Nutrition Information. Table IV lists information normally contained in a "nutrition label". A 2-oz serving (two cookies) provided 15% of the U.S. RDA for protein and copper, 10% for phosphorus, iron, and magnesium, 8% for riboflavin and niacin, 6% for zinc, and 4% for calcium. Contribution of nutrients not measured could also be substantial. Since no animal product was used in the cookie formula, presumably these cookies contained no cholesterol.

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α-Amylase Activity and Preharvest Sprouting Damage in Kansas Hard White Wheat

Grace Huang and E. Varriano-Marston*

α-Amylase activity, sprouting damage, and α-amylase gel electrophoretic patterns of two varieties of Kansas hard white wheat were compared with a standard hard red wheat. Varietal differences significantly ($P < 0.025$) affected the variables measured, with one white wheat (KS73256) showing significantly higher α-amylase activity and sprouting damage than the other varieties. Two methods for determining α-amylase activity were also studied. The falling number method was more highly correlated with sprouting damage than was α-amylase activity, as measured by the production of reducing sugars from a soluble starch substrate.

Work is currently being conducted on the feasibility of developing a variety of hard white wheat in Kansas. Such a wheat should have a higher price than hard red wheats do because of its higher yield of flour and increased marketability (Schruben, 1976). Past experiences with white wheat, however, indicate that they are more susceptible to preharvest sprouting than the red wheats (Everson and Hart, 1961; Greer and Hutchinson, 1945; Miyamoto and Everson, 1958; Miyamoto et al., 1961; McEwan, 1976).

Sprouting is associated with the synthesis and increased activity of a variety of enzymes. One particular enzyme, α-amylase, when present in excessive amounts in flour produces breads with a doughy crumb and poor eating quality (Reed and Thorn, 1971).

α-Amylase activity has been used extensively to estimate the degree of wheat sprouting in the field or in storage, and a close relationship between α-amylase activity and preharvest sprouting has been demonstrated by a number of authors (Bingham and Whitmore, 1966; Derera et al., 1976). Bingham and Whitmore (1966), in a study of fourteen wheat varieties, found that the varieties differed considerably in susceptibility to germination and that germination was always associated with increased α-amylase activity.

A study of Australian white-grained wheats by Derera et al. (1976) also showed that the varieties highly susceptible to sprouting were high in α-amylase activity. By selecting varieties that had low α-amylase activity, Persson (1976) was able to develop a new variety, OTELLO, which was resistant to preharvest sprouting.

In this study, α-amylase activity and preharvest sprouting damage in Kansas hard white wheats were compared with a standard hard red wheat grown under the same environmental conditions. During flowering we also subjected the samples to different levels of nitrogen fertilization in order to determine if this had an effect on amylase activity in the grain. In addition, two methods of measuring α-amylase activity were studied to determine which was the better predictor of sprouting damage.

MATERIALS AND METHODS

Materials. Three varieties of hard winter wheat were studied: Eagle (standard red) and Clark's Cream and KS73256 (white wheats). The wheats were sown at Hutchinson, KS, in October, 1976.

During the flowering stage, wheat in different plots received nitrogen in these amounts: 0, 30, 60, 90, and 120 lb/acre. Three replicates for each treatment were grown, and a split plot design (3 × 5 × 3) was used.

Department of Grain Science and Industry, Kansas State University, Manhattan, Kansas 66506.

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Table I. Protein Content (%) of Eagle, Clark's Cream, and KS73256 Grown under Different Levels of Nitrogen Fertilization[a]

N level, lb/acre	variety		
	Eagle	Clark's Cream	KS73256
0	14.1	15.1	13.6
30	14.6	15.2	14.1
60	14.7	15.4	14.1
90	15.0	15.5	14.5
120	14.9	15.4	14.7

[a] The LSD across varieties was 0.3 ($P < 0.05$), and the LSD of nitrogen level within each variety was 0.1 ($P < 0.05$).

The wheat cultivars were harvested on July 5, 1977. The environmental conditions at harvest were conducive to sprouting. The total precipitation in Hutchinson, KS, in May and June was 15.8 in., which was about two times the normal level.

α-Amylase Assay. The samples were ground on a Tekmar analytical mill, Model A-10 (Tekmar Scientific Apparatus, Cincinnati, OH). One-half-gram portions of the ground samples were mixed with 10 mL of 0.04 M acetate buffer, pH 5.5, containing 0.01 M $CaCl_2$. The extracts were centrifuged (15000*g*, 10 min), and the supernatant solutions were heated for 15 min at 70 °C to inactivate β-amylase (Marchylo et al., 1967). Samples were analyzed in triplicate for α-amylase activity by using the method of Robyt and Whelan (1968); reducing sugars were determined by Nelson's colormetric copper method (1944). Enzyme activity was expressed as micromoles of maltose produced per gram of meal per minute.

