

# Microscopic Evaluation of the Digestibility of Sorghum Lines that Differ in Endosperm Characteristics<sup>1</sup>

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## ABSTRACT

Sorghum grains that differ in endosperm texture and endosperm type were compared to evaluate the usefulness of microscopy to account for differences observed in the feeding properties of these grains. The waxy sorghum kernel sections had the smallest proportion of peripheral endosperm area of the four grains examined. The waxy sections were also more rapidly solubilized by pronase and  $\alpha$ -amylase enzymes and by buffered rumen fluid than the nonwaxy sections. The corneous, floury, and intermediate texture kernels were nonwaxy grains and contained more peripheral endosperm than the waxy sorghum. The increased solubilization of waxy sections probably occurs because waxy starch is more susceptible to enzymes, and because there is less peripheral endosperm area in the kernel. In addition, the protein matrix of waxy grain may be more susceptible to enzymes. These findings may account for observations of feeding trials in which steers fed nonwaxy sorghum grain diets required 8 to 20% more feed to produce a pound of gain than steers fed waxy sorghum grain diets. We believe that microscopy can be used to study and evaluate kernel properties related to feeding quality of sorghum.

Several hundred million bushels of sorghum grain are consumed by livestock annually in the U.S. It is estimated that an increase of 5% in feed efficiency of the grain fed in Texas cattle feedlots would mean a savings of \$14.7 million, annually. This large economic incentive, coupled with the availability of genetic material from the World Sorghum Collection, has stimulated serious efforts to breed sorghum grains with improved nutritive value.

Recent feeding trials have demonstrated consistent improvement in performance of animals fed grains with waxy and heteroyellow endosperm compared to the usual commercial sorghum grain. When fed to steers, waxy sorghum has given 8 to 20% better feed efficiency than nonwaxy sorghum grain (1,2,3). Digestibility of waxy sorghum grain was better than that of nonwaxy grain when fed to steers (3) and sheep (4). Heteroyellow endosperm hybrids may have improved feed efficiency and digestibility (3).

Some of the differences in feedlot performance of sorghum grains can probably be explained by differences in structure of the kernels. The difference in structure of the peripheral endosperm of the kernel may affect digestibility more than any other single factor. The peripheral endosperm is that area just beneath the aleurone cell layer and contains small starch granules well embedded in a dense amorphous proteinaceous matrix (5). Seckinger and Wolf (6) illustrated the structure of the peripheral endosperm area in their transmission and scanning electron microscopy studies of the ultrastructure of the sorghum kernel. Using light microscopy, Sullins et al. (7) found that the increased feedlot efficiency of reconstituted sorghum grain fed to steers was directly related to the partial breakdown of the protein matrix, especially that in the peripheral endosperm area of the kernel. These papers suggest to us that there are differences in

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sorghum kernel structure and that these differences may be related to the ease in which a sorghum ration is digested by animals.

The objective of this study was to make a detailed comparison of a few sorghum grains with wide differences in endosperm type and texture in order to evaluate the potential of microscopic techniques in the development of improved sorghums.

## MATERIALS AND METHODS

### Grain Samples

The sorghum lines used in this study were selected to provide grain with different endosperm texture and type. The different textures that were examined included all corneous (SC 301), floury (NSA 740), and intermediate (Kafir 60). The intermediate endosperm grain contained approximately equal quantities of corneous and floury endosperm. The three lines previously mentioned were normal or regular starch types (approximately 30% amylose and 70% amylopectin), whereas the fourth line (TX 615) used in this study contained a waxy-type endosperm (100% amylopectin). Three of the selections were Kafir lines which included the intermediate (Kafir 60), the floury texture (NSA 740), and the waxy type (TX 615). The corneous line (SC 301) was similar to the Kafir lines in shape, pigmentation, and chemical properties, and was selected from the Texas Agricultural Experiment Station-U.S. Department of Agriculture sorghum conversion program (8). All lines were grown under comparable conditions on the Texas A&M University plantation at College Station in 1970.

