XCIV. ON THE CHANGES OF WHEAT PROTEINS UNDER THE ACTION OF FLOUR AND YEAST ENZYMES.

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THE observation of Blagoveschenski and Sossiedov [1933] of the occurrence in wheat flour of an enzyme with the specific power of disaggregating gluten made it desirable to study in greater detail the changes undergone by wheat proteins in the process of bread making.

In a preliminary orientating experiment an examination was made of the distribution of nitrogen in the flour itself and in the mixture at various stages up to baking; the wheat proteins were separated into fractions by successive extractions with (1) water, 50 parts, (2) 72 % alcohol, 30 parts, (3) 10 % NaCl, 30 parts and (4) 2 % NaOH, 50 parts; these fractions contained respectively (1) leucosin and non-protein nitrogen, (2) gliadin, (3) globulin, (4) glutenin. In the water-soluble fraction additional determinations were made of the nitrogen precipitable with lead acetate and of the free amino-nitrogen by the method of Linderström-Lang [1928].

The results obtained in this manner from two samples of flour are shown in Table I from which it appears that during fermentation of the dough there is

Table I. *Distribution of nitrogen in the different fractions.*

mg. N per g. flour.										
Flour No. 1						Flour No. 2				
	Flour	Sponge		Dough		Flour	Sponge		Dough	
		Before making	At making	After mixing	Before baking		Before making	At making	After mixing	Before baking
Nitrogen of water-soluble fraction	4.37	5.95	8.23	8.57	4.50	3.05	4.85	6.17	5.81	3.43
Nitrogen in the lead precipitate	4.25	5.09	8.26	8.38	4.50	3.00	4.30	6.08	5.80	3.46
Nitrogen of free amino-groups of water-soluble fraction	0.26	0.62	0.56	0.84	0.84	0.25	0.50	0.30	0.67	0.78
Gliadin	6.37	5.15	5.10	5.02	7.90	5.04	4.18	4.21	4.20	6.22
Globulin	3.86	3.62	2.26	3.57	3.55	3.50	3.62	2.09	3.23	3.15
Glutenin	11.59	11.43	11.43	11.43	11.43	9.21	8.18	8.18	8.18	8.18
Total nitrogen	25.11	26.07	26.46	27.53	26.46	19.8	20.73	20.65	21.29	20.98

a marked decrease in the water-soluble nitrogen and an increase in the gliadin-nitrogen. The glutenin remains constant as does also the total nitrogen if allowance be made for the slight increase of the latter due to addition of flour during making. The completeness of precipitation of the water-soluble nitrogen by lead acetate indicates the absence of any appreciable degree of proteolysis except in the sponge before making, and any increase which occurs in this fraction is therefore to be ascribed to increased solubility of the proteins.

Action of flour enzymes on wheat proteins.

Preparations of flour enzymes were made by extracting 10 g. of flour with 100 ml. of *M*/6 citrate-phosphate buffer at varying p_H and 37° for 24 hours; the filtered extracts were mixed with equal volumes of glycerol. For the experiments 1 g. of freshly prepared wheat proteins was mixed with 20 ml. of buffer solution, 5 ml. of enzyme extract and 1 ml. of toluene; fractional analyses of the products were made after digestion for 48 hours at 37°. The results of these analyses are shown in Table II, Experiment (A).

