SIGNIFICANCE OF WEDGE PROTEIN OF RYE FLOUR FOR DOUGH PROPERTIES[1]

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ABSTRACT

Wedge protein of rye flour influences the mechanical properties of dough, especially its coherence. It is significant for the water-absorption of flour. Its presence is indispensable for formation of normal rye dough. The significance for dough properties probably lies in an interaction between protein fractions and soluble high-molecular gums. The possibility of formation of complexes between rye proteins and gums was evidenced by determination of the intrinsic viscosity of the solutions of model mixtures of rye gluten and rye gums.

It is well known that rye dough represents a viscous system consisting essentially of two phases, the liquid and the solid. Viscosity and surface tension of the liquid phase seem to have decided significance in regard to mechanical properties of rye dough (1).

Of special importance to the properties of the liquid phase are the water-soluble high-molecular gums (2,3), which give more viscous solutions than gums of other cereal origin (4). On the other hand, almost no importance for properties of rye dough was attributed to proteins, in view of the fact that they are partly dissolved in the liquid phase (whereas it was taken for granted that they only negligibly influence the viscosity of the latter, compared with gums) and partly dispersed in the form of swelled solid particles. The circumstance that rye proteins do not form any elastic structure in dough has been explained by Fellenberg (5), on the basis of properties of rye gums, which in his conception envelop the protein particles, preventing their mutual linkage.

The conclusions of Fellenberg were later confirmed and extended (6,7). However, it was also found that even rye proteins are able to form a more or less elastic gel (8,9). The finding of wedge protein, the existence of which in wheat endosperm was demonstrated by Hess (10,11) and the presence of which in rye endosperm was demonstrated by Koz'mina *et al.* (12), made it possible to obtain in a simple way a natural concentrate of rye proteins in the native state, having but a low content of gums (7). Gluten can be washed out from rye wedge protein in the usual manner, its properties being little different from those of wheat gluten (6,12).

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Of importance to the question of the mutual relations of proteins and gums are the observations made by Udy (13) on wheat flour components. He established that interaction occurs between wheat gluten and wheat gums with formation of complexes, which are the cause of flour extract viscosities exceeding the theoretical value determined by the character and concentration of the individual components. The question is of importance also for rye flour component solutions, the more so, that the viscosity of the liquid phase of rye dough is one of the most important factors affecting the properties of the dough.

The work reported in the present paper was an investigation of 1) the influence of wedge protein on properties of rye dough and 2) the possible formation of complexes between proteins and gums contained in the rye flour.

Materials and Methods

The wedge protein and the water-soluble flour fraction were isolated from rye flour T 930 designed for bakery production, containing 0.775% ash and total nitrogenous substances (N × 5.7) 6.54% (both dry basis).

Also used were several preparations of rye glutens and gums which had been isolated from commercial flours of the same type (T 930), average grade.

Isolation of Wedge Protein. Substantially by the method of Hess (10), flour was ground for 10 hr. in a ball mill, then was stirred up with a nonpolar separation liquid (a mixture of carbon tetrachloride and benzene) having density $d_4^{20} = 1.37$, in the ratio 1:5. The suspension was centrifuged (2,100 × *g*) and the particles of wedge protein were collected by filtration of the supernatant. The whole procedure was repeated five times altogether. The wedge protein and the residue of flour after separation were dried at room temperature. Composition of the wedge protein is given in the table below.

Composition of Isolated Wedge Protein

	% dry basis
Total nitrogenous substances (N × 5.7)	42.76
Water-soluble nitrogenous substances	18.00
Globulins (extracted with 5% potassium chloride)	3.67
Prolamins (extracted with 70% ethanol)	6.98
Glutelins (extracted with 60% isopropanol + 0.2% sodium bisulfite)	4.02
Unextractable nitrogenous substances	10.09

Isolation of Gluten. The wedge protein was made into dough with a requisite amount of 2% sodium chloride solution and, after 30 minutes' standing, gluten was washed out by manual rubbing.

Dry Gluten. This was prepared by immediate freezing of the freshly washed gluten to −20°C. and freeze-drying.

Isolation of Water-Soluble Fraction. Flour tempered to 0°C. was mixed with five parts of water cooled equally to 0°C. After 15 minutes' stirring the suspension was centrifuged. Both the extract and the residue were immediately frozen to −20°C. and freeze-dried.

Farinograph Determinations. These were performed with the Brabender Farinograph (50-g. bowl).

