Proteolytic Activity of Triticale[1]

R. L. MADL and C. C. TSEN[2], Kansas State University, Manhattan 66506

ABSTRACT

A modified Ayre-Anderson procedure was used to determine the proteolytic activity of the flours of several Triticale samples, four wheat samples, and three rye samples; and in addition, to determine the proteolytic activity of the various milling fractions of another Triticale sample. The results indicated that the proteolytic activity of the Triticale flours was greater than that of wheat flours, and close to that of rye flour; and that the proteolytic activity of the milling fractions of the Triticale increased as the proportion of flour from the outer endosperm increased.

Recent developments with the synthetic cereal species Triticale, a genomic combination of wheat and rye, indicate that it may be an important new cereal grain for use in the food industry (1).

Baking quality studies have indicated that Triticale flour possesses higher enzymatic activity than does wheat flour (2). The observed properties could be explained by high content of amylase or protease (3). Finney and Shogren (4) have shown through baking studies that malted Triticale exhibits higher diastatic power than wheat, rye, or barley. Klassen and Hill (5) have shown that unmalted Triticale has high amylase activity comparable to its rye parent, as measured by amylograph viscosity. Our own enzymatic analysis has shown that the amylase activity of unmalted Triticale is high, but the level of α-amylase is undetectable. Therefore, the baking properties of Triticale flours are probably best explained as a result of Triticale protein and protease characteristics.

Chen and Bushuk (6) found that both the quantitative distribution of the

---

[1]Contribution No. 787, Kansas Agricultural Experiment Station, Kansas State University, Manhattan.

[2]Respectively: Graduate Assistant and Professor, Department of Grain Science and Industry.

soluble protein fraction and the amino acid composition of Triticale proteins were intermediate between analogous properties of its durum wheat and rye parents. Here we report on the distribution of protease in wheat, rye, and various Triticales, as well as on the relative proteolytic activity in the various milling fractions of Triticale.

MATERIALS AND METHODS

Sample Preparation

The samples of wheat, rye, and Triticale were selected to provide a range of protein content for each cereal. Except for the Mexican Triticale varieties and one variety of rye, all samples were harvested from experimental plots in Kansas. The Mexican varieties were obtained from the International Maize and Wheat Improvement Center experimental plots in Mexico.

The grain samples were tempered overnight at 15% moisture before being milled on a Brabender Quadrumat experimental mill. The flour fraction consisted of particles passing a 56-mesh sieve. The remaining bran fraction was passed through a 40-mesh screen in a micro Wiley mill. A Miag experimental mill was used to provide samples of the various milling fractions.

Proteolytic Assay

The proteolytic activity was measured by a modified Ayre-Anderson method (7,8,9). An 8.00-g. (dry basis) sample of the flour and 2.50 g. of hemoglobin substrate were suspended in 50 ml. of 0.10M sodium acetate buffer at pH 4.45 in a 40°C. water bath. Preliminary tests on samples of wheat, rye, and Triticale indicated a common pH optimum of 4.45. The data, as shown in Fig. 1, compare favorably with that of McDonald and Chen (7).

After 15 min. the enzymatic reaction was stopped in one set of duplicate samples of each flour by adding 10 ml. of 36% TCA solution. At the end of 2.25 hr., 10 ml. of the TCA solution was added to another set of duplicates of each flour. The samples in which the reaction was stopped after 15 min. were used as blanks (in that the supernatant should contain all proteins soluble prior to enzymatic degradation).

All suspensions, after an additional 30 min. in the water bath, were centrifuged at 3,000 × g for 5 min. The supernatant liquid obtained commonly was cloudy. Therefore, all solutions were clarified by heating to boiling and filtering. The colorimetric Folin method of Lowry et al. (10) was used to determine the soluble protein content of the filtrates. Bovine serum albumin was used for the standard protein curve. Proteolytic activity was expressed in milligram of soluble protein per milliliter of supernatant liquid.

RESULTS

Sample Characteristics

Ten grain samples (listed in Table I) were harvested from experimental plots at Manhattan or Garden City, Kans.; two from plots in Mexico; and one rye sample from a plot in Missouri. The Kansas Triticales tended to exhibit decreasing flour yield with increasing protein content. Both Mexican varieties showed comparatively good flour yields (Table I). The wheat samples exhibited the most consistent milling characteristics while the Triticales were the most variable among the samples studied.

