Effect of Nitrogen Fertilization on Quantity and Composition of Wheat Flour Protein

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ABSTRACT

A fractionation method was developed to determine whether the composition of wheat flour protein is related to total protein content. Starch gel electrophoresis showed that two extractions with 70% ethanol and one extraction with 40% ethanol + 0.1 M Na_2SO_4, extracted all of the gliadins, albumins, and globulins from flour. Dialysis of the combined extracts against 0.4 M Na_2SO_4 precipitated the gliadins specifically. The protein in the residue was glutenin. Application of the method to a series of flour samples with increasing protein contents, obtained from nitrogen-fertilized wheat cultivars, showed that only the gliadin content (expressed in milligrams per gram of flour) increased. All the flour samples, regardless of cultivar or total protein content, had the same average amounts of albumin and globulin, ie, 1.6 mg of N per gram of flour. The glutenin contents of the flour samples were also independent of the total protein contents, but differed among cultivars. Glutenin content proved to be related to both baking quality and grain hardness. In each cultivar, the gliadin-to-glutenin ratio increased with loaf volume.

An interesting question is whether the composition of wheat flour protein changes when the total protein content is varied, such as when the wheat plant is fertilized with nitrogen. Reports on this subject have been contradictory. Prugar and Šašek (1970) reviewed the findings of several researchers of the 1960s and found that application of N fertilizer consistently led to a large increase in the gliadins, a smaller increase in the glutenin, and a slight increase in the albumins and globulins, such that the relative proportions of these protein fractions changed. The reviewers obtained similar results with a wide range of fertilizer compositions and applications. By contrast, Feillet and Bourdet (1967) found that the albumin and globulin content of flour did not depend on total protein content but did vary among wheat cultivars. Tanaka and Bushuk (1972) found that all protein fractions varied in proportion to the total protein content of the flour, with the composition of the protein showing no net change.

Many of the contradictory reports probably have arisen because of the differences between the fractionation methods used and the different quantities of protein found. Therefore, a prerequisite to answering the above question is finding a method through which wheat flour protein can be separated quantitatively into glutenin, gliadins, albumins, and globulins. A satisfactory new method is described in this article.

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MATERIALS AND METHODS

We used five samples each of selected cultivars grown on trial sites of the Foundation for Agricultural Plant Breeding, Wageningen, The Netherlands, in 1971. The cultivars we used were: Orca, a soft-grained wheat with poor baking characteristics; Ring, a semihard wheat with good baking properties; 6225-18-5-3, a soft-grained breeding line of intermediate baking quality (henceforth referred to as A); 6589-14-3, a hard breeding line with good baking properties (B); and H.85, a hard wheat with good baking characteristics (C). The wheats were dressed at tillering with 30 kg of calcium ammonium nitrate per hectare and with 0, 30, or 60 kg at both stem extension and at the beginning of flowering. This program yielded series of samples with increasing protein contents.

Flour of approximately 70% extraction was obtained with a Bühler laboratory mill. A Kjeldahl method was used to determine N contents. Standard baking tests were conducted by the method described by Smak (1972); the optimum $KBrO_3$ addition was determined by preliminary baking tests. The amounts added varied

between 15 and 55 mg per kilogram of flour. Electrophoresis on starch gels in aluminum lactate-lactic acid buffer, pH 3.1, was performed by a previously described method (Doekes 1968), but with 15% starch in the gels instead of 13%. The gels were stained with amido black 10 B.

RESULTS AND DISCUSSION

The Fractionation Method

In the extraction procedure, 2 g of flour was suspended in 12 ml of solvent with a Potter-Elvehjem homogenizer; the suspension was centrifuged for 20 min at 6,000 × *g*. The same quantity of solvent was used in subsequent extractions of the residue. The fraction yields varied least when the temperature did not fluctuate. Extractions and centrifugation were therefore performed in a climate-controlled chamber at 25 ± 1°C. Yields were determined by freeze-drying and weighing the fractions and determining the N contents. Albumins + globulins, gliadins, and glutenin were defined on the basis of solubility and electrophoretic migration (Doekes 1968).

Gliadins and most of the albumins and globulins were dissolved by three successive extractions of flour with 70% ethanol. The remaining albumins and globulins were extracted with salt solutions, particularly Na_2SO_4. The extraction procedure could be simplified by extracting twice with 70% ethanol, then once with 40% ethanol to which 0.1*M* Na_2SO_4 had been added. The completeness of the extraction was established as follows: the flour residue was further extracted either with the solvents mentioned or with aluminum lactate buffer, 3*M* urea solution, dimethyl formamide, or cetyl trimethyl ammonium bromide, but electrophoresis of the extracts, immediately or after dialysis, produced no migrating bands of protein. The protein remaining in the residue consisted solely of glutenin, as defined here.

