

The Influence of Protein Composition on Spaghetti Quality¹

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ABSTRACT

Research was conducted to establish the influence of protein composition on quality by investigating the proteins of eight wheat varieties which represented a wide range of spaghetti properties. Proteins from each wheat variety were separated by quantitative Maes column extraction and Sephadex G-200 gel-filtration chromatography into general classes (albumins, globulins, gliadins, glutenins, and base-solubles). To show relations between protein fractions and quality factors, analysis of variance and regression analysis of the data were conducted. The resulting correlation matrix indicated that protein composition was related (significant at the 1% level) to several spaghetti-quality factors. Poor spaghetti color was shown for varieties which had high albumin and glutenin contents. Moreover, high spaghetti firmness was associated with high glutenin but low gliadin contents. In addition, a new method for precise measurement of cooked spaghetti firmness was developed. To test for firmness, the shearing characteristics of cooked spaghetti were measured with a specially equipped Instron Testing Instrument. The work (g. X cm.) required to shear a single cooked spaghetti strand was used as a measure of firmness.

Amber durum is a specialty class of wheat which is the raw material of choice for production of high quality spaghetti and other pasta products. Spaghetti made from good quality durum semolina is bright yellow in the dry form, and when cooked, resists disintegration and has a firm texture. Many factors, such as processing conditions, age, handling, and storage can influence spaghetti quality. However, one of the most important factors determining quality is wheat variety. It is known that certain durum wheat varieties result in spaghetti of distinctly superior quality when compared with other similarly grown and processed varieties. Although similar to the poor quality varieties in total protein content, the superior varieties apparently have a unique composition which results in better quality spaghetti.

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Recent workers have suggested that wheat proteins influence spaghetti quality. Sheu et al. (1) interchanged the biochemical constituents of durum and hard red spring wheat. The results showed that the gluten as well as the water-soluble fractions of durum added to the yellow color of macaroni. Furthermore, the gluten fraction showed a marked influence on cooking quality. In a subsequent publication, Matsuo and Irvine (2) demonstrated the influence of gluten strength on spaghetti cooking quality. Through reconstitution studies, gluten from semolina was shown as a major factor which determined the tenderness of cooked spaghetti. Gluten of medium strength, not as strong as the type needed in bread dough, reportedly resulted in cooked spaghetti of optimum tenderness.

In the present study, the influence of wheat-protein composition on spaghetti quality was investigated. The major protein fractions (albumins, globulins, gliadins, and glutenins) were quantitatively separated by classes according to solubility and molecular size. Eight wheat varieties which represented a wide spectrum of spaghetti quality were tested, and statistical analysis of analytical results was conducted to reveal relations between protein composition and spaghetti quality.

MATERIALS AND METHODS

Samples

Samples of eight pure durum and common wheat varieties were grown under comparable conditions at the North Dakota State University Agricultural Experiment Station at Langdon, N. D. Included were Leeds and Mindum, amber durum varieties known for good quality; Stewart 63, a Canadian amber durum variety; Yuma, Golden Ball, and Peliss, poor quality amber durum varieties; Pentad, a red durum used primarily for feed; and Selkirk, a hard red spring wheat used generally for bread baking and included for comparative purposes.

Quality Tests

Semolina or farina was experimentally milled from each variety and stored at 5°C. prior to testing. Spaghetti was processed in duplicate from each semolina by extruding the pasta through a 0.073-in. brass spaghetti die with a previously described method (3).

Spaghetti color scores were determined by the light-reflectance method of Walsh et al. (4) on a Hunter Color Difference meter, Model D 25, equipped with a D 25-A optical unit. To measure color, the entire 2-in. diameter specimen area was covered with spaghetti. Reflectance readings were taken against a black background. Reflectance values were converted to color scores by using a spaghetti color map (5).

In the cooking tests, spaghetti (10 g.) was cooked 20 min. in boiling water (300 ml.), removed, and drained. Cooking water was evaporated to dryness in an air oven at 95°C. and the remaining solids weighed to determine cooking losses. Cooked strands of spaghetti were placed in 20°C. water to cool for 10 min. prior to taking firmness measurements.

