SIGNIFICANCE OF GLUTEN CONTENT AS AN INDEX OF WHEAT AND FLOUR QUALITY

by

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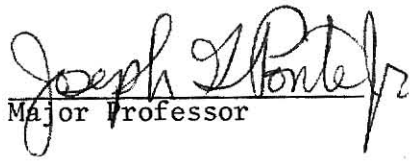

Major Professor

DEDICATED TO MY PARENTS

TABLE OF CONTENTS

LIST OF TABLES

Page

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INTRODUCTION

Flour quality means different things to the ultimate users of the product. It usually represents conformance to several measurable characteristics which experience has suggested to be significant in terms of the end use. In a rather broad sense flour strength is quite synonymous with flour quality. The presence or absence of strength factors governs the suitability of the flour for a specific application. Strength is usually associated with wheat or flour proteins and encompasses both quality and quantity measurements.

Both protein quantity and quality are thus considered primary factors in measuring the potential of a flour. Protein quantity is measured by the classic Kjeldahl nitrogen analysis while the quality criteria are related to the gluten portion of the flour protein and quality is appraised largely by subjecting the flour to several physical testing devices which measure various rheological characteristics of flour water doughs and by baking tests (Pratt, 1978).

Although the terms protein and gluten are frequently used as synonyms, they do not designate the same thing. Protein comprises all the nitrogenous material in the flour while gluten constitutes the residue obtained by working and washing a small piece of dough in water to remove most of the starch, but also some of the water soluble protein substance.

Gluten washing is practiced for two purposes. First, the weight of any gluten is a fair index to the protein content of the flour, and secondly, the cereal chemist is able to obtain an index of the flour strength from the physical properties of the washed out gluten (Dill and Alsberg, 1924).

Although gluten washing has been practiced for more than a century, the "Personal Factor" has been important in contributing to variations in the results obtained in the process of hand washing of gluten (Fisher and Halton, 1936). Several mechanical devices have been experimented to improve the performance of gluten washing e.g. the Berliner and Ruter washer, the Henry Simon washer, etc. The latest development has been the Glutomatic Gluten Washing System developed by the Falling Number Company in Sweden and satisfactory performance and reproducibility of results has been reported with this system (Greenaway and Watson, 1975; Redman and Burbridge, 1979).

The objective of this study was firstly to determine the gluten contents of some Hard Red Winter and Hard Red Spring flours and wholemeals by the Glutomatic 2100 gluten washing system and study the correlations between the glutens and other flour parameters determined by the Farinograph, Mixograph, Resistograph and Rheograph. Secondly, the aim has been to detect the effect of fermentation on the gluten content of a commercial bread flour as determined by the Glutomatic system.

LITERATURE REVIEW

Wheat is unique among cereals in that its milled product, flour, is alone capable of forming a dough that will retain gas evolved during fermentation and on baking will yield a high, well aerated bread. This unique characteristic is imparted to wheat by its proteins which on combining with water result in the formation of gluten, the actual substance that confers on dough the properties of gas retention. The crude gluten is a cohesive, extensible and rubbery mass containing 65-70% water, while its solid matter consists of 75-80% protein, 5-15% residual starch, 5-10% lipids and a small amount of mineral salts (Pyler, 1973).

The proteins of wheat can be separated into four main fractions on the basis of their solubility in different solvents (Osborne, 1907). The fractions include the gluten forming proteins gliadin and glutenin which constitute some 80% of the total wheat flour proteins, and the non gluten forming proteins, leucosin, which is a globulin, and edestin which is an albumin. These non gluten proteins, which are soluble in either water or dilute salt solutions are assumed to be non essential to the formation of gluten and are largely removed in the gluten washing process.

When crude gluten is treated with 70% alcohol, the gliadin fraction dissolves and can be obtained in a fairly pure form. The remainder of the protein consists of glutenin which is soluble in dilute acetic acid and alkali solution. The gliadins and glutenins have been shown to contain an exceptionally high level of glutamic acid and relatively high content of proline, and glutamic acid is principally responsible for the cohesive, elastic properties of gluten (McDonald and Gilles, 1967; Dimler, 1963).

Glutenin forms a very tough rubbery mass when hydrated while gliadin produces a viscous fluid mass, and normal gluten in contrast exhibits physical characteristics of glutenin, gliadin and native gluten. The nitrogen solubility of native vital gluten is low through pH 4 to 7 and this is attributed to its high molecular weight and presence of inter-polypeptide disulfide bonds. An increase in ionic strength does not seriously affect solubility or the water uptake of the gluten (Sarkki, 1979).

A high correlation between gluten with protein and water absorption has been found. Gliadin has been shown to influence loaf volume while glutenin influences mixing time and dough development (Pomeranz, 1977). The type and extent of milling has an important bearing in gluten evaluation (Skalinska, 1969). Grinding to a point where 50-60% of the material passes through a #230 silk gauze gives gluten values in close agreement with the levels actually present.

As reviewed earlier, gluten washing has been attempted in various forms to get an index of protein content and flour strength. In an early work on hand washing it has been shown that important factors affecting the results are length of period dough is allowed to set, the period gluten is allowed to set, temperature, length of washing period, mechanical manipulation, nature of wash water, H ion concentration of flour, concentration and kind of electrolytes in flour, gluten quality and quantity (Dill and Alsberg, 1924). Prolonging the gluten washing period when tap water is used continually decreases the non-nitrogenous constituents of gluten but there is only a slight loss of nitrogenous constituents, while prolonged washing with boiled distilled water results in considerable dispersion of protein. Variations in concentrations of a sodium phosphate buffer solution (pH 7.6) does not

greatly influence gluten quality or yield.

In spite of these findings there was no reproducibility of results as the personal variation causes errors in the washing process (Fisher and Halton, 1936). An earlier mechanical washer developed by Berliner and Ruter consists of four curved blades revolving on a vertical axis inside a cylindrical metal kneading vessel, the flat bottom of which is perforated and covered with a silk gauze. The Henry Simon gluten washer also works on the same principle and the gluten is removed by a constant feed of water.

Initial work on the Glutomatic System has shown that there is much less variation in the gluten:protein ratios for different types of flours and the correlations are much higher when compared to hand washing (Waltl, 1972, 1975). Deviations of 0.11 to 0.35% for glutens in the range of 22 to 38% and mean standard deviations of 0.25% have been obtained and correlations up to 0.956 have been reported (Perten, 1977; Petzold and Bartsch, 1978).

The AACC method for gluten determination involving hand washing requires dough hydration for 1 hour, washing for 12 minutes, placing the dough ball in water for 1 hour, taking wet weight, drying at 104 C for 24 hours and taking dry weight. This is a time consuming test and the coefficient of variation is high (10.02) as compared to the low value for the Glutomatic (3.62) which therefore is more advantageous (Greenaway and Watson, 1975). Better reproducibility and superior performance has been obtained with the Glutomatic while working with spring, winter and soft wheats by these workers and they have distinguished the wheat classes by the differences in the mean ratios of (dry gluten:protein) x 100, which could be attributed to varying ratios of soluble:total protein. The correlation coefficient has been found to be higher for Hard Red Winter and White wheats as compared to that for Hard Red Spring wheat samples.

Gluten contents of both flours and wholmeals have been determined using the Glutomatic 2100 system that was used in this study, and results have been compared with the Simon gluten washer (Redman and Burbridge, 1979). The Simon washer has been shown to give higher values of wet and dry gluten but the gluten is lower in protein indicating the presence of large amounts of residual starch. Once again, high correlation coefficients for wet and dry glutens with protein have been observed. According to the authors, the particle size and the gluten quality does not apparently influence these relationships. Sieving of the wholemeals seems to improve the correlations and in general, the Glutomatic system is more rapid and reasonably reproducible.

MATERIALS AND METHODS

Flour Samples: 40 samples of Hard Red Winter Wheat flours (comprising of 16 Large scale and 24 Small scale samples) and 17 samples of Hard Red Spring Wheat flours were selected. The "Large scale" denoted that these were available in large quantities for the collaborative study of the Wheat Quality Council and milled on the Pilot mill at the Department of Grain Science and Industry, Kansas State University, Manhattan, Kansas. The "Small scale" on the other hand, were available in smaller amounts and thus milled on the Miag-Multomat Mill also at the Department of Grain Science, KSU, Manhattan. The Hard Red Spring Wheat flour samples were from North Dakota milled on the Double-Multomat Mill and made available by Mr. Vernon Young.

The same Hard Red Winter Large scale wheats were used for wholemeal gluten determinations.