### Physical and Chemical Analyses of Whole Grain and Starch

Thousand-kernel weight, test weight, hardness, and density were described by Rooney and Sullins (9). Starch was extracted from the grain by the wet-milling procedure outlined by Norris and Rooney (10). Moisture, protein, fat, and ash were determined by AACC methods (11). Starch was determined by the heat gelatinization-glucoamylase hydrolysis method outlined by Norris (12). The procedure was as follows:

1. Weigh 140 to 200 mg. ground grain into centrifuge tubes containing 20 ml. water. Place samples in boiling water bath for 3 min.; transfer to autoclave at 15 p.s.i. (250°F.) for 5 hr.

2. Add 25 ml. 0.1N acetate buffer, cool to 60°C., add 1 ml. of Diazyme L 30 glucoamylase<sup>3</sup>, and incubate for 30 min. Add 3 ml. of 1:9 sulfuric acid:water and 2 ml. 12% sodium tungstate. Let stand for 5 min. and centrifuge at 1,340 × g.

3. Dilute 2 ml. of supernatant to 100 ml. with distilled water and determine sugars in 1 ml. of diluted supernatant by glucose-oxidase method (13).

Amylose was determined by the method of Gilbert and Spragg (14).

### Preparation and Examination of Endosperm Sections

Paraffin-embedded sections for microscopic examination were prepared as described by Sullins et al. (7). Plastic-embedded sections for enzymatic studies were prepared as outlined by Sullins (15). This method was as follows:

1. Whole kernels were fixed in 6% acrolein for 3 to 4 hr. at 4°C., dehydrated in four changes of 100% ethanol for 10 min. per change followed by three changes of 10 min. each in acetone.

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TABLE I. KERNEL CHARACTERISTICS AND PHYSICAL PROPERTIES OF THE GRAIN FROM FOUR SORGHUM LINES

Lines	Endosperm Texture	Endosperm Type	1,000-Kernel Wt. g.	Test Wt. lb./bu.	Hardness %	Density g./cc.
NSA 740	Floury	Normal	22.0	46.2	7.2	1.223
Kafir 60	Intermediate	Normal	24.0	53.1	15.7	1.370
SC 301	Corneous	Normal	20.0	53.7	33.5	1.400
TX 615	Intermediate	Waxy	26.0	56.4	17.1	1.325

2. Specimens were embedded by a gradual change from acetone to Araldite/Epon plastic.

3. Specimens were placed in pure Araldite/Epon plastic and left overnight.

4. Kernels were put in fresh plastic and placed in a 40°C. oven for 24 hr. to polymerize. Final polymerization was at 60°C. for 2 to 3 days.

5. Specimens were trimmed, glued to lucite rods, and sectioned with a glass knife.

Sequential sections were cut from each of the four lines for enzymatic treatments. Starch was removed by treating the sections with hog pancreas  $\alpha$ -amylase<sup>4</sup> as described by Wolf and Khoo (16). Sections were also treated with pronase<sup>5</sup> to determine susceptibility of peripheral endosperm protein. Other sections were treated with buffered<sup>6</sup> rumen fluid to observe the susceptibility of starch and protein. A Zeiss universal microscope equipped with phase contrast as well as bright field optics and a Zeiss automatic camera were used for the photomicrographs.

#### Susceptibility of Starch to Amyloglucosidase

The susceptibility of starch to enzymatic digestion was determined on ground whole grain and isolated starch. Carbon dioxide gas produced from fermentation of glucose was measured manometrically (17) as millimeters of Hg. The whole grain was ground through a laboratory hammer mill with a 0.010-in. screen. The sample weight of each grain was adjusted so that each sample placed in the manometers contained 1.25 g. of starch. One gram of baker's compressed yeast in 10 ml. of water, 10 mg. of CaCl<sub>2</sub> in 4 ml. of water, and excess enzyme (1.0 ml. of amyloglucosidase<sup>7</sup>), was added to each manometer. The control manometer contained the same materials, except 450 mg. of glucose was substituted for the grain. The manometers were placed in a circulating-water bath at 30°C. with readings taken at 2-hr. intervals for 70 hr.

