ENZYMATIC HYDROLYSIS OF DISPERSED WHEAT GLUTEN[1]

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ABSTRACT

Gluten from hard red spring wheat dispersed in 8% sodium salicylate with sodium bisulfite added (pH 6.8) was partially digested by papain and by an enzyme in a commercial enzyme preparation. Similar gluten dispersed in aluminum lactate (pH 3.05) was partially digested by pepsin and by a second enzyme in the commercial preparation. There were qualitative, and probably quantitative, differences in the action of the enzymes. Precipitates formed in all digests of sodium salicylate dispersions. The amino acid distribution of these precipitates was quite different from that of whole gluten, there being much higher proportions of lysine, arginine, aspartic acid, and alanine and much lower proportions of glutamic acid and proline in the precipitates.

The addition of fungal proteolytic enzymes to aged flour improves the quality of bread that can be produced from such flour. In this laboratory, the improvement in quality has been evidenced by improved handling quality of the doughs, improved elasticity and texture of the gluten washed from such doughs, and substantial increases in loaf volume of the bread. It is clear that the physical properties of the gluten are altered by the enzyme action, and it was decided to try to determine the nature of the changes. It was first necessary to study the action of these enzymes on normal wheat gluten, and this paper reports the results of preliminary studies.

The role of proteases in relation to bread-baking was reviewed by Hildebrand (1) in 1946. The early work was largely concerned with the presence of such enzymes in wheat and flour and the effects of very small additions of enzymes in the baking process. The effects of above-

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normal concentrations of proteolytic enzymes have been reported as generally deleterious to bread quality (2,3). The action of pepsin was reported to be different from that of papain (4,5).

Harris (6) added various proteases to dough balls from which gluten was washed immediately. He found greater dispersibility of gluten from such doughs, and these effects increased with added increments of the enzymes. Swanson and Andrews (7) found that the amount of gluten that could be recovered from doughs treated with papain decreased as the amount of papain was increased or the rest period of the doughs was lengthened. This effect was not obtained with doughs treated with yeast water, cysteine, or glutathione.

One would expect that the immediate effect of proteolytic enzymes on gluten would be the hydrolysis of peptide bonds in the amino acid chains of the proteins. Johnson and Miller and their associates (8,9) carried out extensive experiments on the effects of various enzyme preparations on doughs and gluten. They suggest (8) that the increased extensibility of doughs to which proteolytic enzymes have been added is the result of the breakage of peptide bonds.

The action of pepsin on glutenin has recently been studied by Oka *et al.* (10). They reported that the cleavage of a few peptide bonds rapidly produced large molecular polypeptides. Further pepsin action caused a substantial increase in small peptide fractions.

The action of specific proteolytic enzymes on dough and gluten is affected by conditions such as temperature and pH. It is pertinent to the work reported in this paper to note that investigators (9) have found that the optimum pH for enzymes such as pepsin and some fungal proteases is as low as 3.0; for papain, 5.5; and for trypsin near 7–8. Oka *et al.* (10) gave 2.0 as the optimum pH for pepsin action on glutenin.

Materials and Methods

All gluten samples used in this study were prepared from ether-extracted flour from the variety Red Bobs, a hard red spring wheat grown at Edmonton, and milled on an experimental Buhler mill. Details of preparation of gluten and dispersing agents and of the method of dispersion are given in an earlier paper (11).

Enzymes used included the following:

Fermex MT, a commercial preparation of enzymes produced by *Aspergillus oryzae,* containing two apparently distinct proteases, was obtained from Wallerstein. According to information provided by the supplier in a private communication, the pH optimum for one of these is at 3.5 and of the other at 7.0.

Crystalline pepsin (2x) was obtained from Worthington Biochemicals.

Papain was obtained from Fisher Scientific Co.

