A STUDY OF ALPHA AMYLASE ACTIVITY IN KANSAS HARD WHITE WHEATS

by

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E. Varriano-Marston

Major Professor

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The author dedicates this work to the memory of her mother who encouraged her to further her education, and to her father for his continuous support and prayers.

INTRODUCTION

Work is currently being conducted on the feasibility of developing a variety of hard white wheat in Kansas. Such a wheat would have an increased price value over hard red wheats because of its higher yield of flour and increased marketability (Schruben, K.S.U. Dept. of Ag. Econ.). However, past experiences with white wheat indicate that they are more susceptible to sprouting than the red wheats (Everson & Hart, 1961; Greer & Hutchison, 1945; Miyamoto & Everson, 1958, 1961; McEwan, 1976).

Sprouting susceptibility of wheat has been related to alpha-amylase activity (Persson, 1976; Derera et al., 1976), sensitivity of seeds to the release of gibberellic acid from the scutellum (Freed et al., 1976; Derera et al., 1977), the presence of inhibitory substance in the seed coat (McEwan, 1976; Miyamoto & Everson, 1958), and many other factors. A large number of the published papers have dealt with the first factor mentioned above, i.e. the relationship between alpha-amylase activity and sprouting susceptibility. However, many questions remain unanswered.

The objectives of this study are (1) to compare the alpha-amylase activity of two varieties of Kansas hard white wheat with that of a standard hard red wheat, (2) to define the relationship between wheat protein content and alpha-amylase activity, (3) to compare two different methods for determining alpha-amylase activity, and (4) to define the microscopical (SEM & Light) differences among the Kansas white wheats and a standard hard red wheat that might translate into differences in the milling and baking characteristics of the flours.

REVIEW OF LITERATURE

In keeping with the objectives of this study, the following topics will be briefly discussed: (1) the importance of alpha-amylase in breadmaking, (2) the relationship between alpha-amylase activity and sprouting damage, (3) tests used to determine alpha-amylase activity in wheat, and (4) structural factors related to sprouting susceptibility.

Alpha-Amylase Activity and Breadmaking

Alpha-amylase activity in wheat is important to millers and bakers because high activity due to sprouting can lower the quality of bread products. The alpha-amylase activity in flour must be controlled if optimum bread is to be produced.

Alpha-amylase is thought to be important in gas production. In addition, its action results in the production of residual sugars which contribute to bread flavor and crust color. However, dough containing excessive levels of alpha-amylase activity due to excessive malt supplementation or use of flours milled from sprout-damaged wheat produces breads with a doughy crumb and poor eating quality (Geddes, 1946; Pomeranz, 1971).

Alpha-Amylase Activity and Sprouting Susceptibility

Alpha-amylase activity has been used extensively for estimating the degree of wheat sprouting in the field or in storage (Pomeranz, 1971). Bingham and Whitmore (1966) studied the relationship between alpha-amylase activity and resistance to germination of fourteen varieties of wheat. They found that there was considerable varietal difference in susceptibility to germination and such germination was always associated with an increase in alpha-amylase activity.

A study of Australian white-grained wheats by Derera et al. (1976) also

showed that the varieties which were highly susceptible to sprouting were high in alpha-amylase activity. By selecting the varieties which had low alpha-amylase activity, Persson (1976) was able to develop a new variety called OTELLO which was resistant to pre-harvest sprouting. A close relationship between alpha-amylase activity and pre-harvest sprouting was demonstrated. However, a cause and effect relationship was not established.

Tests Used to Determine Alpha-Amylase Activity in Wheats

A number of methods have been developed to determine alpha-amylase activity in grain and flour. These methods involve measuring one of the following effects of enzyme action: (1) an increase in reducing power of a starch solution, (2) a decrease in the iodine color of beta-limit dextrin, (3) a decrease in the viscosity of a starch solution, or (4) the increase in concentration of soluble dye-labelled products. Below is a brief review of the various approaches.

Reducing value methods. Bernfeld (1951) developed a method using alkaline 3,5-dinitrosalicylate to measure alpha-amylase activity by reducing power values. However, the method was found to be inaccurate because this reagent did not give equal reducing values for equimolar quantities of maltodextrins (Robyt & Whelan, 1968). In addition, there was not a direct relationship between the amount of maltose produced and enzyme quantity.

Robyt and Whelan (1968) developed a method using Nelson's colorimetric copper method which overcame the problems encountered with 3,5-dinitrosalicylate. In this method, the apparent maltose produced in an amylase reaction was found to be directly proportional to concentration of enzyme. Therefore, it is possible to obtain a quantitative measurement of alpha-amylase activity which gives values in standard enzyme units.

Iodine-staining methods. A method based on starch-iodine color was

first described by Wohlgemuth (1908), and later, Sandstedt, Kneen, and Blish (1939) modified the method and established a standard SKB unit. Further modification by Briggs (1961) and MacGregor et al. (1971) resulted in expressing alpha-amylase activity in wheat by I.D.C. units. One I.D.C. unit is the amount of enzyme required to lower the absorbance of a standard digest from 0.6 to 0.4 at 540 nm in 100 min. As you can see, this method measures enzyme activity in arbitrary units so it is difficult to compare the results with methods which report standard enzyme units. Some authors (Barnes & Blakeney, 1974) also indicate that this method lacks precision and sensitivity.

Viscosity tests. The falling number method developed by Hagberg (1960) and Perten (1964) measures alpha-amylase activity by using flour as the native substrate. It is based on the rapid gelatinization of a flour suspension and subsequent measurement of the degradation of the starch paste by alpha-amylase under conditions similar to those encountered during baking. The method is quick and accurate when large differences exist among varieties; it is less sensitive to small differences. Again, the data obtained from this method gives an arbitrary measure of alpha-amylase activity, and therefore, makes it difficult to compare with standard enzyme methods.

The amylograph has also been used to give an indication of alpha-amylase activity. It is less sensitive than the falling number method and is considerably more time-consuming.

Dye-labelled substrates. The Phadebas method (Barnes & Blakeney, 1974) employs a cross-linked potato starch covalently bound with Cibacron Blue. Amylase activity is determined as the amount of soluble dye-labelled products produced during a 15 min. reaction time. Results are expressed in mEU/10 ml.

The Phadebas method was originally developed for clinical diagnostic tests

on human serum and urine. When applied to cereals it is considerably less sensitive for samples with low alpha-amylase activity. In addition, considerable variability in the results of duplicate trials has been observed (personal observation).

Recently, a new colorimetric method called the modified Cibacron Blue Amylose (CBA) was developed by Mathewson and Pomeranz (1978). They showed that this test was sensitive to smaller amount of alpha-amylase activity (3-30 mDU), and hence can be used to a wider extend to evaluate sprout damage in wheats.

Further comparisons of the various aspects of these methods described above are presented in Table 1.

Wheat Structural Characteristics and Sprouting Susceptibility

The structural characteristics of wheat varieties, rather than the level of alpha-amylase activity, may play a role in sprouting susceptibility. Krauss (1933) found that the structure in the area of the micropyle was different for red and white grains and that these differences might relate to differences in sprouting resistance. In a white grain, the narrow micropylar opening was directly sealed off by tissue made up of swollen cell walls originating from the nucellus and from the embryonic appendage. In a red grain, the wide micropylar pore was plugged up with corky and woody nucellus joining the embryonic appendage on the inside.