## RESULTS AND DISCUSSION

#### Physical and Chemical Properties of Whole Grain and Starch

Kernel characteristics, physical properties, and chemical analyses are presented in Tables I and II. The floury-textured grain has low density and test weight and is soft compared to the other grains. The low density and test weights

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<sup>5</sup>Source of pronase was Calbiochem, La Jolla, Calif.

<sup>6</sup>McDougall's buffer for remen inoculum.

<sup>7</sup>Diazyme L 30 = 30 diazyme units per ml.

TABLE II. CHEMICAL PROPERTIES OF THE GRAIN FROM FOUR SORGHUM LINES<sup>a</sup>

Lines	Protein %	Fat %	Ash %	Starch %	Amylose %
NSA 740	16.6	3.4	1.9	66.7	28.7
Kafir 60	11.8	2.6	1.3	75.2	29.0
SC 301	12.9	2.8	1.6	73.4	29.0
TX 615	12.7	2.6	1.8	73.8	0.2

<sup>a</sup>Values calculated on a moisture-free basis. All values for amylose are expressed as a percent of the total starch dry weight.

are serious defects of floury-textured grains, making their commercial use extremely unlikely. Chemical composition of the grains was similar except the protein content of the floury variety was higher. Its grain yield was lowest which probably accounts for part of the difference. In general, we do not believe that floury grain has higher protein than the other grains; other comparisons of floury Kafir with intermediate-textured Kafir have not shown significant differences in protein content. There was essentially no major difference in starch-granule size among the four varieties.

#### Differences in Kernel Structure

General anatomical comparisons of the four lines are illustrated in Fig. 1. The waxy line has less peripheral endosperm and amorphous protein matrix than the other grains. Examination of plastic-embedded sections after treatment with  $\alpha$ -amylase and pronase confirmed that the waxy-grain kernels had less peripheral endosperm than the other three lines. The starch and amorphous protein were digested, leaving the protein bodies clearly visible (Fig. 2). These protein bodies become smaller and fewer in number progressively toward the center of the endosperm. Thus, the greater concentration and larger size of protein bodies beneath the aleurone layer is a good index of the amount or concentration of peripheral endosperm. The waxy with fewer protein bodies and less amorphous protein in the peripheral area (Fig. 2) would therefore have less peripheral endosperm. Thus, the greater concentration and larger size of protein bodies beneath the aleurone layer are a good index of the amount or concentration of the starch except in the peripheral endosperm area where the starch granules were completely encapsulated by the protein matrix. Apparently, the rumen fluid had difficulty in penetrating the protein matrix of the peripheral endosperm. Only small differences in the rate of digestion among the sections from floury, intermediate, and corneous endosperm grains were observed. However, all our studies consistently indicated that the waxy endosperm sections contained the smallest proportion of peripheral endosperm and that they were the most rapidly solubilized of all the grains.

#### Susceptibility of Starch to Amyloglucosidase

We measured the rate of hydrolysis of starch in the ground whole grain and starches isolated from each of the grains to provide additional data to clarify our microscopic observations. The waxy grain and starch were more rapidly hydrolyzed by the amyloglucosidase than were the other ground grains and starches (Figs. 4 and 5). Therefore, one might conclude that the increased