Combining the three ethanol extracts and dialysis against water produced a precipitate. To separate the supernatant, the intact dialysis tube was placed in a centrifuge tube and centrifuged for 30 min at 1,600 × *g*, then was carefully cut open in a beaker. The precipitate remained in the tube. The supernatant consisted mainly of albumins and globulins and the precipitate mainly of gliadins, but the separation was imperfect. Better separations were obtained by dialysis at 4°C against three successive portions of 10 L of Na_2SO_4 solution; 0.4*M* was the optimum Na_2SO_4 concentration and 18 hr the optimum duration of dialysis. Longer dialysis led to the partial return of gliadins into solution. Figure 1 summarizes the fractionation method.

Electrophoresis showed that the albumin and globulin fraction was virtually free of gliadins and that the gliadin fraction still contained some albumins and globulins. However, probably no more than a trace was involved, because albumin and globulin bands have a relatively high dye-binding strength (Lawrence et al 1970). The quality of separation was not influenced by varietal differences in hardness and baking quality. After doing several replications, we found standard deviations of 0.18 for albumin and globulin N; 0.26 for gliadin N; and 0.18 mg per gram of flour for glutenin N.

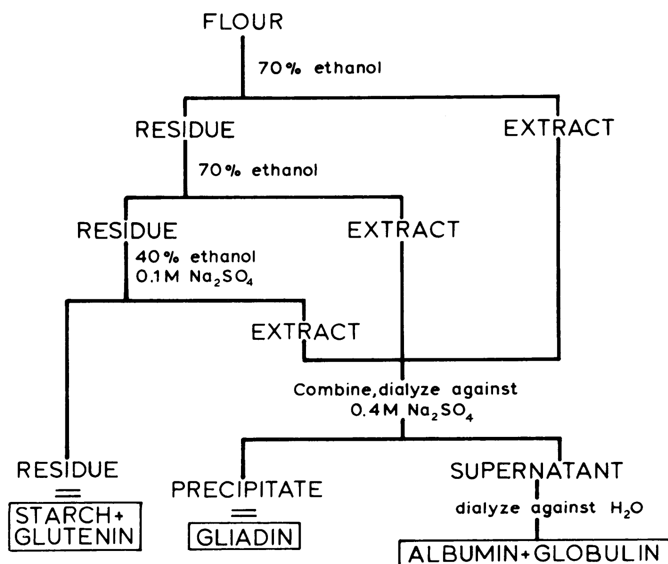

Fig. 1. Fractionation method for wheat flour protein.

Fractionation of Flour Samples

The results of the fractionation tests on flours of increasing protein contents are shown in Table I and Fig. 2. Each point in Fig. 2 is the average result of at least two fractionations and four N determinations.

As shown in Fig. 2, only the gliadins increased as the total protein content rose; the quantities of glutenin and albumins + globulins did not change. This result contrasts with the observations of other authors (Feillet and Bourdet 1967, Prugar and Šašek 1970, Tanaka and Bushuk 1972).

Another interesting point is that the quantity of glutenin per gram of flour can vary among cultivars: Orca contained 3.5, A 3.6, B 4.6, Ring 4.7, and C 5.7 mg of N per gram of flour. Only the differences between Orca and A and between B and Ring are not significant at $P = 0.05$.

The observation that all flour samples contained the same amount of albumins + globulins, ie, 1.6 mg of N per gram of flour, is new.

Table I also gives the results of the baking tests. As expected, the loaf volume for each cultivar increased more or less linearly with the total protein content. Only the gliadins increased; thus, a relationship between gliadin content and loaf volume clearly existed within each cultivar. This observation agrees with the conclusion drawn by Hoseney et al (1969) from reconstitution experiments.

TABLE I
Protein Content, Protein Composition, and Baking Quality for Some Wheat Cultivars and Selected Lines

Flour		Nitrogen[a] Albumin + Globulin	Gliadins	Glutenin	Totals	Ratio of Gliadin to Glutenin	Loaf Volume[b]
Orca	14.2	1.6	9.4	3.4	14.4	2.8	430
	16.1	1.6	11.0	3.4	16.0	3.2	460
	17.0	1.6	11.5	3.7	16.8	3.1	470
	17.7	1.5	12.2	3.5	17.2	3.5	480
	18.3	1.5	12.4	3.6	17.5	3.4	490
A	16.5	1.6	11.4	3.5	16.5	3.3	500
	18.2	1.4	12.5	3.7	17.6	3.4	520
	19.6	1.6	13.9	3.6	19.1	3.9	630
	20.2	1.4	15.0	3.5	19.9	4.3	650
	21.6	1.5	15.5	3.6	20.6	4.3	690
Ring	17.7	1.5	11.7	4.7	17.9	2.5	570
	20.4	1.6	14.3	4.6	20.5	3.1	660
	21.8	1.5	15.3	4.9	21.7	3.1	690
	22.3	1.4	16.0	4.7	22.1	3.4	700
	23.5	1.3	16.6	4.7	22.6	3.5	770
B	16.3	1.9	10.1	4.5	16.5	2.2	580
	18.2	1.6	11.5	4.4	17.5	2.6	630
	19.3	1.7	12.4	4.9	19.0	2.5	640
	20.0	1.5	13.7	4.5	19.7	3.0	700
	21.2	1.5	14.5	4.7	20.7	3.1	760
C	20.5	2.0	13.3	5.7	21.0	2.3	630
	22.1	1.4	15.3	5.6	22.3	2.7	690
	23.0	1.8	15.6	5.7	23.1	2.7	700
	23.3	1.4	15.9	5.8	23.1	2.7	700
	24.9	1.8	17.0	5.7	24.5	3.0	760