Table II. *The action of flour proteinases upon wheat proteins.*

	p_H 5.1				p_H 4.8				p_H 3.8			
	Water sol. fract.	Gliadin	Globulin	Glutenin	Water sol. fract.	Gliadin	Globulin	Glutenin	Water sol. fract.	Gliadin	Globulin	Glutenin
Experiment (A)	22.2	15.6	12.8	51.5	22.4	15.6	12.7	51.0	20.1	15.8	14.9	51.6
Fresh enzyme (a)	2.4	2.4	2.4	2.4	2.4	2.4	2.4	2.4	2.4	2.4	2.4	2.4
Fresh gluten (b)	16.1	16.4	14.3	51.2	16.0	16.0	14.3	51.0	15.8	16.4	14.5	51.2
Difference: $A - (a + b)$	+3.7	-3.2	-1.5	-2.1	+4.0	-2.8	-4.0	-2.4	+1.9	-4.0	-2.0	-2.0
Enzyme and gluten both heated	18.3	16.5	14.6	51.5	18.1	16.3	14.5	51.04	18.0	16.2	14.4	51.1
Heated enzyme and fresh gluten	18.6	16.7	14.8	51.4	18.4	16.4	14.7	51.1	18.2	16.2	14.5	51.1
Fresh enzyme and heated gluten	18.6	16.5	14.4	51.2	18.1	16.3	14.4	51.1	17.9	16.3	14.5	51.2

The possibility of adsorption of proteinases by the wheat protein preparation has to be considered. An attempt to eliminate this factor in the usual way by heating the preparation failed, since, as is shown by the figures in the second, third and fourth lines of Table II, heating rendered the proteins resistant to the action of the flour enzymes. Determinations were therefore made of the changes occurring in the protein and enzyme preparations separately on digestion under the above conditions (*a* and *b* in Table II); the differences between the figures obtained in the original experiment (A) and the sum of those obtained in experiments (*a*) and (*b*) were taken to represent the true action of the flour enzymes. It is evident that there is an increase in the water-soluble nitrogen and a decrease in the various protein fractions, this decrease being most marked with gliadin and least with glutenin.

Effect of p_H . The effect of p_H on the action of the flour enzymes on the wheat proteins is shown in Table III. The experimental mixtures consisted of 1 g. wheat proteins, 20 ml. buffer and 5 ml. enzyme extract; in the controls 5 ml. of buffer were substituted for the enzyme. It is seen that the maximum increase in water-soluble nitrogen occurs at p_H 8.5 and the minimum at p_H 7.5; furthermore these reactions correspond with the maxima and minima of the decreases in the various protein fractions.

It is noteworthy that glutenin remains unaffected during the first 24 hours, suggesting that it may be protected by the other proteins so long as these remain intact.

Changes in the amino-nitrogen. Table IV gives the results of amino-nitrogen determinations in the various fractions during digestion of wheat proteins with flour enzymes. Increases occur in both experimental and control solutions but the differences between the experiments and their corresponding controls are significant only in the case of the water-soluble fraction, and here only at the

Table III. *Effect of action of flour enzymes on wheat proteins.*

mg. N per g. wheat proteins.									
p_{H}	24 hours		48 hours		72 hours		144 hours		Increase or decrease in 144 hours
	Exp.	Control	Exp.	Control	Exp.	Control	Exp.	Control	
Water-soluble fraction.									
3.8	20.1	18.4	36.6	19.0	43.8	19.9	59.4	20.0	+39.4
4.0	21.8	18.4	38.5	19.0	45.2	20.0	58.8	19.9	+38.9
4.8	22.4	18.4	36.2	19.0	45.5	19.9	58.8	19.9	+38.9
5.1	22.2	18.6	38.0	18.7	45.4	19.9	58.7	19.9	+38.8
6.5	23.4	18.1	—	—	—	—	—	—	—
7.5	19.8	18.1	29.0	20.0	39.1	20.0	43.7	19.9	+23.8
8.5	27.2	19.0	47.8	20.3	67.3	20.4	81.0	20.4	+60.6
Gliadin.									
3.8	15.8	16.4	14.8	16.6	10.9	15.5	5.6	15.6	-10.0
4.0	15.9	16.4	14.0	16.7	9.8	15.4	5.9	15.5	-9.6
4.8	15.6	16.4	14.9	16.7	10.0	15.5	6.0	15.5	-9.5
5.1	14.6	16.7	14.5	16.7	10.0	15.6	6.0	15.6	-9.5
6.5	16.3	20.7	—	—	—	—	—	—	—
7.5	18.9	20.7	16.1	17.9	12.1	17.9	11.1	15.6	-4.5
8.5	15.5	20.7	11.4	17.9	5.5	16.9	3.6	16.9	-13.3
Globulin.									
3.8	13.9	14.5	7.3	14.8	6.4	14.7	2.3	14.7	-12.4
4.0	13.0	14.7	6.5	14.8	6.0	14.7	4.0	14.7	-10.7
4.8	12.7	14.7	7.5	14.8	6.2	14.7	3.4	14.7	-11.3
5.1	12.8	14.8	7.1	14.8	6.2	14.7	3.4	14.7	-11.3
6.5	9.4	10.5	—	—	—	—	—	—	—
7.5	11.4	10.5	7.3	11.6	5.9	11.6	4.8	14.6	-9.8
8.5	7.7	10.4	6.5	12.6	4.5	13.1	2.6	13.2	-10.0
Glutenin.									
3.8	51.62	51.1	42.4	51.4	40.1	51.4	34.1	51.4	-16.3
4.0	51.12	51.1	42.6	51.5	40.1	51.4	31.9	51.4	-19.5
4.8	51.0	51.1	42.5	51.4	40.1	50.1	31.9	51.3	-19.4
5.1	51.5	51.5	42.6	51.6	40.1	50.1	31.9	51.3	-19.4
6.5	51.0	51.0	—	—	—	—	—	—	—
7.5	51.0	51.1	48.7	51.8	45.0	51.8	42.4	51.3	-8.9
8.5	51.0	51.1	36.6	51.6	26.7	50.6	18.1	50.5	-32.4