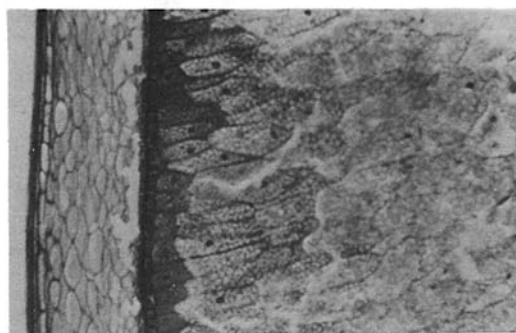
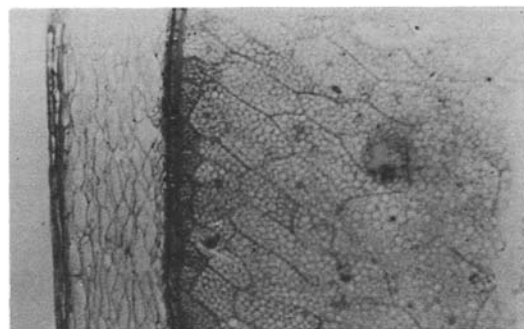
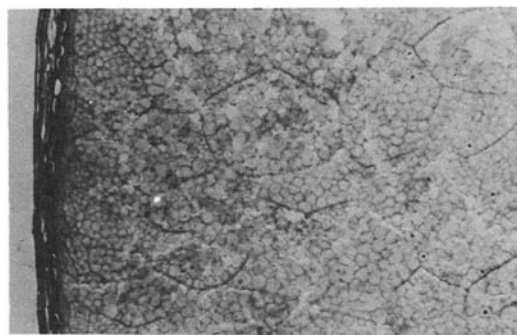
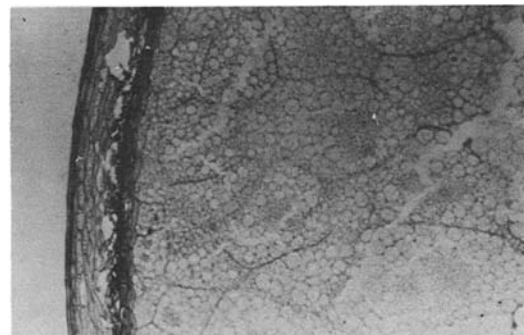
**A****B****C****D**

Fig. 1. Paraffin sections stained with Safranin and fast green. A, floury (NSA 740); B, intermediate (Kafir 60); C, corneous (SC 301); and D, waxy (TX 615).