Enzymatic digestion was carried out either in Erlenmeyer flasks or in cellophane tubing. Weighed amounts of the enzyme to be used were added to specific volumes of the dispersed gluten which had been brought to the reaction temperature in a thermostatically controlled water bath. In some experiments, dialysis was carried on simultaneously with the digestion; in all such experiments, cellophane tubing was used. A bag made from the tubing was filled, the enzyme added, and the bag tied to a rotary dialyzer and immersed in a vessel containing a large volume of the dispersing medium at the reaction temperature.

Since the enzymes could not be inactivated by adding chemicals or changing pH without seriously altering the solubility properties of the protein and its degradation products, no inactivation was attempted. Subsequent procedures were carried out as promptly as possible after the end of the reaction period and, where necessary to hold the samples, the reaction products were kept in a cold room at 3°C.

Aliquots of some digests were subject to precipitation by bringing them to half-saturation with magnesium sulfate. The precipitate was separated by centrifugation at 9,500 × *g* for 20 min.

Sodium salicylate (8%), with or without added bisulfite, has been used by many workers as a dispersing agent for gluten (7,11). Aluminum lactate has also been frequently used in recent years (11). These and other dispersing agents were tested by McCalla and Verma (11) and shown to give essentially similar results. Since the pH values of these agents are roughly equivalent to the optimum for the action of three of the four enzymes to be tested, it was decided to make preliminary trials using each enzyme with gluten dispersed in aluminum lactate (0.02*M* in respect to aluminum), sodium salicylate (8%), and 8% sodium salicylate to which 50 mg. sodium bisulfite per 100 cc. had been added.

Results

Digestion Studies. As already noted, Harris (8) added various proteases to dough balls during mixing and then immediately washed out the gluten. In the early stages of the present study, a somewhat similar procedure was tried. It was found that, if the usual practice of allowing the dough ball to stand under water for 1 hr. was followed, the gluten could not be recovered by washing. It also seemed likely that

some of the enzyme might be removed by the washing procedure. It was therefore decided to carry out all digestion studies on dispersed gluten.

These preliminary experiments showed that 1) Fermex MT digested gluten dispersed in all three reagents; 2) papain digested gluten dispersed in sodium salicylate plus bisulfite but not in aluminum lactate nor in sodium salicylate by itself; 3) pepsin acted only when the gluten was dispersed in aluminum lactate.

Olcott *et al.* (12) reported that gluten dispersions in sodium salicylate were not affected by proteases. It is certain that the pH of these dispersions is not suitable for some proteases, *e.g.*, pepsin; activation of others may be necessary, as was found with papain in this study.

It was decided to study the effects of papain and the neutral protease of Fermex on gluten dispersed in 8% sodium salicylate to which had been added 50 mg. of sodium bisulfite per 100 ml. (pH = 6.8), and the effects of pepsin and the acid protease of Fermex on gluten dispersed in 0.02*M* aluminum lactate (pH = 3.05). It is to be understood that, in the remainder of this paper, all dispersions referred to as "in sodium salicylate" had sodium bisulfite added.

Quantitative estimates were obtained by determining the loss of digestion products, either by dialysis or by the recovery of partially digested material by precipitation of the digest, using half saturation with magnesium sulfate. Examples of the results obtained are given in Table I. These have been selected to illustrate the various proce-

TABLE I

EXAMPLES OF QUANTITATIVE RESULTS OBTAINED IN DIGESTION STUDIES

Enzyme	Enzyme Conc.	Dispersing Agent	Digestion Period	Temperature	Percentage of Total Gluten Protein Digested	
					Determined by Loss Due to Increased Solubility [a]	Determined by Loss by Dialysis
	mg./100 ml.		*hr.*	*°C.*	%	%
Fermex	100	Sodium salicylate	4	37	32	..
			12	37	40	..
			36	37	58	..
Papain	2		4	37	27	..
			12	37	34	..
			36	37	52	..
Fermex	100	Aluminum lactate	12	37	..	10
			36	37	..	26
Pepsin	20		12	25	..	7
			36	25	..	24
			60	25	..	56

[a] The figures in this column give the percentage of total protein remaining in solution at half-saturation with magnesium sulfate. It is clear that some of the precipitated material had been partially digested and that at least some of the precipitated products were relatively small in size.

dures used. Direct comparisons of the effects of the different enzymes cannot be made, since it was not possible to standardize the quantity of active enzyme added to the dispersions. Further quantitative studies are being carried out, since qualitative results have indicated that the differences in action of the different enzymes on gluten are significant.

