THE INFLUENCE OF ENDOSPERM TYPE AND PROCESSING

ON THE CHARACTERISTICS OF CORN AND

SORGHUM GRAIN STARCH

Ву

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CHAPTER I

INTRODUCTION

Sorghum grain is an important crop in Oklahoma and is the major feed grain for finishing cattle in the Southwest. In the feedlot industry, feed grains commonly comprise 80 to 90% of the finishing ration. Cereal grains usually contain 70 to 80% starch; therefore, the efficient utilization of the starch portion of the ration is of major importance in the utilization of the entire ration.

Sorghum grain has long been considered a much more variable and lower quality feed grain than corn. Sorghum grain varieties have been genetically selected and bred for traits such as resistance of the open grain head to weathering, lodging, bird damage, insect attack and various other agronomic traits. Development of sorghum grain varieties suitable for a wide range of environmental conditions has resulted in sorghum grain being a much more variable product than other grains. It has also been shown that sorghum grains of various endosperm types differ in their feeding value for cattle.

Although sorghum grain and corn are rather similar in chemical composition, previous work indicates sorghum grain has a lower feeding value for cattle. Normally dry ground or rolled sorghum grain has only about 85 to 90% of the nutritive value of corn for finishing cattle.

It has been demonstrated that certain grain processing procedures, conducted under proper conditions, will increase the nutritive value of

sorghum grain for cattle. The digestibility of the starch, in particular, is increased by processing. It is imperative that grains be used more efficiently for livestock feeding in the future; the efficient utilization of sorghum by livestock is of major economic importance. However, the physical and chemical effects of grain processing on the grain components and their digestion in the animal are poorly understood. Additional improvements should be possible through a better understanding of the nature of processing effects and by greater emphasis on nutritive value in sorghum breeding.

The objectives of this study were to: 1) determine the starch characteristics of sorghum grain as compared to corn and the influence of sorghum endosperm type on them; 2) determine the effects of processing on corn and sorghum grain of various endosperm types; and 3) characterize the structural and chemical properties associated with increased starch availability.

CHAPTER II

LITERATURE REVIEW

Recent trends in the beef feeding industry have encouraged the extensive use of cereal grains in finishing rations. In the feedlot industry, cereal grains commonly comprise 80 to 90% of finishing rations. Because most cereal grains contain 70 to 80% starch (Rooney and Clark, 1968; Greenwood, 1970), the efficiency of utilization of the starch portion of the ration is of major importance in the utilization of the entire ration.

It is generally agreed that sorghum grain must be processed in some manner to obtain efficient utilization by feedlot cattle. This appears to be more true for sorghum grain than other grains.

Sorghum grain varieties have been genetically bred and selected for traits such as resistance of the open grain head to weathering, insect attack, bird damage, and lodging. Development of sorghum grain varieties suitable for a wide range of environmental conditions has resulted in sorghum grain being a much more variable product than other grains. Moreover, a lower feeding value for sorghum grain than suggested by its chemical composition compared to corn or barley (Pope et al., 1961; Totusek et al., 1963; Buchanon-Smith et al., 1968; Crampton and Harris, 1970). The lower feeding value of sorghum appears to be due, in part, to a reduced starch availability (Buchanon-Smith et al., 1968; Totusek et al., 1967). This probably explains why sorghum grain appears to be

improved more by processing than other grains.

Processing Methods

Heat Treatment

Grinding or dry rolling have been the conventional or traditional methods of sorghum grain processing over the years and much sorghum grain is still fed in this manner.

In recent years, steam flaking has proven beneficial for increasing the efficiency of sorghum grain utilization over grinding and rolling. Steam flaking is accomplished by subjecting the grain to steam at atmospheric pressure for 15 to 30 minutes or exposure for a short time under high pressure prior to rolling.

Totusek et al. (1967) reported improved feed efficiency with steam flaked milo compared to milo ground with a hammer mill using a 3/16 inch screen. Steam flaking resulted in a 3.5% improvement in feed efficiency as well as increased intake and faster rate of gain compared to the ground treatment. Martin and Wagner (1974) reported feed efficiencies of 5.77 and 6.82 pounds of feed per pound of gain for steam flaked and dry rolled sorghum grain, respectively. No differences were observed in rate of gain, however, average daily feed consumption was 19.2 and 16.2 pounds for dry rolled and steam flaked, respectively.