Belderok (1976) also observed structural differences in the testa layers between white and red grains. He found that in an unripened and highly sprouting susceptible white grain, the color layer and the outer cuticle are clearly visible; they consist of a dense, homogeneous material. However, in a sprouting susceptible red grain, the testa consists of a dense, homogeneous layer in which no subdivision can be seen between the color

Table 1. A Comparison of the Various Aspects of the Methods to Determine Alpha-Amylase Activity in Wheats.

Method	Unit	Sample Size (g)	Digestion Time (min.)	Temperature (^{o}C)
Robyt & Whelan	mU[1]	Variable	10	25
SKB	SKB[2]	1.0	60	30
Falling Number	Sec.	7.0	Varied	100
Phadebas	mEU[3]	1.0	15	50
CBA	mDU[4]	0.25	5	60

[1] mU = μmole maltose produced/ml/min. x 1,000.

[2] SKB = number of grams of soluble starch which under the presence of an excess of beta-amylase are dextrinized by one gram of malt in one hour at 30^{o}C.

[3] mEU = converted from absorbance obtained by a factor provided with the tablet, into milli enzyme unit of wheat alpha-amylase per 10 mls of colored solution.

[4] mDU = "quantity of alpha-amylase which dextrinize soluble starch in the presence of an excess of beta-amylase at the rate of one gram per hour at 20^{o}C x 1,000."

layer and the outer cuticle. In both cases, one week after maturation, there was an increase in the granular material in the testa. Conversely, in a sprout resistant red variety, two thick homogeneous layers, the color layer and the outer cuticle, were observed in the unripened grain. One week after maturation, the cuticle was so fused with the color layer that it was undetectable; the testa was fairly thick and showed a homogeneous structure.

X-ray microanalysis was used by Belderok in conjunction with the SEM to study sulphur distribution in white and red wheats. Previous work by this author (1961) had suggested that substances containing thiol groups may be capable of initiating sprouting. It was postulated that the sulphydryl containing compounds facilitated the removal of the inhibitory influence exerted by the covering layers. He found that the amount of soluble -SH groups present in the grains was not the determining factor in the increase in germinative energy; a decrease in the content of bound disulphide linkages in the covering layers, however, might be associated with the termination of dormancy.

MATERIALS AND METHODS

Materials

Three varieties of hard wheat were studied: Eagle (standard hard red wheat), Clark's Cream and KS73256, the latter two are white wheats. The wheats were grown by the Agronomy Department (K.S.U.) in Hutchison under different levels of nitrogen fertilization. For this study, we used the grain which was fertilized at flowering. The nitrogen was applied at five rates: 0, 30, 60, 90, and 120 lbs/acre. Three replicates for each treatment were grown, and a split plot design (3x5x3) was used (Fig. 1). The wheats were sown in October 1976, and harvested on July 5, 1977. The temperature and precipitation records for Hutchison during this period are shown in Table 2.

Methods

Alpha-amylase assay. The samples were ground on a Tekmar Analytical Mill, Model A-10 (Tekmar Scientific Apparatus, Cincinnati, Ohio). Portions of the ground samples were mixed with 0.04M acetate buffer, pH 5.5, containing 0.01M $CaCl_2$. The extracts were centrifuged (15,000 x g for 10 min.), and the supernatant solutions were heated for 15 min. at 70°C to inactivate beta-amylase (Kreen et al., 1943; Tarrago et al., 1976). Samples were analyzed in triplicate for alpha-amylase activity using the method of Robyt and Whelan (1968); reducing sugars were determined by Nelson's colormetric copper method (1944). Enzyme activity was expressed as μmoles maltose produced per gram of meal per minute.

Protein determination. Protein in the supernatant was determined by Miller's modification (1959) of the Lowry et al. (1951) method. Specific activity was expressed as μmole maltose produced per miligram of nitrogen

Figure 1. A Split Plot Design of the Wheats Used in this Study (Hutchison-1977).

* Samples used for this study.

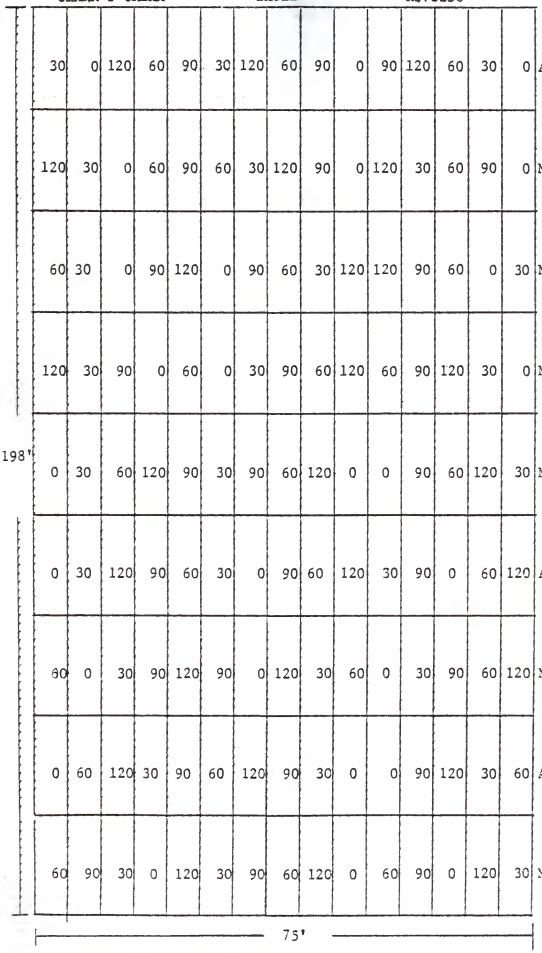

CLARK'S CREAM
EAGLE
KS73256
30 0 120 60 90 30 120 60 90 0 90 120 60 30 0 Apr.
120 30 0 60 90 60 30 120 90 0 120 30 60 90 0 May*
60 30 0 90 120 0 90 60 30 120 120 90 60 0 30 Mar.
120 30 90 0 60 0 30 90 60 120 60 90 120 30 0 May*
0 30 60 120 90 30 90 60 120 0 0 90 60 120 30 Mar.
0 30 120 90 60 30 0 90 60 120 30 90 0 60 120 Apr.
0 0 30 90 120 90 0 120 30 60 0 30 90 60 120 May*
0 60 120 30 90 60 120 90 30 0 0 90 120 30 60 Apr.
60 90 30 0 120 30 90 60 120 0 60 90 0 120 30 Mar.
198'
75'
Rep.III
Rep. II
Rep. I

Table 2. Temperature and Precipitation Records During the Growing Season for Three Hard Wheats*.

	Temperature °F			Precipitation
	High	Low	Average	(in.)
October	66.4	39.7	53.1	2.87
November	53.1	25.7	39.4	0.08
December	50.8	20.3	35.6	0.03
January	35.5	12.9	24.2	0.66
February	57.4	26.0	41.7	0.05
March	64.2	35.2	49.7	3.13
April	69.4	47.1	58.3	3.86
May	78.6	57.5	68.1	7.63
June	90.0	64.1	77.1	8.15
July	97.3	69.4	83.4	1.86

* Climatological Data, National Oceanic and Atmospheric Administration Environmental Data Service. National Climate Center Asheville, N.C.

per minute. Total protein content in the wheat was determined by the Kjeldhal method and expressed on a 14% moisture basis.

Falling Number. The AACC method 56-81 B (1973) was used to determine the falling number. It is 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.

