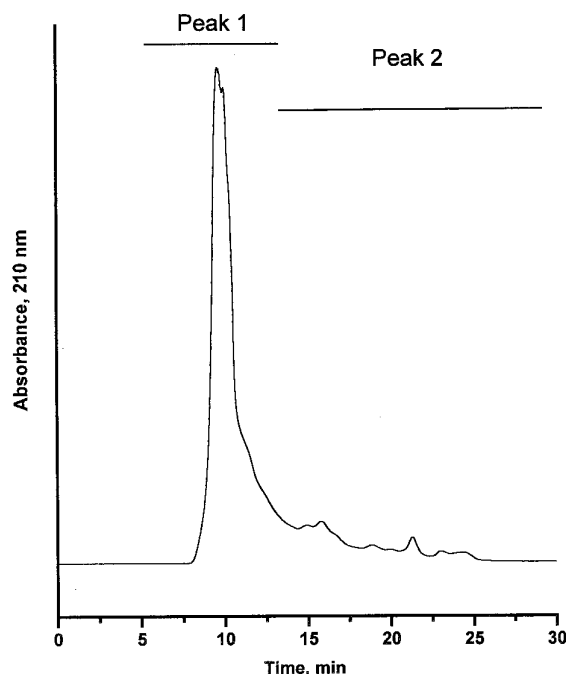


Fig. 4. Size-exclusion chromatography (SEC) pattern of sonicated extract from pellet after three 50% 1-propanol extracts. SEC analysis performed with a Waters ProteinPak 300SW column as utilized by Batey et al (1991).

meric proteins. The SDS-insoluble polymeric proteins should consist of polymers that are slightly larger than the 50% 1-propanol. In any case, 50% 1-propanol performed well in this procedure and accomplished the goals of this project.

TABLE I
Reproducibility Data from Nitrogen Combustion Analysis of Consecutive Extracts of TAM 105

	Pellet Protein (%)		
	Replicate 1	Replicate 2	Replicate 3
	6.906	6.922	6.904
	6.951	6.712	7.025
	7.016	6.647	6.706
	6.792	6.827	6.897
	6.888	6.913	6.805
	6.872	6.888	7.095
	6.947	6.829	6.935
	6.875	7.028	6.967
	6.821	6.866	6.511
	6.828	7.069	6.836
	7.022	6.934	6.932
	7.109	7.154	7.155
	6.867	6.933	6.960
	6.951	7.272	6.933
	7.023	6.989	7.222
Average	6.924	6.932	6.926
Standard deviation (SD)	0.089	0.159	0.174
Relative SD, %	1.29	2.29	2.52

Urea, which would also be a good solvent to use, was not tested for fear of residual urea in the pellet interfering with the nitrogen analysis. Additionally, Fu and Sapirstein (1996a) described a method for precipitating polymeric glutenin extracted in 50% 1-propanol for further study, making 50% 1-propanol an attractive solvent to use, should it be necessary to study soluble polymeric proteins in future projects.

Reproducibility

To test the reproducibility of the extraction procedure, three sets of 15 samples of TAM 105 were extracted on three separate days. Results are presented in Table I. RSD (%) ranged from 1.29–2.52% for all three sets, demonstrating the reproducibility within a given extraction set. To test reproducibility between sets, four sets of 1996 WQC samples were extracted on separate days by two different laboratory personnel. Results are shown in Table II. RSD (%) varied from <1 to 4.8% for the 28 samples.

Quality Correlations

To test the methodology, the amounts of insoluble polymeric proteins from 28 samples were correlated with several baking quality parameters (Table III). Relative amount of insoluble polymeric protein was determined by dividing the insoluble polymeric protein value by flour protein (%) (Gupta et al 1993). Flour protein was highly correlated to the amount of insoluble polymeric protein, that is, a flour with high protein will also produce high levels of insoluble polymeric protein. However, the relative amount of insoluble polymeric protein (insoluble polymeric protein/flour protein)

TABLE II
Reproducibility Data from Nitrogen Combustion Analysis Gathered from Four Replicate Data Sets