A typical commercial bread flour (HRW flour, ex Ross Mills) was selected for the Glutomatic-Mixograph correlation study and to evaluate the effect of fermentation on gluten as determined by the Glutomatic system. The flour had the following characteristics: Protein = 11.6%, ash = 0.45%, moisture = 13.7%, farinograph absorption = 64.8%, mixograph mixing time = 4.0 min.

Analytical Methods: Moisture and protein analyses were performed using approved AACC Methods 44-15 A and 46-11. All analyses were carried out in duplicate. Starch damage was determined by AACC Method 76-30 A. Farinographs were run on the flours using AACC Method 54-21. Data for flour mixograph, resistograph and rheograph parameters was obtained through the courtesy of the Wheat Quality Council collaborative study.

Gluten Determination: This was done with the Glutomatic gluten washer (Model 2100) manufactured by the Falling Number Company in Sweden. The system consists of 3 separate components: 1) the combined dough mixer and washer (2100), 2) the centrifuge (2012), and 3) the glutork drier (2020). The mixing chamber is a plastic cylinder, the bottom of which is closed by a sieve of 80μm aperture size supported by a perforated stainless steel plate. The sieve is first moistened to achieve a capilliary water bridge to prevent flour loss.

A) Gluten washing from flours. Ten grams of flour was introduced in the plastic cylinder and 5.2 ml of 2% sodium chloride solution was added by means of an inbuilt syringe. The chamber was attached to the mixing head by means of a bayonet fixing and mixing was activated by the appropriate push button control. After a mixing time of 20 seconds (controllable by a programming device) the mixing head comprising of a rotating stainless steel hook was automatically lowered a little further into the chamber and washing sequence with 2% sodium chloride solution was started. The wash time (also adjustable) was kept for 5 minutes and flow rate was maintained at 50-60 ml per minute.

The centrifuge is fitted with 2 diametrically opposite metal spikes on which metal grids are placed. To remove excess water from the washed gluten, it was divided into two portions which were then impaled on the spikes. The rotation of the centrifuge was at 6000 rpm for a pre set time of 1 minute. The weight of "wet" gluten retained on the grids was determined at this stage.

The glutork drier consists of two p.t.f.e. coated hot plates between which the gluten was flattened and dried for 4 minutes to give a thin sheet of dried gluten which was weighed and recorded as "dry" gluten.

B) Gluten washing from wholemeal: The wholemeals were ground in a Girmi grinder similar to a KT mill as used with the Falling Number Apparatus. Sub samples were hand sieved through a 65 mesh (210/μm) hand sieve (0.0082 inch) to get between 55 to 60% throughs as sieved wholemeals.

The gluten washing process was similar to that for flours except that the cycle was interrupted at 2 minutes (i.e. 20 seconds mixing and 100 seconds washing) and the half washed gluten was transferred with all the bran into a second test chamber fitted with a 800μm aperture sieve instead of the 80μm one. The program was recommended and the majority of the bran particles were thus removed. The other steps were similar to those for flour washing.

C) Gluten washing from doughs: Firstly, doughs were mixed with 100 grams flour and 451.5 ml water (64.5% predetermined water absorption) in the Hobart Mixer with the following variations: a) yeast levels of 0.0 and 2.5% based on flour weight, b) mixing times of 2, 4, and 6 minutes and fermentation times of 0, 1, 3, and 5 hours were selected for these doughs under the conditions of 85 +/-2% Relative Humidity and 85 +/-1^{o}F temperature in the humidity cabinet. After the requisite fermentation period, 16.7 grams of yeasted dough and 16.4 grams of unyeasted dough (considering 10 grams flour in dough basis) were washed in the Glutomatic system. Here the mixing stage was only 5 seconds while the washing was carried out for 10 minutes and wet and dry glutens were determined as before. A term "Gluten Absorption Factor (GAF)" was defined to indicate the water absorption capacity of the gluten and

$$GAF = \frac{(\text{wet gluten} - \text{dry gluten})}{\text{dry gluten}} \times 100.$$

Glutomatic-Mixograph Correlation Study: In this study, 10 gm of flour was run in a mixograph following Finney's procedure (Finney and Shogren, 1972). Doughs were removed at 1, 2, 3, 4, 5, and 6 minute intervals after mixing and both wet and dry glutens were determined after each mixing period by the Glutomatic system.

Statistical Analysis: This included Analysis of Variance procedure, Correlation study, and General Linear Model Procedure.

RESULTS AND DISCUSSION

1) Correlation Study and Prediction Equations for Flours

The wet and dry gluten values, protein content and farinograph parameters for Hard Red Winter (HRW) Large Scale flour samples are shown in Table 1. Both the wet and dry gluten contents increased with increase in protein content of the flours and good reproducibility was observed in duplicating the gluten readings. Table 2 shows the correlation coefficients for the glutens with protein and farinograph parameters. A highly significant correlation coefficient was obtained for both wet and dry gluten with flour protein, while significant values were also observed for farinograph absorption and mixing time. The General Linear Model procedure was used to obtain prediction equations for Protein (PT), Farinograph Absorption (ABS), and Farinograph Mixing Time (MT) based on gluten values as follows:

$$PT = 0.3010(WG) + 2.5471$$

$$ABS = 0.4271(WG) + 48.1417$$

$$MT = 0.8844(WG) - 21.9218$$

$$PT = 0.8272(DG) + 2.4293$$

$$ABS = 1.1065(DG) + 48.7996$$

$$MT = 2.2776(DG) + 20.3908$$

This set of equations could be used to predict the dependent variables like protein, etc. by rapidly and simply determining the gluten values.

TABLE 1

Gluten Contents, Protein and Farinograph Parameters of Hard Red Winter Large Scale Flours

Flour	Protein (%)	Wet Gluten (%)			Dry Gluten (%)			ABS (%)	MT (min)	AT (min)	DT (min)	ST (min)	MTI (min)	VAL
		1	2	Mean	1	2	Mean							
716	12.6	33.4	33.6	33.5	12.6	12.4	12.5	61.9	6.25	2.25	16.0	13.75	21	68
717	12.6	33.4	33.6	33.5	12.3	12.5	12.4	61.9	8.75	3.00	20.5	17.5	20	77
718	13.0	34.9	34.8	34.85	12.8	12.4	12.6	62.6	9.00	2.75	21.5	18.75	15	78
719	12.0	32.3	32.1	32.2	12.2	12.3	12.25	63.5	10.5	2.25	22.5	20.25	21	81
720	12.3	31.5	31.8	31.65	11.6	11.7	11.65	60.9	7.5	2.25	14.5	12.25	30	71
721	11.4	31.1	30.9	31.0	11.4	11.2	11.3	60.2	7.5	2.00	12.5	10.50	40	69
722	11.8	29.9	30.1	30.0	11.9	12.0	11.95	59.2	2.75	1.25	24.50	23.25	35	59
723	12.0	30.5	30.8	30.65	11.4	11.6	11.5	61.8	5.0	1.80	23.60	21.8	17	68
724	14.6	39.9	40.1	40.0	14.6	14.3	14.45	63.4	14.25	8.75	20.25	11.5	40	87
725	14.5	38.6	38.4	38.5	14.6	14.4	14.5	65.0	15.0	11.50	21.50	10.0	21	89
726	14.5	39.6	39.7	39.65	14.5	14.2	14.35	65.6	10.5	4.00	18.75	14.75	25	81
727	12.6	35.6	35.4	35.5	12.7	12.4	12.55	64.7	6.15	3.15	11.25	8.10	45	64
728	13.0	33.8	34.0	33.9	12.4	12.3	12.35	64.1	8.50	2.75	19.25	16.50	15	75
729	11.9	30.8	30.5	30.65	11.3	11.2	11.25	62.0	2.25	0.90	9.25	8.35	25	53
730	11.2	29.8	30.0	29.9	10.6	10.7	10.65	60.2	6.00	1.65	16.15	14.5	18	66
731	12.0	30.2	30.0	30.1	11.8	11.5	11.65	62.0	3.00	1.50	9.75	8.25	25	56

ABS: Farinograph Absorption; MT: Farinograph Mixing Time; AT: Farinograph Arrival Time;

DT: Farinograph Departure Time; ST: Stability; MTI: Mixing Tolerance Index; VAL: Valorimeter Reading

TABLE 2

Correlation Coefficients Between Wet and Dry Gluten and Flour Protein and Farinograph Parameters for HRW Large Scale Flours

	Protein	Absorption	Mixing Time
Wet Gluten	0.96**	0.79**	0.82**
Dry Gluten	0.97**	0.75**	0.80**

** significantly different from zero at the 5% level

The data for gluten contents, proteins and Farinograph characteristics of Hard Red Winter "Small" scale samples is shown in Table 3, while that for Hard Red Spring is presented in Table 4. Once again, a similar trend was observed i.e. the gluten contents were correspondingly higher for flours with higher protein contents, and there was good reproducibility of results. The correlation coefficients for the two classes of flours are shown in Tables 5 and 6, respectively. From these coefficients, it was seen that there was high significant correlation between the glutens and proteins but only fair correlation of glutens with absorption and non-significant correlation with mixing time. Therefore for all the three classes of flours, HRW Large Scale, HRW Small scale and HRS, there was high significant correlation between glutens and protein contents. However, only in the case of HRW Large scale samples, was a fairly high significant correlation obtained between gluten and absorption. At this stage it was decided to study the effect of starch damage since it is considered to be another important factor in flour water absorption determination. The aim was to show that inclusion of starch damage factor in addition to the gluten content could improve correlation and predictability for absorption and explain the deviations observed in the correlation coefficients between gluten and absorption for the classes of flours.