Assessment of sprouting. Three hundred kernels of each sample were assessed. Sprouted kernels were defined as those in which the germ end 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. Alpha-amylase from the three varieties was extracted by mixing 7 grams of freshly ground meal with acetate buffer (contained 0.003M $CaCl_2$, pH 5.5) and centrifuging at 48,000 x g for 10 min. The extracts were heat-treated at 70°C for 20 min. to inactivate beta-amylase. Acetone fractionation of the heat-treated alpha-amylase extracts from the three varieties was by the method of Kruger (1966). Polyacrylamide gel electrophoresis was then carried out on both crude extracts and acetone (35%-50%) precipitates using the method of Davis (1964) with the 7% small pore gel at pH 8.9. After electrophoresis, the multiple forms of alpha-amylase were detected by incubating the gels for two hours at 35°C against a starch film attached to a glass plate and subsequently staining the films with 0.2% potassium iodide and 0.02% iodine solution. The acrylamide films were prepared as described by Doane (1967) and modified by MacGrefor et al. (1974) and Marchylo et al. (1976).

Microscopy. Samples of wheat was soaked in water for two hours and then frozen on a sample stub. Frozen sections (12 μm) were obtained using an IEC Cryotome. Sections were collected on glass slides and stained with Ponceau

2R. Photomicrographs were taken on a Reichert (Austria) light microscope.

Samples for scanning electron microscopy were prepared by fracturing kernels with a dull razer blade, mounting then on aluminum stubs, and coating with carbon and gold-palladium. Micrographs were taken on a ETEC U-1 SEM operating at 10 KV.

Wheat kernels were prepared for X-ray microanalysis as described above but the samples were only coated with carbon. X-ray analysis was done with a Kevex Energy Dispersive X-ray analyzer operating at 20 KV.

Data analysis. Statistical analysis included Anovas as well as the determination of simple and pooled correlation coefficients.

RESULTS AND DISCUSSION

Protein Content As Affected by Variety

The protein content of the wheat varieties is shown in Table 3. Varietal differences significantly ($P < 0.025$) affected protein content which was, in turn, influenced by the level of nitrogen application. The 90 lbs/acre nitrogen application produced the highest wheat protein content; however, this level was not significantly different from the 60 and 120 lbs/acre levels. Others have observed that as nitrogen level is increased, protein content also increases and that the optimum level of nitrogen is 90 lbs/acre (Hucklesby et al., 1971; Hunter et al., 1961; Stanford et al., 1972; Hunter, 1973; Pendleton et al., 1960).

The response to nitrogen application was dependent on variety. Linear correlations between nitrogen level and protein content were 0.72, 0.53, and 0.65 for Eagle, Clark's Cream and KS variety, respectively.

Sprout-Damaged Wheat

The percent visible sprouting damage recorded for the wheat varieties is shown in Table 4. Sprouting damage was not affected by the level of nitrogen applied to the soil during flowering. However, the LSD values indicated that all three varieties were significantly different ($P < 0.05$) from each other with respect to sprouting damage: Eagle had the lowest ($\sim$1-2%) and KS variety had the highest ($\sim$35-42%) values. These results show that under the growing conditions of this experiment, KS hard white wheat was more susceptible to sprouting than Clark's Cream or Eagle. In addition, we observed extensive shriveling of the kernels of the KS variety. High levels of sprout damage and shriveling would tend to limit the acceptability of the grain.

Table 3. Protein Content (%)[1] of Eagle, Clark's Cream and KS73256 Grown at Different Levels of Nitrogen Fertilization.

	Varieties[2]		
Nitrogen Level[3](lbs/acre)	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

[1] 14% moisture basis.

[2] LSD = 0.3 at $P < 0.05$ level.

[3] LSD = 0.1 at $P < 0.05$ level.

Table 4. Sprouting Damage (%) of Eagle, Clark's Cream and KS73256 Grown at Different Levels of Nitrogen Fertilization.

	Varieties[1]		
Nitrogen Level (lbs/acre)	Eagle	Clark's Cream	KS73256
0	2	6	35
30	1	5	38
60	2	5	39
90	1	4	42
120	1	4	38

[1] LSD = 2 at $P < 0.05$ level.

Alpha-Amylase Activity

The next goal was to define the relationship between alpha-amylase activity and sprout damage. The first step was to determine alpha-amylase activity. Since the falling number method is widely used as an indirect measure of alpha-amylase activity, this test was run on our samples. In addition, the Robyt and Whelan (1968) method was also used to give a quantitative measure of alpha-amylase activity.

The falling numbers of the three hard wheat varieties which were studied are shown in Table 5. The KS variety had a low falling number (FN=72) while Eagle and Clark's Cream had comparably high values (FN $>$ 400). Based on the U.S.D.A. flour standards (Greenaway et al., 1967) the KS variety would not be acceptable for breadmaking. On the other hand, malt supplementation of the other two varieties may be necessary to produce optimum bread characteristics.

Alpha-amylase activity, as determined by the Robyt and Whelan method, is shown in Table 6. Varietal differences were highly significant ($P < 0.004$). The KS variety had the highest alpha-amylase activity, and was significantly different from the other two varieties. Eagle and Clark's Cream showed essentially the same low level of enzyme activity.

Data on specific alpha-amylase activity are shown in Table 7. These results paralleled the data on enzyme activity presented in the previous paragraph and indicate that the high enzyme activity exhibited by the KS variety was due to a greater extraction of alpha-amylase from that grain.

In order to relate the alpha-amylase activity to method of determination and sprouting damage, linear correlations across varieties were determined and are shown in Table 8. Low but significant correlations were found between protein content and alpha-amylase activity, protein content and falling number, and protein content and sprouting damage. A plot of these relationships (Figs. 2-5) suggests that varietal differences played a major

Table 5. Falling Number Values (Sec.) of Eagle, Clark's Cream and KS73256 Grown at Different Level of Nitrogen Fertilization.

	Varieties[1]		
Nitrogen Level (lbs/acre)	Eagle	Clark's Cream	KS73256
0	398	414	75
30	431	405	74
60	420	418	73
90	405	424	68
120	363	418	70

[1] LSD = 29 at $P < 0.05$ level.

Table 6. Alpha-Amylase Activity[1] of Eagle, Clark's Cream and KS73256 grown at Different Levels of Nitrogen Fertilization.

	Varieties[2]		
Nitrogen Level (lbs/acre)	Eagle	Clark's Cream	KS73256
0	2.89	3.16	4.75
30	2.91	3.15	4.80
60	2.99	3.23	4.77
90	2.90	3.13	4.81
120	2.98	3.18	4.79

[1] μmole/min/g meal.

[2] LSD = 0.22 at $P < 0.05$ level.

Table 7. Specific Alpha-Amylase Activity[1] of Eagle, Clark's Cream and KS73256 Grown at Different Levels of Nitrogen Fertilization.

	Varieties[2]		
Nitrogen Level (lbs/acre)	Eagle	Clark's Cream	KS73256
0	1.55	1.64	2.24
30	1.51	1.67	2.23
60	1.55	1.67	2.23
90	1.52	1.66	2.27
120	1.56	1.63	2.22

[1] μmole/min/mg soluble Nitrogen.

[2] LSD = 0.18 at $P < 0.05$ level.

Table 8. Linear Correlations Across All Varieties.

	PC [1]	A[2]	SPA[3]	FN[4]	SPD[5]
PC	--	-.49**	-.46**	.55**	-.52**
A	--	--	.99**	-.85**	.86**
SPA	--	--	--	-.79**	.79**
FN	--	--	--	--	-.97**

[1] PC = Protein Content

[2] A = Alpha-Amylase Activity

[3] SPA = Specific Alpha-Amylase Activity

[4] FN = Falling Number

[5] SPD = Visible Sprouting Damage

** = Significant at $P < 0.01$ level

role in determining the correlations.

