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Effect of Protein Content and Wheat Variety on Solubility and Electrophoretic Properties of Flour Proteins[1]

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ABSTRACT

Quantitative distribution of the flour proteins of two hard red spring wheat varieties among five solubility groups was not affected by protein content ranging from 10.5 to 15.6%. Of the two varieties, the one with the longer farinograph dough-development time contained less acetic acid-soluble protein and more insoluble residue protein. Polyacrylamide-gel electrophoretic patterns of three of the soluble groups were not affected by protein content. This study showed that, in sound wheat, the endosperm protein is not affected qualitatively by protein content as discerned by the techniques used. Solubility distributions of proteins of flours from widely different varieties of about the same protein content showed differences that might be related to breadmaking quality. There were no major intervarietal differences in the electrophoretic patterns of the water- and salt-soluble proteins. Distinct varietal differences were observed in the patterns for the alcohol-soluble group of proteins. Such differences have been observed by others but as yet have not been related to breadmaking quality.

Studies with a number of different techniques have shown that breadmaking quality of flour from mature sound wheat is directly related to the protein content (1,2). Some investigators have observed a nonlinear relationship between loaf volume and protein content with a gradually decreasing slope with increasing protein (3). The latter observations raised the possibility of the existence of qualitative differences in the proteins of wheat samples of the same variety but of differing protein contents. This possibility was investigated with two sets of samples of pure varieties grown under identical field conditions but differing in protein content over a fairly wide range. Fractionation by solubility and polyacrylamide-gel electrophoresis were adopted as the techniques to investigate possible qualitative differences in the endosperm proteins of the various samples. Part I of this paper deals with this investigation.

Part II deals with analogous comparative studies of the proteins of three varieties generally regarded as having "poor" breadmaking potential and two bread-wheat

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varieties considered to have acceptable breadmaking quality. In this investigation an attempt was made to compare the proteins of different varieties having approximately the same protein content in the flour.

MATERIALS

Samples of two pure varieties of Canadian hard red spring wheat were composited by protein content from the material harvested from rod rows of the same crop. These samples were grown under the same climatic conditions, on three closely spaced plots measuring 36 X 16.5 ft. The variability in protein content resulted from widely variable soil fertility, which was verified by soil nitrogen analyses. The two varieties were Manitou and 11-463A (an experimental line related to the Canadian variety Pembina).

The three varieties, normally considered poor in breadmaking quality, were grown in 1968 in various locations in Canada. Pitic 62, a soft spring wheat of Mexican origin, was grown in Manitoba; Garnet, a hard red spring wheat, was grown in Alberta; and Talbot, a soft white winter wheat, was grown in Ontario.

The wheats were milled into straight-grade flour on the Buhler experimental mill after tempering overnight to 16.5% moisture. Tables I and II list the samples used, together with extensive technological data. Although some of these data are only indirectly relevant to the present study, they are included since they might be of interest to some readers.

All chemicals used were of standard reagent grade.

METHODS

Analysis of Wheat and Flour

The wheat and flour data of Tables I and II were obtained through approved methods of the AACC (5). The remix baking test (6) was used to determine loaf volumes.

Fractionation of Flour Proteins

The proteins in the flour were extracted and fractionated according to solubility by a modified Osborne fractionation as described by Chen and Bushuk (7) and summarized in Fig. 1. This fractionation gives five solubility classes: a) water-soluble proteins; b) salt-soluble proteins; c) alcohol-soluble proteins; d) acetic acid-soluble proteins; and e) insoluble residue proteins. The four soluble groups of proteins are generally referred to as albumins, globulins, gliadins, and glutenins. All extractions were made in a cold room (4°C.). The distribution of proteins among the five fractions was quantitated by the Nessler procedure (8) to determine the protein content. The water-, salt-, and alcohol-soluble fractions were freeze-dried after dialysis against distilled water as required.

