THE GLUTO-MATIC FOR SEMIAUTOMATIC DETERMINATION OF WET AND DRY GLUTEN CONTENT OF WHEAT FLOUR[1]

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ABSTRACT

The Gluto-Matic is a semiautomated device for determining per cent of wet and dry crude gluten in wheat flour. In the manual method, the starch and water solubles are kneaded from the dough with the fingers. In the Gluto-Matic method, the kneading is done by a 3/16" thick metal loop which rotates and mixes the dough in a small plastic cup. Leaching (washing) is also automated. Ten experiments were performed with the Gluto-Matic to explore sources of experimental error and to determine their significance. The results show that the Gluto-Matic method, with a coefficient of variation of 3.62, is significantly superior to the manual method which had a coefficient of variation of 10.02. About 9 min is required to determine per cent wet gluten and 12 min to determine per cent dry gluten by the semiautomatic method. Starch remaining in the dry gluten ranged between 1.6 and 6.4%.

In some countries, such as Austria, Poland, Soviet Union, and Japan emphasis is still placed on the determination of gluten content of wheat. The simplicity of the gluten test (1) and its low cost led to its wide use as a measure of wheat quality in many countries.

A gluten test is satisfying to many analysts because in separating the starch and water solubles, the actual gluten is obtained which may be easily weighed and its elasticity noted by stretching it. Hence, an idea of gluten quality is learned. Moreover, the weight of the wet gluten is a measure of water-binding capacity which is recognized as an important wheat quality factor.

A gluten test takes on special significance where insects are known to have attacked the wheat. Stinkbugs of the genera *Eurygaster* and *Aelia* pierce the kernel and inject proteolytic enzymes which cause loss of water-binding capacity of the protein. Both the gluten and sedimentation tests (2,3) can determine the extent of damage.

The time required for making a gluten test and the variability in results among laboratories and analysts prompted a search for more accurate tests on which a market value for wheat might be based. The most notable method developed was the Kjeldahl nitrogen test which measures the amount of total nitrogen in the wheat. Continued research and improvement of this test have made it one of the most accurate for nitrogen content of wheat. Other protein tests, such as the Udy and biuret, are geared to the accuracy of the Kjeldahl. Different laboratories are expected to check within 0.2% on the same sample by the Kjeldahl method. Gluten results are not that accurate.

AACC Approved Methods (1) describes a hand-washing method for gluten. The flour is treated with water to form a dough ball which is allowed to hydrate 1 hr. Starch and water solubles are kneaded from the dough in 12 min. The ball is placed in distilled water 1 hr, pressed, and weighed as wet gluten. Weight of the dough ball after drying in a 104° C oven for 24 hr is taken as dry gluten. Described

[1]Use of a company or product name does not imply approval or recommendation of the product by the U.S. Department of Agriculture to the exclusion of others which may also be suitable.

also is a mechanical-washing method using the German Theby Gluten Washer. This method requires about 15 min to obtain per cent wet gluten.

Since water-soluble protein, which constitutes about 20% of the total protein, is removed by leaching, a good estimate of gluten is about 80% of the total protein content. When gluten content exceeds protein content, which always happens in manual methods, it is best explained by incomplete removal of starch. Removal of all starch is impossible and about 3% remains as a constant bias, even by the Gluto-Matic method. However, the method should still have an important relative value.

MATERIALS AND METHODS

Approximately 30 samples each of Hard Red Spring (HRS), Hard Red Winter (HRW), and White Wheat (WW) were obtained from the U.S. Department of Agriculture, Grain Division, Beltsville, Maryland, and from the Agricultural Research Service, Pullman, Washington.

