ELECTROPHORETIC COMPOSITION OF GLUTENS FROM AIR-CLASSIFIED FLOURS[1]

R. W. JONES AND R. J. DIMLER

ABSTRACT

Gluten was separated from the flour and from the high- and low-protein fractions of a hard red winter, a soft red winter, and a club wheat. These glutens were compared, using both starch-gel and moving-boundary electrophoresis. Glutens from a given wheat flour and from its high- and low-protein fractions were electrophoretically identical and exhibited the same components in the same relative concentrations.

Wheat flour can be separated by fine grinding and air classification into fractions containing levels of protein ranging from a high around 28% to a low of about 2% (1,5,13,14). In many ways this process is analogous to the Hess (6–9) procedure for separating flour protein into wedge and adhering proteins by differences in specific gravity. Microscopic examination of the high-protein fraction after air-classification shows that it contains a great amount of wedge protein, whereas the low-protein fraction contains very little. This paper describes an experiment designed to determine whether the gluten in these two fractions has the same electrophoretic components.

Methods

Gluten could not be separated from very low-protein fractions by conventional flour-washing techniques, because a gluten ball failed to form. For uniformity, an adaption of Cunningham's acetic acid dispersion method (3) was used on all samples. Flours and flour fractions prepared as described by Pfeifer and Griffin (13) were defatted by suspending 25 g. of flour in 60 ml. of n-butyl alcohol (10), stirring for 5 minutes, and centrifuging. Four such extractions were made. The centrifuge cake was then washed with petroleum ether, filtered, and equilibrated with atmospheric moisture. The flour was extracted with distilled water (3) to remove salts and water-soluble proteins. This extraction was made by stirring a suspension of 25 g. of flour in 200 ml. of distilled water for 10 minutes with a magnetic stirrer. The suspension was centrifuged at 2,000 × *g.* and the wet centrifuge cake dispersed in 175 ml. of 0.01*M* acetic acid in a Waring Blendor[2] (5 minutes at

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[2]The mention of firm names or trade products does not imply that they are endorsed or recommended by the Department of Agriculture over other firms or similar products not mentioned.

top speed followed by 10 minutes at low speed) and again centrifuged. After the centrifuge cake (starch portion) was re-extracted with 175 ml. of acetic acid, it contained some protein and was the source of the first loss of gluten (Table I). The opaque supernatant was centrifuged in a

TABLE I
GLUTEN DISTRIBUTION IN FLOUR EXTRACTS

TOTAL GLUTEN PERCENTAGE	COMANCHE (HRW)			MAYFLOWER (SRW)			OMAR (CLUB)		
	Flour	High	Low	Flour	High	Low	Flour	High	Low
Recovered	86	81	83	84	91	75	73	80	63
Lost in starch	7	5[a]	7	9	2	15	13	9	21
Lost in ultracentrifuge	7	6	10	7	6	10	10	12	15

[a] An additional 3% was lost in foam.

Spinco preparative ultracentrifuge at 20,000 *g*. Some more gluten was lost in the residue (Table I). The clear supernatant was lyophilized and subjected to electrophoretic analysis. All nitrogen remaining in the flour after water extraction was considered to be gluten nitrogen. All calculations of gluten recovery are based on this figure. The extraction with distilled water admittedly not only may fail to remove all globulins but also may dissolve some gliadin, while the acetic acid may not disperse all the gluten protein. However, essentially identical gluten yields and compositions were obtained with a representative flour by the modified Cunningham procedure and by the standard dough-washing procedure (10). Flours used and their protein contents are listed in Table II.

TABLE II
PROTEIN DISTRIBUTION IN FLOUR FRACTIONS

PROTEIN PERCENTAGE	COMANCHE (HRW)			MAYFLOWER (SRW)			OMAR (CLUB)		
	Flour	High	Low	Flour	High	Low	Flour	High	Low
Total	12.2	26.1	6.9	8.6	28.2	3.1	6.5	18.4	1.7
Water-soluble	1.3	1.8	1.2	1.1	2.0	0.6	0.9	1.5	0.5
Gluten (by diff.)	10.9	24.3	5.7	7.5	26.2	2.5	5.6	16.9	1.2

Results and Discussion

The distribution of gluten in the three portions is shown in Table I. Loss of gluten appears to be great, especially in the low-protein fractions, but the gluten extraction is really very efficient. Starch recovered from low-protein fractions contained only 0.4% protein, that from straight flours contained 0.9%, and that from high-protein fractions had 1.5% protein. Even after washing with alkali, wheat starch

contains 0.3–0.6% protein (4). This residual protein is probably enzyme inside the granule rather than being gluten protein. For example, purified dwarf-bean starch contains 0.3% protein and has starch-synthesizing power (11).