Husted et al. (1968) compared the digestibility of steam flaked and dry rolled milo in ration containing 77% grain. Dry matter digestibility was 65.2% and 74.4% for dry rolled and steam flaked grain, respectively in the first trial. Digestibilities were 68.8% and 76.3% for dry rolled and steam flaked, respectively in a second trial. The estimated

total digestible nutrients of the steam flaked milo was 89.9% as compared to 72.1% for dry rolled milo.

Buchanon-Smith et al. (1968) also observed increased dry matter digestibility of steam flaked sorghum grain compared to ground sorghum in a ration containing 78.3% grain.

White (1964) showed in a summary of 7 trials comparing steam flaked and ground milo that steam flaked milo increased rate of gain 10% and feed efficiency 8% compared to dry coarsely ground milo.

In an Arizona study, Hale et al. (1966) compared dry rolled and steam flaked milo in a 77% milo ration. An increase in daily gain of .12 kg was observed. Feed intake increased by an average of .45 kg per steer daily due to steam flaking. Feed efficiency was improved by 4.8% by steam flaking. The improvement in feed efficiency from steam flaking paralleled the increase in nitrogen free extract digestibility of the steam flaked ration.

Osman et al. (1970) reported an <u>in vitro</u> study comparing steam flaked to untreated sorghum grain in which starch digestibility approximately tripled when grain was flaked to a thin high quality flake.

In a feeding trial consisting of five processing methods, Franks et al. (1972) observed increased rate of gain, feed intake and feed efficiency for steam flaked compared to coarsely ground sorghum grain. Finely ground and reconstituted treatments, although similar in rates of gain, were superior in feed per unit gain.

Findings of Newsom et al. (1968) were very similar except that the finely ground treatment was not as efficiently utilized as was the steam flaked sorghum grain. Brethour and Duitsman (1969) also found steam flaked superior to dry rolled sorghum grain in average daily gain and

feed efficiency. Steam flaking of sorghum grain also proved superior to coarse grinding in rate of gain, feed consumption and efficiency of utilization in a study by Totusek et al. (1967).

Summarizing several Texas studies Riggs (1971) noted more efficient utilization of energy in steam flaked and reconstituted grain than in dry ground grain sorghum.

More recently, dry heat treatment or micronization has also been shown to enhance the starch availability and feeding value of sorghum grain. Micronization is usually accomplished by passing the cleaned grain over a reciprocating steel table. The grain is heated by infrared generators suspended above the table.

Croka and Wagner (1975) reported micronized milo to be more efficient than dry rolled milo requiring .65 and .75 pounds less feed per pound of gain in two trials. These reductions in feed per unit gain correspond to 11.1 and 9.8% improvements in feed efficiency (total ration) for the micronized milo over the dry rolled milo in trials 1 and 2, respectively. Assuming that the improvements in feed utilization can be attributed to the processing of the milo fraction of the ration (80% in both rations), the corresponding improvement would be 13.9 and 12.2% for the micronized grain in trials 1 and 2, respectively. In another trial using three densities of micronized milo obtained by varying the exposure and intensity of heat in the micronizing apparatus, treatment differences for feed intake, gain or feed efficiency were not significant. Nevertheless, certain trends existed. All three micronized treatments produced gains slightly higher than dry rolled milo. Feed efficiency favored the micronized treatments over dry rolled milo, with little difference among the three densities of micronized milo.

Micronized treatments tended to decrease feed consumption compared to dry rolled milo. Generally, there was a consistent trend in all trials for reduced intakes with similar gains and an improved feed/gain ratio with micronized milo compared to dry rolled milo.

Hinman and Johnson (1973) compared dry rolled and micronized sorghum grain by <u>in vitro</u> and digestibility trials. There were no significant differences in the amount of starch digested in the rumen. A lower total tract digestion of starch was observed with the dry rolled treatment. Twelve hour <u>in vitro</u> dry matter disappearance was increased by the micronization of sorghum grain.

