

# Proteolytic Activity of Maturing Wheat Grain<sup>1</sup>

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## ABSTRACT

Proteolytic activities of the grain of three varieties of wheat of the hard red spring, amber durum, and soft white winter types were determined at various stages of maturity from 10 days after flowering to full ripeness. All three varieties were characterized by a gradual decline in activity during maturation; however, the ripe grain still retained considerable activity. The amber durum and soft white winter varieties were about 40% higher in activity than the hard red spring wheat.

The literature contains a number of reports on the increase in proteolytic activity of wheat during germination (1 and references therein). So far as the authors are aware, there have been only two reported studies of the activity of developing grain (2,3). This note reports proteolytic activities at various stages of maturity for three widely different wheats grown under identical conditions.

## MATERIALS AND METHODS

Grain of the wheat varieties Manitou (hard red spring), Stewart 63 (amber durum), and Talbot (soft white winter) was harvested at various stages of development from greenhouse beds. It was freeze-dried and finely ground for analysis.

For the proteolytic-activity assay, 1% hemoglobin in 0.2M acetate buffer of pH 3.8 was the substrate solution. An appropriate amount of ground grain (2 to 5 mg. of protein) was suspended in 5 ml. of substrate solution and incubated for 2 hr. at 37°C. The reaction was then stopped by adding 5 ml. of 5% trichloroacetic acid solution, and the mixture was clarified by centrifugation (20 min. at 12,000 X g). Tyrosine content of 1 ml. of supernatant was determined by the procedure of Lowry et al. (4), using tyrosine to obtain the standard curve. Proteolytic activity was expressed in  $\mu$ moles of tyrosine per mg. protein per min. Two types of blank determinations were made for each activity measurement. In the first blank, the ground grain was added to the hemoglobin after the addition of the trichloroacetic acid solution. In the second, 5 ml. of acetate buffer was used instead of the substrate solution. The blank corrections, most of which were contributed by the hemoglobin substrate blank, were about 30% of the experimental value. A relatively high blank value was anticipated for the immature grain, but was not found.

## RESULTS AND DISCUSSION

The results are summarized in Table I. Figure 1 shows the decrease in activity

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TABLE I. ABSOLUTE AND RELATIVE PROTEOLYTIC ACTIVITIES OF MATURING WHEAT GRAIN

Maturity (days after flowering)	Manitou				Stewart 63				Talbot			
	Protein % (d.b.)	Moisture %	Activity <sup>a</sup>	Relative activity	Protein % (d.b.)	Moisture %	Activity	Relative activity	Protein % (d.b.)	Moisture %	Activity	Relative activity
10	17.0	70.0	0.89	3.28	15.6	72.0	1.27	3.43	15.3	71.5	1.23	3.24
14	16.9	63.2	0.75	2.78	17.1	66.3	0.83	2.24	13.9	64.0	0.81	2.14
18	17.1	58.3	0.52	1.93	14.1	57.4	0.63	1.70	14.4	56.3	0.68	1.79
22	17.2	48.2	0.43	1.59	14.5	51.4	0.52	1.40	15.4	47.7	0.51	1.34
26	18.6	44.6	0.36	1.33	13.7	48.2	0.51	1.38	15.8	48.4	0.44	1.16
30	19.0	31.5	0.39	1.45	15.5	42.0	0.40	1.08	16.3	40.6	0.39	1.03
34	20.1	13.2	0.31	1.15	15.3	36.5	...	...	16.5	15.6	0.36	0.95
38	18.3	9.9	0.31	1.15	15.3	26.6	0.37	1.00	...	...	...	...
Ripe	20.8	10.5	0.27	1.00	16.1	13.1	0.37	1.00	19.0	10.8	0.38	1.00

<sup>a</sup> $\mu$ Moles tyrosine per mg. protein per min.  $\times 10^3$ .

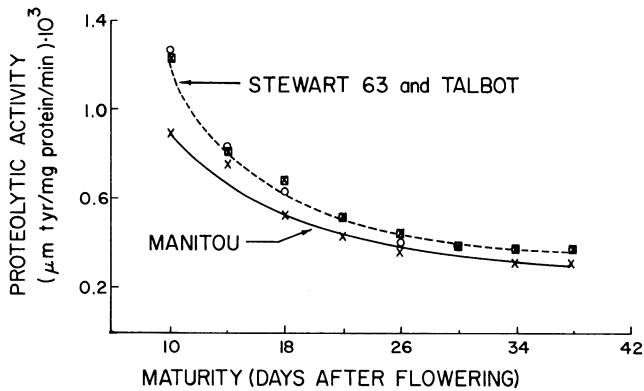


Fig. 1. Proteolytic activity of developing wheat grain.

with maturity. From 10 days after flowering to full ripeness, the drop in activity was about 3.4-fold for the three wheats. In general, the result agrees with those reported by Sharp and Elmer (2). However, no direct comparison was made of absolute activities obtained in the two studies since different assay methods were used. Activity at 10 days was approximately the same as the activity of wheat that had been germinated for 8 days. The activity for ripe Manitou was comparable to that of field-grown Manitou determined by the same method (5). From a technological point of view, it is interesting to note that the three wheats contained considerable activity at the full-ripeness stage. Stewart 63 and Talbot, which are not suitable for bread-baking, partly because of their highly extensible glutens, had significantly higher proteolytic activities than the high-quality Manitou.

#### Literature Cited

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