During digestion of gluten dispersed in sodium salicylate it was noted that a precipitate gradually formed in the digestion vessels. This occurred in digestions with either papain or Fermex. These precipitates were most abundant in the experiments in which simultaneous dialysis was carried on. The precipitates were loose and bulky. When separated by centrifugation, they proved to be insoluble in the usual dispersing agents including 0.1*N* acetic acid and 0.1*N* hydrochloric acid, but they were soluble in dilute sodium hydroxide. The precipitates included 10–15% of the total nitrogen of the original dispersions.

Samples of the original gluten and of the precipitates from the digests were hydrolyzed in 20% hydrochloric acid for 24 hr. and the hydrolysate was analyzed on an amino acid analyzer. The results are presented in Table II, together with the results for whole gluten obtained by Woychik (13).

There are minor differences in the results obtained for whole gluten in this study as compared with those obtained by Woychik (13),

TABLE II

AMINO ACID COMPOSITION OF CRUDE WHOLE GLUTEN AND OF PRECIPITATES FROM ENZYME DIGESTS

(Expressed as g. of amino acid per 100 g. of protein (N × 5.7))

	CRUDE GLUTEN		PRECIPITATE	
	Woychik [a]	Present Study	Papain Digest	Fermex MT Digest
Lysine	1.3	1.6	4.2	4.6
Histidine	2.4	2.0	2.9	3.1
Ammonia	5.6	4.3	3.0	3.0
Arginine	2.6	3.4	7.0	7.2
Aspartic acid	3.2	2.8	7.1	7.4
Threonine	2.7	2.5	3.6	3.7
Serine	5.7	4.6	5.4	5.4
Glutamic acid	40.8	37.1	27.1	25.0
Proline	15.0	12.7	8.1	8.0
Glycine	3.4	3.4	4.2	5.4
Alanine	2.6	2.5	4.6	4.9
Cystine (½)	1.2	1.3	1.4	1.5
Valine	4.5	3.8	5.7	5.5
Methionine	1.3	1.5	2.3	2.4
Isoleucine	4.4	3.7	4.6	4.7
Leucine	7.4	6.8	9.2	8.9
Tyrosine	4.2	3.5	4.7	4.7
Phenylalanine	5.4	5.0	5.6	5.6
Total	113.7	102.5	110.7	111.0

[a] See ref. 14.

but most of these are accounted for by the difference in "total recovery." For example, adjustment of the values for glutamic acid to bring them to the same proportion of 100% reduces the difference between Woychik's and the present values to less than 0.5 g./100 g. protein.

On the other hand, the composition of the precipitates from both the papain and Fermex digests is quite different. The two precipitates give essentially similar results, with the values for lysine, arginine, aspartic acid, and alanine much higher than those for whole gluten whereas those for ammonia, glutamic acid, and proline are much lower. Even when the results for "total recovery" are adjusted, these differences are quite definite.

The values for ammonia (derived from amide groups) and arginine suggest that the precipitate may represent either the most insoluble part of the "glutenin" fraction of whole gluten or the distinctly different fraction found in all dispersed gluten studied in this laboratory (*e.g.*, 14). Under conventional methods of precipitation of the dispersed gluten the latter fraction appears to be the *most* soluble, however, and it seems doubtful that it would be precipitated during enzymatic digestion. No definite conclusion can be reached on the fraction that the precipitate represents. It is possible, of course, that it is a fraction produced as a result of partial hydrolysis of part of the gluten by the enzyme. Further studies of this material are being carried out, but so far no identification of its origin can be made.