Protein Determination. Protein in the supernatant was determined by the method of Miller (1959). Specific activity was expressed as micromole of maltose produced per milligram of nitrogen per minute. Total protein content in the wheat was determined by the Kjeldahl method and expressed on a 14% moisture basis.

Falling Number. The AACC method 56-81 B (1973) was used to determine the falling number, defined as the time in seconds required to stir and allow the stirrer to fall a measured distance through a hot aqueous flour gel undergoing liquefaction.

Sprouting Assessment. Three hundred kernels of each sample were assessed. Sprouted kernels were defined as those in which the germ ends had been opened by germination and exhibited a sprout, or those in which the sprouts had been broken off leaving only the socket.

Gel Electrophoresis. α-Amylase from the three varieties was extracted by mixing 7 g of freshly ground meal with 10 mL of acetate buffer (0.003 M $CaCl_2$, pH 5.5) and centrifuging at 48000*g*. The extracts were heat-treated at 70 °C for 20 min to inactivate β-amylase. Acetone fractionation of the heat-treated α-amylase extracts from the three varieties was also done according to the method of Kruger and Tkachuk (1966). Gel electrophoresis was then carried out on both crude extracts and acetone (35–50%) precipitates by using the method of Davis (1964) with the 7% small pore gel at pH 8.9.

After electrophoresis, the multiple forms of α-amylase were detected by incubating the gels for 2 h at 35 °C against a starch acrylamide film attached to a glass plate and subsequently staining the films with 0.2% potassium iodide and 0.2% iodine solution. The acrylamide films were prepared as described by Marchylo et al. (1976).

Data Analysis. Statistical analysis included Anovas and least significant differences (LSD) as well as the determination of simple and pooled correlation coefficients.

Table II. Sprouting Damage (SD)[a] and Falling Number (FN)[b] of Eagle, Clark's Cream, and KS73256 Grown under Different Levels of Nitrogen Fertilization

N level, lb/acre	variety					
	Eagle		Clark's Cream		KS73256	
	SD, %	FN, s	SD, %	FN, s	SD, %	FN, s
0	2	398	6	414	35	75
30	1	431	5	405	38	74
60	2	420	5	418	39	73
90	1	405	4	424	42	68
120	1	363	4	418	38	70

[a] The LSD across varieties was 2 ($P < 0.05$). [b] The LSD across varieties was 29 ($P < 0.05$).

RESULTS AND DISCUSSION

Protein Content as Affected by Variety. The protein content of the wheat varieties is shown in Table I. Varietal differences significantly ($P < 0.025$) affected protein content, which was in turn influenced by the amount of nitrogen applied. Applying nitrogen at 90 lb/acre produced wheat with the highest protein content; the content, however, was not significantly different from that of wheat produced by applying 60 or 120 lb/acre. Other investigators have observed that as nitrogen level increased, protein content also increased and that the optimum level of nitrogen was 90 lb/acre (Hunter and Stanford, 1973).

The response to nitrogen was variety dependent. Linear correlations between nitrogen level and protein content were 0.71, 0.53, and 0.65 for Eagle, Clark's Cream, and KS variety, respectively.

Sprout-Damaged Wheat. The percentage of visible sprouting damage recorded for the wheat varieties is shown in Table II. Sprouting damage was not affected by the level of nitrogen applied to the soil during flowering. The LSD values, however, indicated that all three varieties were significantly different ($P < 0.05$) from each other with respect to sprouting damage: Eagle had the lowest (~1–2%) value, KS the highest (~35–42%). These results show that under the growing conditions of wheat in this experiment, KS hard white wheat was more susceptible to sprouting than was Clark's Cream or Eagle. In addition, we observed extensive shriveling of the kernels of the KS variety. Extensive sprouting damage and shriveling would tend to lower the market value of this grain.

α-Amylase Activity. It is quite possible that wheat varieties may have high α-amylase activity without exhibiting considerable sprouting damage. Therefore, we determined the relationship between sprouting damage and α-amylase activity for the Kansas wheat varieties. The falling number method was used as an indirect measure of α-amylase activity and sprouting damage. In addition, the Robyt and Whelan method (1968) was used to give a more quantitative measure of α-amylase activity.

The falling numbers of the three hard wheat varieties studied are shown in Table II. The KS variety had a low falling number (FN ~ 72); Eagle and Clark's Cream had comparable high values (FN > 350). On the basis of the USDA flour standards (Greenaway, 1967), the KS variety would not be acceptable for breadmaking. On the other hand, the other two varieties may require a malt supplement to produce optimum bread characteristics.