TABLE 3

Gluten Contents, Proteins and Farinograph Parameters of Hard Red Winter "Small" Scale Flours

Flour	Protein (%)	Wet Gluten (%)			Dry Gluten (%)			Absorption (%)	Mixing Time (min)
		1	2	Mean	1	2	Mean		
739	11.7	31.9	32.2	32.0	11.5	11.5	11.5	59.2	6.2
740	10.6	25.9	25.9	25.9	9.4	9.4	9.4	61.8	8.0
741	10.4	26.7	27.3	27.0	9.8	10.0	9.9	62.3	7.25
742	11.8	34.1	32.4	33.3	11.9	11.9	11.9	63.6	3.75
743	11.6	31.7	32.8	32.2	11.3	11.3	11.3	63.1	5.6
744	14.6	40.2	40.0	40.1	14.5	14.5	14.5	60.6	15.2
745	13.9	37.3	37.5	37.4	14.0	14.0	14.0	67.0	16.5
746	14.6	37.6	37.2	37.4	13.9	14.1	14.0	65.6	23.75
747	15.0	42.0	41.8	41.9	15.2	15.0	15.1	71.6	12.0
748	12.3	33.4	33.4	33.4	12.0	11.8	11.9	69.0	7.0
749	13.4	37.1	37.1	37.1	13.2	13.2	13.2	69.6	8.5
750	12.7	31.8	31.6	31.7	12.1	11.9	12.0	65.2	16.0
751	11.8	30.0	29.8	29.9	11.4	11.0	11.2	63.3	9.5
752	12.3	35.4	35.4	35.4	12.4	12.4	12.4	69.1	5.0
753	11.9	31.2	31.6	31.4	11.7	11.5	11.6	62.7	3.5
754	12.6	36.4	35.4	35.9	12.9	12.7	12.8	63.1	5.25
755	11.3	29.4	29.4	29.4	10.4	10.4	10.4	59.1	3.6
756	11.0	29.8	29.2	29.5	10.6	10.4	10.5	61.8	6.25
757	10.8	29.2	29.2	29.2	10.8	10.8	10.8	61.0	6.0
758	14.2	42.3	41.9	42.1	15.2	15.0	15.1	65.9	6.0
759	13.9	39.2	39.6	39.4	14.2	14.4	14.3	62.9	5.5
760	13.1	35.3	35.5	35.4	13.6	13.4	13.5	59.8	8.5
761	13.8	36.7	36.3	36.5	13.9	13.7	13.8	61.8	15.0
762	13.0	38.0	37.6	37.8	14.6	14.6	14.6	61.0	6.0

TABLE 4

Gluten Contents, Proteins and Farinograph Parameters for Hard Red Spring Flours

Flour	Protein (%)	Wet Gluten (%)			Dry Gluten (%)			Absorption (%)	Mixing Time (min)
		1	2	Mean	1	2	Mean		
CRCK	14.6	38.9	39.1	39.0	14.3	14.1	14.2	64	5.5
CR 3	12.4	35.0	35.1	35.05	12.8	12.9	12.85	66	7.0
M 11	14.7	41.1	41.1	41.1	14.9	14.9	14.9	61	5.5
MCK	13.9	38.2	38.2	38.2	13.65	13.75	13.7	63	4.0
M 10	13.4	37.3	36.9	37.1	13.45	13.35	13.4	60	10.0
CR 4	12.1	30.9	31.1	31.0	11.75	11.55	11.65	65	3.5
M 13	11.8	34.4	34.9	34.65	12.2	12.3	12.25	58	5.0
M 14	12.7	34.4	34.5	34.45	12.8	12.8	12.8	64	6.0
CR 5	12.0	34.4	34.6	34.5	12.4	12.5	12.45	65	7.5
CA 8	11.8	31.1	31.4	31.25	11.8	12.0	11.9	59	4.5
CR 6	12.6	35.2	35.5	35.35	13.2	13.4	13.3	62	8.5
CA 7	14.4	40.4	40.7	40.55	14.6	14.8	14.7	63	7.0
CR 1	15.0	41.7	42.0	41.85	15.2	15.4	15.3	65	8.5
CA 9	12.5	33.7	33.5	33.6	12.35	12.45	12.4	66	8.0
CR 2	12.3	32.7	32.6	32.65	12.2	12.5	12.35	54	25.5
CACK	14.5	38.8	39.2	39.0	14.2	14.6	14.4	62	4.0
M 12	13.3	34.4	34.0	34.2	13.4	12.9	13.15	58	15.0

TABLE 5

Correlation Coefficients Between Wet and Dry Gluten and Protein and Farinograph Parameters for HRW Small Scale Flours

	Protein	Absorption	Mixing Time
Wet Gluten	0.94**	0.43**	0.32
Dry Gluten	0.95**	0.36	0.41

** = significantly different from zero at the 5% level

TABLE 6

Correlation Coefficients Between Wet and Dry Glutens and Protein and Farinograph Parameters for HRS Flours

	Protein	Absorption	Mixing Time
Wet Gluten	0.92**	0.59**	-0.12
Dry Gluten	0.96**	0.43	-0.003

** = significantly different from zero at the 5% level

The data for starch damage for the different classes of flours is shown in Table 7. The data show that the extent of starch damage was higher for HRS wheat flours than the HRW varieties. It was therefore apparent that starch damage factor would have a more prominent effect in predicting absorption in case of the Spring wheat flours. A correlation study was done to determine the degree of correlation and the results are given in Table 8 which show that there was significant correlation between absorption and starch damage for the HRS flours but not HRW ones. This was again confirmed by comparing the R^2 (Correlation Coefficient)^2 values with and without the inclusion of starch damage as shown in Table 9. It is seen that the value of R^2 was 0.18 based on wet gluten alone, but this value increased to 0.60 ABS when both wet gluten and starch damage were considered in the case of HRW small scale samples. Similarly, the corresponding values for HRS flours were 0.35 and 0.63, but there was no significant improvement in the R^2_{ABS} value for the HRW large scale samples.

Therefore it can be concluded that the inclusion of starch damage improved the correlation and predictibility of flour absorption for both HRW small scale and HRS samples but not significantly for HRW large scale ones. This also explains the fact that both the small scale and spring varieties did not show a strong correlation as seen in Tables 5 and 6 for absorption.