Alpha-amylase activity (by Robyt and Whelan) was negatively correlated with falling number (r= -0.85) and positively correlated with sprouting damage (r= 0.86). Again, the major contribution to the high correlations was varietal differences (Figs. 6 & 7). On the other hand, the high correlations between alpha-amylase activity and specific activity (r= 0.99) was independent of variety (Fig.8).

The falling number method was highly correlated with sprouting damage (r= -0.97) (Fig. 9). Therefore, this method is a better predictor of sprouting damage than the quantitative measure of alpha-amylase activity as determined by the Robyt and Whelan method. These results may suggest that other factors besides alpha-amylase activity influence falling number determinations. On the other hand, the Robyt and Whelan method may not be measuring the total alpha-amylase activity that is present in the whole meal. The latter method does not take into consideration the following contributors to alpha-amylase activity: (1) the presence of enzyme activating factors in the whole wheat meal, (2) differences in the rates of activity towards native substrate and soluble starch, (3) the presence of tightly bound enzymes which increase amylase activity in situ, and (4) the role of beta-amylase in starch degradation.

Gel Electrophoresis of Alpha-Amylases

Gel electrophoretic patterns were run on the enzyme extracts to determine (1) which isozymes were inactivated by the heat treatment employed in the Robyt and Whelan method, and (2) if there were any varietal differences in alpha-amylase isozymes.

Polyacrylamide gel electrophoretic separations of heat treated crude alpha-amylase extracts from the three varieties shown that each wheat possessed

Figure 2. Protein Content Versus Alpha-Amylase Activity: Eagle (□). Clark's Cream (○), and KS73256 (△).

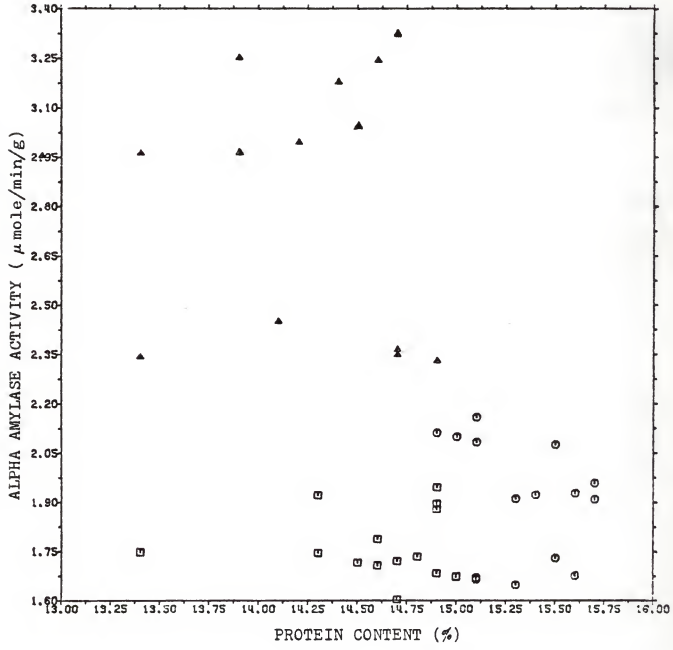

ALPHA AMYLASE ACTIVITY (μmole/min/g)
3.40
3.25
3.10
2.95
2.80
2.65
2.50
2.35
2.20
2.05
1.90
1.75
1.60
13.00
13.25
13.50
13.75
14.00
14.25
14.50
14.75
15.00
15.25
15.50
15.75
16.00
PROTEIN CONTENT (%)

Figure 3. Protein Content Versus Specific Activity: Eagle (□), Clark's Cream (○), and KS73256 (△).

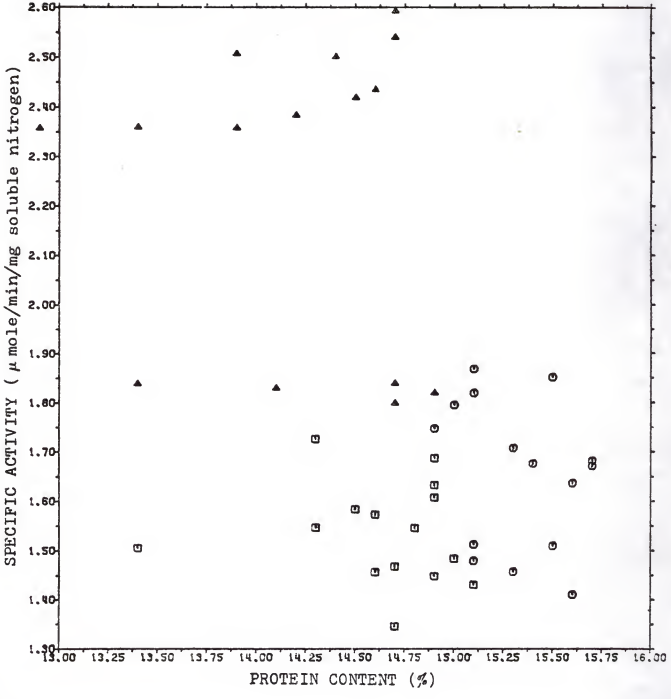

SPECIFIC ACTIVITY (μ mole/min/mg soluble nitrogen)
2.60
2.50
2.40
2.30
2.20
2.10
2.00
1.90
1.80
1.70
1.60
1.50
1.40
1.30
13.00
13.25
13.50
13.75
14.00
14.25
14.50
14.75
15.00
15.25
15.50
15.75
16.00
PROTEIN CONTENT (%)

Figure 4. Protein Content Versus Falling Number: Eagle (□), Clark's Cream (○), and KS73256 (△).

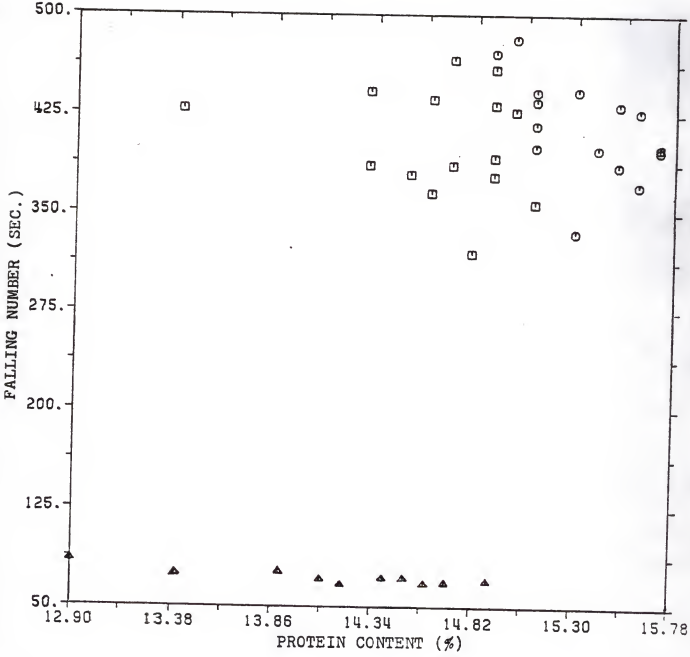

500.
425.
350.
275.
200.
125.
50.
FALLING NUMBER (SEC.)
12.90
13.38
13.86
14.34
14.82
15.30
15.78
PROTEIN CONTENT (%)

Figure 5. Protein Content Versus Visible Sprouting Damage: Eagle (□), Clark's Cream (○), and KS73256 (△).