more acid reactions; at p_H 8.5 where the maximum solution of proteins occurs the changes in amino-nitrogen are negligible. It is clear therefore that, except at the acid reactions (where some hydrolysis of the dissolved proteins must be assumed), the increased solubility of the proteins which has been shown to occur under the influence of the flour enzymes is not accompanied by true proteolysis.

Action of yeast enzymes on wheat proteins.

During the process of bread making the wheat proteins are exposed to the action of yeast enzymes as well as to that of the flour enzymes already considered. Table V gives the results of the action on wheat proteins under conditions similar to those employed in the experiments described above, of preparations made by extracting 10 g. of fresh pressed yeast with 100 ml. of buffer in presence of toluene at 37° for 24 hours, the filtered extract being mixed with an equal volume of glycerol. It will be observed that in contrast with the flour enzymes the yeast enzymes have no action at p_H 8.5; moreover the figures for amino-nitrogen in Table VI indicate that at the point of maximum activity (p_H 4.8-6.5) the action of the yeast enzymes consists of a true proteolysis.

Table IV. *The nitrogen of free amino-groups.*

pH	24 hours		48 hours		72 hours		144 hours		Difference at 144 hours
	Control	Exp.	Control	Exp.	Control	Exp.	Control	Exp.	
(a) Water-soluble fraction.									
3.8	2.75	6.3	5.4	18.3	8.2	27.0	10.0	29.0	+19.0
4.0	2.4	6.7	5.1	18.7	8.1	27.2	10.1	29.2	+19.1
4.8	2.4	5.9	5.2	14.4	8.0	25.6	9.8	26.8	+17.0
5.1	2.4	5.9	4.9	12.6	8.1	24.1	9.8	25.8	+16.0
7.5	2.4	2.5	2.5	4.0	2.6	5.3	5.3	8.2	+2.9
8.5	2.45	3.5	3.6	4.3	8.5	8.7	15.0	15.9	+0.9
(b) Gliadin fraction.									
3.8	4.7	2.8	4.0	1.0	3.7	0.4	0.2	0.2	0
4.0	4.5	2.8	4.0	1.0	3.6	3.6	2.0	0.2	-1.8
4.8	4.4	3.1	4.0	1.1	3.6	3.6	2.6	0.4	-2.2
5.1	4.4	3.1	4.0	1.2	3.6	3.6	4.2	3.5	-0.7
7.5	4.5	4.5	4.4	4.4	4.5	4.5	4.2	3.5	-0.7
8.5	4.5	4.6	4.4	4.5	3.5	3.5	2.0	2.0	0
(c) Globulin fraction.									
3.8	4.6	2.8	4.0	1.1	3.7	0.4	0.2	0.2	0
4.0	4.5	2.9	4.0	1.1	3.6	0.5	2.0	0.2	-1.5
4.8	4.5	3.2	4.0	1.1	3.5	0.7	2.6	0.4	-2.2
5.1	4.5	3.2	4.1	1.2	3.6	0.8	2.7	0.4	-2.3
7.5	4.5	4.5	4.4	4.4	4.5	4.1	4.2	3.5	-0.7
8.5	4.5	4.9	4.4	4.7	3.4	3.5	1.5	1.5	0
(d) Glutenin fraction.									
3.8	5.2	3.8	4.8	2.2	2.2	1.2	2.2	0.6	-1.6
4.0	5.1	3.8	4.7	2.3	2.3	2.0	2.2	0.6	-1.6
4.8	4.8	3.9	4.7	2.4	2.2	2.0	2.3	0.6	-1.7
5.1	4.8	4.0	4.7	2.4	2.3	2.1	2.3	0.7	-1.6
7.5	5.1	5.1	5.1	5.1	5.1	5.0	5.1	4.3	-0.8
8.5	5.1	5.1	4.9	4.9	3.3	3.3	1.4	1.4	0