[a] Milligrams in 1 g of flour.
[b] Milligrams per 100 g of flour.

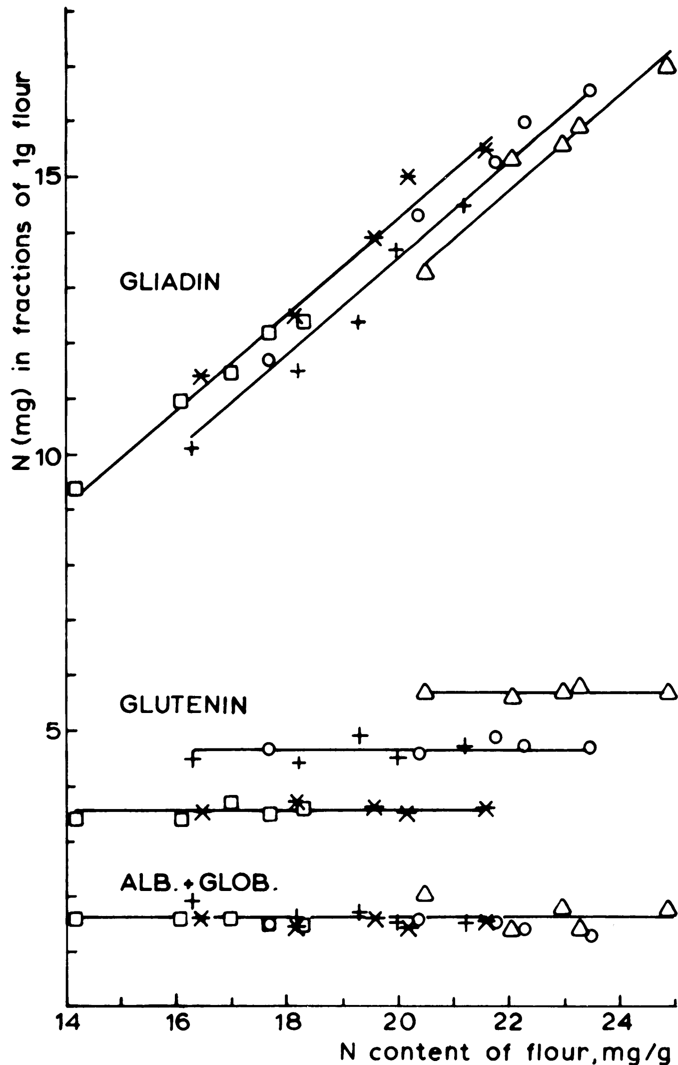

Fig. 2. Effect of increasing flour protein content on protein fractions of some wheat varieties. □ = Orca, * = A, O = Ring, + = B, and △ = C.

The same cannot be said of the glutenin fraction; various samples with equal gliadin contents but different glutenin contents, such as A 5, Ring 3, and C 3, had the same loaf volumes. Feillet (1965), Mattern et al (1968), Orth and Bushuk (1972), and Jeanjean and Feillet (1979) found correlations between the baking quality of flour and the quantity of residual protein remaining after extraction of albumins, globulins, and gliadins, although a very wide variety of methods was used. Our results, however, show only a very general relationship between glutenin content and overall baking quality. A relationship between glutenin content and grain hardness is just as warranted: the hard wheats Ring, B, and C have higher glutenin contents than the soft wheats Orca and A. The difficulty in deciding whether the glutenin content should be related to baking quality or hardness apparently is caused by the fact that both of these properties were either present or absent in our material. Soft wheats with good baking quality or hard wheats with poor baking quality were not used in this study.

Fleurent's (1896) assertion that, for good baking quality, an optimum ratio must exist between the quantities of gliadins and glutenin in flour, may also be examined in the light of our results. Table I shows that, within a cultivar, this ratio increases with loaf volume but without any apparent optimum ratio.

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