ELECTROPHORETIC PROPERTIES OF WEDGE PROTEIN AND GLUTEN OF RYE FLOUR[1]

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ABSTRACT

Wedge protein was isolated from rye flour by sedimentation in a nonpolar liquid, the suspension being exposed to a field of centrifugal force. By moving boundary electrophoresis the presence of eight or nine migrating components was established. Gluten isolated from wedge proteins was subjected to electrophoresis under the same conditions. The mobility values of the two main components are in agreement with those of the two migrating components mostly represented in wedge protein. This fact confirms results obtained by determining the content of proteinaceous fractions in wedge protein wherein a relatively high proportion of prolamin and glutelin fractions had been found. No correlation could be found between the swelling power of rye gluten and its electrophoretic properties.

As has been demonstrated by Hess (1,2), the proteins of wheat endosperm exist in two different forms having distinguishable properties. They are the wedge protein, which occupies the interstices between starch grains and is mechanically separable from starch, and

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adhesive protein, which adheres tightly to the surface of starch grains and is resistant to mechanical separation.

Isolation of wedge protein from the crushed endosperm is possible owing to differences in specific gravities of protein particles, starch, and the starch bearing adhesive protein.

	d_4^{20} (g. cm.$^{-3}$)
Starch	1.5005 ± 0.005 [a]
Proteins	1.345 ± 0.02 [a]
Starch with adhesive protein	$1.470 \div 1.495 \pm 0.03$ [b]
Wedge protein from wheat	1.329 ± 0.003 [b]
Wedge protein from rye	1.341 ± 0.003 [b]

[a] After Hess.
[b] After Koz'mina *et al.*

Hess established that adhesive protein was not able to form gluten; he did not succeed in isolating gluten by wash-rubbing (washing) the adhesive protein whereas isolation of gluten by washing out wedge protein proceeded very easily. Later Koz'mina *et al.* (3) showed, however, that gluten can be washed out even from adhesive protein, though with appreciable difficulty; this statement is confirmed in a publication of this Institute (4). The different character of the wedge and adhesive proteins was demonstrated by Hess, by means of potentiometric titration and determination of the terminal amino acids. The dough-forming ability of these protein fractions also is different, as demonstrated by Hess through farinograph tests.

The presence of wedge and adhesive protein has been demonstrated in rye endosperm (5). Rye gluten can be washed out from the wedge protein, whereas this operation, applied directly to flour, fails. The possibility of washing out rye gluten is probably due to the higher amount of protein in the respective fraction and to the fact that, compared with the initial flour, the wedge protein shows a substantially lower amount of high-molecular-weight gums (4) which impede washing out of the gluten, as has been demonstrated by Fellenberg (6).

Up to this time the electrophoretic properties of rye gluten and rye wedge protein have not been examined.

Materials and Methods

For the isolation of wedge protein and examination of its electrophoretic properties four flours were used: flour 1 was a commercial grade; flours 2, 3, and 4 were prepared from the identical starting material by different extraction in laboratory milling apparatus (constructed at the High Technical School, Prague). Detailed characteristics of these four flours are given in Table I; the characteristics of the other flours examined are described in the text.

TABLE I
CHARACTERISTICS OF FLOURS USED IN FUNDAMENTAL WORK

FLOUR NO.	EXTRACTION	MOISTURE	ASH	TOTAL NITROGENOUS SUBSTANCES (N × 5.7)
	%	%	% dry basis	% dry basis
1	65	12.57	0.775	6.54
2	50	11.10	0.477	4.35
3	75	11.85	0.853	7.34
4	Whole-grain flour	11.06	1.808	8.99

Isolation of Wedge Protein. The wedge protein was isolated from rye flour essentially by the method of Hess (1). The flour was ground in a ball mill for 10 hr., then stirred with the nonpolar separation liquid (a mixture of carbon tetrachloride and benzene) having density $d_4^{20} = 1.37$ in the ratio 1 + 5, and centrifuged at 2,100 × *g*. The particles of wedge protein were separated by filtration and the sediment was again stirred with the separation liquid; this procedure was repeated five times altogether. The wedge protein was dried by evaporation of the separation liquid at normal temperature.