Table V. *The effect of yeast ferments on the total nitrogen of various fractions.*

mg. N per g. wheat proteins.									
pH	24 hours		48 hours		72 hours		144 hours		Difference at 144 hours
	Control	Exp.	Control	Exp.	Control	Exp.	Control	Exp.	
(a) Water-soluble fraction.									
4.0	18.4	25.3	18.9	39.2	19.9	43.9	19.9	55.2	+35.3
4.8	18.4	36.9	19.0	47.2	19.9	54.0	19.9	72.5	+52.6
6.5	18.0	33.6	18.7	42.9	18.6	50.8	18.7	71.7	+53.0
8.5	19.0	19.1	20.3	20.4	20.4	20.4	20.4	20.5	+0.1
(b) Gliadin fraction.									
4.0	16.3	13.1	16.7	7.1	15.4	6.6	15.5	4.7	-10.8
4.8	16.4	8.6	16.7	4.1	16.5	3.0	15.5	2.2	-13.3
6.5	20.8	10.2	20.4	5.5	20.4	4.4	20.4	2.3	-18.1
8.5	20.6	20.7	17.9	17.9	16.9	17.9	16.9	16.7	-0.2
(c) Globulin fraction.									
4.0	14.7	12.2	14.8	6.8	14.7	5.3	14.7	4.2	-10.5
4.8	14.7	6.1	14.8	2.5	14.7	1.1	14.7	1.1	-13.6
6.5	10.6	7.7	10.4	5.3	10.4	4.1	10.4	2.1	-8.3
8.5	10.4	10.4	12.5	12.5	13.1	13.5	13.2	13.2	0
(d) Glutenin fraction.									
4.0	50.9	50.0	51.5	49.7	50.4	45.6	50.4	37.8	-12.6
4.8	51.1	50.1	50.0	47.7	50.1	43.5	51.3	24.9	-26.4
6.5	50.1	50.0	50.6	49.1	50.6	44.4	50.1	25.8	-24.3
8.5	51.1	51.1	51.5	51.5	50.5	50.5	50.5	50.5	0