Disc Electrophoresis

The water-, salt-, and alcohol-soluble proteins were examined by disc electrophoresis using the procedure of Davis (9) and an apparatus manufactured by Buchler Instruments Inc. Solutions for electrophoresis of each protein were prepared by dissolving 10 mg. of the freeze-dried protein in 1 ml. of 0.1N acetic acid solution containing 4N dimethylformamide. Thirty-five μliters of the solution was applied to each electrophoresis tube. Methyl-green dye was used as the visual

TABLE I. TECHNOLOGICAL PROPERTIES OF THE FLOUR SAMPLES USED IN PART I

	Manitou					11-463A				
Protein (N X 5.7), % (14% m.b.)	10.5	11.2	12.5	14.0	15.6	11.0	11.4	11.8	12.7	14.2
Wet gluten, %	29.7	33.5	37.0	46.0	51.0	34.0	35.5	37.2	38.6	46.9
Ash, % (14% m.b.)	0.48	0.48	0.42	0.37	0.55	0.56	0.56	0.57	0.55	0.49
Sedimentation value, cc.	39	43	51.5	60.5	66	45	46	43.5	46.5	61
Starch damage, F.U.[a]	24	23	22	19	17	21	20	21	19	20
Farinograph										
Absorption, % (14% m.b.)	65.8	65.4	66.0	66.9	69.2	62.1	60.8	62.0	62.5	64.3
Development time, min.	1.2	2.2	3.0	6.5	7.0	1.5	2.0	2.2	9.0	9.0
Stability, min.	2.0	2.8	7.2	14.0	12.5	8.0	7.0	12.0	16.0	16.0
Extensigraph										
Length, cm.	14.9	15.8	15.8	19.3	20.6	18.8	18.9	18.4	19.5	20.3
Maximum height, B.U.	437	477	445	370	335	547	782	580	580	617
Area, sq. cm.	98	116	111	120	124	210	226	186	210	232
Remix loaf volume, cc.	585	660	790	860	895	573	558	568	583	688

[a]Farrand units (4).

TABLE II. TECHNOLOGICAL PROPERTIES OF THE FLOUR SAMPLES USED IN PART II

	Pitic 62	Manitou	11-463A	Talbot	Manitou	11-463A	Garnet	Manitou	11-463A
Protein (N X 5.7), % (14% m.b.)	10.8	10.5	11.0	11.4	11.2	11.4	12.9	12.5	12.7
Wet gluten, %	37.0	29.7	34.0	37.7	33.5	33.5	38.8	37.0	38.6
Ash, % (14% m.b.)	0.47	0.48	0.55	0.44	0.48	0.56	0.50	0.42	0.55
Sedimentation value, cc.	34	39	45	36	43	46	36	52	46
Starch damage, F.U.[a]	3	24	21	4	23	20	32	22	19
Farinograph									
Absorption, % (14% m.b.)	57.7	65.8	62.1	55.2	65.4	60.8	64.3	66.0	62.5
Development time, min.	2.5	1.2	1.5	2.5	2.2	2.0	5.5	3.0	9.0
Stability, min.	2.0	2.0	8.0	2.5	2.8	7.0	7.5	7.2	16.0
Extensigraph									
Length, cm.	21.0	14.9	18.8	21.3	15.8	18.9	18.5	15.8	19.5
Maximum height, B.U.	130	437	547	242	477	782	385	445	580
Area, sq. cm.	45	98	210	86	116	220	123	111	210
Remix loaf volume, cc.	605	585	573	638	660	558	790	790	583

[a]Farrand units (4).

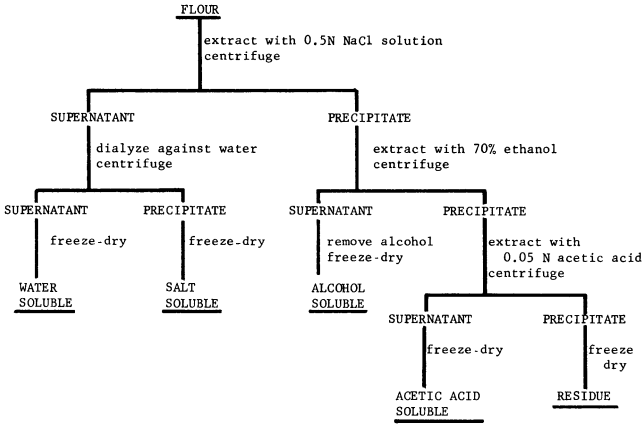


Fig. 1. Summary of protein fractionation procedure.