The Gluto-Matic device was purchased from Foss America, Inc., Rt. 82, Fishkill, NY 12524 (Fig. 1). The device (approximately 11" × 14" × 16") consists of a small plastic cup unit into which is weighed 10 g of flour. A push button injects 7 ml of 2% NaCl solution for hydration and the automatic kneading process is started. This lasts 20 sec. The automatic leaching process then starts and 880 ml of distilled H_2O is used to wash out the starch from the gluten. After 8

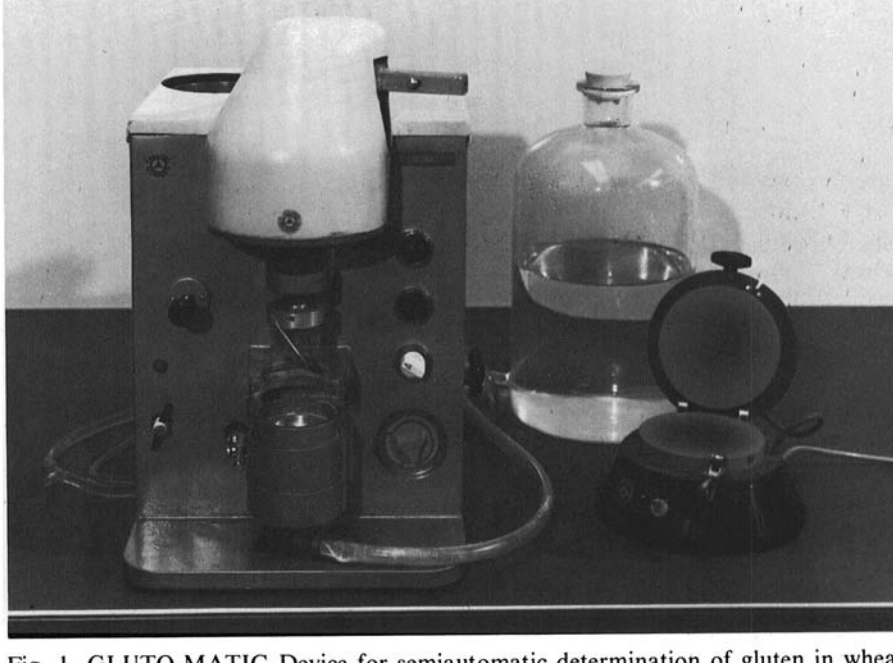

Fig. 1. GLUTO-MATIC Device for semiautomatic determination of gluten in wheat flour.

min the gluten ball is split in half and placed in the small centrifuge contained in the unit, whirled 1 min, then weighed as wet gluten. It is then placed on the Teflon® hot plate for 3 min and weighed as dry gluten.

Reagents are a) tap water, b) distilled water, and c) 2% NaCl solution.

After hydrolysis, starch was determined by the Lane Eynon method (4): Weigh dry gluten disc (0.8–1.9 g) into a 1-l. reflux flask. Add 40 ml dilute HCl (1:3), 150 ml distilled H_2O, and reflux 4 hrs. Cool and neutralize with about 15 ml 20% NaOH solution. Bring to 250 ml and titrate while boiling against 2 ml Fehling solution using a few drops of 0.1% methylene blue indicator.

$$\% \text{ starch in gluten} = \frac{\frac{250}{\text{Titre}} \times 0.012 \times 0.9}{\text{Sample weight}} \times 100$$

The Kjeldahl protein method was used throughout the experiments to determine protein content of samples (N × 5.7).

RESULTS

To develop a standardized procedure for the semiautomatic determination of gluten in hard and soft wheats, possible sources of experimental error in the test were examined. Results of the experiments are shown in Tables I through IV.

Table I shows wet and dry gluten content, protein content, and the ratio of dry gluten:protein content × 100 for the three classes of wheat. In comparing the mean values, note especially the wide differences in the ratio of dry gluten:protein content.

TABLE I
Comparison of Protein and Gluten Contents (Wet and Dry) of Classes of Wheat

Class and Parameter	Range	Mean
Hard Red Spring:		
Wet gluten (%)	22.2– 47.6	36.2
Dry gluten (%)	10.1– 16.6	12.8
Protein (%)	11.9– 16.9	14.3
(% Dry gluten: % protein) × 100	77.7–105.9	89.5
Hard Red Winter:		
Wet gluten (%)	18.5– 33.9	26.5
Dry gluten (%)	7.2– 12.5	9.8
Protein (%)	10.6– 13.1	12.5
(% Dry gluten: % protein) × 100	76.8– 90.1	78.4
White Wheat (soft and hard):		
Wet gluten (%)	16.3– 29.5	26.4
Dry gluten (%)	6.0– 11.6	8.7
Protein (%)	9.8– 14.4	12.4
(% Dry gluten: % protein) × 100	61.2– 85.2	70.2

Analysis of variance by split-plot method showed a highly significant difference between the wheat classes of Table I, the leaching liquids (tap water, distilled water, and 2% NaCl solution), and their volumes (440 ml, 660 ml, and 880 ml), and the effect of hydration liquid (same liquids as for leaching) and volumes (5 ml, 7 ml, and 9 ml) on the amount of gluten obtained.