Micro Baking Tests. Doughs prepared from mixtures of model systems were leavened with rye whole sour (1,204 g. of whole sour, 688 g. of mixture, and 258 g. of water). Rising interval was 30 min. at 26°C.; bread was baked first at 280°C. for 15 min. and then at 200°C. for 45 min.

Amylograph Determinations. There were performed with a Brabender Amylograph. The suspensions were prepared in the ratio 80 g. material to 450 ml. water.

Diastatic Activity. This was estimated by determining the content of maltose formed in aqueous suspension in the ratio 1:10 at 27°C. during 1 hr. Maltose was determined by Schoorl's method (14), after inactivation of amylases by zinc sulfate and potassium ferrocyanide.

Isolation of Rye Gums. Flour was inactivated by threefold boiling in 80% ethanol, and dried at room temperature. It was then mixed with water in a ratio of 1:10 (wt.:vol.). After several hours' extraction, the suspension was centrifuged. The deproteination of the extract was carried out by addition of zinc sulfate (0.5*M* solution) followed by potassium ferrocyanide (0.25*M* solution). The liquid was centrifuged and the extract was dialyzed against water. The gums were precipitated with ethanol in the ratio 1:3 and dried by washing with acetone and ether.

In another modification of the procedure, the gums were salted out by saturating the extract with ammonium sulfate. The precipitate was dissolved in water and the solution was freed from nitrogenous matter as above and dialyzed against water. The gums were precipitated with ethanol and dried in the given way.

Composition of Isolated Gums. This was determined by the phenol-sulfuric acid method used by Gilles and Smith (15) in an investigation of wheat gums. The gums were hydrolyzed; the monosaccharides were separated on Whatman No. 1 paper, eluted, and determined (after addition of phenol solution and sulfuric acid) by means of a Carl Zeiss spectrophotometer. Relative content of monosaccharides of the gums used is shown in Table I.

TABLE I

RELATIVE MONOSACCHARIDE CONTENT OF GUMS PREPARED FROM DIFFERENT FLOURS OF 75% EXTRACTION

GUM	GALACTOSE	GLUCOSE	ARABINOSE	XYLOSE
	%	%	%	%
Precipitated with ethanol				
No. 1	3.5	18.2	35.1	43.2
No. 2	2.5	21.0	31.9	44.6
No. 3	2.3	20.4	33.7	43.6
Precipitated with ammonium sulfate	0	2.0	37.0	61.0

Viscosity Determinations. These were carried out with a Höppler rheoviscosimeter at 20°C. and at shearing force 20 g.cm.$^{-2}$. Solutions of model mixtures of proteins and gums were measured immediately after their preparation.

Results and Discussion

The protein content of the isolated rye wedge protein was considerably lower than that of the wedge protein from wheat flour isolated by Hess (10) under the same conditions. However, since mixtures with only a limited amount of wedge protein were to be used, it was considered unnecessary to prepare products of a higher purity. This could be achieved by using separation liquid of lower density (7,12). Microscopic observation of the isolated crude wedge protein showed the presence of free protein particles not attached to starch, and of a certain amount of small broken starch granules.

Influence of Wedge Protein on Rye Dough Properties. Model mixtures of wedge protein and of the flour residue after its separation were used. The initial content of wedge protein in flour was 5.34%; the mixtures were prepared in the following combinations (mixture No. 3 corresponds to the original flour):

Mixture	*Wedge Protein*	*Residue*
	%	%
1	0	100
2	2.67	97.33
3	5.34	94.66
4	10.68	89.32
5	21.36	78.64

(a) *Farinograph determinations.* It is obvious from Table II that dough prepared from the residue alone had poor firmness and, contrarily, the highest stability. However, it was very incoherent and resembled starch paste, although this circumstance did not become perceivable on the farinogram.

TABLE II

FARINOGRAPH TESTS WITH MIXTURES OF WEDGE PROTEIN AND FLOUR RESIDUE

MIXTURE No.	WATER ABSORPTION	FIRMNESS OR ELASTICITY	WEAKENING OF DOUGH
		f. units [a]	*f. units*
1	63	50	120
2	61	80	150
3	63	70	160
4	66	60	150
5	74	55	150

[a] Farinograph units.

The most firm and elastic dough resulted from mixture No. 2 in which the content of wedge protein was half of its original content in flour. It is evident that wedge protein acts in the dough to some extent as a structural factor. It is also of great significance for water-absorption of the mixtures.