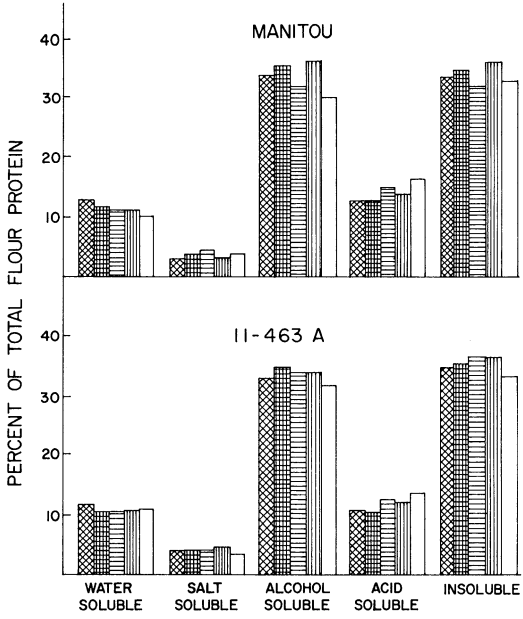


Fig. 2. Distribution of flour proteins among five solubility fractions for Manitou and 11-463A. Standard deviations based on eight complete fractions are as follows: water-soluble, 0.8%; salt-soluble, 0.8%; alcohol-soluble, 3.3%; acetic acid-soluble, 2.2%; and residue, 3.6%. Bars from left to right represent: for Manitou, 10.5, 11.2, 12.5, 14.0, and 15.6% protein flours; and for 11-463A, 11.0, 11.4, 11.8, 12.7, and 14.2% protein flour.

marker to ascertain the termination of the electrophoresis. The separated protein bands were stained with amido black dissolved in 7% acetic acid solution. Unadsorbed dye was removed by electrophoresis.

RESULTS AND DISCUSSION

Part I

The different yields of proteins of each solubility class from flours varying in protein content derived from the two varieties are compared in Fig. 2. Standard deviations based on eight complete fractionations are as follows: water-soluble, 0.8%; salt-soluble, 0.8%; alcohol-soluble, 3.3%; acetic acid-soluble, 2.2%; and residue, 3.6%. The reproducibility of this fractionation procedure is considered adequate for the purpose of the present study.

All samples contained relatively low quantities of water- (9.5 to 12.6%) and salt- (2.9 to 4.3%) soluble proteins. Together these made up approximately 15% of the flour proteins. For the variety Manitou, the water-soluble protein showed a slight decreasing trend with increasing protein content. The difference between lowest and highest protein samples, although small, is slightly greater than twice the standard deviation. A similar decrease with protein content was obtained by Bell and Simmonds (10) for pyrophosphate-extractable protein of flours from widely different varieties grown in different locations. This trend was not observed for the second variety, 11-463A. The amounts of salt-soluble protein did not show any trend with protein content. The differences between the two varieties for the water- and salt-soluble fractions were insignificant.

Alcohol-soluble protein (gliadin) content did not show any significant change with protein content. For both varieties, the amount of acetic acid-soluble protein increased slightly with increasing protein content, although the differences in each case were within experimental error. The small number of samples (five) did not permit a valid statistical test of the significance of this trend. The amounts of residue protein remained essentially constant over the protein-content range examined. The sum (expressed as percentage of total) of the alcohol-soluble, acetic acid-soluble, and residue proteins, which form the gluten proteins, increased slightly with increasing protein content for both varieties. This trend was more evident for Manitou than for 11-463A; however, again the increase was not significant within the experimental error. For the same protein content, samples of the two varieties had the same amounts of gluten, although the longer-mixing variety contained a slightly lower proportion of acetic acid-soluble protein and a slightly higher proportion of the insoluble residue protein. From these results it is concluded that the solubility properties of the proteins of flours milled from similar wheats grown under identical conditions are not affected by protein content.

Three of the soluble-protein fractions for the ten samples were examined by disc electrophoresis. Results for the samples with lowest, medium, and highest protein contents are shown in Figs. 3–5. For each variety, protein content had no effect on the electrophoretic patterns. The patterns for the water-soluble fractions are essentially the same for the two varieties. There are some minor differences in the patterns for the salt-soluble fractions that can only be discerned by very close examination. Varietal differences in the patterns for the alcohol-soluble proteins (gliadins) were readily obvious.

Figs. 3–5. Disc electrophoretic patterns for the water-, salt-, and alcohol-soluble proteins of Manitou and 11-463A wheats.

Part II

Figure 6 compares the solubility distributions for each of the three varieties normally considered as having poor overall breadmaking quality (Pitic 62, Garnet, and Talbot) with those for the two varieties of acceptable breadmaking quality used in Part I of this study (Manitou and 11-463A). For these comparisons, the data for Manitou and 11-463A were selected from Part I for the samples with approximately the same protein content as the named poor-quality variety. For example, the results for Pitic 62 of 10.8% protein are compared with the data for the 10.5%-protein Manitou and the 11.0%-protein 11-463A, and so on for Talbot and Garnet.