Shake et al. (1970) evaluated micronized and steam flaked sorghum grain under commercial feedlot conditions in the Texas Panhandle. Steers fed micronized grain went on feed more rapidly and feed intake was slightly higher than steers fed steam flaked grain. Although feedlot performance tended to favor the cattle fed micronized grain, those fed steam flaked grain were slightly more efficient in feed conversion. Starch gelatinization values were higher for steam flaked compared to micronized treatments.

Gelatinization of the starch occurs during heat treatment via steam flaking or micronization and appears to be increased by rolling of the heated grain. Gelatinization, although not uniformly defined, has been described as hydration or rupturing of starch granules (Smith, 1959). It has been further described as complete rupturing of the starch granule resulting from pressure, heat, moisture, and mechanical shear or strain (Anstaett et al., 1969). When an aqueous suspension of starch is heated, reversible swelling of the starch granule occurs until the gelatinization temperature is reached. When the gelatinization temperature is

reached, however, the micellular structure of the starch granule is weakened, resulting in an irreversible loss of crystalline structure. The internal structure of the granule is disrupted and it loses its birefringence.

Gelatinization temperature varies with the type of starch and degree of molecular association. In general, the gelatinization temperatures are higher for grain sorghum than corn (Leach, 1965). Starch is corn generally gelatinizes between 60 and 72° C; grain sorghum generally gelatinizes between 68.5 and 75° C. It must be remembered that gelatinization does not occur at a specific temperature, but occurs in a range of 8 to 10° C from initiation to total gelatinization (Schock and Maynald, 1956). Moreover, the swelling powers vary with the type of starch as evidenced by the fact that waxy sorghum starch has two to three times the swelling properties of ordinary (non-waxy) sorghum starch as measured in Brabender units (Horan and Heider, 1946).

Gelatinization of starch has been measured by various methods, including microscopic structure, congo red staining, alpha-amylase digestion, and susceptability to beta-amylase attack.

Gelatinized sorghum starch is more susceptable to enzymatic attack than raw starch (Anstaett et al., 1969; Leach and Schock, 1961; Hale, 1973). Steam flaking has been shown to increase digestibility of the NFE fraction in grain sorghum (Buchanon-Smith et al., 1968; Husted et al., 1968; Hale et al., 1966). More recently Hinman and Johnson (1973) demonstrated an increase in ruminal starch digestion and ever larger increase in post-ruminal starch digestion when sorghum grain was micronized. Possibly ruminants have a much more limited enzymatic capability for digestion of large quantities of starch in the intestinal tract than do non-ruminants. Thus, processing of grains, particularly those in which starch availability may be low, may be more beneficial to ruminant than non-ruminant animals. Moreover, in grains where starch availability is low, processing may produce more improvement than in grains where starch availability is better.

High Moisture Processing

High moisture processing has been used during the past few years to enhance the nutritive value of sorghum grain for cattle. High moisture processing includes both high moisture harvesting and reconstitution. High moisture harvesting involves early removal of the grain from the field while the grain still contains a high level of moisture followed by storage of the grain under anerobic conditions for a period of time prior to feeding. Sorghum grain appears to be physiologically mature at a moisture content of approximately 38-40% or less in that the total dry matter deposition in the kernel does not increase beyond this point. It is not known why high moisture harvested sorghum has a higher feeding value, but the nutrients stored in the kernel may be present in a different chemical and/or physical form than in the dry grain. Presumably chemical and physical differences exist in the endosperm making the starch and other nutrients more available, enhancing digestibility and nutritive value. Reconstitution is accomplished by adding water to air dry grain to obtain a moisture content of 30% or more. The high moisture product is then stored 20 days or more under anerobic conditions until fed.

Although published results have been rather variable, both high moisture harvested and reconstituted grain sorghum have shown improve-

ments in feed efficiency.

McGinty et al. (1966) observed increased digestibility for all components of reconstituted sorghum significantly higher than dry rolled sorghum grain. This improvement amounted to approximately 20% for all components except protein. Digestibility of protein in the reconstituted grain was 16% higher than dry grain. Parrett et al. (1966) noted an average increase of 11% in feed efficiency for reconstituted and high moisture harvested over dry rolled sorghum grain. The reconstituted treatment was utilized slightly more efficiently than was the high moisture harvested.