α-Amylase activity, as determined by the Robyt and Whelan method, is shown in Table III. Varietal differences were highly significant ($P < 0.004$). The KS variety had the highest α-amylase activity; it differed significantly from that of the other two varieties, which showed essentially the same low level of enzyme activity.

Data on specific α-amylase activity (also shown in Table

Table III. α-Amylase Activity[a] and Specific Activity[b] of Eagle, Clark's Cream, and KS73256 Grown at Different Levels of Nitrogen Fertilization

	variety					
	Eagle		Clark's Cream		KS73256	
N level, lb/acre	μmol min^{-1}	μmol min^{-1} mg^{-1}	μmol min^{-1}	μmol min^{-1} mg^{-1}	μmol min^{-1}	μmol min^{-1} mg^{-1}
0	2.89	1.55	2.16	1.64	4.75	2.24
30	2.91	1.51	3.14	1.67	4.80	2.23
60	2.99	1.55	3.23	1.67	4.77	2.23
90	2.90	1.52	3.13	1.66	4.81	2.27
120	2.98	1.56	3.18	1.63	4.79	2.24

[a] The LSD across varieties was 0.23 ($P < 0.05$). [b] The LSD across varieties was 0.18 ($P < 0.05$).

Table IV. Linear Correlations Across All Varieties

	PC[a]	A[b]	SPA[c]	FN[d]	SPD[e]
PC		−0.49**[b]	−0.46**	0.55**	−0.52**
A			0.99**	−0.85**	0.86**
SPA				−0.79**	0.79**
FN					−0.97**

[a] PC = protein content. [b] A = α-amylase activity (Robyt and Whelan, 1968). [c] SPA = specific α-amylase activity. [d] FN = falling number. [e] SPD = visible sprouting damage. [f] (**) Significant at the $P < 0.01$ level.

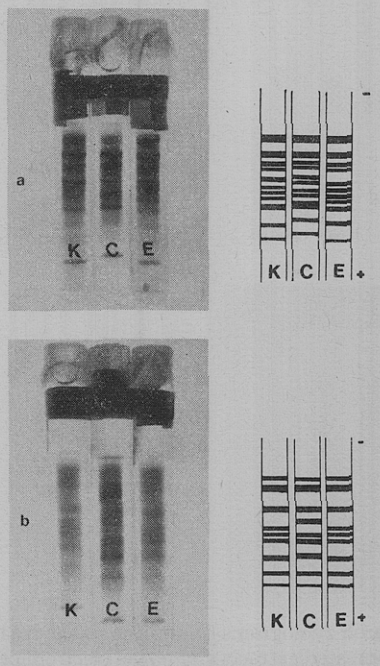

Figure 1. Gel electrophoretic patterns of (a) heat-treated crude extracts and (b) acetone precipitates from Eagle (E), Clark's Cream (C), and KS73256 (K).

III) paralleled the data on enzyme activity. They indicated that the high enzyme activity exhibited by the KS variety was due to a greater extraction of α-amylase from that grain than from either of the other varieties.

To relate α-amylase activity to the other variables studied, we determined linear correlations across varieties (Table IV). α-Amylase activity (by Robyt and Whelan) was negatively correlated with falling number ($r = -0.85$) and positively correlated with sprouting damage ($r = 0.86$). The major contribution to those high correlations was varietal differences. In addition, a low negative correlation was found between grain protein content and α-amylase activity ($r = -0.49$), suggesting that increased protein synthesis in the grain does not necessarily parallel increased enzyme synthesis.

In our study, the falling number method was highly correlated with sprouting damage (r = −0.97). Therefore, this method was a better predictor of sprouting damage than was the quantitative measure of α-amylase activity as determined by the Robyt and Whelan method. Our results suggest that other factors besides α-amylase activity influence falling number determinations. On the other hand, the Robyt and Whelan method may not be measuring the total α-amylase activity present in the whole meal; it does not take into consideration these contributors to α-amylase activity: (1) the presence of enzyme activating factors in the whole wheat meal, (2) differences in the rates of activity toward native substrate and soluble starch, (3) the presence of tightly bound enzymes, which are not readily extracted by acetate buffer but which increase amylase activity in situ, and (4) the role of β-amylase in starch degradation.

Gel Electrophoresis of α-Amylases. Gel electrophoretic patterns were run on the enzyme extracts to determine (1) which isozymes were inactivated by the heat treatment used in the Robyt and Whelan method, and (2) if any varietal differences existed in α-amylase isozymes.