The distributions for each of the three poor-quality varieties showed parallel differences from the distributions for the two better varieties. Each poor variety had slightly more water-soluble protein and less salt-soluble protein; however, the differences were not significant when the experimental error was taken into account. The sum of these two fractions for each set of three varieties was essentially constant. Of the gluten proteins, each poor-quality variety contained less

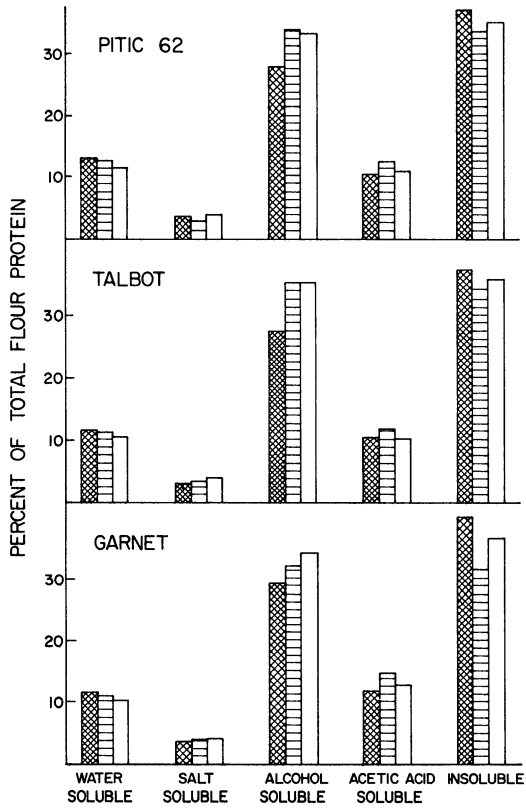
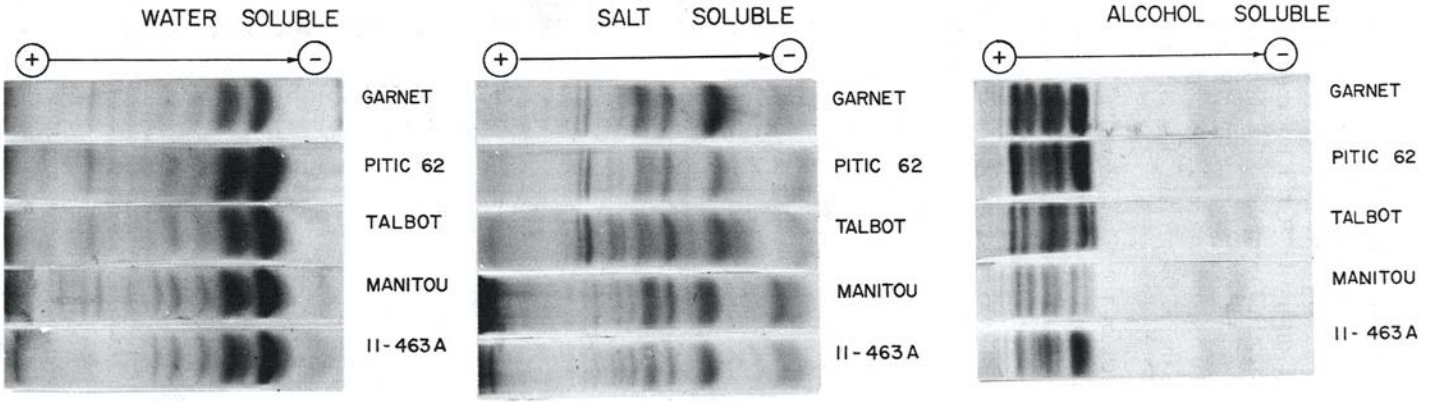


Fig. 6. Distribution of flour proteins among five solubility fractions for each of the three varieties Pitic 62, Talbot, and Garnet, as compared with values for Manitou (horizontal hatching) and 11-463A (open).



Figs. 7–9. Disc electrophoretic patterns for the water-, salt-, and alcohol-soluble proteins examined in Part II.

alcohol- and acetic acid-soluble proteins and more of the insoluble residue protein. The largest differences for each solubility group were significant within experimental error. The sum of the last three protein fractions (gluten) was not significantly different for the two good varieties as compared with the three poor varieties. The variety Garnet, which is normally characterized by a relatively short (low-extensibility) extensigram, contained an unusually high proportion of insoluble residue protein.