Totusek et al. (1967) found reconstituted-rolled sorghum grain improved feed efficiency with no sacrifice in rate of gain, accompanied by a decreased grain intake, compared to coarse grinding. Newsom et al. (1968) also observe equal gains, decreased feed consumption and increased feed efficiency with reconstituted-rolled compared to coarsely ground sorghum grain. Reconstituted-rolled was approximately 14% more efficiently utilized that coarsely ground sorghum grain. Buchanon-Smith et al. (1968) observed digestibilities of dry matter and energy greater in diets containing reconstituted grain compared to finely ground or coarsely ground sorghum grain.

In a Texas study, Schake et al. (1969) found reconstituted milo yielded both slightly increased gain and increased feed efficiency with reconstituted milo compared to steam flaked milo. In a series of seven feeding trials Riggs and McGinty (1970), compared high moisture harvested and reconstituted with dry ground sorghum grain. Cattle fed the moist grains required 7 to 15% less total dry matter per unit gain (average of 11% over all trials) than did cattle fed dry ground grain.

High moisture harvested grain was usually slightly more efficiently utilized than reconstituted grain. Reconstituting the grain to 25 to 30% moistured followed by storage for at least 21 days and grinding prior to feeding increased the digestibility of protein 16 to 22% and dry matter 17 to 29%.

Franks et al. (1972) found that reconstituted sorghum grain produced similar gains with coarsely and finely ground sorghum grain. Reconstituted and finely ground grain were similar in feed per unit gain and both treatments were superior to steam flaked and coarsely ground sorghum grain.

Much work has been done on the physical form of the grain in the reconstituting process. Brethour and Duitsman (1962, 1963, 1964, 1970) ground sorghum grain before reconstituting and observed similar gains with dry ground grain. The moist grains produced very similar increases in feed utilization. In their 1962 study, reconstituted grain that was ground before storage produced increased gains and better feed efficiencies than reconstituted whole and then ground grain.

Other researchers (Penic et al., 1968; White te al., 1969; Sullins and Rooney, 1971; Riggs, 1971; Neuhaus and Totusek, 1971), indicate that sorghum grain must be stored in the whole kernel from rather than the ground form during reconstitution to obtain an improvement in feed utilization. Moreover, it appears that reconstituted sorghum grain should be stored at least 10, and preferably 20 days (Pantin et al., 1969; Neuhaus and Totusek, 1971; Riggs, 1971; Schneider, 1971) and contain approximately 30% moisture or more during storage (White and Totusek, 1969; Neuhaus and Totusek, 1971; Riggs, 1971). The reason reconstituted sorghum must be stored in the whole form to obtain an im-

provement in feeding value is not well understood, but may be related to the endogenous production of enzymes within the kernel. A connection appears to exist between the embryo and aleurone portions of the seed for the production of an amylase during seed germination (van Overbeek, 1966). Perhaps somewhat similar enzymatic activity occurs during the reconstitution process which would explain the need for maintaining an intact kernel. In the malting of barley, the embibed embryo releases gibberellin-like hormones which migrate to the aleurone layer stimulating the release of hydrolytic enzymes, including amylolytic enzymes for starch solubilization and proteases for protein hydrolysis (Luchsinger, 1966). Sullins and Rooney (1971) observed evidence of an alteration in the structure of the endosperm in reconstituted grain facilitating the release of more free starch granules and protein bodies due to an apparent weakening of the proteinoceous matrix surrounding the starch granules. The alterations observed may be due to enzymatic hydrolysis during storage of the high moisture grains. Similarities are likely to exist in the nature of the endosperm and the carbohydrates and protein fractions, in particular, in reconstituted and high moisture harvested sorghum grain.