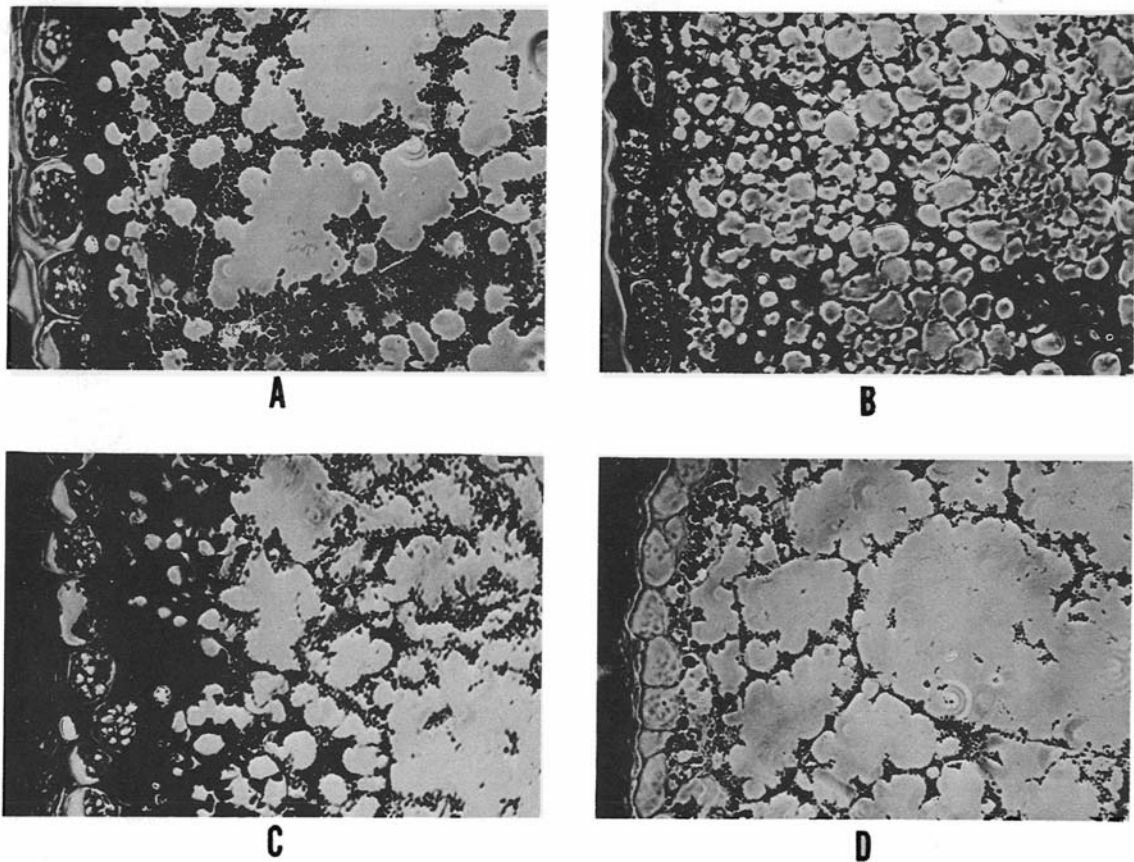


Fig. 2. Plastic sections treated with  $\alpha$ -amylase enzyme for 15 min. at 38°C. followed by pronase for 2 hr. at 28°C. A, floury (NSA 740); B, intermediate (Kafir 60); C, corneous (SC 301); and D, waxy (TX 615). Phase contrast.

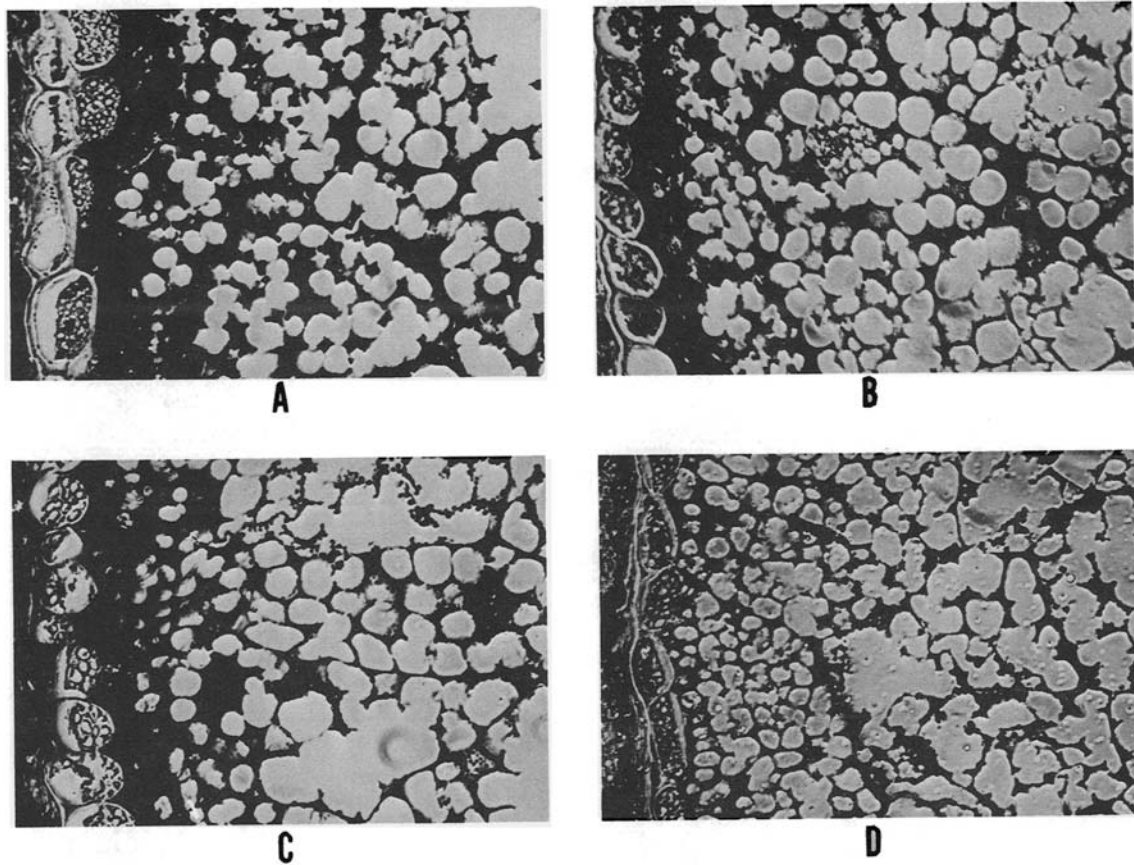


Fig. 3. Plastic section treated with rumen fluid for 20 hr. at 39° C. A, floury (NSA 740); B, intermediate (Kafir 60); C, corneous (SC 301); and D, waxy (TX 615). Phase contrast.