At the same time, however, the farinograph tests confirmed theories attributing the utmost significance for the properties of rye dough to the liquid phase, and hence to components dissolved therein. The farinograph examination was performed with the water-insoluble fraction and with its mixtures with the water-soluble fraction, as shown in

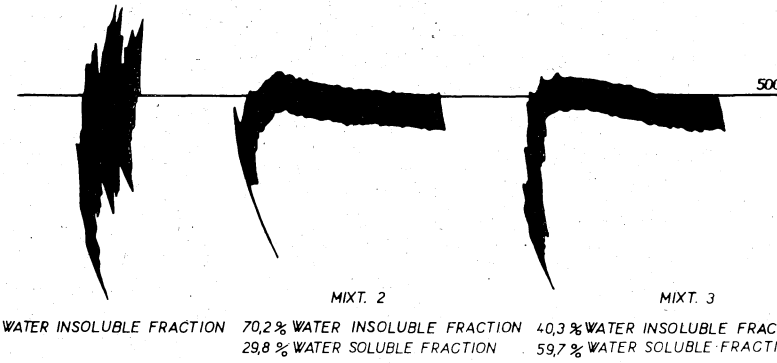

Fig. 1. Farinograms of the water-insoluble fraction and of its mixtures with the water-soluble fraction. Water absorption of mixture 2, 75.5%; of mixture 3, 78.0%.

Fig. 1. In the absence of the water-soluble fraction, the loss in normal dough character was more extensive than in the absence of wedge protein and was evidenced even on the farinogram. The dough thus obtained was entirely incoherent and showed the character of a starch paste.

(b) *Amylograph determinations.* Maximum viscosity was determined with water suspensions of the mixtures in the ratio 80 g.:450 ml. water.

Diastatic activity was determined at the same time to ascertain whether differences in amylographic values might be due to differences in diastatic activity of the mixtures.

Maximum viscosity decreased with increasing content of wedge protein in the mixture. This decrease was not connected with changes of diastatic activity of the mixtures, which was essentially constant (3.0% maltose), and might be due to reduced concentration of starch.

This suggestion was confirmed by an experiment in which residue alone (prepared from another flour) was used. Instead of wedge protein the appropriate amount of water was added. The results are shown in Fig. 2.

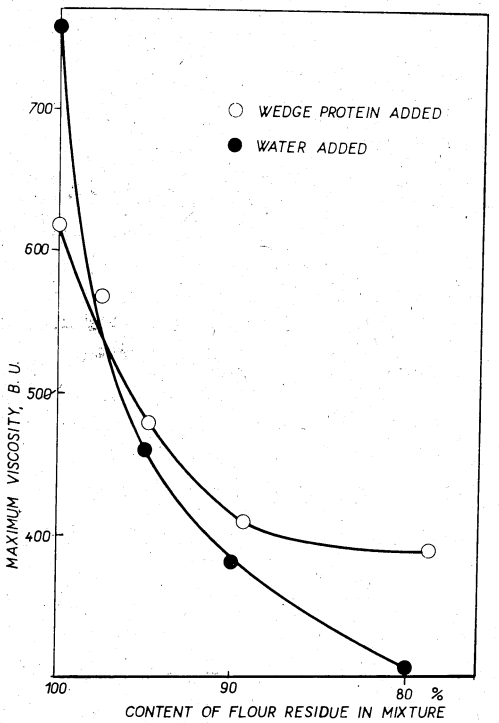

Fig. 2. Maximum viscosities of model mixtures of residue of flour with wedge protein and of residue of flour diluted with different amounts of water.

The lesser decrease of maximum viscosity by addition of wedge protein might be due to the presence of a certain amount of starch in the wedge protein.

(c) *Micro baking tests.* Doughs prepared from model mixtures were leavened with rye whole sour (1,204 g. of whole sour, 688 g. of mixture,

258 g. of water). The rising interval was 30 min. at 26°C. The bread was baked first at 280°C. for 15 min. and then at 200°C. for 45 min. Farinograph determinations were confirmed by the results of the micro baking tests. Crumb of the bread prepared from flour residue only was dry, crumbling, and cracked, because of the low coherence of the dough caused by absence of wedge protein. Bread made from mixtures Nos. 2 to 5 showed no remarkable differences from normal products.

It was thus confirmed that the presence of wedge protein was indispensable for formation of a normal dough. Dough prepared without it had poor mechanical qualities, which were especially manifested by crumbling of the dough and by lack of coherence.

Although the wedge proteins were less significant for dough properties than the water-soluble fraction, the important role they played in the structure of rye dough could not be denied. This conclusion is contrary to theories existing until now on the role of proteins in rye dough. The mechanism of action of the wedge protein is not explainable as merely influencing the viscosity of the liquid phase by simple dissolution of their soluble components, or by absorption of a certain portion of water, due to swelling of the insoluble components, respectively. As will be evident from further results, the viscosity of wedge protein solution is rather low, compared with the viscosity of rye gums, which are a significant component of the water-soluble fraction.