Determination of Contents of Protein Fractions in Flour, and in the Wedge Protein and Adhesive Proteins. A modified method of Lund and Sandstrøm (7) was used, differing from the original procedure in the method of extraction of glutelins. Wedge protein (0.2 g.) or flour residue (0.5 g.) after their separation (comprising essentially starch with adhering protein), or 0.5 g. flour, was successively extracted with water (water-soluble nitrogenous substances), with a 5% potassium chloride solution (globulins), and 70% ethanol (prolamins). Instead of extracting glutelins with 0.2% potassium hydroxide as in the original method, the sample, after extraction with ethanol, was extracted with 60% isopropanol, the extract was combined with the ethanolic extract, and the glutelins were extracted with 60% isopropanol containing 0.2% sodium bisulfite. This reagent was used by Waldschmidt-Leitz *et al.* (8) for isolating barley glutelin. In all instances the extraction was carried out for 1 hr. with three 25-ml. portions of the extracting reagent.

Isolation of Rye Gluten, and Determination of Its Swelling Power. Gluten was isolated by washing dough prepared from wedge protein with 2% sodium chloride solution after a resting period of 30 min. The swelling power was determined in the usual way, according to Berliner-Koopman (9).

Electrophoretic Analysis of Wedge Protein and Gluten. Wedge protein or gluten freshly washed out was dissolved in a solution of

aluminum lactate, about 0.017*M*, as used in electrophoretic analysis of wheat gluten (10). The aluminum lactate solution used had pH 3.2 and specific conductivity 591.7 mho/cm. at 1°C. The protein solution was dialyzed against aluminum lactate solution, centrifuged, and analyzed by moving-boundary electrophoresis using the apparatus *Makroelektrophoresegerät* (Carl Zeiss, Jena). The electrophoresis was carried out at 2.0°C.

Results and Discussion

Determination of individual fractions in the original flour (No. 1), in the wedge protein, and in the adhesive protein gave the results shown in Table II. In comparison with the original flour, the participa-

TABLE II
CONTENT OF PROTEIN FRACTIONS IN FLOUR, WEDGE PROTEIN, AND ADHESIVE PROTEIN

	CONTENT OF FRACTIONS					CONTENT OF FRACTIONS (% OF EXTRACTABLE NITROGENOUS SUBSTANCES)			
	Water-Soluble Nitrogenous Substances	Globulins	Prolamins	Glutelins	Insoluble Residue of Nitrogenous Substances (N × 5.7)	Water-Soluble Nitrogenous Substances	Globulins	Prolamins	Glutelins
	% d.b.[a]	% d.b.	% d.b.	% d.b.	% d.b.				
Flour	2.90	0.88	0.57	0.39	1.89	61.18	18.56	12.03	8.23
Wedge protein	18.00	3.67	6.98	4.02	10.09	55.10	11.23	21.37	12.30
Adhesive protein	2.45	0.79	0.13	0.15	1.46	69.60	22.45	3.69	4.26

[a] Dry basis.

tion of the prolamin and glutelin fractions in wedge protein is increased, whereas in adhesive protein the content of these fractions is very low. Nevertheless, in the rye wedge protein both the total protein content and the participation of prolamins and glutelins are relatively low compared with analogous preparations from wheat; this is clear from comparison of the data in Table II with the results presented by Hess (2). The total protein content in crude wedge protein from rye flour was 42.76%. The content of gluten fractions (prolamin and glutelin) was 25.72% of the total protein. In the crude wedge protein from wheat flour, prepared under the same conditions, the content of total protein was more than 87%, the gluten fractions content being 90% of the total protein.

By electrophoresis of the extract of wedge protein from flour 1 in

aluminum lactate at a field strength 9.68 V. cm.$^{-1}$, the presence of eight migrating components was established. Analogous results were obtained also by electrophoresis of wedge protein originating from flours 2, 3, and 4. A typical electrophoretic pattern is shown in Fig. 1.

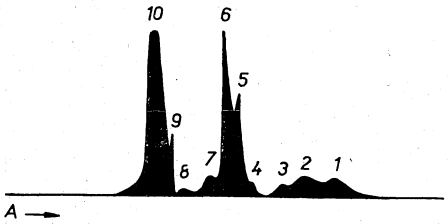

Fig. 1. Electrophoresis of wedge protein from flour 2; pH = 3.2, field strength 8.00 V.cm.$^{-1}$ Ascending boundary. Peak 10 represents the δ boundary.

TABLE III

ABSOLUTE MOBILITY VALUES OF WEDGE PROTEIN COMPONENTS OF FLOURS 1, 2, 3, AND 4

FLOUR NO.	ABSOLUTE MOBILITY VALUES OF COMPONENTS, cm.^{2} V^{-1} sec.$^{-1}$ × 10^{-5}								
	1	2	3	4	5	6	7	8	9
1	9.5	8.0	6.4	[a]	4.5	3.8	2.8	2.3	1.2
2	9.5	8.2	6.7	4.8	4.1	2.7	2.5	1.6	1.3
3	8.9	8.0	6.7	5.0	4.0	2.9	2.7	1.8	1.1
4	9.0	7.8	6.5	4.9	3.9	3.2	2.3	[a]	1.2

[a] Not observed.