Firmness of cooked spaghetti was measured with a specially equipped Instron Universal Testing Instrument, Type TM (Fig. 1). The instrument consisted of a drive mechanism which moved a crosshead in a vertical direction by means of twin screws, a load-sensing cell (Type CB), a strip chart recorder which was



Fig. 1. Instron Universal Tester (Type T.M.) equipped with a special "tooth" used in firmness measurements of cooked spaghetti.

synchronously driven with the crosshead drive system, and an automatic integrator. To measure firmness, a single strand of cooked spaghetti was placed on a Plexiglas plate covering the load-cell table and sheared at a 90° angle by lowering a specially designed Plexiglas tooth (Fig. 2) at the rate of 0.018 cm. per sec. A continuous recording and integration of force vs. distance was made during the operation and the area ($\text{g.} \times \text{cm.}$) of the shearing curve was considered a measure of spaghetti firmness. When sheared with a tooth of this design, firm spaghetti gave a high sharp peak (force vs. distance curve), whereas soft or "mushy" spaghetti showed a low peak. When tough strands were sheared, however, a rather wide peak resulted, which indicated that a portion of the spaghetti was crushed beneath the flat edge of the tooth.

Protein Extraction

The proteins derived from semolina or farina were extracted according to solubility classes with the column-extraction method described by Maes (6) which was modified for use with semolina. Powdered semolina (20 g.) was ground in a mortar with Filteraid (20 g.) blended thoroughly with sand (240 g. Fisher S-25) and water (150 ml.). The mixture was deaerated and poured into a chromatographic column (3.0 \times 30.0 cm.). Elution of protein fractions was accomplished with the step-wise gradient procedure described by Mattern et al. (7) with the following solvent sequence: water, 200 ml.; 2% NaCl, 200 ml.; water, 100 ml.; 40% isopropanol, 200 ml.; water, 100 ml.; 3.85% lactic acid, 200 ml.; water, 100 ml.; and 0.5% KOH, 200 ml. The progress of the extractions was followed continuously

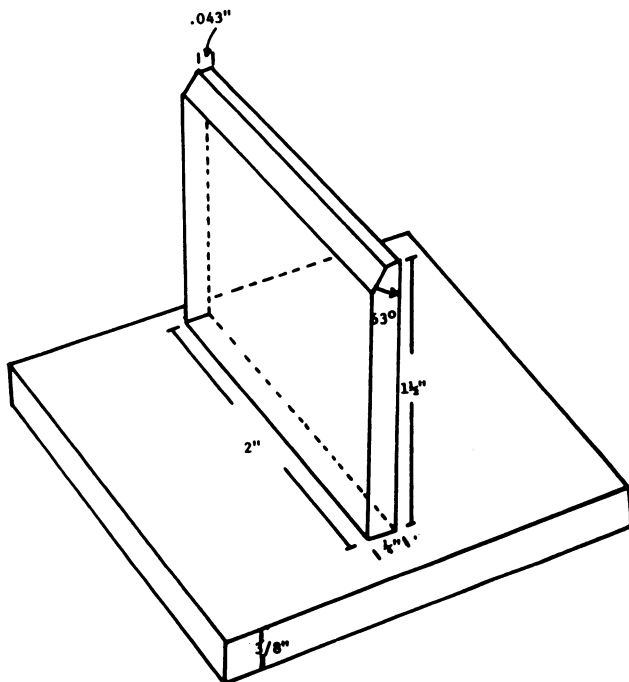


Fig. 2. Plexiglas "tooth" used for shearing cooked spaghetti in firmness determinations.

with a recording U.V. monitor (Gilson S.B.). Protein fractions were collected, dialyzed at 4°C., freeze-dried, and measured for protein content by the micro-Kjeldahl procedure (8).