TABLE 7

Starch Damage Values for Hard Red Winter and Hard Red Spring Flours

A) Hard Red Winter Large Scale Samples

Flour	Starch Damage (%)	Flour	Starch Damage (%)	Flour	Starch Damage (%)
716	3.81	721	4.40	726	4.67
717	3.87	722	5.38	727	5.38
718	4.13	723	4.76	728	5.72
719	4.84	724	4.19	729	5.07
720	5.23	725	4.64	730	4.77
				731	5.33

B) Hard Red Winter Small Scale Samples

Flour	Starch Damage (%)	Flour	Starch Damage (%)	Flour	Starch Damage (%)
739	3.80	747	4.35	755	4.86
740	5.42	748	5.37	756	4.72
741	5.77	749	4.73	757	4.48
742	5.67	750	4.13	758	4.12
743	5.28	751	4.50	759	4.08
744	2.75	752	6.05	760	3.50
745	3.97	753	4.17	761	2.82
746	4.23	754	4.17	762	3.15

C) Hard Red Spring Samples

Flour	Starch Damage (%)	Flour	Starch Damage (%)	Flour	Starch Damage (%)
MCK	6.847	M 12	5.679	CRCK	6.215
CA 9	6.745	CR 4	5.433	CACK	7.724
CR 1	7.150	M 13	8.052	CA 8	8.154
CR 2	6.847	CR 5	7.949	CA 7	8.052
M 11	7.093	M 14	4.374	M 10	8.974
CR 3	6.0006	CR 6	4.664		

TABLE 8

Correlation Coefficients Between Starch Damage and Flour Protein, Glutens, Absorption and Mixing Time for HRW and HRS Flours

Damaged Starch	ABS	Mixing Time	Protein	Wet Gluten	Dry Gluten
HRW Large Scale	0.03	-0.40	-0.24	-0.31	-0.28
HRW Small Scale	0.34	-0.42**	-0.59**	-0.51**	-0.62**
HRS	0.66**	-0.41	-0.13	0.25	0.15

** = significantly different from zero at the 5% level

TABLE 9

R^2 (Correlation Coefficient)^2 Values With and Without Starch Damage

A) Without Starch Damage

	HRW Large Scale	HRW Small Scale	HRS
With Wet Gluten			
Absorption	0.63	0.18	0.35
Mixing time	0.66	0.10	0.01
Protein	0.92	0.88	0.85
With Dry Gluten			
Absorption	0.56	0.13	0.18
Mixing time	0.58	0.17	0.0001
Protein	0.92	0.91	0.92

B) With Starch Damage

	HRW Large Scale	HRW Small Scale	HRS
With Wet Gluten			
Absorption	0.72	0.60	0.63
Mixing time	0.68	0.19	0.17
Protein	0.93	0.90	0.92
With Dry Gluten			
Absorption	0.62	0.64	0.54
Mixing time	0.67	0.21	0.17
Protein	0.94	0.91	0.92

This difference is explained by the fact that the three classes of flours were milled by three different processes which caused varying degrees of starch damage. Where there was low starch damage, the prediction for absorption could be based on the gluten alone, whereas with higher values of starch damage, both gluten and the damaged starch play significant roles. Therefore, by gluten and starch damage determinations, it was possible to show that the type and extent of damaged starch can affect correlation and predictibility of flour absorption, as shown by statistical models.

From Table 9 it was also seen that the correlation for protein was not significantly improved by inclusion of the starch damage factor and this is expected since protein is highly correlated with the gluten itself. The correlation for mixing time is fairly improved in the case of Spring wheat samples.

Based on both the gluten contents (WG/DG) and starch damage values (STDM) the prediction equations for absorption (ABS), mixing time (MT) and protein (PT) were obtained using the General Linear Models procedure as follows:

Hard Red Winter "Large" scale flours

ABS = 41.635 + 0.479(WG) + 0.998(STDM)

MT = -15.212 + 0.83(WG) - 1.030(STDM)

PT = 1.764 + 0.307(WG) + 0.12(STDM)

ABS = 42.249 + 1.304(DG) + 0.851 (STDM)

MT = -15.839 + 2.372(DG) - 1.221(STDM)

PT = 1.136 + 0.904(DG) + 0.064(STDM)

Hard Red Winter "Small" scale flours

ABS = 29.737 + 0.617(WG) + 2.919(STDM)

MT = 11.873 + 0.164(WG) - 1.976(STDM)

PT = 4.768 + 0.258(WG) - 0.231(STDM)

ABS = 24.814 + 1.864(DG) + 3.541(STDM)

MT = 5.986 + 0.754(DG) - 1.50(STDM)

PT = 3.011 + 0.764(DG) + 0.009(STDM)

Hard Red Spring flours

ABS = 34.817 + 0.481(WG) + 1.495(STDM)

MT = 20.254 - 0.023(WG) - 1.68(STDM)

PT = 2.348 + 0.319(WG) - 0.101(STDM)

ABS = 36.548 + 1.085(DG) + 1.671(STDM)

MT = 15.861 + 0.295(DG) - 1.734(STDM)

PT = 0.248 + 0.983(DG) - 0.018(STDM)

As before, these prediction equations are useful in predicting the above parameters by gluten and starch damage determinations for the different classes of flour samples.

2) Correlation Study for Wholemeals and Sieved Wholemeals

The data for the gluten contents of wholemeals and sieved wholemeals is shown in Table 10. The reproducibility was fairly good and duplicate readings were within +/- 0.6 difference range, but this was not as good as one for flours where the range was +/- 0.3 units. The readings for both wet and dry glutens were higher in case of sieved wholemeals than ones for unsieved ones. This may be accounted for by the removal of bran by sieving to give more protein in the ten gram sample. However, the dry gluten values were also found to be higher than the wheat proteins and this was probable since it was not possible to remove 100% bran in the sieving operation but minute traces could be probably present.

A correlation study was done between the wholemeal and sieved wholemeal glutens with both wheat and flour protein, flour glutens and flour farinograph parameters and the results are shown in Table 11. Firstly, a significant

correlation was observed between the wholemeal glutens and wheat protein, flour protein and flour gluten. The same correlations were also true for sieved wholemeals and in fact were higher. Thus the sieving process improved the correlations. Significant correlation was also present between sieved wholemeal glutens with farinograph absorption, but not in the case of unsieved wholemeals and again, the absence of bran attributes to this improvement.

Thus, determining the wholemeal glutens enables one to predict other parameters like wheat and flour proteins, and flour glutens, as these are all significantly correlated and the results are improved by sieving the wholemeals.

TABLE 10

Gluten Contents of Wholemeals and Sieved Wholemeals of Hard Red Winter Large Scale Samples

Sample	Protein (%)	Wholemeals						Sieved Wholemeals					
		Wet Gluten (%)			Dry Gluten (%)			Wet Gluten (%)			Dry Gluten (%)		
		1	2	Mean	1	2	Mean	1	2	Mean	1	2	Mean
716	13.32	35.3	35.3	35.3	13.0	13.0	13.0	42.1	41.4	41.75	15.3	15.1	15.2
717	13.91	36.4	36.2	36.3	14.0	13.9	13.95	40.0	39.5	39.75	15.4	15.1	15.25
718	14.14	36.8	36.6	36.7	14.1	13.9	14.0	37.8	38.1	37.95	14.4	14.0	14.2
719	13.14	32.9	33.5	33.2	11.8	12.2	12.0	38.0	37.6	37.8	13.8	13.4	13.6
720	13.26	32.9	32.4	32.65	12.7	12.3	12.5	37.3	37.6	37.45	13.7	14.0	13.85
721	12.12	34.0	34.7	34.35	11.9	12.0	11.95	36.6	36.0	36.3	12.8	12.3	12.55
722	12.94	31.0	31.5	31.25	11.4	11.9	11.65	36.0	35.3	35.65	12.8	13.0	12.9
723	12.7	29.2	28.4	28.8	10.5	9.9	10.2	35.1	35.6	35.35	13.6	13.3	13.45
724	12.55	40.3	40.1	40.2	14.7	14.4	14.55	48.8	48.6	48.7	18.0	17.7	17.85
725	14.57	35.7	35.2	35.45	13.4	13.0	13.2	43.5	44.0	43.75	15.3	15.9	15.6
726	14.90	37.3	37.0	37.15	13.3	13.2	13.25	45.8	46.2	46.0	16.1	16.6	16.35
727	13.50	33.3	33.1	33.2	11.6	11.5	11.55	41.3	42.0	41.65	14.4	14.5	14.45
728	13.66	28.8	28.0	28.4	10.7	10.3	10.5	33.4	33.6	33.5	12.5	12.6	12.55
729	12.85	29.1	28.9	29.0	10.5	10.1	10.3	36.3	36.8	36.55	13.0	13.0	13.0
730	11.92	27.4	28.0	27.7	9.8	10.0	9.9	34.8	34.7	34.75	12.3	12.3	12.3
731	12.76	26.5	26.6	26.55	9.8	9.6	9.7	34.2	34.5	34.35	12.6	12.5	12.55

TABLE 11

Correlation Coefficients Between Wholemeal and Sieved Wholemeal Glutens and Proteins, Flour Glutens and Farinograph Parameters for HR Winter Large Scale Samples

	Wheat Protein	Flour Protein	Flour Wet Gluten	Flour Dry Gluten	Abs	Mixing Time
Wholemeal						
Wet gluten	0.76**	0.68**	0.74**	0.74**	0.36	0.72**
Dry gluten	0.76**	0.66**	0.71**	0.71**	0.29	0.71**
Sieved wholemeal						
Wet gluten	0.83**	0.83**	0.79**	0.87**	0.59**	0.70**
Dry gluten	0.89**	0.86**	0.81**	0.87**	0.56**	0.71**

**significantly different from zero at the 5% level

3) Correlation Study Between Glutens and Farinograph, Mixograph, Rheograph, and Resistograph Parameters for HRW Large Scale Flours

Every flour has an optimum mixing time and absorption which yields doughs possessing optimal properties, especially with respect to their machinability and their capacity to produce loaves of desired physical characteristics. Physical dough testing instruments have proved useful in assessing the mixing requirements of different flours. By correlating the data obtained by these various instruments such as the farinograph, mixograph etc. with each other as well as with baking tests, it is frequently possible to arrive at dependable conclusions concerning the baking quality of the flour under consideration. Thus in this study, it was decided to study the correlations between the glutens and other parameters measured by the different instruments as well as between the various parameters themselves to observe how well the readings agree or correlate.