Polyacrylamide gel electrophoretic separations of heat-treated crude α-amylase extracts from the three varieties showed that each wheat possessed at least 11 components (Figure 1a). Comparing these gel patterns with the gels obtained with the 35–50% acetone precipitates (a more pure α-amylase extract) showed that only nine of the 11 components shown in gels of the crude extract were present (Figure 1b). The three wheat varieties showed the same number of gel electrophoretic components but slightly different mobilities (Figure 1). In addition, there were differences among varieties with respect to the density of the various gel components. Several authors have identified eight α-amylase components in hard red spring wheats (Kruger, 1972a,b). The data suggest that during the procedure other enzymes and/or proteins were extracted which were not affected by the 70 °C heat treatment.

If some β-amylase survived the heat treatment, then the results of the Robyt and Whelan method could not be strictly considered as measuring α-amylase activity only. The same would be true if phosphorylase enzymes were not heat inactivated.

Starch zymograms of the crude nonheat-treated and crude heat-treated extracts were run to determine which gel bands exhibited amylase activity. The results are shown in Figure 2. The zymograms of crude extracts which were not heat treated showed extensive digestion of the starch film along the entire gel; that was undoubtedly due to the action of both α- and β-amylases.

Gels run with the crude heat-treated extract produced entirely different starch zymograms (Figure 2a). The KS variety exhibited two areas of amylase activity. According to Kruger's notation (1972a,b), we would designate the fast moving components as β-amylase and the slower components as α-amylases (possibly α-1 through α-3). On the other hand, only the slower moving components (α-amylase) could be detected in the gels for Eagle and Clark's Cream.

The presence of active β-amylase in the KS variety suggests that the enzyme might be more heat stable than the β-amylases of Eagle or Clark's Cream. Again, that suggests that for some varieties, the Robyt and Whelan method is not solely a measure of α-amylase activity.

The zymograms also indicate that more α-amylase components were active in the KS variety than in the two

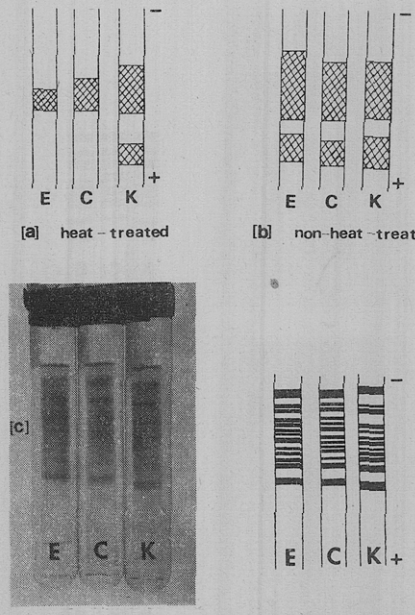

Figure 2. Starch zymograms of (a) crude heat-treated and (b) crude nonheat-treated extracts and (c) gel electrophoretic pattern of nonheat-treated crude extracts. Eagle (E), Clark's Cream (C), KS73256 (K).

other varieties. That was expected because it exhibited the highest level of sprouting damage (Table II) and, according to Kruger (1972a,b), as germination time increases, more α-amylase components become active.

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Comparison of the Amino Acid Composition of Tomato Pulp Recovered from Caustic Peelings to That of Conventionally Processed Tomato Pulp and Fresh Tomatoes

John E. Schade,* William G. Schultz, Harry J. Neumann, James P. Morgan, and John W. Finley

Tomato pulp recovered from caustic (sodium hydroxide) peelings obtained from commerical peelers has been considered as a potential food material. The nonsulfur amino acids of reacidified recovered pulp were compared to the amino acids of both conventionally processed tomato pulp and fresh tomatoes to determine the extent of modification in composition caused by the exposure to hot alkali. Arginine was the only amino acid that decreased appreciably in the peeling process; the recovered pulp product contained 25–83% of that found in fresh tomatoes, depending upon the severity of the process. This degradation of arginine can be minimized by proper control of process conditions. The decrease in arginine content appears to be a sensitive indicator for the severity of exposure of tomato to alkali.

Tomatoes, like many other fruits and vegetables, are often peeled commercially by immersion in hot caustic solution, followed by removal of the skin and adhering pulp either by spraying with water or by rubbing with rubber disks (Hart et al., 1974). The use of rotating rubber disks to remove peels reduces water consumption and results in peelings that have about the same solids content as whole tomatoes. This byproduct can be discarded, as is the current practice, or the pulp can be economically recovered for food use by acidification and separation of the desirable pulp from the less desirable skin, as reported previously by Schultz et al. (1977).

Alkaline treatment of proteins has been reported to lower their nutritional value (deGroot and Slump, 1969; Gorill and Nicholson, 1972). Alkali and heat converts some of the amino acid residues in proteins to unnatural amino acids and also can cause racemization of amino acid res-

Western Regional Research Center, Science and Education Administration, U.S. Department of Agriculture, Berkeley, California 94710.