Gel Filtration

To separate proteins into classes according to molecular size, the Sephadex gel-filtration method described by Meredith and Wren (9) was modified for use with semolina and farina extracts. Semolina or farina (6 g.) was blended with 60 ml. AUC solvent (0.1M acetic acid, 3M urea, and 0.01M cetyltrimethylammonium bromide in water) in a Waring Blender for 2 min. at high speed. The mixture was centrifuged for 30 min. at 140,000 \times g to remove undissolved solids and haze. Three milliliters of clear centrifugate was applied to a column of Sephadex G-200 (1.75 \times 56 cm.) and eluted with the AUC solvent. Fractions were analyzed at 280 nm. and the absorbance converted to protein content with standard curves derived from micro-Kjeldahl determinations for each fraction.

RESULTS AND DISCUSSION

The average quality data for the wheat varieties investigated are shown in Table I. As indicated earlier, the varieties selected represented a wide range of spaghetti quality. Leeds showed the best overall quality, followed by Mindum and Stewart 63, which had lower color scores but were otherwise similar to Leeds. In the next lower category for quality were Golden Ball, Yuma, and Peliss. Yuma and Peliss

TABLE I. SUMMARY OF QUALITY DATA FOR DURUM AND COMMON WHEAT VARIETIES

Variety	U.S. Grade	Test Weight lb./bu.	Wheat Protein ^a %	Semolina		Farinogram Score ^b	Absorption %	Spaghetti		Cooking Loss %	Cooked Spaghetti Weight g./10 g.	Overall Quality
				Yield ^a %	Protein ^a %			Color ^c	Firmness g. X cm. ^d			
Leeds	2HAD	61.3	14.8	55.4	13.8	3	34.3	10.5	8.1	5.0	37.0	Excellent
Mindum	2HAD	59.3	13.6	56.9	12.8	5	35.0	9.5	9.1	5.6	40.2	Good
Stewart-63	2HAD	61.2	15.0	55.0	14.1	5	34.1	8.0	8.3	4.3	36.3	Fair
Yuma	3HAD	57.4	15.5	51.0	13.3	6	39.3	7.5 Br.	8.1	5.0	33.0	Poor
Golden Ball	3HAD	57.5	15.0	56.6	13.8	3	35.2	7.0	8.4	5.1	39.4	Poor
Peliss	3HAD	57.4	15.1	54.0	13.9	7	38.0	7.0 Br.	8.7	4.5	37.0	Poor
Pentad	3RD	56.7	16.0	51.9	14.6	6	39.3	4.0 Rd.	8.1	4.4	37.5	Very poor
Selkirk	2DNS	57.6	16.4	43.6	13.6	7	39.0	4.0 Gr.	9.3	3.8	34.2	Very poor

^aValues are stated on a 14% moisture basis.

^bValues are an overall classification incorporating mixing time and general characteristics which are assigned so that a high value indicates strong gluten.

^cAbbreviations used: Br. = brown; Rd. = red; and Gr. = gray.

^dFirmness values are reported as the work (g. X cm.) required to shear a single strand of cooked spaghetti. Values between 7.5 and 9.0 g. X cm. are considered acceptable
g. X cm. are considered acceptable with 8.0 as the optimum firmness. Firmness above 9.0 g. X cm. is considered excessive and termed as "tough spaghetti".

were of particular interest since brown, off-color spaghetti was made from these varieties. Poorest in quality were Pentad (the red durum) and Selkirk (the hard red spring wheat variety), which were characterized by poor spaghetti color and low milling yield. Furthermore, spaghetti made from Selkirk had the highest firmness score (9.3 g. X cm.) of the varieties tested. Since firmness above 9.0 g. X cm. was considered excessive, Selkirk spaghetti was termed as "too tough". Still another quality factor which showed a wide range among varieties was the farinograms score. Other workers (2) have suggested that low farinogram scores indicated weak gluten strength, which was related to low firmness in cooked spaghetti. However, with the data for the eight varieties tested, no trend relating mixing strength to firmness was apparent.