Structure and Importance of Starch

Cereal grains usually comprise 80 to 90% of cattle finishing rations and account for the major expense of such rations. Methods to improve the efficiency of utilization of these grains are, therefore, of considerable importance. Cereal grains usually contain 70 to 80% starch (Rooney and Clark, 1968; Greenwood, 1970). Most of the starch of cereal grains is located in the endosperm as small granules embedded in a

proteinaceous matrix (Greenwood, 1970). The endosperm of the sorghum kernel is composed of an aleurone layer and the peripheral, corneous, and floury endosperm portions. The peripheral endosperm is located beneath the aleurone layer and consists of a layer of several cells thick which is distinguishable from the remaining endosperm because the cells are small and contain very small starch granules which are enmeshed in a thick protein matrix. The thickness of the peripheral endosperm is influenced mainly by variety and to some extent environment. The floury endosperm is located in the center of the kernel and is surrounded by the horny or corneous endosperm (Rooney and Clark, 1968).

The storage of starch in the granule form serves to make it less soluble in water (Greenwood, 1970). The starch granule is composed of linear and branched chain starch molecules associated by hydrogen bonding to form radially oriented micelles or crystalline areas. The overall strength of the micellar network is dependent upon the degree of association and molecular arrangement (Leach, 1965). All starches, when grown under natural conditions show a layered or shell structure (Badenhuizen, 1965). The size, shape and striations of the granules are characteristic of the type and variety of starch (Greenwood, 1970).

Starch granules in cereal grains usually contain a mixture of two polysaccharides. Amylose is a linear polymer of glucose units joined by alpha-(1+4) linkages to yield chains of several hundred glucose units. Amylopectin is a branched chain glucose polymer with alpha-(1+4) and alpha-(1+6) linkages. Branch points are located at the alpha-(1+6) bonds and each branch normally contains 20 to 30 glucose units (Pazur, 1965). The relative ratio of these fractions vary with the type of starch. Most cereal starches contain 25 to 30% amylose and 75 to 80%

amylopectin (Rooney and Clark, 1968), although some starches contain nearly 100% amylopectin. Variations of this magnitude can be found in sorghum grains of different types (waxy types) and varieties.

Enzymatic Degradation of Starch

The complete in vitro degradation of starch is generally accomplished with the enzymes alpha-amylase, beta-amylase, and glucoamylase. Alpha-amylase randomly hydrolyzes alpha- $(1\rightarrow4)$ glucan linkages of the starch molecule yielding dextrins, maltose and glucose. Beta-amylase hydrolyzes alpha- $(1\rightarrow4)$ glucan links in polysaccharides so as to remove successive maltose units from the non-reducing ends of the chains (Herp et al., 1970). Glucoamylase or amyloglucosidase hydrolyzes alpha- $(1\rightarrow6)$, alpha- $(1\rightarrow4)$ and alpha- $(1\rightarrow3)$ linkages. The enzyme hydrolyzes starch, amylose, amylopectin, and dextrins to glucose from the non-reducing end of the molecule (Nisizawa and Hashimoto, 1970).

In Vitro Systems

<u>In vitro</u> techniques have been employed extensively in the past to evaluate forages and their relative nutritive values. The extent of <u>in</u> <u>vitro</u> techniques as a tool for measuring relative energy availability for concentrates has not been as great, but Kumeno et al. (1967), Albin et al. (1966), Klett (1967) and Schneider (1971) agree that their use is helpful in searching for feeds which would be utilized more efficiently.

Kumeno et al. (1967) used an <u>in vitro</u> technique for estimating the nutritive value or digestibility of high energy mixed rations, dry matter disappearance was used as the criterion. The values obtained after a 48 hr fermentation were highly correlated with the estimated

energy digestibilities of a wide range of mixtures of orchardgrass or alfalfa with ground corn. Dry matter disappearance in vitro at 48 hours was correlated (r = .85) with dry matter digestibility in vivo. Correlations of dry matter disappearance with total acid production resulted in r values of approximately .90.

Albin et al. (1966) used the <u>in vitro</u> techniques to study digestibilities of rations and their relationship to feedlot performance of steers fed all-concentrate rations. His technique involved the use of both whole rumen fluid and resuspended bacterial cells as the inoculum. Samples were incubated in 50 ml test tubes at 39° C for 24 hours. The criteria used for detecting differences in fermentation rates were digestion of dry matter, ether extract, starch, and gross energy. When compared to field trials, significant correlations were not found within each <u>in vitro</u> period, but the most consistent correlation coefficients were between per cent digestible dry matter <u>in vitro</u> and daily feedlot gain (r = .88); and per cent digestible dry matter <u>in vitro</u> and feedlot efficiency of feed utilization (r = .99).