The gluten isolated as described is representative enough to establish whether fractions from a given flour are alike in terms of electrophoretic components. For the comparison both starch-gel (15) and moving-boundary (10) electrophoresis were used. The starch-gel technique yields greater resolution of components, thereby giving a more adequate comparison of the number of constituents in different samples. Moving-boundary electrophoresis is at present the more suitable for quantitative measurement of amounts of the components that are resolved by this procedure, although each electrophoretic peak is likely to contain several of the proteins which are resolved by the starch-gel technique.

The starch-gel electrophoresis patterns were essentially identical for the glutens from a given flour and its high-protein and low-protein fractions. Not only were the same components present in each of the three gluten samples, but their relative concentrations were similar, as judged by depth of staining of the protein bands. Obvious differences between the wheat varieties, however, confirmed the ability of starch-gel electrophoresis to detect differences in glutens. These differences included both relative amounts of components, as noted by Woychik *et al.* (15), and number of components. Detailed information on differences in starch-gel electrophoresis patterns of glutens from several varieties of wheat will be published separately.

Quantitative calculation of the concentrations of components resolved by moving-boundary electrophoresis also showed that the glutens from a given flour and its fractions were the same. In moving-boundary electrophoresis of a typical hard wheat gluten (from Ponca wheat) in aluminum lactate buffer, the glutenin and alpha-gliadin components detected in starch-gel electrophoresis appear as a single peak or electrophoretic component (10,15) comprising some 66% of the total proteins. The four beta-components account for a second broad peak comprising about 15% of the protein; and the single gamma-component yields a third peak comprising another 15% of the protein. Minor components are detectable with mobilities greater than and less than the main components. While the quantities making up the major electrophoretic peaks were alike for the flour and its fractions, different flours differed in the relative amounts, as had been shown earlier by Cluskey *et al.* (2) in comparing the glutens from

different varieties of soft and hard wheats.

The data from starch-gel and moving-boundary electrophoresis indicate that glutens in the wedge and adhering protein of Hess, as concentrated in high- and low-protein air-classified fractions respectively, do not differ electrophoretically. Other methods of detection and analysis, of course, might be found which would reveal differences. Profound influences on properties of wedge and adhering protein might occur also as a result of differences in amounts of nongluten protein present either in the wedge and adhering protein or otherwise associated with the high- and low-protein fractions.

There is, for example, a striking difference in the percentage of water-soluble protein in the air-classified fractions (Table III). Low-

TABLE III

WATER-SOLUBLE PROTEIN AS PERCENTAGE OF TOTAL PROTEIN

COMANCHE (HRW)			MAYFLOWER (SRW)			OMAR (CLUB)		
Flour	High	Low	Flour	High	Low	Flour	High	Low
10.6	6.9	17.4	12.7	7.1	19.3	13.8	8.2	29.4

protein fractions contain more water-soluble nitrogen than do high-protein fractions.[3] This water extract may contain gliadin as well as albumin and globulin (12). Water-solubles do have components with electrophoretic mobilities similar to those of gliadin components, but mobility alone does not prove that they are true gliadin. Differences between wedge and adhering protein might be found in the water-solubles rather than in the gluten.

An interesting difference was noted between the high-protein fraction of Comanche flour and the other flours and protein fractions studied. During initial blending of this Comanche fraction in acid, a foam formed that could not be destroyed on standing or by centrifuging or adding defoaming agent. The foam contained 3% of the total protein and was insoluble in all reagents studied. An attempt to extract gluten by gentle stirring instead of blending resulted in formation of the same insoluble material.

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[3] This result was also obtained by J. W. Pence (private communication), Western Utilization Research and Development Division, Albany, California.

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