TABLE II
Comparison of Correlation Coefficients and Standard Errors of Estimate for Gluten Content from Different Wheat Classes *vs.* Other Quality Indexes

Statistic	r	Syx	Regression Equation[a]
Hard Red Spring (n = 29)			
% Prot. *vs.* ml Sed. Val.	0.52**	9.07	Y = 18.36 + 3.91X
% Prot. *vs.* % W G[b]	0.70**	3.86	Y = 2.75X − 2.52
% Prot. *vs.* % D G[c]	0.73**	1.18	Y = 0.19 + 0.89X
ml Sed. Val. *vs.* % W G	0.54**	4.58	Y = 15.81 + 0.28X
ml Sed. Val. *vs.* % D G	0.53**	1.45	Y = 6.48 + 0.09X
% W G *vs.* % D G	0.97**	0.38	Y = 1.68 + 0.31X
Hard Red Winter (n = 30)			
% Prot. *vs.* ml Sed. Val.	0.75**	8.40	Y = 6.57X − 33.03
% Prot. *vs.* % W G	0.93**	2.18	Y = 3.90X − 22.23
% Prot. *vs.* % D G	0.94**	0.70	Y = 1.29X − 6.29
ml Sed. Val. *vs.* % W G	0.84**	3.25	Y = 7.10 + 0.40X
ml Sed. Val. *vs.* % D G	0.83**	1.09	Y = 3.51 + 0.13X
% W G *vs.* % D G	0.93**	0.21	Y = 1.17 + 0.33X
White (n = 27)			
% Prot. *vs.* ml Sed. Val.	0.68**	8.86	Y = 6.75X − 53.38
% Prot. *vs.* % W G	0.81**	2.56	Y = 2.93X − 12.36
% Prot. *vs.* % D G	0.83**	0.85	Y = 1.01X − 3.83
ml Sed. Val. *vs.* % W G	0.82**	2.50	Y = 14.98 + 0.30X
ml Sed. Val. *vs.* % D G	0.84**	0.82	Y = 5.58 + 0.10X
% W G *vs.* % D G	0.99**	0.18	Y = 0.60 + 0.33X

[a]Y is the dependent variable, the second listed under the statistic column.
[b]W G = wet gluten.
[c]D G = dry gluten.

TABLE III
Comparison of Manual *vs.* Automatic Gluten Methods

Method and Parameter	Range	Mean ($\bar{X}$)	Standard Deviation	Coefficient of Variation
Manual Method				
Wet gluten (%)	32.1 − 40.7	38.7	9.89	25.5
Dry gluten (%)	9.0 − 27.2	17.8	6.34	35.6
% W G : % D G[a]	1.9 − 2.7	2.32	0.23	10.0
(% D G : % protein) × 100	75.6 −170.0	123.4	25.0	20.2
Automatic Method				
Wet gluten (%)	17.6 − 47.6	30.0	7.83	26.1
Dry gluten (%)	7.1 − 16.6	10.6	2.50	23.6
% W G : % D G	2.48− 2.86	2.76	0.11	3.7
(% D G : % protein) × 100	67.4 − 98.5	78.4	10.5	13.4

[a]W G = wet gluten. D G = dry gluten.

Because the kinds of treatment and volumes were found to cause variations in the results, the test was standardized by using 880 ml distilled water for leaching. This yielded dry gluten containing about 80% protein, a commonly accepted figure (5). To reduce variability in results 9 ml of 2% NaCl solution was used as the standard hydration treatment. These selections produced results which were in best agreement with accepted estimates.

Statistical relation between various quality indexes and gluten content were examined. Simple correlation coefficients were calculated within each wheat class and are shown in Table II along with standard errors of estimate and linear regression equations. All correlation coefficients were highly significant; however, the magnitude of the r values was greatest for the HRW wheats and smallest for the HRS.