Proteolytic Activity

Results of the proteolytic assays on the flours (summarized in Table I) showed the proteolytic activity of rye to be significantly higher than that of wheat. The Kansas Triticales seemed to possess activities only slightly lower than those of the two samples of Balbo rye tested; those of the Mexican varieties were well above those of the Balbo samples while at a level comparable to the Minn. II variety.

Assays revealed that proteolytic activities of the brans (summarized in Table I) were considerably higher than those of the flours. A trend similar to that of the flours was observed: Wheat brans exhibited the lowest activities; rye and Triticale brans showed more activity with a higher degree of variability than was observed with the flours.

Assays of the milling fractions (summarized in Table II) indicated that proteolytic activity of the fractions increased steadily as the protein content of the fractions increased.

DISCUSSION

Results of the milling fractions were essentially as expected, considering our prior knowledge of the individual flour-stream characteristics. It is known that protein content increases from the center to the periphery of the endosperm in the grain. Therefore, enzyme content and activity would be expected to increase as flour is extracted from areas close to the aleurone layer. The flours from the later-breaks and later-middlings contain higher proportions of endosperm from that region.

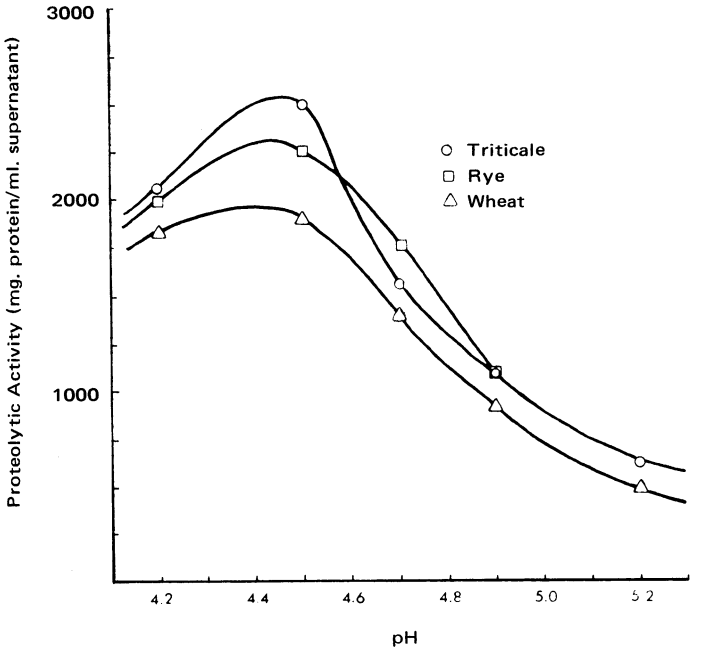

Fig. 1. Proteolytic activity vs. pH for Triticale, rye, and wheat.

The work of Chen and Bushuk (6) has indicated that the protein content of Triticale is intermediate between the protein content of wheat and rye. Interestingly, this study showed proteolytic activity of the Triticales to be comparable to the activity of rye. Klassen and Hill (5) have shown a similar relationship for amylase activity.

TABLE I. RELATIVE PROTEOLYTIC ACTIVITIES OF FLOURS AND BRANS OF WHEAT, RYE, AND TRITICALE

	Whole Grain		Flour		Bran	
	% Protein (d.b.)[a]	% Flour Yield	% Protein (d.b.)[a]	Activity[b]	% Protein (d.b.)[a]	Activity[b]
Eagle[c] (wheat)	12.7	70	11.8	1.74	14.3	4.31
Centurk[c] (wheat)	12.7	73	11.4	1.75	15.2	4.87
Scout-I[d] (wheat)	13.6	72	13.1	1.73	16.7	5.37
Scout-M[e] (wheat)	14.6	72	13.9	1.65	17.7	4.80
Minn. II[f] (rye)	10.0	51	7.1	2.61	13.9	6.48
Balbo[c] (rye)	12.6	45	8.1	2.07	15.7	5.77
Balbo[e] (rye)	16.3	48	12.7	2.08	19.5	5.75
Triticale-I[e]	13.7	64	12.6	1.98	18.1	6.60
Triticale-F[d]	15.1	61	14.1	2.01	18.6	6.15
Triticale-385[d]	15.4	62	13.6	2.00	20.0	5.70
Triticale-298[e]	17.5	58	15.5	1.93	21.1	5.96
Armadillo[g] Triticale	16.2	67	13.9	2.68	21.3	6.35
Bronco[g] Triticale	19.9	69	17.6	2.53	23.3	6.45

[a]Protein determined as (% N X 5.7).

[b]Expressed as mg. protein per ml. of supernatant.

[c]Harvested from Manhattan experimental plots in 1972.