Neuhaus (1968) and Christiansen (1973) demonstrated that a definite relationship existed between <u>in vitro</u> fermentation digestibilities of processed grains and feed efficiency of feedlot cattle receiving the same grains in high concentrate rations, although no correlations were calculated due to lack of numbers.

Trei et al. (1969) described an <u>in vitro</u> system employing gas production by rumen microorganisms to evaluate processed grains. A mixed 'suspension of rumen microorganisms was used as the source of inoculum and calibrated manometric tubes were used to measure gas production. High correlations were found between gas production and dry matter dis-

appearance (r = .95), volatile fatty acid production and $\underline{in vitro}$ starch digestion.

Although <u>in vitro</u> systems do not provide a measure of all the parameters of importance in studying processed grains for feedlot cattle, they can be extremely useful in 1) determining processing effects on large numbers of grain samples in a relatively short period of time and 2) providing estimates of nutrient availability in variously processed grains.

Variations in Nutritive Value of Different Varieties of Sorghum Grain

Until the present time the primary method of increasing the efficiency of grain sorghum utilization by cattle has been through various processing techniques. While these techniques all bring about some alteration of the physical and/or chemical properties of the grain and are generally effective in improving its utilization, they provide only an immediate solution to an existing problem rather than an attack on the problem of changing some characteristics of the grain so the improvement in efficiency of utilization may become a permanent characteristic of the grain.

Many researchers (Brethour and Duitsman, 1965; Breuer, 1966; Breuer et al., 1967; Nishimuta et al., 1969; McCollough et al., 1970, 1972a,b,c,d; Samford et al., 1970, 1971; Maxon et al., 1973) have observed differences in feeding value and efficiency of utilization due to endosperm types and varieties. <u>In vitro</u> techniques have also shown differences between endosperm types and varieties (Saba et al., 1972; Davis and Harbers, 1974). In general, waxy types (nearly 100% amylo-

pectin) and yellow endosperm types produced superior feed efficiencies. Bird resistant or corneous types were consistently inferior in all studies.

Samford et al. (1970), in studying ruminal digestibilities sorghum grains of floury, normal, corneous and waxy endosperm types found floury significantly more digestible than all other types. The digestibility of the floury endosperm type was considerably higher than that of ground yellow corn.

These results indicate the wide variation in digestibilities of sorghum grain. The fact that some are higher in digestibility than corn leads to the conclusion that the past acceptance of sorghum grain as being somewhat inferior to corn in feeding value was based on average values for common sorghum varieties. Sorghums of distinctly superior feeding value probably exist.

Laboratory wet milling techniques have also shown distinct differences in varieties (Watson et al., 1955; Watson and Hirata, 1955). Different types and varieties of sorghum grain exhibited differences in solubles yield, gluten yield and protein content of the finished starch.

It is not known whether sorghums of different endosperm types would display similar improvements in starch availability from the various processing methods. Possibly sorghum endosperm types which are less digestible in the raw state would be improved to a great extent by the different processing methods. Limited evidence by Hinders and Freeman (1969) and Saba et al. (1972) suggests that different sorghum endosperm types respond differently to processing. In the majority of data reported to date, the variety or type of sorghum investigated was not reported, in most cases, probably unknown.

Although it is known that processing can improve nutrient utilization and the changes occurring during processing are not well understood. Published information on the results or processing have been quite variable, due likely to variations in the conditions or procedures during processing, type of rations fed, type of sorghum and the analytical techniques employed. There is a need to characterize and define the chemical and physical changes associated with improved nutrient utilization in sorghum processed in various ways. Moreover, there is a need to define the relationship between sorghum grain types and processing effects. Undoubtedly, significant improvements in the nutritive value of sorghum could also be obtained through selection and plant breeding efforts. Most previous efforts along this line have been devoted largely in obtaining desirable agronomic characteristics in the sorghum plant with only limited regard for feeding value.