Table III shows results of an experiment comparing the automatic with the manual method for gluten content. A paired t-test made on the observations showed a highly significant value of 8.56. The automatic method gave lower gluten weights and a lower coefficient of variation. This indicates that the automatic method removed more starch and water solubles and was significantly more precise than the hand method.

A chemical analysis of the constituents was made on a composite of gluten wafers saved from the many tests on each of the three wheat classes. The results are shown in Table IV.

Other experiments performed were as follows: 1) The time for centrifuging the wet gluten was examined to note the effects of 0-, 30-, 60-, and 90-sec centrifuging periods. Although no significant effects were found, the mean wet gluten values were 30.8, .29.6, 28.4, and 28.8 respectively. The time of 60 sec was selected as standard. 2) The effect of hot plate drying time was examined using 2, 3, 4, and 5 min. The mean dry gluten values were 11.72, 10.66, 10.72, and 10.78 respectively. Three min was selected as standard. 3) The effect of 150- and 90-μ sieve openings on the removal of starch during leaching was examined. Mean dry gluten values of 10.92 and 11.33 g respectively, were obtained with a significant F value of 31.11. The 150μ sieve was selected as standard. 4) The effect of sample size was

TABLE IV
Components in Crude Dry Gluten Obtained from Wheat Flour by the Gluto-Matic Method

Components	Hard Red Spring	Hard Red Winter	White
Moisture content (%)	7.06	8.30	8.91
Ash (%)	0.54	0.49	0.53
Starch (%)	3.30	6.36	1.64
Fat (%)	0.09	0.08	0.89
Other organic compounds (by difference) (%)	6.01	2.77	8.03
Protein (Kjeldahl) (%)	83.00	82.00	80.00
Average original wheat protein (%)	14.40	12.60	12.40
Average dry gluten (%)	12.90	10.00	8.70
Ratio $\frac{\text{dry gluten}}{\text{protein}} \times 100$	90.20	79.40	70.40

examined using 5.0, 7.5, and 10.0 g. A significant F value of 8.62 indicated that size of sample was significant. The 10.0-g size was selected because of ease in computing results.

Recommended Method

The method recommended for a gluten determination using the Gluto-Matic consists of the following steps:

1. Weigh 10 g flour into sample cup.
2. Hydrate 20 sec with 9 ml 2% NaCl solution.
3. Leach 8 min with 880 ml distilled water.
4. Split gluten ball after leaching for easy centrifuging.
5. Centrifuge 1 min.
6. Weigh wet gluten and record per cent.
7. Place on hot plate 3 min.
8. Weigh resulting gluten wafer and record per cent.

Because only one machine was available, a comparison of different machines was not possible.

DISCUSSION AND CONCLUSIONS

Good reproducibility of results, the relative rapidity of analysis (especially if several machines are used at the same time), and extremely low cost per analysis are important factors which may revive interest in the gluten determination as it is performed by the Gluto-Matic device—particularly when results are compared to the manual method. Also, no harsh chemicals are required; this is an important factor to a laboratory concerned about chemical waste pollution.

The mean ratios of dry gluten:protein in Table I are 89.5, 78.4, and 70.2 for HRS, HRW, and WW respectively. This indicates a distinct class difference and may be due to the ratio of water-soluble:total protein. Pratt (6) quotes Greenberg and associates as having noted that the ratio of soluble:total protein is considerably higher in soft that in hard wheat flours.

In Table II, the correlation coefficients for gluten *vs.* protein and gluten *vs.* sedimentation value are not as high for HRS as for HRW and WW. For the last-mentioned, correlations were sufficiently high for prediction of protein content from gluten content by means of a simple regression equation within the standard error shown.

A comparison of statistics in Table III reveals that the automatic method is more precise than the manual method. This is demonstrated by a lower standard deviation and coefficient of variation for all factors listed except wet gluten.

Distinct differences in the ratio dry gluten:protein $\times$ 100 were observed among wheat classes studied. It would be of interest to investigate whether these observed differences among commercial wheat classes reflect inherent differences among wheat cultivars.

Standardizing the automatic gluten method decreased the standard error of the gluten estimate. In addition, the gluten:protein $\times$ 100 ratio of about 80% indicated almost complete removal of nonprotein components (mainly starch). Such removal was not possible by the hand-washing method which yielded excessively high nonreproducible results.

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