

Effects of Gibberellic Acid on the Amylase Activity of Malted Wheat and Comparison of Methods for Determining Amylase Activity¹

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ABSTRACT

Adding 50 p.p.m. gibberellic acid (GA_3) to steep water substantially increased the amylase activity as measured by the maltose, α -amylase, and diastatic power methods in five wheat varieties from two locations. Increased response to GA_3 treatment ranged from 20 to 126% when measured by the maltose method, 90 to 213% as measured by the dextrinogenic α -amylase method, and 15 to 34% as determined by the diastatic power method. The response to GA_3 varied with variety. There was a significant interaction of location by variety. Comparisons were made among several widely used conventional laboratory methods for measuring amylase activity in cereals and their products. In addition, loaf volumes from a bread-baking test using a no-sugar formula were compared with the conventional methods. Correlations found among the methods ranged from 0.53 to -0.97. The bread-baking test provided a highly sensitive and consistent assay procedure for evaluating low levels of α -amylase.

Gibberellins have been successfully applied to malting of barley (1). Fleming and Johnson (2) steeped several wheat varieties in potassium gibberellate prior to and during germination. An optimum concentration of 50 p.p.m. greatly increased the amylase activities of these malts. The response depended on variety. Moro et al. (3) found α -amylase activity of whole seeds or endosperm incubated with 10 p.p.m. gibberellic acid to be substantially higher than those incubated without gibberellic acid. Bloch and Morgan (4) followed α -amylase development in wheat and barley in the presence of gibberellic acid by inhibiting the germination with turbulent agitation during steeping. α -Amylase was increased two to threefold in malt treated with 2 p.p.m. gibberellic acid over malt steeped conventionally.

The effects of environment, variety, and class of wheat on amylase activity have been studied extensively. Classes and varieties of wheat vary in ability to produce malts with satisfactory amylase activities and other malt qualities (5). However, the effect of gibberellic acid on amylase production by wheat varieties grown in different locations under controlled conditions has not been studied.

The need for determining the activity of amylolytic enzymes in cereal grains, flour, and malt preparations has led to development of various analytical methods which measure the physical changes or chemical transformations produced by enzymes on selected substrates. Among the more widely used procedures for β -amylase determination are the diastatic power (6), the maltose method, and the gassing-power technique (7). For α -amylase determination, the

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dextrinogenic α -amylase method (6), the Brabender amylograph procedure, and the Hagberg falling number method (7) are widely accepted.

Loaf volume in a straight-dough baking method (8) has been evaluated with regard to its sensitivity to respond to low levels of amylase activity in malts. This involved a formula using variable amounts of malt, depending on α -amylase activity, in a common base flour (CBF) with no added sugar. The data indicated that the bread-baking test could be used to evaluate low levels of α -amylase that could not be measured precisely by conventional methods.

In the present study, we compared amylase activities of malts treated and untreated with gibberellic acid-3 (GA_3) in five varieties of wheat grown under controlled conditions in two locations. Several conventional laboratory methods for measuring amylase activity were compared with a bread-baking test for evaluating enzyme activity in these varieties. Correlations among the conventional methods and baking results were determined.

MATERIAL AND METHODS

Malting Material

Five commercially important wheat varieties of the Pacific Northwest grown in 1970 nurseries of the Washington State University Agricultural Experiment Station plots at Pullman and Lind were studied. Table I gives the varieties, the market classes of wheat, weight per bushel, and percent protein.

Malting Procedure

Preliminary results with Nugaines wheat from Pullman, Wash., using 0, 10, 50, and 100 p.p.m. GA_3 in water steeping solutions showed that optimum concentration for amylase production was about 50 p.p.m. This value, which agreed with the optimum concentration found by Fleming and Johnson (2) was used in this study.

Sixty-five grams of cleaned wheat was steeped at $15^\circ \pm 1^\circ C$. for 24 hr. with GA_3 solution or distilled water in a germination cabinet and germinated for 4 days at $20^\circ \pm 1^\circ C$. in a controlled germination cabinet.

The wheat malt was dried for 24 hr. at $40^\circ \pm 5^\circ C$. in a forced-air convection-type oven on rectangular wire baskets. After drying, the rootlets and coleoptiles were removed by rubbing the dried malts in a plastic bag. Malted wheat was separated from the rootlets and coleoptiles by sifting the material on a No. 8 wire sieve.