Fractionation of Semolina Proteins

Maes Column. To seek differences related to the variation in quality of wheat varieties, the proteins of semolina were fractionated in three replicate determinations according to solubility on a Maes column (6) and according to molecular size with Sephadex G-200 gel filtration (9). The average results of the Maes-column extractions where proteins were separated into general classes (albumins, globulins, gliadins, glutenins, and base-solubles) are shown in Table II.

TABLE II. PROTEIN FRACTIONS EXTRACTED FROM SEMOLINA BY STEPWISE ELUTION ON MAES COLUMN^a

Variety	Water (Albumin) %	2% NaCl (Globulin) %	40% Isopropanol (Gliadin) %	3.85% Lactic Acid (Glutenin) %	0.5% KOH (Base Soluble) %
Leeds	12.4	11.9	46.7	18.6	10.2
Mindum	12.1	12.4	32.4	27.0	16.1
Stewart 63	14.7	11.3	37.1	27.0	16.1
Yuma	14.1	13.7	32.7	22.1	17.5
Golden Ball	13.9	12.7	43.8	15.4	14.2
Peliss	12.0	10.2	46.1	18.7	13.0
Pentad (red durum)	14.2	11.8	37.2	27.2	9.6
Selkirk (HRS)	16.2	9.4	34.2	34.6	5.5

^aData are reported as percentage of total semolina protein, and are the averages of three replicate determinations.

On the average, the durum proteins contained 13.2% albumins, 12.2% globulins, 39.8% gliadins, 20.5% glutenins, and 14.3% base-soluble material (Fig. 3). When compared with Selkirk (HRS), the amber durum varieties contained more gliadins and fewer glutenins. In addition, the proteins of Pentad (red durum) contained more albumins and less base-soluble material than the average for the amber durum varieties.

To show varietal differences, an analysis of variance (AOV) of the analytical data was conducted which used a randomized block design of three replicates and eight varieties. The results of the AOV (Table III) showed that variety was a highly significant source (1% level) of variation in all protein fractions, indicating that differences of protein composition were, indeed, detected among the varieties tested. The next and perhaps more important question was this: How were these differences in protein related to quality? To show relations between protein

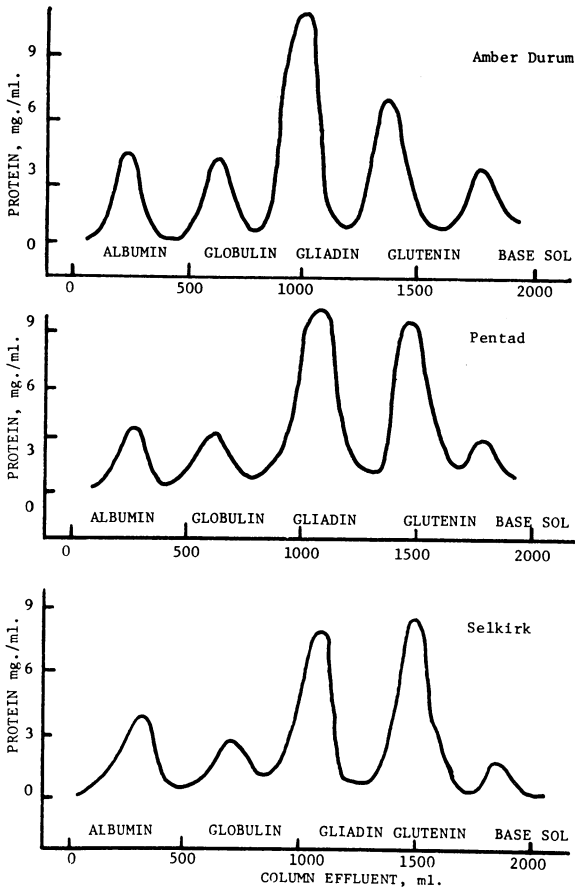


Fig. 3. Maes-column elution patterns showing the average protein composition of semolina or farina.