That is why the possibility of formation of complexes between proteins and gums of rye flour was examined, in an analogous way, as demonstrated by Udy (13) with these components of wheat.

Complex Formation between Proteins and Gums of Rye Flour. Intrinsic viscosity of the solutions of wedge protein and gums. The logarithm of relative viscosities of wedge protein solutions, gluten solu-

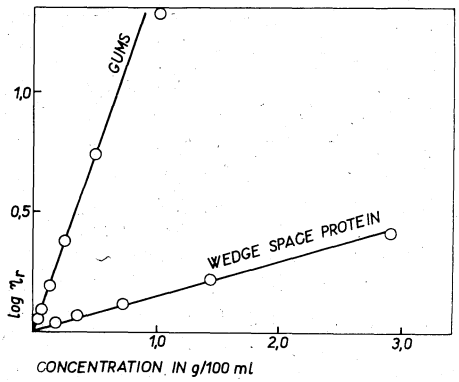

Fig. 3. Relation between relative viscosity and concentration of the solutions of gums and of wedge protein.

tions, and gum solutions, respectively, in 0.02*N* lactic acid showed linear dependence on concentration of the substances. This is shown by an example of wedge protein solution and gum solution (Fig. 3).

The determination of intrinsic viscosities of model mixed solutions of wedge protein and gums showed that no interaction occurred between these substances. The intrinsic viscosities were complying with the condition:

$$[\eta] = \sum_{i=1}^{n} [\eta]_i W_i$$

where $[\eta]_i$ was intrinsic viscosity of the component i, and W_i its weight fraction in the mixture. (See Table III.)

TABLE III

INTRINSIC VISCOSITIES OF GUMS (PRECIPITATED WITH ETHANOL) AND WEDGE PROTEIN SOLUTIONS IN 0.02*N* LACTIC ACID

WEIGHT FRACTION		INTRINSIC VISCOSITY	
Gums	Wedge Proteins	Measured	Calculated
1.0	0	3.338	....
0	1.0	0.357	
0.508	0.492	1.829	1.872
0.345	0.655	1.352	1.385
0.104	0.896	0.669	0.666
0.064	0.936	0.551	0.549

Results were similar with solutions of both substances in 0.05*N* acetic acid.

The effective intrinsic viscosity of solutions was conformable to the calculated theoretical values. Consequently, formation of complexes between wedge protein and gums could not be assumed.

In the further course of the work rye gluten, both wet and freeze-dried, was used in place of wedge protein. The latter contained a relatively large amount of polysaccharides; hence, the possibility of interaction and engagement of active centers during dissolution of wedge protein could not be excluded.

The results obtained in determining viscosity of both lyophilized and wet gluten and of several preparations of rye gums are given in Table IV.

With model mixed solutions of gums and gluten a higher intrinsic viscosity was observed than would correspond with the theoretical values. Thereby it was demonstrated that the gluten proteins of rye flour were able to form complexes with the gums of the flour. The formation of such a complex might explain (among other probable

TABLE IV

INTRINSIC VISCOSITIES OF RYE GLUTENS AND GUM SOLUTIONS

SOLVENT	PRODUCT USED	WEIGHT FRACTION		INTRINSIC VISCOSITY	
		Gums	Gluten	Measured	Calculated
Lactic acid, 0.02*N*	Gums precipitated with ethanol, No. 2, lyophilized gluten	1.0	0	9.488	....
		0	1.0	0.610	....
		0.385	0.615	4.203	4.028
Aluminum lactate, about 0.017*N*	Gums precipitated with ethanol, No. 3, lyophilized gluten	1.0	0	5.662	....
		0	1.0	0.816	....
		0.326	0.674	2.588	2.396
Lactic acid, 0.02*N*	Gums precipitated with ethanol, No. 3, freshly washed-out gluten	1.0	0	5.662	....
		0	1.0	1.018	....
		0.187	0.813	2.021	1.887
Lactic acid, 0.02*N*	Gums precipitated with ammonium sulfate, freshly washed-out gluten	1.0	0	4.698	....
		0	1.0	0.259	....
		0.339	0.661	1.805	1.429
Lactic acid, 0.02*N*	Gums precipitated with ammonium sulfate, freshly washed-out gluten	1.0	0	4.698	....
		0	1.0	0.514	....
		0.237	0.763	1.625	1.505

explanations) the fact that rye wedge protein proved to be significant for the rye dough structure.

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