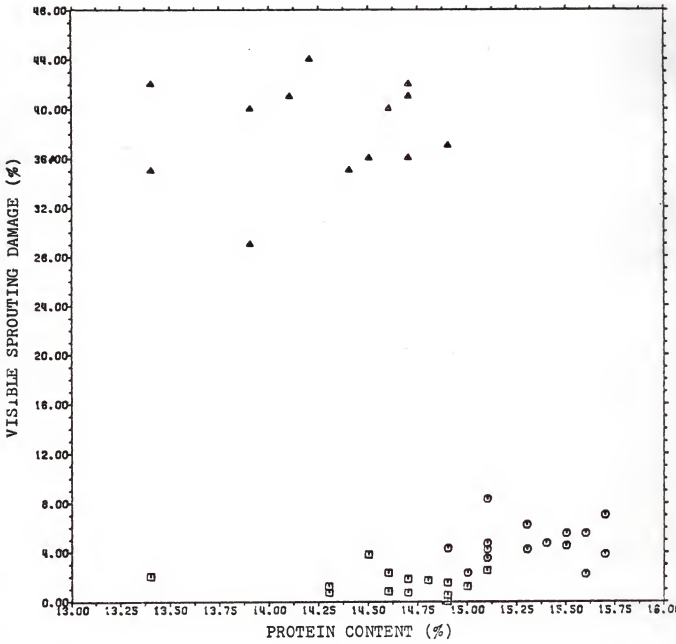

VISIBLE SPROUTING DAMAGE (%)
46.00
44.00
40.00
36.00
32.00
28.00
24.00
20.00
16.00
12.00
8.00
4.00
0.00
13.00
13.25
13.50
13.75
14.00
14.25
14.50
14.75
15.00
15.25
15.50
15.75
16.00
PROTEIN CONTENT (%)

Figure 6. Alpha Amylase Activity Versus Falling Number: Eagle (□) Clark's Cream (○), and KS73256(△).

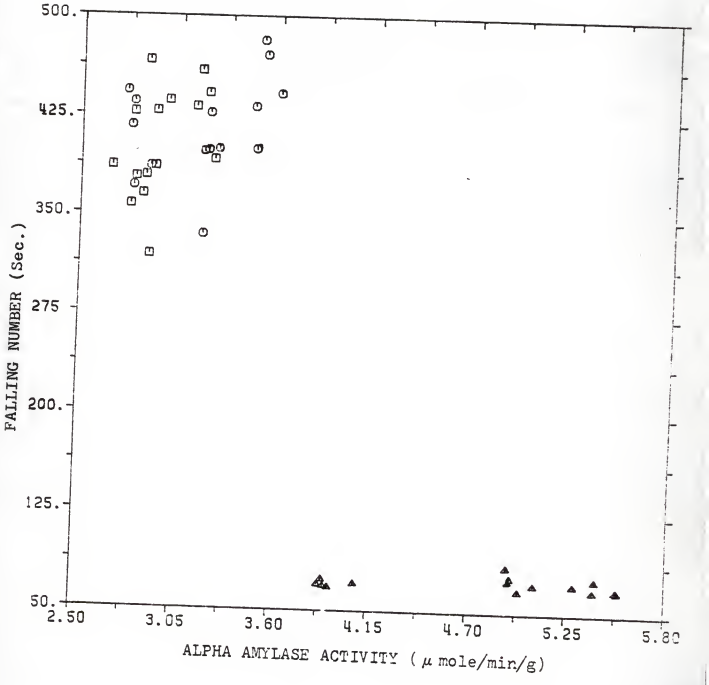

500.
425.
350.
275
200.
125.
50.
FALLING NUMBER (Sec.)
2.50
3.05
3.60
4.15
4.70
5.25
5.80
ALPHA AMYLASE ACTIVITY (μ mole/min/g)

Figure 7. Alpha Amylase Activity Versus Visible Sprouting Damage: Eagle (□), Clark's Cream (○), and KS73256 (△).

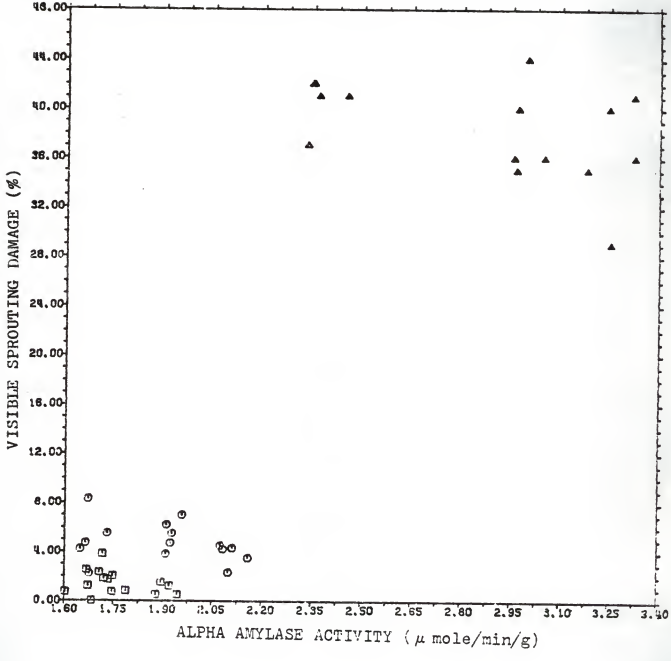

VISIBLE SPROUTING DAMAGE (%)
48.00
44.00
40.00
36.00
32.00
28.00
24.00
20.00
16.00
12.00
8.00
4.00
0.00
1.60
1.75
1.90
2.05
2.20
2.35
2.50
2.65
2.80
2.95
3.10
3.25
3.40
ALPHA AMYLASE ACTIVITY (μ mole/min/g)

Figure 8. Alpha Amylase Activity Versus Specific Activity: Eagle (□), Clark's Cream (○), and KS73256 (△).

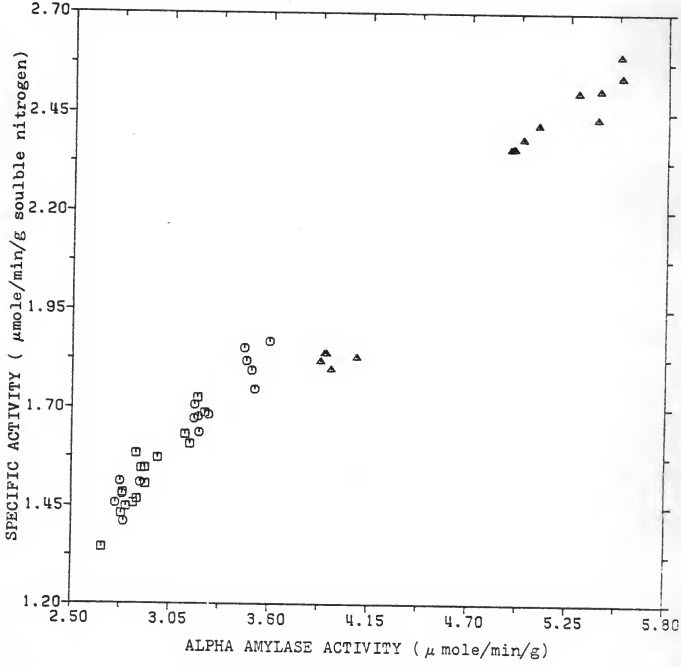
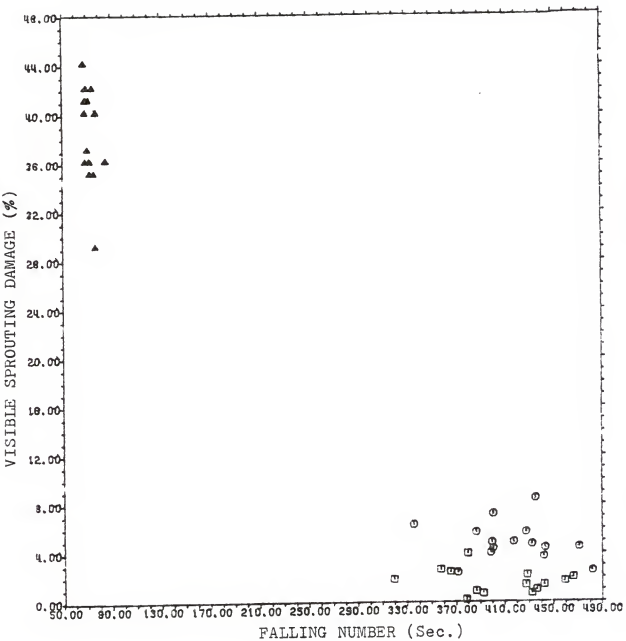