[d]Harvested from Garden City experimental plots in 1971.

[e]Harvested from Manhattan experimental plots in 1971.

[f]Harvested from Columbia, Mo., experimental plots in 1972.

[g]Harvested from Mexican experimental plots in 1971.

TABLE II. PROTEOLYTIC ACTIVITY IN VARIOUS MILLING FRACTIONS OF A KANSAS TRITICALE

Mill Fraction	Moisture %	Protein (d.b.) %	Proteolytic Activity[a]
Straight grade	12.7	11.2	1.78
Ist break	14.5	7.5	1.33
2nd break	14.0	9.1	1.43
3rd break	12.6	12.6	2.07
Ist middlings	13.0	12.0	1.55
3rd middlings	11.6	15.5	2.80
4th middlings	10.3	16.5	3.31
5th middlings	9.5	18.0	4.16
Reduction shorts	8.2	14.8	6.10
Red dog	9.0	15.6	6.40
Break shorts	11.0	18.4	6.43
Germ	9.6	21.0	5.20
Whole grain	9.7	13.3	...

[a]Milligram protein per milliliter of supernatant.

No direct correlation between protein content and proteolytic activity was observed. In fact, some instances seem to imply an inverse relationship. It is notable that the wheat and Triticale flours of highest protein content contained the lowest proteolytic activity in their respective groups while the rye of lowest protein content contained the highest proteolytic activity of the rye samples. A calculation of specific activities (proteolytic activity per gram of sample protein) produced no discernible trends in the samples. For this reason the simpler enzyme unit was chosen. The unit reflects the relative activity of a given weight of flour independent of the protein content. Therefore, the simpler unit is more useful than specific activity in assessing the effect of the enzyme activity on the baking performance of flour on a weight basis.

The recently developed Mexican varieties of Triticale exhibited improved characteristics (higher flour yields and higher protein contents) over the Kansas-grown varieties. The Mexican varieties also had higher proteolytic activity, which may be considered a disadvantage for many of the intended uses of Triticale. For instance, proteolytic activity may have a detrimental influence on the baking quality of the flour. That disadvantage, however, may be overcome by using inhibitors or reducing fermentation proof time (2). Increasing the percentage of salt (commonly used as an inhibitor of proteolytic enzyme systems) in the formulation would be a convenient way to control the activity in a dough system. Of course, there is a practical limitation on the amount of salt which may be used.

Triticales seem to exhibit characteristically more variable flour properties than do wheat flours. The variability should be lessened through genetic improvement to encourage acceptance of Triticale for increased use in the food industries.

Acknowledgments

T. Walters, F. J. Zillinsky, R. J. Robinson, and Dale Sechler supplied samples for the study and A. Ward and E. Farrell aided in their milling. Financial support came from the Agency for International Development (Contract No. 1586) and the National Science Foundation. We gratefully acknowledge all assistance.

Literature Cited

1. ZILLINSKY, F. J., and BORLAUG, N. E. Progress in developing Triticale as an economic crop. Research Bull. 17, International Maize and Wheat Improvement Center, Mexico.
2. TSEN, C. C., HOOVER, W. J., and FARRELL, E. P. Baking quality of Triticale flours. Cereal Chem. 50:16 (1973).
3. REDMAN, D. G. Softening of gluten by wheat proteases. J. Sci. Food Agr. 22: 75 (1971).
4. FINNEY, K. F., and SHOGREN, M. D. Cereal malts in breadmaking. (Abstr.) Cereal Sci. Today 16: No. 77 (1971).
5. KLASSEN, A. J., and HILL, R. D. Comparison of starch from triticale and its parental species. Cereal Chem. 48: 647 (1971).
6. CHEN, C. H., and BUSHUK, W. Nature of proteins in Triticale and its parental species. II. Gel filtration and disc electrophoresis results. Can. J. Plant Sci. 50: 15 (1969).
7. McDONALD, C. E., and CHEN, L. L. Properties of wheat-flour proteinases. Cereal Chem. 41: 443 (1964).
8. WANG, C. C., and GRANT, D. R. The proteolytic enzymes in wheat flour. Cereal Chem. 46: 537 (1969).
9. BUSHUK, W., HWANG, P., and WRIGLEY, C. W. Proteolytic activity of maturing wheat grain. Cereal Chem. 48: 637 (1971).
10. LOWRY, O. H., ROSEBROUGH, N. J., FARR, A. L., and RANDALL, R. J. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193: 265 (1951).

[Received May 10, 1972. Accepted October 23, 1972]