TABLE I. WHEAT CLASSES, TEST WEIGHT, AND PROTEIN OF FIVE WINTER WHEAT VARIETIES FROM TWO LOCATIONS

Variety	Class or Subclass	Test Weight		Protein ¹	
		Pullman lb./bu.	Lind lb./bu.	Pullman %	Lind %
Nugaines	Soft common white	62.4	60.5	10.1	13.4
Wanser	Hard red winter	61.9	62.0	11.6	13.8
Paha	White club	61.0	59.8	10.2	13.6
Luke	Soft common white	63.0	59.7	9.8	13.6
Coulee	Hard common white	62.2	61.3	10.6	13.3

¹14% m.b. (N \times 5.7).

Amylase Methods

Wheat malts were ground on a Udy Cyclone hammermill with a slotted 0.010-in. screen and then thoroughly blended. The amylase activity of the malted wheat was determined by the maltose method (7), and the α -amylase (dextrinogenic) and diastatic power methods of the American Society of Brewing Chemists (6). In addition, CBF supplemented with 0.05% wheat malt was analyzed by the maltose, amylograph, gassing power, and falling number methods according to AACC *Approved Methods* (7). The CBF was an unmalted bread flour obtained from a commercial mill. Adding 0.05% malt produced a flour in which α -amylase could be determined by all conventional amylase assay methods.

Baking Procedure

A straight-dough baking method was used to evaluate α -amylase activity. A common base unmalted flour was used as a standard. Two levels of each of the malts were added to this flour (0.019 g. per 100 g. flour and 0.038 g. per 100 g. flour) and the amylase content was evaluated by the loaf volume response. The standard flour had protein ($N \times 5.7$) of 13.8%, moisture of 12.2%, and a falling number of 693 sec. The baking method and formula were as described by Finney et al. (8).

RESULTS AND DISCUSSION

Effect of GA₃-Treatment on Amylase Activity

GA₃ greatly increased the amylase activity of all five wheat varieties grown at both locations (Table II). The increase from GA₃-treatment was 20 to 126% in amylase activity as measured by the maltose method, 90 to 213% as measured by

TABLE II. EFFECT OF GIBBERELIC ACID AND LOCATION ON THE AMYLASE ACTIVITY

Location	Variety	Amylase Activity								
		Maltose ¹			α -Amylase ²			Diastatic power ³		
		N-T ⁴	GA-T ⁵	% Increase	N-T	GA-T	% Increase	N-T	GA-T	% Increase
Pullman	Luke	1,395	3,158	126	24	75	213	134	179	34
	Coulee	2,018	3,042	51	49	110	124	222	267	20
	Paha	2,458	2,960	20	52	115	121	172	211	23
	Wanser	2,464	3,345	36	71	135	90	258	296	15
	Nugaines	2,510	3,180	27	57	117	105	192	228	19
Lind	Luke	1,648	3,100	88	44	114	159	184	243	32
	Coulee	2,194	2,950	34	66	143	117	285	344	21
	Paha	1,919	2,640	38	50	120	140	204	255	25
	Wanser	2,410	3,168	31	78	158	103	298	361	21
	Nugaines	2,169	2,875	33	70	146	109	217	281	29

¹mg. maltose per 10 g. malt as measured by the maltose method (7) (mean of four determinations).

²20° units, dry basis, as measured by the α -amylase method (6) (mean of two determinations).

³Degrees, dry basis, as measured by the diastatic power method (6) (mean of two determinations).

⁴N-T = Nontreated.

⁵GA-T = Gibberellic acid-treated.

the dextrinogenic α -amylase method, and 15 to 34% as measured by the diastatic power method. The cultivar Luke showed the greatest response. The overall responses to GA₃ were within the range previously reported by Fleming and Johnson (2) using other varieties of wheat. Analysis of variance (Table III) showed a significant ($P > 0.05$) effect in all of the treatments as measured by the maltose test. The diastatic power and α -amylase tests also showed significant effects in all of the treatments with the exception of variety \times GA₃ and variety \times location \times GA₃ interactions. The α -amylase method used measured dextrinogenic activity of malt (9), while diastatic power values indicate malt ($\alpha + \beta$) saccharifying activity (10). This may account for significant effects in the variety \times GA₃ interaction using the α -amylase and diastatic power methods. The maltose method measures primarily action by β -amylase. Because the response to GA₃ varied with variety and location, prediction of the response of a particular variety is difficult. Interaction of location \times variety was significant, probably because of the difference in adaptability of certain varieties for particular locations. Lind has only 9 to 11 in. annual precipitation and Pullman has nearly twice that amount. Both locations have a winter-precipitation pattern. Varieties perform differently from one location to another and the location \times variety interaction confirmed this variation.