2.70
2.45
2.20
1.95
1.70
1.45
1.20
SPECIFIC ACTIVITY (μmole/min/g soulble nitrogen)
2.50
3.05
3.60
4.15
4.70
5.25
5.80
ALPHA AMYLASE ACTIVITY (μ mole/min/g)

Figure 9. Falling Number Versus Visible Sprouting Damage: Eagle (□), Clark's Cream (○), and KS73256 (△).


48.00
44.00
40.00
36.00
32.00
28.00
24.00
20.00
16.00
12.00
8.00
4.00
0.00
VISIBLE SPROUTING DAMAGE (%)
50.00
90.00
130.00
170.00
210.00
250.00
290.00
330.00
370.00
410.00
450.00
490.00
FALLING NUMBER (Sec.)

at least eleven components (Fig. 10 a). A comparison of these gel patterns with the gels obtained with the 35%-50% acetone precipitates (a more pure alpha-amylase extract) showed that only nine of the eleven components shown in gels of the crude extract were present (Fig. 10 b). Others have identified eight alpha-amylase components in HRS wheats (Kruger, 1972; Warchalewshi et al., 1978). Our data suggest that other enzyme and/or proteins were extracted during the procedure which were not affected by the 70°C heat treatment.

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

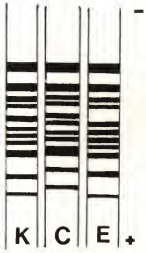
Starch zymograms of the crude, nonheat treated and heat treated extracts were run to determine which of the gel bands exhibited amylase activity. The results are shown in Fig. 11. The zymograms of crude extracts which were not heat treated showed extensive digestion of the starch film along the entire gel which was undoubtly due to the action of both alpha and beta amylases.

Gels that were made with the crude heat-treated extract produced entirely different zymograms (Fig. 11 a). The KS variety exhibited two areas of amylase activity. According to Kruger's notation (1972), we would designate the fast moving components as beta-amylase and the slower components as alpha-amylases (possibly alpha-1 through alpha-3). On the other hand, only the slower moving components (alpha-amylase) can be detected in the gels for Eagle and Clark's Cream.

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

Figure 10. Gel Electrophoretic Patterns of Heat Treated Crude Extracts (a) and Acetone Precipitates (b)from: Eagle (E), Clark's Cream (C), and KS73256 (K).

a



b

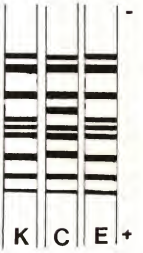
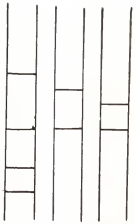
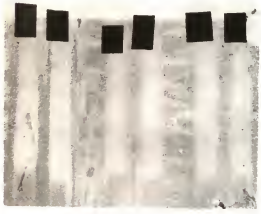


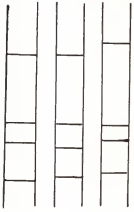
Figure 11. Starch Zymograms of Crude, Heat Treated (a), and Nonheat Treated Extracts (b), and Gel Electrophoretic Pattern of Nonheat Treated Crude Extracts (c) from: Eagle (E), Clark's Cream (C), and KS73256 (K). And alpha (α) and beta (β) amylases identified by Kruger (1972).

a

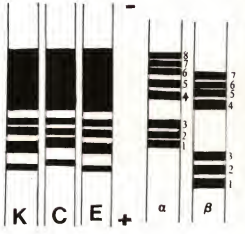


b





c



The zymograms also indicate that more alpha-amylase components were active in the KS variety than in the other two varieties. This was expected since it exhibited the highest level of sprouting damage, and according to Kruger (1972), as germination time increases, more alpha-amylase components become active.

It should be noted that the three wheat varieties showed the same number of gel electrophoretic components but slightly different mobilities (Fig. 10). In addition, there were differences among varieties with respect to the density of the various gel components. It is not known whether these differences would contribute to variations in alpha-amylase activity. More extensive studies are needed.

Microscopy

A study done by Kruger (1972) showed that wheat alpha-amylases were found largely in the pericarp, with small amounts present in the seed coat and aleurone layer. This led us to investigate possible structural and elemental differences in the outer layers that might contribute to sprouting susceptibility.

Covering layers. Scanning electron and light microscopical studies were done on the outer layers of the three varieties; those micrographs are shown in Figs. 12 & 13. No differences were found between sound and sprouted kernels within each variety. However, differences were observed among varieties. Eagle had a more sturdy structure compare to that of Clark's Cream or KS variety. Both of the latter varieties exhibited some air spaces in the pericarp and showed a less compact structure (Fig. 12 d, e, & f). In addition, the pericarp of the white wheats was damaged more readily than the Eagle variety during thin sectioning (Fig. 13). Again, this suggests a less rigid pericarp structure in the white wheats. One other structural

Figure 12. SEM's of Hard Endosperm (a, b, c) and Outer Layers (d, e, f) of Three Wheat Varieties: Eagle (a, d),Clark's Cream (b, d) KS73256 (c, f).