The data for the various observations for HRW large scale flours is as shown in Table 12. The "absorption" term denotes the water absorption of the flour to give doughs of optimum consistency as determined by arbitrary but standardized reference levels and procedures which however vary with each type of instrument. The farinograph mixing time or peak time, which is a measure of the time needed for the curve to reach its peak or point of maximum dough consistency, varies with different flours and is indicative of gluten quality, strong flours generally giving a longer development time than weak ones. The mixogram peak time is the distance from the start of the curve to its peak and is that part of the curve that is indicative of the period during which dough formation and gluten development take place. The rheogram fatigue time is also a characteristic similar to the mixing time and also affected by protein content, gluten strength, milling intensity etc.

TABLE 12

Farinograph, Mixograph, Rheograph and Resistograph Data for Hard Red Winter Large Scale Flours

Flour	Farinograph Absorption (%)	Farinograph Mixing Time (min)	Mixograph Peak Time (min)	Rheograph Absorption (%)	Rheograph Fatigue Time (min)	Resistograph Absorption (%)	Resistograph Break Point (min)
716	61.9	6.25	4.25	66	29.2	58.7	9.7
717	61.9	8.75	4.625	65	37.0	58.8	11.0
718	62.6	9.00	4.50	65	34.3	60.2	11.0
719	63.5	10.50	4.50	66	22.7	60.6	8.5
720	60.9	7.50	5.75	65	31.0	58.5	14.0
721	60.2	7.50	3.75	64	20.0	57.6	7.3
722	59.2	2.75	6.25	64	39.2	57.3	17.5
723	61.8	5.00	5.37	64	40.6	57.4	16.5
724	63.4	14.25	4.62	64	37.7	57.1	11.4
725	65.0	15.0	5.12	65	35.8	58.6	12.0
726	65.6	10.50	3.75	66	28.4	60.7	9.5
727	64.7	6.15	3.12	66	26.4	60.2	8.0
728	64.1	8.50	5.6	67	31.4	60.7	15.3
729	62.0	2.25	5.375	65	26.5	58.3	14.0
730	60.2	6.00	4.25	65	28.3	57.4	13.5
731	62.0	3.00	6.50	66	32.5	57.5	18.0

Finally, the resistogram breaking point is also the time indicating the final softening of the dough. Thus all these times are a measure of the extent of dough development and indicative of the gluten content and quality and thus the strength of the flour.

The different correlations, i.e. between the glutens and the various parameters and between the parameters themselves are shown in Tables 13 and 14 respectively. Firstly it was seen that the farinograph, rheograph and resistograph absorptions all have a significant correlation with each other. As far as the mixing times were concerned, however, the mixograph peak time, the rheograph fatigue time and the resistograph breaking point were correlated to each other, but not to the farinograph mixing time. Finally, only in the case of the farinograph were the absorption and mixing time correlated, but for the rheograph or the resistograph, no correlation between either absorption and fatigue time or absorption and breaking point was observed.

There was significant correlation between the gluten/protein and farinograph absorption and mixing time but not with the other parameters. Since the farinograph mixing time was not correlated with the peak time, fatigue time or breaking point, it is probable that the latter three therefore did not show the correlations with glutens, as did farinograph mixing time.

The correlation study thus showed that determining any one of the absorptions could be used as an index for estimating the other absorptions since they are significantly correlated. Similarly, the peak time could be a predictive factor for the fatigue time or breaking point. The results indicated that the different instruments basically test flour strength and quality factors on a similar basis. As mentioned before, the farinograph mixing time did not correlate with the mixograph peak time significantly and this is in agreement

TABLE 13

Correlation Coefficients Between Glutens and Farinograph, Mixograph, Rheograph, and Resistograph Parameters for Hard Red Winter Large Scale Flours

	Farinograph		Mixograph	Rheograph		Resistograph	
	ABS	MT	MPT	ABS	FT	ABS	BPT
Wet gluten	0.79**	0.82**	-0.43	0.14	0.15	0.38	-0.45
Dry gluten	0.75**	0.80**	-0.23	0.11	0.28	0.32	-0.32
Protein	0.77**	0.78**	-0.17	0.14	0.32	0.31	-0.23

**significantly different from zero at the 5% level

TABLE 14

Correlation Coefficients Among Farinograph, Mixograph, Rheograph, and Resistograph Parameters for Hard Red Winter Large Scale Flours

	Farinograph		Mixograph	Rheograph		Resistograph	
	ABS	MT	MPT	ABS	FT	ABS	BPT
Farinograph							
ABS		0.60**	-0.36	0.56**	-0.06	0.68**	-0.37
MT			-0.35	0.02	0.08	0.29	-0.49
Mixograph							
MPT				-0.11	0.52**	-0.39	0.90**
Rheograph							
ABS					-0.36	0.74**	-0.12
FT						-0.35	0.60**
Resistograph							
ABS							-0.45

ABS = absorption; MT = mixing time; MPT = peak time; FT = fatigue time; BPT = breaking point

**significantly different from zero at the 5% level

with the results obtained by Miller et al. (1956). Experimental baking mixing time is more highly correlated with mixograph peak time than with farinograph mixing time (Miller et al., 1956). Further, the resistograph breaking point has a good correlation with the rheograph fatigue time (Brabender, 1973) and this was also confirmed in this study. Thus the rheograph, mixograph and resistograph show better correlations with each other than the farinograph. With the help of the correlation data obtained in this study, it would be possible to estimate the mixograph, rheograph or resistograph absorptions or mixing times if one knows any single parameter of the above group and one can then use the data to evaluate and correlate these characteristics to the actual baking parameters.

4) The Mixograph-Glutomatic Correlation Study for a Commercial Bread Flour

Mixing to the correct degree is of critical importance for the eventual behavior of the dough during subsequent processing and for the ultimate quality of the final bread. In conventional mixing, the dough development is achieved in four differentiated stages. During the initial stage, the main action is the incorporation of the dough ingredients and at this point the dough is slack. With continued mixing the dough enters the second stage called "pick up" when the gluten structure begins to form. The third phase known as the "clean up" stage is one where developing dough becomes more elastic and forms a cohesive mass. The final "development" stage involves the dough transformation from a rough appearance to a smooth sheen. Further mixing diminishes the coherence and this is the "let down" stage. Mixing beyond this stage causes dough disintegration and stickiness and gluten cannot be washed from such doughs (Pyler, 1973).

The changes in dough appearance are attributed to a basic alteration in the gluten's flow characteristics (Hlynka, 1962). Whereas the dough in its initial stages behaves as if it consisted of minute units, this stage eventually gives way to laminar flow at which point the gluten exhibits the tendency to form thin films and become highly extensible. This change is explained by the "disaggregation" phenomenon (Tsen, 1970). To achieve dough development, the large protein aggregates in flour must first be hydrated and disaggregated into smaller protein units which will more readily undergo molecular orientation that is necessary for the formation of continuous protein films. This disaggregation can be obtained by the shearing action of mixing as well as the splitting of the disulfide bonds by reducing agents. It was decided to study the effect of different mixing times on the gluten contents of a commercial bread flour. As mentioned before, the Ross mills flour was mixed in the mixograph and glutens were determined by the Glutomatic method. The data for the gluten contents and mixograph heights and mixing times are shown in Table 15.