Table VI. *Effect of yeast proteases upon the free amino-nitrogen of the different fractions of wheat proteins.*

mg. N per g. wheat proteins.									
<i>p</i> _H	24 hours		48 hours		72 hours		144 hours		Difference at 144 hours
	Control	Exp.	Control	Exp.	Control	Exp.	Control	Exp.	
(a) Water-soluble fraction.									
4.0	2.4	10.5	5.1	18.3	8.1	27.2	10.0	29.2	+19.2
4.8	2.0	18.7	2.4	27.5	5.1	40.7	10.1	51.1	+41.0
6.5	2.5	15.7	2.5	24.9	5.6	39.2	9.7	48.5	+38.8
8.5	2.5	3.5	3.6	4.3	8.5	8.7	15.9	15.8	-0.1
(b) Gliadin fraction.									
4.0	4.5	5.7	4.0	3.8	3.6	2.3	2.0	2.0	+1.8
4.8	3.6	4.2	4.4	3.9	4.0	2.5	2.0	2.0	0
6.5	4.5	5.3	4.5	5.0	4.0	4.4	2.6	2.0	-0.6
8.5	4.5	4.5	4.4	4.5	3.5	3.5	2.0	2.0	0
(c) Globulin fraction.									
4.0	4.5	5.3	4.0	3.0	3.6	2.5	2.0	2.8	+0.8
4.8	3.5	3.6	4.5	2.1	4.0	1.1	2.0	1.0	-1.0
6.5	4.5	4.5	4.5	5.4	3.7	4.0	2.7	2.0	-0.7
8.5	4.8	4.8	4.4	4.7	3.4	3.5	1.5	1.5	0
(d) Glutenin fraction.									
4.0	5.1	5.8	4.5	8.0	2.3	10.1	2.2	11.4	+9.2
4.8	5.0	5.8	4.8	8.9	4.7	11.2	2.2	12.0	+9.8
6.5	5.0	6.1	4.9	8.0	4.8	10.3	2.5	12.7	+10.2
8.5	5.1	5.1	4.9	4.9	3.3	3.3	1.4	1.4	0

The effects of temperature on the activities of the flour and yeast enzymes are shown in Table VII from which it will be seen that whilst the flour enzymes exhibit an optimum at 37–40° the yeast enzymes are equally active over the range 20–37° and only show a falling off when a temperature of 45° is reached.

Table VII. *The effect of temperature upon the action of the proteolytic ferments of flour (A) and yeast (B) upon wheat proteins during 24 hours at p_H 4.8.*

mg. N per g. wheat proteins.												
A.	20°			37°			40°			45°		
	Begin- ning	End	Difference	Begin- ning	End	Difference	Begin- ning	End	Difference	Begin- ning	End	Difference
Water-soluble fraction	18.4	31.9	+13.5	18.98	36.24	+17.26	18.99	36.25	+17.26	19.5	31.2	+11.7
Gliadin	17.19	15.13	-2.06	16.59	14.9	-1.8	16.71	14.93	-1.78	16.68	15.43	-1.25
Globulin	14.8	9.50	-5.3	14.8	7.5	-7.3	14.8	7.45	-7.35	14.8	9.55	-5.25
Glutenin	51.4	44.5	-6.9	51.4	42.5	-8.9	51.4	42.5	-8.9	51.32	44.9	-6.42
B.	20°			30°			37°			45°		
	Begin- ning	End	Difference	Begin- ning	End	Difference	Begin- ning	End	Difference	Begin- ning	End	Difference
Water-soluble fraction	18.4	36.9	+18.5	18.4	36.9	+18.5	18.4	37.0	+18.6	18.4	28.8	+10.4
Gliadin	16.4	8.6	-7.8	16.4	8.6	-7.8	16.4	8.6	-7.8	16.4	12.5	-3.9
Globulin	14.7	6.1	-8.6	14.7	6.2	-8.6	14.7	6.2	-8.5	14.7	12.1	-2.6
Glutenin	51.1	50.1	-1.0	51.1	50.0	-1.1	51.1	50.0	-1.1	51.0	50.0	-1.0

SUMMARY.

1. Enzymes are present in flour which exercise a definite solvent effect on wheat proteins; this effect is to be regarded as disaggregation rather than proteolysis since it is not accompanied by increase in amino-nitrogen.

2. The effect of the flour enzymes is most marked at p_H 8.5. At this p_H yeast enzymes have no action on wheat proteins. At p_H 4.8–6.5 yeast enzymes also exercise a solvent effect on wheat proteins, but this is due to a true proteolysis.

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