The established components of wedge protein (Table III) include components of all the fundamental protein fractions. Differentiation of gluten components in the electrophoretic pattern of wedge protein was effected by washing gluten from wedge protein and by electro-

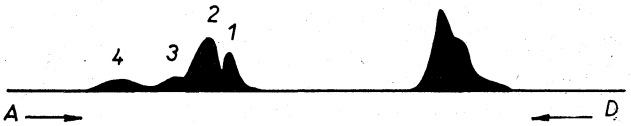

Fig. 2. Electrophoresis of gluten from flour 2; pH = 3.2, field strength 6.08 V.cm.$^{-1}$ Peak 4 represents the δ boundary.

TABLE IV

ABSOLUTE MOBILITY VALUES OF GLUTEN COMPONENTS FROM FLOURS 1 TO 4

FLOUR NO.	ABSOLUTE MOBILITY VALUES OF GLUTEN COMPONENTS, cm.^{2} V^{-1} sec.$^{-1}$ × 10^{-5}		
	1	2	3
1	4.3	3.6	2.1
2	4.1	2.9	1.8
3	4.1	3.0	1.8
4	4.1	3.1	1.9

TABLE V

SWELLING POWER OF GLUTENS, ABSOLUTE MOBILITY VALUES, AND RELATIVE CONTENT OF THEIR COMPONENTS

FLOURS		GLUTENS									
No.	Protein Content (N × 5.7)	Swelling Power	Absolute Mobility Values of Components, cm.^{2} V^{-1} sec.$^{-1}$ × 10^{-5}				Relative Content of Components				
			1a	1	2	3	1a	1	2	3	4
	% *d.b.*						%	%	%	%	%
1	7.11	8		4.5	3.3	2.2		20.9	52.8	9.3	16.9
2	7.21	3	5.4	4.4	3.3	1.9	7.3	19.9	44.1	7.9	20.9
3	7.43	5		4.3	3.1	1.9		23.5	42.2	9.1	25.2
4	8.15	10		4.5	3.3	1.9		27.9	46.9	9.1	16.1
5	8.10	9		4.4	3.2	1.9		26.1	49.8	6.8	17.2
6	7.37	14	5.4	4.4	3.2	1.9	8.1	19.8	39.0	11.2	21.9
7	8.04	4	5.0	4.1	2.9	1.9	9.3	18.5	41.5	11.5	19.2
8	8.11	16		4.4	3.3	2.1		23.1	48.5	8.5	19.9
9	10.61	20		4.5	3.2	1.9		30.5	39.8	9.6	20.1
10	7.58	4		4.8	3.5	2.1		26.3	41.3	10.6	21.8
11	7.14	7		4.2	3.1	1.9		25.0	45.3	10.3	19.3
12	6.99	14		4.5	3.4	1.9		27.3	46.3	9.1	17.3
13	7.13	11		4.5	3.3	2.0		22.6	49.3	8.8	19.4
14	7.43	9		4.5	3.3	1.9		24.2	49.3	9.0	17.5
15	7.71	9		4.3	3.2	1.8		29.1	44.6	9.7	16.6
16	7.52	19		4.3	3.2	1.8		28.7	46.0	8.5	16.8
17	8.26	14		4.3	3.1	1.7		36.8	39.3	8.2	15.7
18	8.43	12		4.4	3.3	2.0		28.6	44.0	8.4	19.1
19	8.13	5	5.6	4.4	3.2	2.0	11.4	19.6	44.4	8.4	16.2
20	12.72	20	5.6	4.4	3.2	1.9	5.4	21.8	46.2	8.3	19.0

phoretic analysis under the same conditions as above. Figure 2 shows a typical electrophoretic pattern. Glutens from flours 1 to 4 contained three migrating components.

Comparison of mobility values of the components of wedge proteins and the components of the corresponding glutens (Table IV) indicates that the components of wedge proteins designated by numbers 5, 6, and 8 are identical with gluten components.

Twenty commercial rye flours were further examined for possible correlation between the gluten quality expressed by swelling power and its electrophoretic properties (Table V). In spite of clear differences existing in the swelling power of rye glutens, their electrophoretic characteristics were but little different.

In five instances four migrating components have been ascertained; in the other instances, three migrating components. The mobility values of the corresponding gluten components are very close as is the relative content of the individual components. In flours in which four migrating components have been found, there probably occurs an additional partition of the component of greatest mobility, as far as can be judged on the basis of the relative content of components.

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