Sample	Set 1	Set 2	Set 3	Set 4	Average	Standard Deviation	Relative Standard Deviation (%)
1	5.796	5.786	6.206	6.116	5.976	0.217	3.6
2	5.338	5.003	5.606	5.444	5.348	0.255	4.8
3	5.716	5.517	5.795	5.382	5.603	0.188	3.4
4	5.802	6.042	6.136	5.928	5.977	0.144	2.4
5	5.585	5.398	5.688	5.510	5.545	0.122	2.2
6	5.822	5.585	5.729	5.698	5.709	0.098	1.7
7	6.218	6.428	6.128	6.109	6.221	0.146	2.3
8	6.088	6.083	6.240	6.221	6.158	0.084	1.4
9	9.298	9.152	9.408	9.174	9.258	0.119	1.3
10	8.105	8.042	8.550	8.381	8.270	0.238	2.9
11	7.786	7.827	7.880	7.889	7.846	0.048	0.6
12	6.677	6.595	6.595	6.553	6.605	0.052	0.8
13	7.838	7.981	8.033	8.066	7.980	0.101	1.3
14	8.801	8.957	9.249	8.971	8.995	0.186	2.1
15	6.971	7.355	7.009	6.657	6.998	0.286	4.1
16	7.860	7.723	7.836	7.589	7.752	0.124	1.6
17	6.248	6.094	6.314	5.769	6.106	0.243	4.0
18	6.862	6.591	6.718	7.244	6.854	0.283	4.1
19	6.234	6.219	6.253	5.972	6.170	0.132	2.1
20	6.320	6.621	6.330	6.187	6.365	0.183	2.9
21	8.553	8.108	8.277	8.163	8.275	0.198	2.4
22	7.351	7.402	7.579	7.469	7.450	0.098	1.3
23	7.908	7.832	8.063	8.058	7.965	0.114	1.4
24	6.204	5.875	6.018	6.072	6.042	0.136	2.3
25	6.287	6.240	6.419	6.431	6.344	0.095	1.5
26	6.032	6.081	5.989	6.152	6.064	0.070	1.2
27	7.011	6.579	6.930	6.674	6.799	0.205	3.0
28	6.175	6.293	6.373	...	6.280	0.100	1.6

^a Sample lost during processing

TABLE III
Correlation Coefficients (r) for Various Protein Measurements and Selected Quality Parameters^a

	Flour Protein	Loaf Volume	Bake Mixtime	Water Absorption	Mixing Tolerance
Flour protein	...	0.40	0.67	0.69	0.74
Pellet protein	0.94	0.74	0.82	0.71	0.84
Relative amount of pellet protein	0.54	0.41	0.84	0.53	0.75

^a Hard red winter wheat samples (n = 28) from the 1996 Wheat Quality Council. All numbers significant at P ≤ 0.10.

was only weakly correlated with flour protein. Thus, a cultivar with high flour protein does not necessarily produce a large percentage of its protein as insoluble polymeric proteins. Relative amount of polymeric protein is therefore a measure of protein quality, and not simply an extension of protein content (Gupta et al 1993).

Both flour protein and amount of insoluble polymeric protein were well correlated to water absorption and mixing tolerance, but the amount of insoluble polymeric protein was more highly correlated to mixing tolerance than was flour protein ($r = 0.84$ vs. 0.74). Insoluble polymeric protein was also well correlated to loaf volume, much higher than either flour protein or relative amount of insoluble polymeric protein. Both percent and raw amount of insoluble polymeric protein were highly correlated to bake mix time (Table III).

Gupta et al (1993) reported that the relative amount of insoluble polymeric protein (in flour or in total protein extracted) was a more reliable indicator of dough strength properties than total polymeric protein or total insoluble polymeric protein. Our results corroborate those of Gupta et al (1993), as the relative amount of insoluble polymeric protein measured in this study was highly correlated to bake mix time in 28 U.S. wheats. However, we did find that mixing tolerance was most highly correlated with the amount of pellet protein and not to the relative amount. Also, the loaf volume, in the samples used here, was most highly correlated to the amount of insoluble polymeric protein, not the relative amount of these proteins. Gupta et al (1993) found that total polymeric proteins, not percent unextractable, was more highly correlated to loaf volume, although this was somewhat dependent on the baking method.

Soluble Proteins

As a side note, in many research projects it may be desirable to measure the amounts of gliadin as well as soluble polymeric proteins in addition to the amount of insoluble polymeric protein. Fu and Sapirstein (1996a) developed methodology for this, although that method requires that soluble glutenin be precipitated away from gliadin, and both fractions analyzed separately. While this may be desirable in some cases, it may not be well suited for processing large numbers of samples. Gliadins and soluble glutenins can easily be quantified during the procedure described in this article through the use of SEC.

To accomplish this, the first two 50% 1-propanol extracts are simply pooled together and analyzed using SEC. These two extracts contain $\approx 99\%$ of all soluble proteins, the third extract, which contains little to no protein is simply discarded. For the most part, SEC separates the soluble glutenins easily from the gliadins (Gupta et al 1993) and the amounts of these two protein classes can be easily obtained from the chromatograms. This does require the use of SEC, which has certain drawbacks as discussed earlier. By pooling the first two extracts, the reproducibility is fairly good, with an RSD of $<5\%$ for both the gliadins and soluble polymeric proteins.