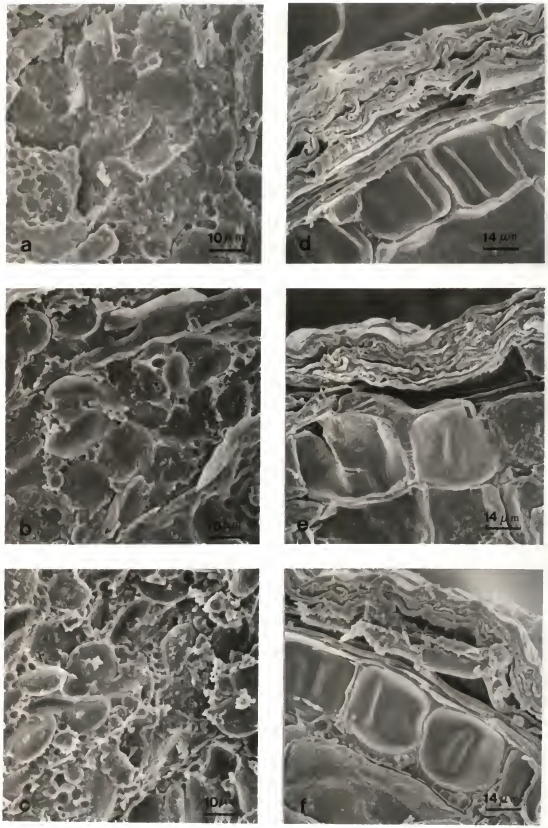
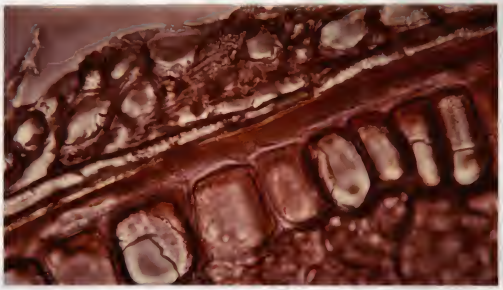
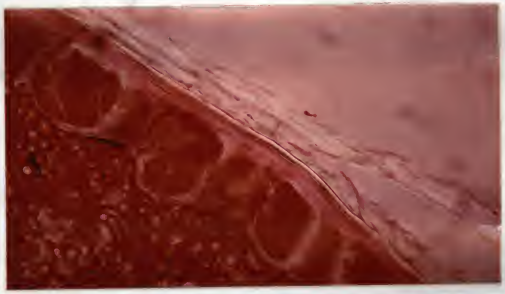
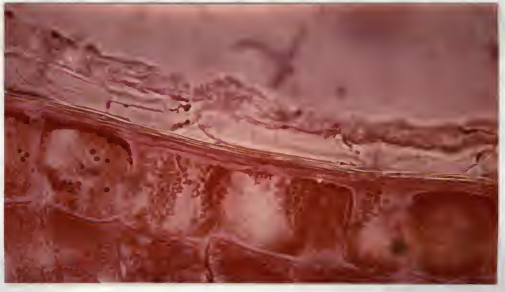

a
10 µm
d
14 µm
b
e
14 µm
c
f

Figure 13. Light Photomicrographs of the Outer Layers of Three Wheat Varieties: Eagle (a), Clark's Cream (b), and KS73256 (c). (x 1,250).







difference was noted among varieties: the nucellar epidermis of the Eagle variety is much thicker than of the white wheats (Fig. 13). This might suggest that water is imbibed much more readily by the white varieties than by the red variety, thus increasing the susceptibility of the white varieties to sprouting.

Several authors (Belderok, 1961; Goodwin & Carr, 1970) have suggested that the presence of certain minerals in cereal grain is related to sprouting susceptibility. Therefore, X-ray microsnalysis was done on the outer layers of the wheat kernels to determine if differences in elemental analysis might be related to the sprouting susceptibility of the various wheat varieties. The X-ray spectra of sound and sprouted grains are shown in Figs. 14 & 15, respectively. Kernels of sound wheat showed distinct X-ray spectra for each variety (Fig. 14). However, kernels of sprouted white wheats showed similar X-ray patterns (Fig. 15 a & b): low sulphur and potassium content.

A sharp decrease in sulphur content was observed in the seed coat of sprouted kernels of the KS variety (Figs. 14 & 15 a). These data agree with Belderok (1961) who observed a decrease in the sulphur content of the pericarp and seed coat of sprouted wheat (as determined by specific stains). Apparently, it is possible that the thiol containing substances are involved in removing the sprouting inhibitory factors present in the covering layers. Clark's Cream and Eagle, both of which exhibited low sprouting damage (Table 4), showed little change in sulphur due to sprouting.

Potassium consititutes the major mineral of wheat (Blanck, 1955) and is thought to be involved in the prevention of water loss by the plant. For sound wheats the X-ray microanalysis showed that the level of potassium present in the seed coat was higher in Clark's Cream than in KS variety. On sprouting there was a great reduction in the potassium content for Clark's

Figure 14. X-ray Spectra of the Seed Coat of Sound Wheats.

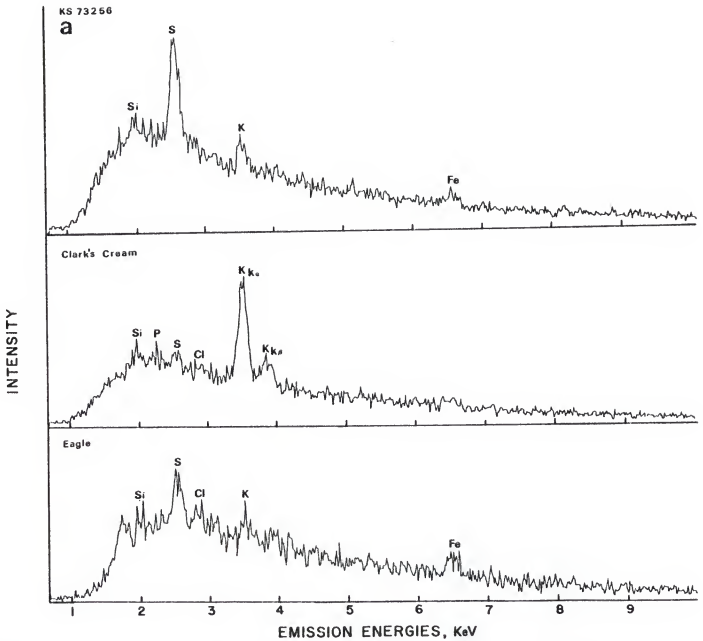
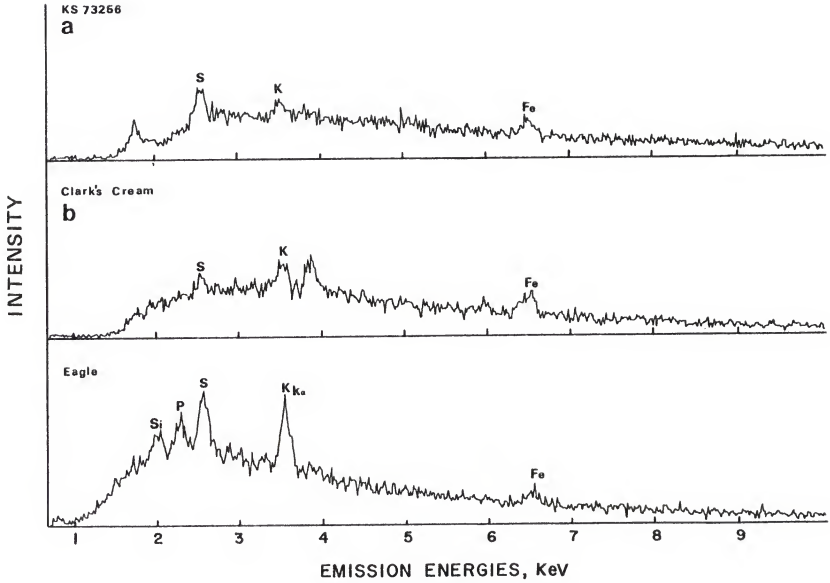

KS 73256
a
S
Si
K
Fe
Clark's Cream
Kkα
Si
P
S
Cl
Kkβ
Eagle
S
Si
Cl
K
Fe
INTENSITY
1
2
3
4
5
6
7
8
9
EMISSION ENERGIES, KeV

Figure 15. X-ray Spectra of the Seed Coat of Sprouted Wheats.

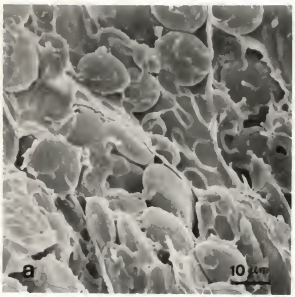

KS 73266
a
S
K
Fe
Clark's Cream
b
S
K
Fe
Eagle
Si
P
S
K Kα
Fe
INTENSITY
1
2
3
4
5
6
7
8
9
EMISSION ENERGIES, KeV

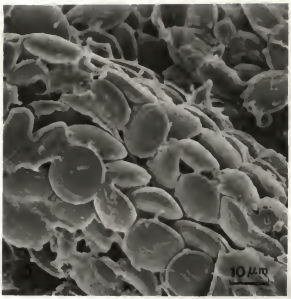
Cream. The low level of potassium in the KS variety may be one factor contributing to the high percentage of shriveled grains that were observed in this variety.