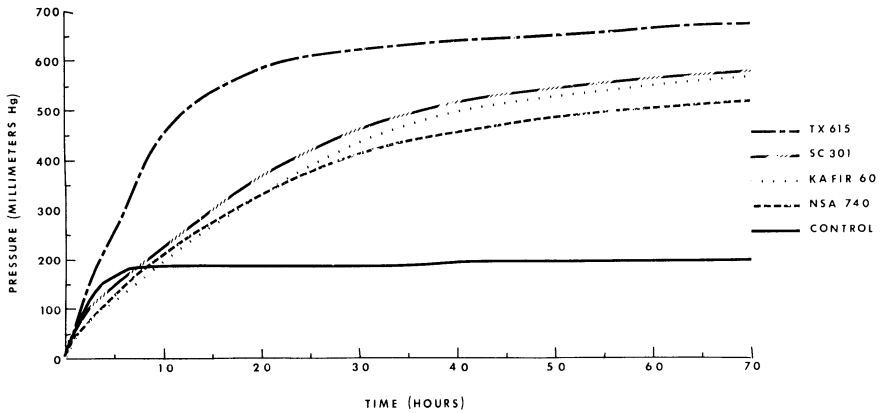


Fig. 4. Carbon dioxide gas production of ground whole grain subjected to amyloglucosidase and yeast for 70 hr. at 30°C. in manometers.

susceptibility of waxy starch accounts for the differences observed in the microscopic studies. Removal of the protein matrix during isolation of the starches should increase the rate of hydrolysis of the starch if the matrix prevents or retards hydrolysis. Waxy starch was more rapidly hydrolyzed to glucose than an equal quantity of starch in ground waxy grain. Lamar (unpublished data, Texas A&M University) found that waxy grain that had been pretreated with pronase to partially degrade the matrix was more susceptible to amyloglucosidase than the control sample of waxy grain that was not treated with pronase.

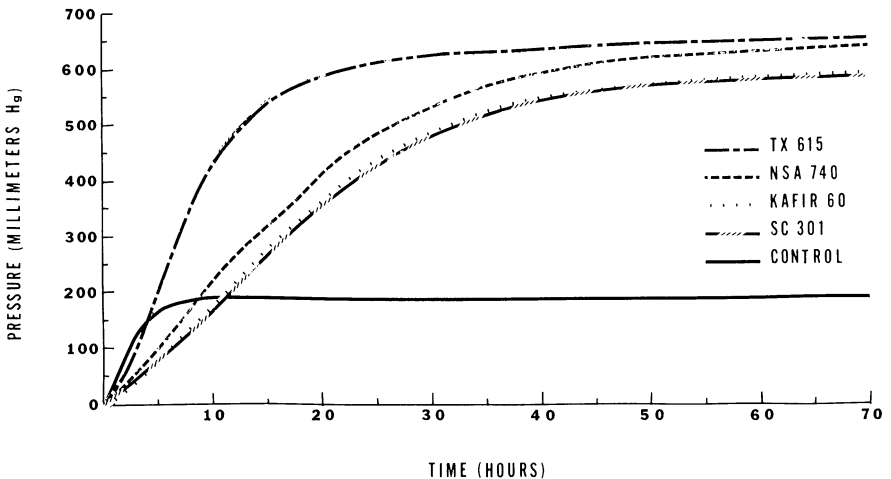


Fig. 5. Carbon dioxide gas production of isolated starches subjected to amyloglucosidase and yeast for 70 hr. at 30°C. in manometers.



We conclude that the improved feed efficiency of feedlot cattle fed waxy sorghum grain diets compared to nonwaxy grain diets is caused by: 1) the increased enzyme susceptibility of waxy starch; 2) the smaller proportion of peripheral endosperm in the waxy kernel; and 3) the increased susceptibility of the protein matrix of the waxy grain to solubilization. We believe that microscopic examination of kernel sections is a powerful tool for use in studying differences in digestibility and feedlot performance of sorghum grains.

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