TABLE III. ANALYSIS OF VARIANCE FOR THE AMYLASE ACTIVITY AS MEASURED BY THE MALTOSE, DIASTATIC POWER, AND α -AMYLASE METHODS OF GA-TREATED AND NONTREATED WHEAT VARIETIES FROM TWO LOCATIONS

Source	D.F.	Mean Squares		
		Maltose	Diastatic power	α -Amylase
Variety	4	620,039*	9,554*	1,168*
Location	1	424,861*	13,158*	1,692*
Variety \times location	4	207,186*	178*	119*
GA	1	17,057,045*	12,450*	22,620*
Variety \times GA	4	632,290*	8	18
Location \times GA	1	40,051*	432*	290*
Variety \times location \times GA	4	48,189*	13	5
	60	5,345		

*Significant at 5% level. All other values significant at 1% level.

TABLE IV. CORRELATIONS AMONG AMYLASE METHODS AND BREAD LOAF VOLUME¹

Method of Analysis	Diastatic Power	α -Amylase	Amylograph	Maltose CBF	Gassing Power	Falling Number	Loaf Volume	
							0.019 g. malt	0.038 g. malt
Malt-maltose	0.53*	0.84	-0.93	0.77	0.89	-0.88	0.90	0.91
Diastatic power		0.77	-0.68	0.73	0.70	-0.75	0.72	0.68
α -Amylase			-0.91	0.90	0.96	-0.96	0.88	0.80
Amylograph				0.91	-0.96	0.95	-0.94	0.90
Maltose					0.91	-0.93	0.81	0.76
Gassing power						-0.97	0.90	0.85
Falling number							0.91	0.87
Loaf volume								
0.019 g. malt								0.96

¹N = 20.

This study indicated that certain classes and varieties of wheat grown at different locations may vary in amylase activity and in response to GA_3 . The wheat and barley breeder, grower, and processor would benefit if further studies could establish whether certain varieties could produce superior quality malts when grown at particular locations.

Comparison of Amylase Activity Methods

Among all of the methods of analysis tested (Table IV), correlation coefficients ranged from 0.53 for diastatic power vs. malt maltose to -0.97 for gassing power vs. falling number.

Methods for determining α - and β -amylase in malt have been developed by Sandstedt et al. (9) and Kneen and Sandstedt (10). These were designed as measures of α -dextrinizing, malt ($\alpha + \beta$) saccharifying (diastatic power), and (by difference) β -saccharifying activity of malt. Kneen and Sandstedt (10) and Olson et al. (11) have shown that the effects of α - and β -amylase are additive in saccharification. Therefore, α -amylase can be determined accurately by suitable dextrinization measurements; a correction can be applied for its action on soluble starch in the malt saccharification (diastatic power) determination, i.e., the difference between the diastatic power and the α -amylase activity is equal to the β -amylase activity. Kneen and Sandstedt (10) found a high degree of correlation between diastatic power of a malt and its β -amylase but reported no

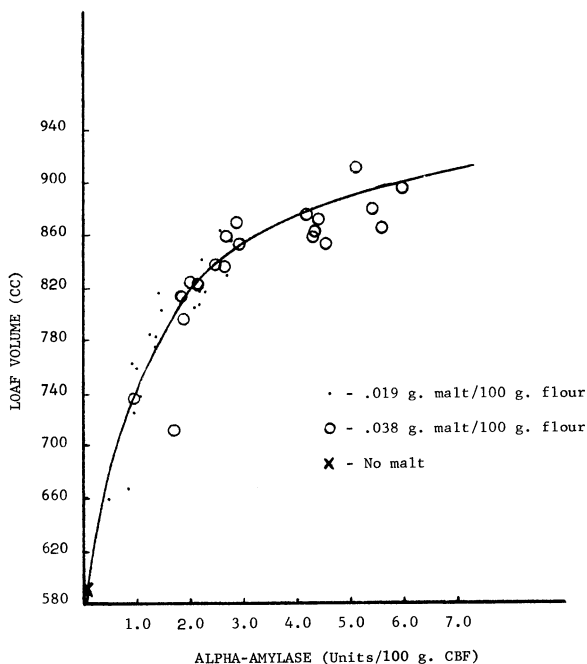


Fig. 1. Effects of malts on loaf volume of bread baked without added sugar and various levels of α -amylase from wheat malt. Full formula gave a loaf volume of 930 cc.