With successive increase in mixing time from 1 to 6 minutes, the wet gluten was found to increase. To compare significant differences, the Duncan's Multiple Range test was done and this is shown in Table 16. Wet glutens were significantly different from each other for mixing times of 1, 2 and 3 minutes while those of 4, 5 and 6 minutes were in one category, but significantly different from the others. A similar pattern was observed in case of the heights. The peak of the mixogram curve was obtained at 4 minutes, and since this is the optimum development time, the highest gluten value was observed at this point. Being a commercial bread flour, with good stability, mixing further for 5 or 6 minutes did not significantly affect the glutens

since hydration and development had already occurred at the optimum mixing time of 4 minutes. Washing in the Glutomatic was not possible when mixing was done beyond 6 minutes.

The correlation coefficients between glutens and mixogram heights and mixing time are shown in Table 17. The height was found to be significantly correlated with both the glutens and the mixing time, while the wet gluten was also significantly correlated with the mixing time. Since both mixing time and gluten have an effect on height, the General Linear Model procedure was used to study if there was a combined effect or interaction of the two parameters on the height and this is shown in Table 18.

TABLE 15

Gluten Contents and Mixograph Data for Hard Red Winter "Ross" Flour

Obs No.	Mixograph Mixing Time (min)	Mixograph Height (mm)	Wet Gluten (%)	Dry Gluten (%)
1	1	36	28.60	10.77
2	1	35	27.64	10.24
3	1	37	28.98	10.13
4	1	37	29.03	10.41
5	2	38	29.22	10.84
6	2	40	30.66	10.47
7	2	41	30.92	10.16
8	2	38	30.60	10.80
9	3	40	31.55	10.89
10	3	43	30.88	10.57
11	3	43	30.65	10.28
12	3	45	30.14	10.21
13	4	49	33.73	11.36
14	4	53	31.68	10.87
15	4	50	33.12	11.21
16	4	50	33.25	11.18
17	5	45	33.65	11.25
18	5	50	33.54	11.22
19	5	50	32.04	10.25
20	5	52	32.86	10.56
21	6	48	33.85	11.15
22	6	50	33.44	11.01
23	6	49	33.72	10.39
24	6	48	33.05	10.42

TABLE 16

Duncan's Multiple Range Test for the Variables Wet Gluten, Dry Gluten and Height

Alpha Level = 0.05 DF = 18 Mean Square = 0.1199

Variable - Wet Gluten		Variable - Dry Gluten		Variable - Height	
Mean	Mixing Time	Mean	Mixing Time	Mean	Mixing Time
33.52 A	6	11.16 A	4	50.50 A	4
33.02 A	5	10.82 AB	5	49.25 A	5
32.95 A	4	10.74 AB	6	48.75 A	6
30.81 B	3	10.57 B	2	42.75 B	3
30.35 B	2	10.49 B	3	39.25 C	2
28.56 C	1	10.39 B	1	36.25 D	1

Means with the same letter are not significantly different at the 5% level.

TABLE 17

Correlation Coefficients Between Glutens, Mixing Time and Mixograph Curve Heights for Hard Red Winter "Ross" Flour

	Mixograph Height	Mixograph Mixing Time	Wet Gluten	Dry Gluten
Mixograph height		0.87**	0.86**	0.41**
Mixograph mixing time			0.91**	0.39
Wet gluten				0.59**

**significantly different from zero at the 5% level

TABLE 18

General Linear Model Procedure for the Variable "Height"

(Mixograph-Glutomatic Study for HRW Ross Flour)

A) Gluten-Mixing Time Interaction

Source	DF	Type 1 SS	F Value	PR > F	Type 4 SS	F Value	PR > F
Mixing time	5	701.208	63.64	0.0001	33.248	3.02	0.0543
Wet gluten	1	6.544	2.97	0.1105	3.508	1.59	0.2310
MT**WG	5	27.763	2.52	0.0880	27.763	2.52	0.0880
Error	12	26.442					
Mixing time	5	701.208	67.97	0.0001	15.168	1.47	0.2699
Dry gluten	1	21.523	10.43	0.0072	23.053	11.17	0.0059
MT**DG	5	14.467	1.40	0.2913	14.467	1.40	0.2913
Error	12	24.758					

B) No Gluten-Mixing Time Interaction

Source	DF	Type 1 SS	F Value	PR > F	Type 4 SS	F Value	PR > F
Mixing time	1	574.289	71.41	0.0001	35.276	4.39	0.0485
Wet gluten	1	18.792	2.34	0.1413	18.792	2.34	0.1413
Error	21	168.876					
Mixing time	1	574.289	65.53	0.0001	450.529	51.40	0.0001
Dry gluten	1	3.617	0.41	0.527	3.617	0.41	0.527
Error	21	184.051					

**denotes interaction
MT = mixing time; WG = wet gluten; DG = dry gluten

From the GLM, Table 18, Part A, it was seen that there was no interaction between the mixing time and gluten to affect the variable height and it was decided to investigate which of the two factors was more important in predicting height. Once again, the GLM procedure was used and the results are shown in Table 18, Part B. These showed that knowing gluten does not help much in predicting height if one knows the mixing time which is the more important as compared to the gluten. However, gluten and mixing time were themselves significantly correlated and it is thus possible that they did not therefore show the effect simultaneously. Hence, although gluten and mixing time are interdependent, the latter plays a more important role in predicting mixogram height.

Another result that was obtained was the set of prediction equations for the wet and dry glutens as follows:

$$WG = 28.0413 + 0.9977(MT)$$

$$DG = 10.373 + 0.0914(MT)$$

(MT = Mixing Time, WG = Wet Gluten, DG = Dry Gluten)

These equations can once again be used to predict the gluten contents of the samples given the specific mixing times in the mixograph. Thus there exists a definite quantitative relationship between the mixing time and the gluten amounts, and there is also a significant correlation between the gluten with both mixing time and height.

5) Effect of Fermentation on Gluten Contents of Commercial Hard Red Winter "Ross" Flour as Detected by the Glutomatic

During fermentation, the yeast brings about the depletion of fermentable substances, there is accumulation of carbon dioxide, alcohols, acids and esters, modification of pH conditions the gluten is rendered more elastic

and springy (Pyler, 1973). During fermentation, the unique film forming and gas retaining properties of dough come into focus. These films, probably lipid complexes in which the starch granules are embedded, form the walls of the gas vesicles that maintain their integrity to a certain extent while subjected to carbon dioxide and moisture vapor diffusion (Cotton and Ponte, 1974). Thus the fermentation time adopted as optimum represents the sum total of interrelated effects produced by such factors as character of flour, amount of yeast, temperature, formula ingredients, level of oxidation etc., and it is important to have optimum fermentation and proofing to get good dough characteristics, loaf volume, texture and quality (Hoseney, 1974).

Although some of the qualitative changes have been reviewed, very few attempts have been made to quantitatively follow the changes in gluten contents during fermentation. Thus, in this study the gluten contents were determined with varying fermentation times and Table 19 shows the values obtained. Since there were several variables e.g. mixing time, fermentation time and yeast level, Analysis of Variance was carried out to detect firstly if there was any interaction and the results are shown in Table 20.

TABLE 19

Gluten Contents of Doughs Made with Hard Red Winter "Ross" Flour

Flour Protein = 11.6%
Flour Wet Gluten = 30.0%
Flour Dry Gluten = 10.75%

Yeast (%)	Mixing Time (min)	Hours Fermentation: 0 hrs		1 hr		3 hrs		5 hrs	
		WG	DG	WG	DG	WG	DG	WG	DG
0	2	33.90	11.30	35.00	11.55	34.80	11.65	35.40	11.65
0	2	33.30	11.25	34.50	11.60	35.10	11.65	35.45	11.60
0	2	33.30	11.35	34.55	11.40	34.70	11.60	35.45	11.70
0	4	35.50	11.35	34.70	11.40	35.20	11.40	35.00	11.45
0	4	35.95	11.55	35.80	11.55	35.80	11.50	35.55	11.55
0	4	35.50	11.35	35.20	11.40	35.65	11.45	36.00	11.30
0	6	36.25	11.50	35.10	11.45	35.25	11.50	34.75	11.50
0	6	36.30	11.50	35.00	11.30	34.65	11.30	34.75	11.40
0	6	36.20	11.45	35.65	11.35	35.70	11.45	36.15	11.55
2.5	2	33.70	11.25	33.10	11.40	33.80	11.65	35.10	12.05
2.5	2	33.95	11.35	35.00	11.50	34.80	11.80	35.40	12.40
2.5	2	33.20	11.00	34.10	11.40	34.75	11.75	35.55	12.25
2.5	4	35.85	11.50	35.20	11.50	35.40	12.05	35.25	12.10
2.5	4	35.15	11.45	35.00	11.45	35.00	11.85	35.10	12.10
2.5	4	34.65	11.55	34.70	11.40	34.70	12.00	35.30	12.05
2.5	6	35.80	11.55	34.40	11.50	34.50	11.90	35.10	12.20
2.5	6	35.80	11.25	34.30	11.45	35.10	12.05	34.90	12.15
2.5	6	35.20	11.55	34.80	11.50	34.70	11.65	35.05	11.95