As was done in Part I, three of the soluble-protein fractions were examined by disc electrophoresis (Figs. 7–9). Since protein content within a variety does not affect the electrophoretic patterns (see Part I), it is justified to use the results for one sample of Manitou and 11-463A for comparison.

The water-soluble fractions (Fig. 7) showed the same major bands for the varieties. Some of the minor bands were considerably more distinct in the patterns for the two good-quality varieties, Manitou and 11-463A. However, their presence in the other varieties could be detected by the original gel.

The salt-soluble fractions (Fig. 8) contained a large number of electrophoretically distinct components. Garnet, Pitic 62, and Talbot had slow-moving bands that were more intense than the bands of the same mobility of Manitou and 11-463A. The high-mobility doublet was considerably more intense for the three poor varieties. However, there were no qualitative intervarietal differences for this fraction.

Electrophoretic patterns for the alcohol-soluble (gliadin) fractions (Fig. 9) showed the most distinct intervarietal differences. Similar intervarietal differences have been observed by other investigators (11–14).

GENERAL DISCUSSION

In Part I of this report, the proteins of the flours of two varieties of hard red spring wheat samples of different protein contents were examined by solubility fractionation and disc electrophoresis. As far as the authors are aware, this is the first study of this type applied to samples of varying protein contents of pure varieties grown under identical climatic conditions in the field. Other studies (10,15) using one or the other technique, but not both, have been reported. However, these studies used a large number of different varieties—even different classes of wheat in some cases—grown under a variety of conditions. These previous studies are extremely useful for the purpose intended by the authors; however, they do not provide an unequivocal answer to the question of whether so-called "protein quality" for breadmaking is affected by protein content. Availability of proper experimental material has been the limiting factor in such studies.

Part I of this study showed that the solubility properties and polyacrylamide-gel electrophoretic patterns of endosperm proteins are not affected by protein content in the range examined. Accordingly, within the limits of the techniques used, it is concluded that the protein content of flour does not have any qualitative effects on the protein. The variety Manitou showed a slight trend for the water-soluble protein to decrease, and acetic acid-soluble protein to increase, with increasing protein. The small number of samples used did not permit a valid test of the significance of these trends. The actual differences obtained were within the experimental error of the fractionation method. These trends observed with Manitou were analogous to those of Bell and Simmonds (10), who showed that for 26 flours from different varieties grown at different locations, the pyrophosphate-soluble protein decreased and

formic acid-soluble protein increased with protein content. On the basis of the results presented in the present paper (Parts I and II), it would appear that major portions of these trends might be due to genotypic factors.

Comparison of the solubility distributions for samples of the same protein content of the two hard red spring varieties used in Part I of this study showed that 11-463A, which had the longer dough-development time, had slightly less water- and acetic acid-soluble protein, and more salt-soluble and insoluble residue protein. The amounts of alcohol-soluble protein did not show a regular difference between the two varieties over the range of protein content examined. The higher content of insoluble residue protein in variety 11-463A could account for the longer dough-development time. Analogous results were obtained by Smith and Mullen (15).

In Part II, three varieties generally considered as being of poor breadmaking quality were compared with two good-quality varieties. As far as was possible, the comparisons were made at the same protein content.

The differences in the protein-solubility observed (Fig. 6) are considered to be sufficient to give rise to distinct differences in the rheological properties of doughs obtained from the flours investigated. The considerably higher relative content of insoluble residue protein of Pitic 62, Talbot, and Garnet, suggests that the glutens of these three varieties are actually "stronger" than that of Manitou. Indeed, at equivalent protein contents, the three so-called poor varieties had longer dough-development times and gave essentially the same loaf volumes as Manitou (see Table II). However, normally they would be rated as inferior in overall breadmaking quality because of their lower water absorption. Presumably this results mainly from the extremely low starch damage. Accordingly, the poor breadmaking performance of varieties such as Pitic 62, Talbot, and Garnet, appears to be due to their normally low protein content, and to factors other than protein, e.g., starch damage, and not to their so-called low gluten quality.

There were no major varietal differences in the electrophoretic patterns for the water- and salt-soluble fractions of the five varieties used in Part II. As found by others (11-14), definite varietal differences were observed for the alcohol-soluble fractions. Although each pattern appears to be a genotypic character, it is difficult to relate it to breadmaking quality.

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