Using two different instruments to measure the amount of gliadins, soluble polymeric proteins, and insoluble polymeric proteins, respectively, does present a problem when trying to compare the data. To overcome this problem, we have developed a procedure to convert all measurements into milligram quantities. From these numbers, it is simple to convert measurements into percentages for easy comparison among the various protein classes.

The first step is to convert the amount of insoluble polymeric protein (residue protein) from a percentage to milligrams. This is done by multiplying the weight of the pellet (which is determined during LECO analysis) by the percent of protein in the pellet. The second step, calculating the amount of total protein in milligrams, is done by multiplying the amount of starting flour (250 mg) by the total flour protein. The next step is to subtract the amount of insoluble polymeric protein (mg) from the amount of total protein (mg). This difference is the total amount of soluble protein in mil-

ligrams and is equivalent to the total area of the SEC chromatogram of the soluble proteins. Finally, the percentage of gliadin and percentage of soluble glutenin in the total soluble protein is determined by dividing the peak area of each fraction by the total area under the SEC chromatogram. Each percentage is then multiplied by the total soluble proteins (mg) and gives amounts (mg) of gliadins and soluble glutenins. Now all three classes of proteins are represented in milligrams, which makes direct comparisons between the classes possible. By dividing the amount of each class of protein, in milligrams, by the total protein, in milligrams, the percentage of each class of protein is readily determined (Table IV). These calculations are easy to do and can be performed in only minutes with any commercial spreadsheet software program.

The values for each class of protein presented in Table IV are similar to those reported by Fu and Sapirstein (1996b) using their two-step precipitation procedure. Note that Fu and Sapirstein (1996b) reported only total monomeric proteins, that is, albumins and globulins were quantified along with gliadins and, accordingly, the values they report for total monomeric proteins are higher than we have reported for gliadins in this article. Similarly, the amount of insoluble glutenin reported here is slightly higher than that reported by Fu and Sapirstein (1996b) as these authors split this category of proteins into insoluble glutenins and residue protein. The values reported in Table IV also match those reported in Lookhart (1991).

The amount of insoluble polymeric protein reported in Table IV is higher than that reported by either Ciaffi et al (1996) or Gupta et al (1993). This is most likely due to the fact that SDS will extract higher amounts of polymeric protein as noted earlier in this article.

It should be noted that the error margin is slightly higher when converting LECO values to milligrams. This is because the milligram data is more sensitive to slight measurement errors, whereas the LECO data, which is a percentage, is not affected by small measurement errors. Finally, when converting to milligrams, the LECO data overestimated the amount of insoluble glutenins by $\approx 10\%$ relative to the milligram data. This is due to changes in the weight of the pellet analyzed following the multiple extraction procedure. However, correlations are essentially identical whether using LECO or milligram data, and LECO values can be safely used for predicting quality attributes. However, the LECO values (especially LECO residue protein/flour protein) should not be compared to values obtained by other methodologies. This problem is alleviated when using milligram data.

CONCLUSIONS

It has been well established that an important parameter in breadmaking properties of wheat lies in the molecular weight distribution of polymeric proteins. However, no method for extraction and analyzing the native state of the polymeric protein exists. Differential solubility combined with SEC has been utilized as an indirect method for estimating the molecular weight distribution with great success. Recent studies using SEC have found good correlations between amounts of insoluble polymeric protein and dough strength and mixing parameters (Gupta et al 1993, Ciaffi et al 1996). Insoluble polymeric protein thus serves as a reliable biochemical marker for predicting some dough and mixing properties. Our results show that similar relationships can be found by simply

TABLE IV
Amounts of Protein in Various Protein Fractions

	Gliadins	Soluble Polymeric Protein	Insoluble Polymeric Protein
Average, %	34.75	12.05	41.19
Standard deviation, %	4.33	1.92	2.62

removing monomeric proteins and soluble polymeric proteins and analyzing the remaining proteins by nitrogen combustion.

Multiple, short (5-min) extractions with 50% 1-propanol were effective in removing the majority of monomeric and soluble polymeric proteins. The proteins remaining insoluble were primarily unextractable polymeric proteins, and the amounts were well correlated with a number of dough strength and mixing properties of selected U.S. hard red winter wheats. The developed extraction procedure is simple to perform and, combined with rapid protein analysis (≈ 3 min/sample), allows for large numbers of samples to be reproducibly processed and analyzed daily.

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