WG = Wet gluten, DG = Dry gluten

TABLE 20

Analysis of Variance Tables to Show Fermentation, Mixing Time and Yeast Interactions (HRW Ross Flour)

A) Dependent Variable = Dough Wet Gluten

Source	F Value	PR > F
MT	25.61	0.0001
Y	13.55	0.0006
MT**Y	0.57	0.5700
FM	4.41	0.0080
MT**FM	11.36	0.0001
Y**FM	0.72	0.5442
MT**Y**FM	0.40	0.8775

Error Mean Square = 0.1799, DF = 48

B) Dependent Variable = Dough Dry Gluten

Source	F Value	PR > F
MT	0.02	0.9766
Y	107.40	0.0001
MT**Y	6.33	0.0036
FM	74.07	0.0001
MT**FM	4.68	0.0008
Y**FM	39.43	0.0001
MT**Y**FM	1.56	0.1779

Error Mean Square = 0.0102, DF = 48

C) Dependent Variable = Gluten Absorption Factor

Source	F Value	PR > F
MT	24.43	0.0001
Y	115.44	0.0001
MT**Y	6.05	0.0045
FM	19.66	0.0001
MT**FM	3.69	0.0043
Y**FM	15.78	0.0001
MT**Y**FM	1.07	0.3912

Error Mean Square = 14.3945, DF = 48
**denotes interaction; Y = yeast; FM = fermentation;
MT = mixing time

It was seen that there was significant interaction between mixing time and fermentation time as well as mixing time-yeast and fermentation time-yeast. Because of these interactions, the two cases should be discussed separately i.e. with and without yeast. However, it was also decided that with the fermentation time = 0, the yeast did not have a chance to affect the dough properties and hence for the case "with yeast" the data for fermentation time = 0 was excluded.

To confirm this, a correlation study between the wet and dry glutens was done and the results are in Table 21. There was no significant correlation between wet and dry gluten when yeast was 0.0% but a significant correlation was observed when yeast was at 2.5% level and fermentation was greater than 0 hours. Hence, the two cases were dealt with separately.

The Analysis of Variance data for the case where yeast = 0% is shown in Table 22 and a significant interaction between mixing time and fermentation time was noted in this case. Therefore we should not average over one of the variables in order to discuss the other.

Any significant differences in the gluten values were obtained by using Fisher's LSD procedure and the results are shown in Table 23 showing the Least Square Means. The wet gluten had a minimum value when mixing time was 2 minutes and there was no fermentation. This was a case of undermixing the dough and thus the gluten did not have a chance to be hydrated or developed. In contrast, with 6 minutes mixing there was an opportunity for gluten hydration and development and thus the highest gluten value was recorded. However, the other values with varying fermentation times were not significantly different from each other. This showed that there was not much effect or change on the glutens since there was no yeast to cause appreciable changes

TABLE 21

Correlation Coefficients Between Dough Glutens and Gluten Absorption Factor

(HRW Ross Flour)

A) Yeast = 0.0%

	Dough Wet Gluten	Dough Dry Gluten	Gluten Absorption Factor
Dough Wet Gluten		0.30	0.87**
Dough Dry Gluten			-0.20

B) Yeast = 2.5% and Fermentation > 0 Hours

	Dough Wet Gluten	Dough Dry Gluten	Gluten Absorption Factor
Dough Wet Gluten		0.63**	-0.08
Dough Dry Gluten			-0.81**

**significantly different from zero at the 5% level

TABLE 22

Analysis of Variance Results for the Case Where Yeast = 0.0%

A) Dependent Variable = Dough Wet Gluten

Source	DF	F Value	PR > F
Mixing time	2	16.82	0.0001
Fermentation time	3	1.19	0.3353
MT**FM	6	7.68	0.0001

Error Mean Square = 0.1702, DF = 24

B) Dependent Variable = Dough Dry Gluten

Source	DF	F Value	PR > F
Mixing time	2	5.79	0.0089
Fermentation time	3	5.83	0.0039
MT**FM	6	4.95	0.0020

Error Mean Square = 0.0056, DF = 24

C) Dependent Variable = Gluten Absorption Factor

Source	DF	F Value	PR > F
Mixing time	2	32.15	0.0001
Fermentation time	3	0.50	0.6861
MT**FM	6	3.20	0.0187

Error Mean Square = 12.2663, DF = 24

**denotes interaction
MT = mixing time; FM = fermentation time

TABLE 23

Least Square Means for Dough Wet and Dry Glutens and Gluten Absorption Factor for the Case Where Yeast = 0.0%

Mixing time (min)	Fermentation time (hours)	Dough Wet Gluten (%)	Dough Dry Gluten (%)	Gluten Absorption Factor
2	0	33.50	11.30 A	196.47 A
2	1	34.68 A	11.52 CD	201.17 ABC
2	3	34.87 AB	11.63 D	199.70 AB
2	5	35.43 B	11.63 D	204.17 BC
4	0	35.47 B	11.37 AB	212.03 CD
4	1	35.28 AB	11.45 BC	208.17 C
4	3	35.55 B	11.45 BC	210.50 CD
4	5	35.52 B	11.43 BC	210.70 CD
6	0	36.25	11.48 BC	215.70 D
6	1	35.25 AB	11.37 AB	210.33 CD
6	3	35.20 AB	11.42 ABC	208.33 C
6	5	35.22 AB	11.48 BC	206.67 C

Means with the same letter are not significantly different at the 5% level.

during fermentation. The same results were observed for the gluten absorption factor.

For the case where 2.5% yeast was used, the Analysis of Variance results are shown in Table 24. Unlike the earlier situation (yeast = 0.0%), there was no significant interaction between the mixing time and fermentation time. Further, fermentation time had a significant effect on the gluten values, but not mixing time. This does not indicate that the mixing time has no bearing in the gluten and dough properties, but that the fermentation time plays a more prominent role in determining and predicting the glutens and thus in this particular study, since there was no interaction we could average over one of the variables in order to discuss the other.

Once again, the significant differences in the gluten contents and gluten absorption figures were obtained by Fisher's LSD procedure and the results are shown in Table 25. It was seen that here also the dough wet and dry glutens and gluten absorption factor were not significantly different for mixing times 2, 4, and 6 minutes, except that wet gluten with 4 minutes mixing time was significantly higher than that with 2 minutes mixing showing that best development occurred with optimum mixing time. Significant differences were observed, however, with varying fermentation times. Thus, it was more important how long the dough underwent fermentation to cause any significant changes than the variations in mixing time. Wet gluten was found to increase as fermentation was increased from 1 to 5 hours, but the value for 5 hours fermentation was significantly different from those with 1 and 3 hours, which were not significantly different from each other. The dry gluten values also increased from 1 to 5 hours fermentation and values for 1, 3, and 5 hours were significantly different from each other. The gluten

absorption factor, on the other hand, decreased from 1 hour to 5 hours fermentation and values were also significantly different from each other.

TABLE 24

Analysis of Variance Results for the Case Where Yeast = 2.5% and Fermentation > 0 Hours

A) Dependent Variable = Dough Wet Gluten

Source	DF	F Value	PR > F
Mixing time (MT)	2	2.61	0.1007
Fermentation time (FM)	2	5.92	0.0106
MT**FM	4	1.29	0.3123

Error Mean Square = 0.1827, DF = 18

B) Dependent Variable = Dough Dry Gluten

Source	DF	F Value	PR > F
Mixing time	2	0.20	0.8217
Fermentation time	2	84.24	0.0001
MT**FM	4	2.42	0.0867

Error Mean Square = 0.0125, DF = 18

C) Dependent Variable = Gluten Absorption Factor

Source	DF	F Value	PR > F
Mixing time	2	1.11	0.8217
Fermentation time	2	25.82	0.0001
MT**FM	4	1.16	0.3605

Error Mean Square = 12.8670, DF = 18
**denotes interaction
MT = mixing time; FM = fermentation time

TABLE 25

Least Square Means for Dough Wet and Dry Glutens and Gluten Absorption Factor for the Case Where Yeast = 2.5% and Fermentation > 0 Hrs.