Small amounts of precipitates were formed in the pepsin digests of gluten in aluminum lactate, but these were not analyzed.

A rough qualitative measure of the differences in the action of the various enzymes was obtained by subjecting the digests to high-speed sedimentation analyses. These showed clearly that pepsin and the acid protease of Fermex acted to produce large quantities of intermediate-sized products but left little if any of the gluten untouched. No attempt was made to determine further similarities in the action of these two enzymes. Papain and the neutral protease of Fermex, on the other hand, appeared to produce large quantities of much smaller products, but at least part of the gluten was apparently not digested to any extent since the rate of sedimentation of this part was unaffected by the digestion. It was also clearly demonstrated that some of the relatively small digestion products which were eliminated by dialysis during digestion were precipitated by half-saturation of the digest with magnesium sulfate.

While the results reported here are preliminary in nature, they indicate that the enzymes used act effectively on dispersed gluten and,

as one would expect, the action of the different enzymes produces different types of digestion products. More detailed studies are being carried out, but these studies are complicated by the relatively poor solubility of the more-insoluble fractions of gluten.

Literature Cited

1. HILDEBRAND, F. C. *In* Enzymes and their role in wheat technology, J. A. Anderson, ed.; pp. 275–294. Interscience: New York (1946).
2. TISSUE, KATHRYN A., and BAILEY, C. H. A study of the proteolytic enzymes of malt preparations. Cereal Chem. **8:** 217–226 (1931).
3. READ, J. W., and HAAS, L. W. Baking quality of flour as affected by certain enzyme actions. Cereal Chem. **11:** 280–298 (1934).
4. RUPP, E., and BAILEY, C. H. The effects of protease increments on the plasticity of doughs. Cereal Chem. **14:** 496–510 (1937).
5. HARRIS, R. H., and JOHNSON, J. A. A study of the effects of proteolytic enzymes and potassium bromate upon the viscosity and allied properties of dispersions of hard red spring wheat gluten. Cereal Chem. **17:** 203–222 (1940).
6. HARRIS, R. H. A study of the effect of proteoclastic enzymes upon wheat gluten solubility in sodium salicylate solutions. Cereal Chem. **15:** 690–707 (1938).
7. SWANSON, C. O., and ANDREWS, A. C. Effects of papain, yeast water, cysteine, and glutathione on gluten dispersion or on disintegration as indicated by gluten recovery and by mixogram patterns. Cereal Chem. **22:** 134–149 (1945).
8. JOHNSON, J. A., and MILLER, B. S. Amylases and proteases in baking. Trans. Am. Assoc. Cereal Chemists **10:** 14–20 (1952).
9. JOHNSON, J. A., MILLER, B. S., BOYER, P. D., and GEDDES, W. F. Properties of certain protease systems used in breadmaking. Cereal Chem. **33:** 1–17 (1956).
10. OKA, S., BABEL, S. J., and DRAUDT, H. N. Proteolytic action of pepsin on glutenin. J. Food Sci. **30:** 212–217 (1965).
11. MCCALLA, A. G., and VERMA, S. C. Sedimentation of gluten in several dispersing reagents. Can. J. Biochem. Physiol. **40:** 1123–1128 (1962).
12. OLCOTT, H. S., SAPIRSTEIN, L. A., and BLISH, M. J. Stability of wheat gluten dispersions toward reducing agents in the presence and absence of a gluten proteinase. Cereal Chem. **20:** 87–97 (1943).
13. WOYCHIK, J. H., BOUNDY, JOYCE A., and DIMLER, R. J. Amino acid composition of protein in wheat gluten. J. Agr. Food Chem. **9:** 307–310 (1961).
14. MCCALLA, A. G., and ROSE, R. C. Fractionation of gluten dispersed in sodium salicylate solutions. Can. J. Research **12:** 346–356 (1935).