composition and specific quality factors, the correlation matrix shown in Table IV was calculated. Several coefficients were highly significant (1% level of confidence). Albumin content was negatively correlated with spaghetti color and cooking loss. Accordingly, varieties high in albumin tended to show low color and cooking loss. Globulins, on the other hand, showed positive correlations with spaghetti color and cooking loss and a negative correlation with firmness—which suggested high-globulin varieties to be high in color and cooking loss but low in firmness. The gliadins showed no significant correlation with quality, whereas glutenin, on the other hand, showed significant (5% level of confidence) negative correlation with color and a highly significant negative correlation with cooking loss. A trend for high-glutenin varieties to have poor color and low cooking loss was indicated. The base-soluble fraction showed positive correlations with cooking loss and color, which, correspondingly, pointed toward a trend for varieties containing large amounts of base-solubles to have high cooking losses and good color.

TABLE III. ANALYSIS OF VARIANCE FOR MAES-COLUMN EXTRACTION DATA

	Degrees of Freedom	Mean Squares for Protein Fraction				
		Albumin	Globulin	Gliadin	Glutenin	Base-Soluble
Replications	2	0.0517	0.5529**	3.6953	0.938	0.0537
Varieties	7	5.5695**	5.3921**	113.2110**	115.2741**	48.3142**
Error	14	0.1488	0.0391	2.5479	0.7995	0.2722

TABLE IV. CORRELATION COEFFICIENTS SHOWING RELATIONS BETWEEN QUALITY AND MAES-COLUMN PROTEIN FRACTIONS

Quality Factors	Protein Fraction				
	Albumins	Globulins	Gliadins	Glutenins	Base-Solubles
Color	-0.7148**	0.5239**	0.1882	-0.5010*	0.5310**
Cooked weight	-0.4960*	0.0102	0.0278	-0.0091	0.0245
Cooking loss	-0.6402**	0.7007**	0.0967	-0.5748**	0.7061**
Firmness	0.3343	-0.5896**	0.0565	0.3602	-0.3785

Sephadex G-200 Gel Filtration. To further characterize the proteins of wheat varieties, gel filtration was used to separate wheat proteins according to molecular size. By means of the AUC solvent described by Meredith and Wren (9), about 97% of the total protein of semolina was extracted. When the AUC extracts of semolina were chromatographed on Sephadex G-200, four main fractions were extracted. Meredith and Wren (9), who standardized a similar Sephadex G-200 column with proteins of known molecules, designated the four main protein fractions as follows:

<i>Fraction</i>	<i>Approximate Molecular-Weight Range</i>
Glutenins	Above 150,000
Gliadins	30,000 to 75,000
Albumins	10,000 to 30,000
Nonprotein nitrogen	Below 5,000

Figure 4 shows typical gel-filtration patterns for eight varieties and Table V shows the amount of protein found in each fraction. The gel-filtration data only roughly agrees with the Maes-column results for the same varieties. However, since the two procedures separated proteins by different means, only approximate agreement among the corresponding fractions was expected. Overall, there was a high degree of variability in protein distribution among wheat varieties (Table VI). Typically, the amber durum varieties contained about 32% glutenins, 50% gliadins, 11% albumins, and 7% nonprotein nitrogen according to the Sephadex data. A unique pattern was shown for Pentad, which had the highest albumin and nonprotein nitrogen contents of the varieties tested (15.9 and 8.2%, respectively). The most striking contrast among varieties, however, was in the pattern for Selkirk, which showed that over 48% of the total protein of this variety was in the glutenin fraction. Furthermore, the high glutenin values for Selkirk were confirmed by the Maes-column data, which also showed more glutenin for Selkirk than for the durum varieties.