Mixing Time (min)	Dough Wet Gluten	Dough Dry Gluten	Gluten Absorption Factor
2	34.62 A	11.80 A	193.50 A
4	35.07 B	11.83 A	195.97 A
6	34.76 AB	11.81 A	194.29 A

Fermentation Time (hours)	Dough Wet Gluten	Dough Dry Gluten	Gluten Absorption Factor
1	34.51 A	11.45 A	201.25 A
3	34.75 A	11.85 B	193.13 B
5	35.19 B	12.14 C	189.37 C

Means with the same letter are not significantly different at the 5% level.

The comparisons so far have been "within" the two groups or cases, considering with and without yeast. A comparative study was also made "between" the two groups i.e. to observe simultaneously any differences in the gluten contents and gluten absorption between the two cases - 0.0% and 2.5% yeast respectively, considering all the mixing times and the fermentation times, and the results are shown in Table 26. Since there was no interaction between yeast and mixing time or yeast and fermentation time, the values for wet gluten could be averaged over the variables, but because of the above interactions such an averaging was not possible in the case of dry gluten and gluten absorption factor. It was seen that the wet gluten value was significantly lower for the process with 2.5% yeast as compared to that with 0.0% yeast. This was also true for the gluten absorption factor, but the dry gluten showed the reverse phenomenon.

It has been reported that during dough making, the wet and dry gluten and gluten hydration capacity are found to decrease (Chizhova, 1965). It has not been proven that proteolytic action in a fermenting dough significantly affects dough properties but a slow but progressive liberation of amino nitrogen has been observed, and although the quantity of amino nitrogen set free from protein by proteolysis in a dough is small, it does not follow that the physical changes are also small (Kent-Jones and Amos, 1967). It has also been observed that a decrease in pH is accompanied by a reduction in the amount of washed gluten and the hydration capacity (Chizhova, 1965).

TABLE 26

Least Square Means Comparison for the Two Processes—
With and Without Yeast

A) Dough Wet Gluten

% Yeast	Dough Wet Gluten
0.0	35.18
2.5	34.82

B) Dough Dry Gluten and Gluten Absorption Factor

Mixing Time (min)	% Yeast	Dough Dry Gluten	Gluten Absorption Factor
2	0.0	11.52	200.37 A
2	2.5	11.65 B	195.17
4	0.0	11.42 A	210.35 B
4	2.5	11.75 C	198.53 A
6	0.0	11.44 A	210.26 B
6	2.5	11.72 BC	198.46 A

C) Dough Dry Gluten and Gluten Absorption Factor

Fermentation Time (hours)	% Yeast	Dough Dry Gluten	Gluten Absorption Factor
0	0.0	11.38 A	208.07 A
0	2.5	11.38 A	205.79 A
1	0.0	11.44 A	206.55 A
1	2.5	11.45 A	201.25
3	0.0	11.50 A	206.18 A
3	2.5	11.86	193.13
5	0.0	11.52 A	207.18 A
5	2.5	12.14	189.37

Means with the same letter are not significantly different at the 5% level.

Therefore it could be inferred that owing to both the action of proteolytic enzymes having a disaggregration effect and the decrease in pH due to fermentation, the values for gluten hydration and thus wet gluten were significantly lower with yeast fermentation.

Hence in this particular study, the effect of fermentation, mixing time and yeast on the gluten content and hydration was observed. The two cases - with and without yeast had to be dealt with separately due to interactions and different results were obtained. Without yeast there was no significant difference in the gluten values or gluten absorption values, probably since there was no active fermentation to cause any appreciable rheological changes. However, undermixing gave lower wet gluten and gluten absorption value when compared to the optimum mixing time, indicating that mixing to the correct degree and for the right time is essential for optimum gluten hydration and development. For the situation with yeast, fermentation time was the more important factor i.e. how long the dough was allowed to ferment made significant changes in the gluten content and hydration. Longer fermentation showed a decrease in gluten hydration as expected, but an increase in the wet glutens. However, a further comparison of the two processes, with and without yeast, indicated that both wet gluten and gluten absorption are significantly lower in the process with yeast and fermentation than the one without. Thus it was possible to show the above changes by gluten determinations.

CONCLUSIONS

The gluten contents of some Hard Red Winter and Hard Red Spring flours, wholemeals and fermented doughs made from a typical commercial bread flour were determined by the Glutomatic 2100 gluten washing system. Various correlation studies between the glutens and proteins, farinograph, mixograph, resistograph, and rheograph parameters were carried out. Prediction equations, models and interrelationships between such parameters as flour absorption and mixing time, protein, and gluten contents were determined by statistical methods.

Results showed a high significant correlation between the glutens and flour proteins for both Hard Red Winter and Hard Red Spring wheat flours. The inclusion of the "starch damage" factor in addition to the "gluten" factor improved the correlation and predictability of flour absorption since these were affected by the degree and type of flour milling.

Significant correlations were also obtained between wholemeal and sieved wholemeal glutens and wheat protein, flour protein and flour glutens. Sieving was beneficial in improving the correlations since a majority of the bran was eliminated in the sieving process.

The farinograph, rheograph and resistograph absorptions were correlated as were mixogram peak time, rheograph fatigue time and resistograph breaking point. Only the farinograph absorption and mixing time exhibited a significant correlation with the glutens and flour protein. Also, only in the case of the farinograph were the absorption and mixing time correlated.

As far as the mixograph-glutomatic correlation study with Hard Red Winter commercial bread flour was concerned, results showed that the wet gluten with the optimum mixing time was significantly higher than that with undermixing,

and the value was significantly increasing for each 1 minute mixing time till the optimum time was attained, thus showing the progressive gluten hydration and development. The mixograph height was significantly correlated with both mixing time and gluten, but mixing time was more important and sufficient in predicting the height than gluten, but this was since gluten and mixing time were again themselves correlated.

To study the effect of fermentation, mixing time and yeast level, it was necessary to classify the two processes as "with yeast" and "without yeast" respectively, as indicated by statistical analyses and interactions. Without yeast, there was little active fermentation, thus resulting in non-significant differences in the wet gluten and gluten hydration values with varying fermentation times. However, undermixing gave a significantly lower wet gluten and gluten absorption value when compared to optimum mixing. For the process "with yeast", fermentation time was the more important factor, and longer fermentation time decreased the gluten absorption but increased the gluten values. On comparison of the two processes it was found that both wet gluten and gluten absorption factor are significantly lower for the process with yeast and fermentation than the one without.

These results therefore emphasize the significance of the gluten content and quality as an important parameter in the assay of flour and wheat quality. The Glutomatic 2100 system enabled rapid gluten determinations with good reproducibility and precision especially for the flours. The study threw some light on the correlations and interrelationships of the glutens with other quality evaluation factors. From the various significant statistical correlations obtained it appears that gluten estimation decidedly leads to the prediction and understanding of the other characteristics. More work is

certainly needed to be done in this area, but it is clear that the gluten estimation test seems indeed promising for wheat and flour quality evaluation.

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SIGNIFICANCE OF GLUTEN CONTENT AS AN INDEX OF WHEAT AND FLOUR QUALITY

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ABSTRACT

Gluten content is one of the important factors in assessing flour quality. In this study, some Hard Red Winter and Hard Red Spring flours, HRW wholemeals, and fermented doughs made from a commercial HRW bread flour were analyzed for wet and dry gluten contents using the Glutomatic 2100 gluten washing system. Statistical correlation studies between glutens and the proteins, farinograph, mixograph, rheograph and resistograph parameters were carried out and prediction equations and interrelationships between glutens and proteins, flour farinograph absorption and mixing time, mixograph mixing time and fermentation factors were derived.

A high significant correlation between gluten and protein was obtained for the Hard Red Winter and Hard Red Spring flours. The inclusion of the starch damage factor in addition to gluten improved the predictability for absorption. Significant correlation was obtained between wholemeal and sieved wholemeal glutens with wheat and flour proteins as well as flour glutens, and sieving improved the correlations. The farinograph, rheograph and resistograph absorptions were correlated as were the mixograph peak time, rheograph fatigue time, and resistograph breaking point. Only farinograph absorption and mixing time showed a correlation with the glutens. For the HRW commercial bread flour, the mixograph height was significantly correlated with mixing time and gluten, but mixing time was more important in predicting height. Both wet gluten and gluten absorption were significantly lower for the process employing yeast and fermentation than the one without these factors.