significant correlation between α -amylase activity and either diastatic power or β -amylase of a malt. Our data gave a significant correlation of 0.77 between α -amylase and diastatic power. We did not use the β -amylase activity method. The diastatic power compared to all of the other methods gave lower correlations (Table IV).

The amylograph correlations given in Table IV were from linear plots and essentially identical correlations were found with semilog plots. Plots of increasing increments of barley malt vs. the falling number (FN) and amylograph values have been shown to be curvilinear (12). The relationship between amylograph and FN values and other analyses was almost linear for the range of values in this study which covered levels normally encountered in commercial practice. With a wider range of enzymatic activity, the relationship would probably be more curvilinear.

The empirical FN method developed by Hagberg and Perten (13-15) is relatively simple, rapid, and quite reliable. This method determines principally α -amylase activity of flour or pulverized grain. Activity is determined by heating rapidly a flour-water suspension and measuring the time required to gelatinize and subsequently liquefy the flour suspension. Medcalf et al. (16) have adapted the FN procedure to direct determination of α -amylase activity in malt. Greenaway and Neustadt (17) examined the sources of and means of minimizing the experimental error in the FN test. Meredith (18) found that the FN method, because of more rapid heating, is much less sensitive than the amylograph to low levels of α -amylase. Our correlation of 0.95 between FN and amylograph values indicated that the sensitivity of the FN for the range covered was highly satisfactory.

Very low levels of α -amylase, which are difficult to ascertain by conventional routine assays, were estimated by their effects on loaf volume of bread baked without added sugar. Effects of small amounts of malts (0.019 and 0.038 g. per 100 g. of CBF) on loaf volume were measured. The α -amylase ranged from about 0.5 to 6.0 units per 100 g. CBF as measured by the α -amylase method. Figure 1 shows the effects of adding malt on loaf volume. The basic formula using CBF without malt and sugar gave a loaf volume of 590 cc. and a full formula 930 cc. From 3.0 to 6.0 α -amylase units per 100 g. CBF gave near optimum loaf volume but differentiation was less than that when 0.5 to 3.0 units α -amylase were added. With this bread test, differentiation was better at the lower levels of α -amylase. Finney et al. (8) reported that it was necessary to add 12.3 and 24.6 α -amylase units to obtain a similar response. Addition of 0.019 g. malt (0.5 to 3.0 units α -amylase) increased loaf volume an average of 199 cc.; 0.038 g. malt (3.0 to 6.0 units) on the average increased loaf volume an additional 53 cc. Correlations were higher between conventional α -amylase methods and the bread-baking test using the lower levels of added malt.

It has been reported that protease activity is increased when GA_3 is used in the steeping of cereals for malt production (1,2). No protease or soluble protein analyses were followed in this study; however, no differences could be observed in either the physical dough or bread-baking properties which would indicate any deleterious effect of higher protease levels as a result of the use of GA_3 .

Correlations among the other methods of analysis compared were generally good (Table IV). Good overall correlations were partly due to use of a single source of CBF, thus eliminating variations in such factors as storage time and

temperature, moisture content (19), particle size, and starch damage (e.g., 20-22). Yeast variability poses a problem in precise experimental baking (23,24). We used the same batch of compressed yeast for the gassing power and baking tests. The gassing power method reflects the contribution of all fermentable sugars, both those present in the flour and those that result from the amylolysis during the test; the maltose method has been criticized on grounds that it does not determine the nonreducing sugars (25).

In testing products, such as cereal grains, flour, and malt, several methods give relative values of amylase activity when used on the appropriate product. Our results indicate that the choice of method or methods could depend upon the laboratory equipment available, personal preference, or the need for specific data.

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