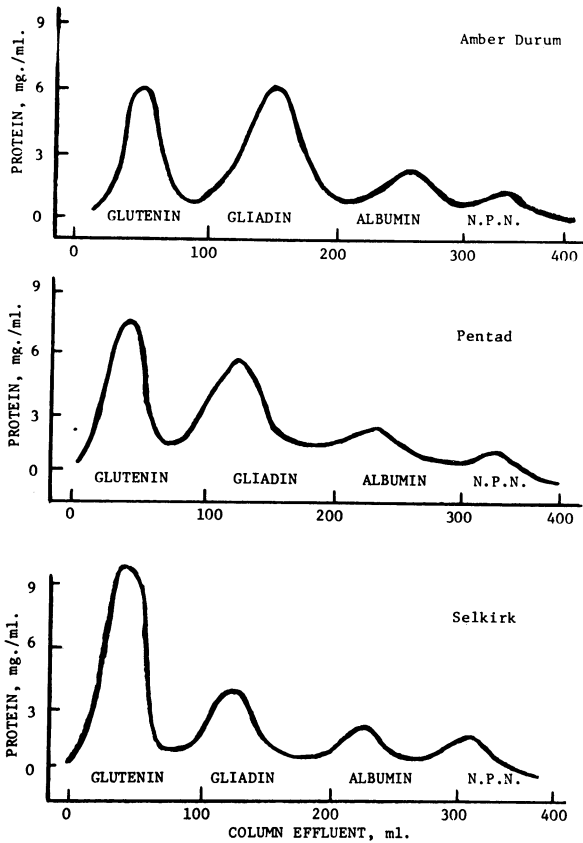


Fig. 4. Sephadex G-200 chromatographic patterns showing the molecular-size distribution of protein extracts of semolina or farina.

TABLE V. SEPHADEX GEL FRACTIONS OF SEMOLINA PROTEINS^a

Variety	Glutenin %	Gliadin %	Albumin %	Nonprotein Nitrogen %
Leeds	27.9	54.9	10.3	6.9
Mindum	27.7	54.5	11.4	6.4
Stewart 63	31.4	52.7	10.4	5.5
Yuma	34.1	49.1	11.4	5.4
Golden Ball	32.6	49.1	10.9	7.4
Peliss	39.6	40.6	12.2	7.6
Pentad	29.9	46.0	15.9	8.2
Selkirk	48.1	34.8	9.0	8.1

^aData are averages of the total extractable proteins of semolina.

TABLE VI. ANALYSIS OF VARIANCE FOR SEPHADEX-COLUMN FRACTIONATION DATA

	Degrees of Freedom	Mean Squares for Protein Fractions			
		Glutenins	Gliadins	Albumins	Nonprotein Nitrogen
Replications	2	2.4465	7.9211	0.4088	0.2138
Varieties	7	113.8817**	209.0473**	12.3894**	2.8085**
Error	14	1.3853	4.8961	0.1802	0.1299

TABLE VII. CORRELATION COEFFICIENTS SHOWING RELATIONS BETWEEN QUALITY AND SEPHADEX-COLUMN PROTEIN FRACTIONS

Quality Factors	Protein Fractions			
	Glutenins	Gliadins	Albumins	Nonprotein Nitrogen
Spaghetti color	-0.8032**	0.8785**	0.1193	-0.4050*
Cooked weight	-0.4314*	0.3318	0.3000	0.0354
Cooking loss	-0.7303**	0.7629**	0.1789	-0.3315
Firmness score	0.6273**	-0.5690**	-0.3802	0.2799

To show how protein composition was related to quality, a linear correlation matrix was calculated between quality factors and the Sephadex chromatographic data (Table VII). In the correlation matrix, there was partial agreement shown with Maes-column data. Both methods showed negative correlations relating glutenin content to color and cooking loss, which strongly suggested that high glutenin was associated with poor color and low cooking loss. Other coefficients which were highly significant showed that the gliadin fraction was positively correlated with color and cooking loss and negatively correlated with firmness. In general, a trend for high-gliadin varieties to have good color, high-cooking loss, and low cooked-spaghetti firmness was indicated.

CONCLUSIONS

A comparison of the protein-fractionation results from solvent extraction and gel filtration leads to the conclusion that differences exist in protein composition among wheat varieties of different quality. Further, the differences in protein composition appear related to spaghetti quality—especially to color and cooking quality. According to chromatographic data, varieties with high contents of glutenin and albumins tend to show poor color but good cooking quality. However, high gliadin contents appear related to good spaghetti color but low cooked-spaghetti firmness.

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