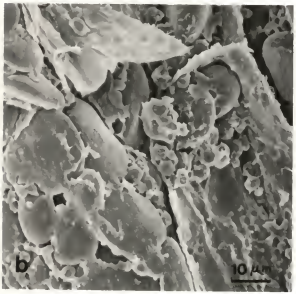
Endosperm. Scanning electron micrographs of the soft endosperm of both sound and sprouted wheats are shown in Fig. 16. The quantity of the matrix protein decreases in sprouted wheats. This observation agrees with the result previously reported by Ponpipom (1975), and may suggest that proteolytic enzymes break down the matrix protein producing a looser structure of starch granules, thus making them more susceptible to alpha-amylase attack.

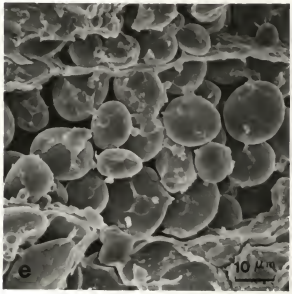
We did not observe any differences in the structure of the hard endosperm for sound and sprouted kernels within each variety. However, KS variety shows a "softer" hard endosperm than the other varieties (Fig. 12 a, b, & c). Whether or not endosperm compactness is related to sprouting susceptibility is not certain. It is undoubtedly a function of the ease of water penetration into the grain (Moss, 1977) and therefore, may be indirectly related to sprouting susceptibility. In addition, work by Ponpipom (1975) indicates that softer endosperm structures are more susceptible to attack by alpha-amylase.

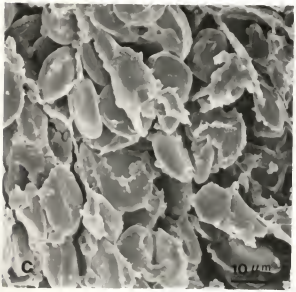
Figure 16. SEM's of the Soft Endosperm of Sound (a, b, c) and Sprouted (d, e, f)Wheat Kernels from : Eagle (a, d) Clark's Cream (b, e), and KS73256 (c, f).

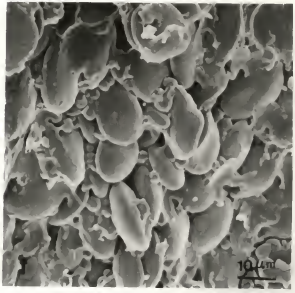

a
10 µm


10 µm


b
10 µm


e
10 µm


c
10 µm


10 µm

SUMMARY

Two varieties of Kansas hard white wheat (Clark's Cream and KS73256) were compared with a standard hard red wheat (Eagle) to determine differences in protein content, alpha-amylase activity, specific activity, falling number, visible sprouting damage, and structural characteristics.

Level of nitrogen application affected the protein content of the wheat; however, protein content did not significantly affect alpha-amylase activity. Variety had a greater effect on the variables that were measured than did rate of nitrogen application.

Sprouting damage and alpha-amylase activity were found to be highly correlated. The KS variety had higher levels of sprouting damage and alpha-amylase activity than the other two varieties; this would make it unacceptable to the growers and may limit its use for breadmaking. Clark's Cream and Eagle varieties showed essentially the same alpha-amylase activity but were significantly different from each other with respect to sprouting damage. The practical significance of the differences observed in sprouting damage between these varieties can not be evaluated without doing test bakes. However, since these two varieties showed essentially the same low level of alpha-amylase activity, as measured by both the Robyt and Whelan method and by falling number, this would suggest that the low level of sprouting damage that occurred with Clark's Cream did not significantly increase the level of alpha-amylase activity in the meal. These data also point out that there may be exceptions to the concept that sprouting damage is related to high levels of alpha-amylase activity.

Of the two methods for determining alpha-amylase activity, falling number appears to be a better predictor of sprouting damage than alpha-amylase activity as determined by the method of Robyt and Whelan.

Gel electrophoresis was used to obtain more information concerning the beta-amylase heat inactivation step of the Robyt and Whelan method. Starch zymograms from the gels indicated that beta-amylase in the KS variety was more heat stable than in the other two varieties. This suggests that beta-amylase contributed, in part, to the amylase activity measured for KS variety. Further studies using gel electrophoresis showed that the gel electrophoretic patterns of all three varieties possessed the same number of bands but with slight differences in mobilities and densities.

Microscopical differences were observed among hard white wheats and the standard hard red wheat. The covering layers of the hard white wheats exhibited some air spaces and a less compact structure which was more readily damaged during the thin sectioning procedure. The KS variety showed a "softer" endosperm structure than the other two varieties. No differences in the covering layers or hard endosperm were observed, however, between sound and sprouted kernels within varieties. Varietal differences in the soft endosperm of sprouted wheats were observed. The quantity of matrix protein appeared to decrease in sprouted grains, and a less compact structure was observed in the soft endosperm.

X-ray spectra of the seed coats of the two white varieties showed similar elemental patterns for the sprouted kernels (low sulphur and potassium content), and these patterns were different from the patterns of the sound counterparts. A decrease in the sulphur content of the seed coat was observed on sprouting. These data may support Belderok (1976) hypothesis that the presence of sulphur containing substances in the covering layers prevents seed germination. More extensive studies are needed to determine whether or not these observed differences affect the milling and baking characteristics of the flour.

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A STUDY OF ALPHA AMYLASE ACTIVITY IN KANSAS HARD WHITE WHEATS

by

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The protein content, sprouting damage, alpha-amylase activity, gel electrophoretic patterns, and structural characteristics of two varieties of Kansas hard white wheats (Clark's Cream and KS73256) were compared with a standard hard red wheat (Eagle). Varietal differences significantly ($P<0.025$) affected protein content which was, in turn, influenced by the level of nitrogen application. Sprouting damage was correlated to two different methods for determining alpha-amylase activity. Falling number was more highly correlated ($r = -0.97$, $P<0.01$) with sprouting damage than alpha-amylase activity ($r = 0.86$, $P<0.01$) as measured by the method of Robyt and Whelan (1968).

All three wheat varieties showed the same number of alpha-amylase components as identified by gel electrophoresis but exhibited different mobilities and densities. Starch film zymogram showed that some beta-amylase components in the KS variety were stable during the 70°C, 15 min. inactivation step employed in the Robyt and Whelan method. This suggested that the latter method did not completely eliminate beta-amylase as a contributor to reducing value determinations.

X-ray spectra of the seed coats of sound wheat showed distinct patterns for each variety. However, sprouted white wheats had similar X-ray patterns:low sulphur and potassium contents. A reduction in the sulphur content on sprouting substantiated Belderok's (1961) hypothesis relating sulphydryl components to the termination of dormancy.

SEM and Light microphotographs of the covering layers showed that white wheats exhibited some air spaces and a looser structure which was readily damaged by thin sectioning. No differences were observed between

sound and sprouted kernels within each variety with regard to the covering layers and the hard endosperm. However, differences were observed between sound and sprouted soft endosperm within each variety. KS variety showed a "softer" endosperm structure than the other two varieties. Variety appeared to be the major contributor to the differences observed in all the variables studied.