CHAPTER III

MATERIALS AND METHODS

Five sorghum grains of various endosperm types were planted in early June, 1973 under similar conditions of fertilization, planting and harvesting under dryland conditions at the Perkins Agronomy Research Station. Two hybrid corns were planted under similar conditions of fertilization, planting and harvesting on irrigated land at the Panhandle Agronomy Research Station. Descriptive characteristics and classification of the varieties are given in Table I. The grains were grouped in this manner because of the large number of varieties available today, and varieties of a common endosperm type should be more alike than varieties of different endosperm types. Grouping in this manner may allow for expanded application of the results to many varieties if endosperm type is known.

Eight sorghum grains of various endosperm types were planted in early June, 1974 under similar conditions of fertilization, planting and harvesting under dryland conditions at the Perkins Agronomy Research Station. Two hybrid corns were planted under similar conditions of fertilization, planting and harvesting on irrigated land at the Panhandle Agronomy Research Station. Descriptive characteristics and classification of the varieties are given in Table II.

Of the varieties in the 1973 drop year produced at the Perkins Station, the Darset variety failed to germinate and had to be replanted

TABLE I

DESCRIPTIVE CHARACTERISTICS AND CLASSIFICATION OF THE 1973 CROP GRAINS

	Seed		Endosperm		
Variety	Coat Color	Color	Hardness	Waxy or Normal	Classification
Pioneer Corn 3149	Colorless	Yellow corn	Yellow dent corn	Normal	> Corn
Pioneer Corn 3306	Colorless	Yellow corn	Yellow dent corn	Normal	corn
Darset (Bird Resistant)	Brown	White	Intermediate	Normal	White-BR
Soft Endo	Brown	White	Soft	Normal 🔨	> White-normal
Redlan Normal	Red	White	Intermediate	Normal	white-normal
OK 612	Red	Hetero-yellow	Intermediate	Normal	Hetero-yellow
Dwarf Redlan	Red	White	Intermediate	Waxy	Waxy

TABLE II

DESCRIPTIVE CHARACTERISTICS AND CLASSIFICATION OF THE 1974 CROP GRAINS

	Seed				ang na ang na pang mang na pang
Variety	Coat Color	Color	Hardness	Waxy or Normal	Classification
Pioneer Corn	Colorless	Yellow corn	Yellow dent corn	Normal	Corn
Northrupt King Corn	Colorless	Yellow corn	Yellow dent corn	Normal	COLI
Darset (Bird Resistant)	Brown	White	Intermediate	Normal	White-BR
Soft Endo	Brown	White	Soft	Normal	White-Normal
Redlan Normal	Red	White	Intermediate	Normal	
OK 612	Red	Hetero-yellow	Intermediate	Normal	Hetero-Yellow
Dwarf Redlan	Red	White	Intermediate	Waxy	
73BCT 1126	White	Yellow	Intermediate	Waxy	
73BCT 1133-2	Brown	Yellow	Intermediate	Waxy	/ Waxy
733CT 1122-2	Red	Yellow	Intermediage	Waxy	

and was later to mature in a different plot at the station. Because of limited rainfall (1" below normal) during the growing season (June through September), the 1973 sorghum varieties grew under moisture stress.

In 1974 at the Perkins Station, the season was more favorable in that rainfall was 1.26" above normal for the growing season. Thus, stands were established in all varieties at the first planting and all varieties were harvested under better conditions.

Grain Processing Methods

All varieties in each crop year were processed for laboratory evaluation as untreated (finely ground through 20 mesh screen), micronized and reconstituted grain.

Untreated

Untreated grain of each variety was used as a control in evaluating the two different processing methods. Untreated grain was prepared for laboratory evaluations by grinding it through a laboratory Wiley mill equipped with a 20 mesh screen.

Reconstituted

In preparing the reconstituted grain, approximately 300g of each cleaned variety of grain was reconstituted to 30% moisture in the whole kernel form and stored 21 days at room temperature under anaerobic conditions in plastic jars. Reconstitution vessels were flooded with CO 2 prior to sealing. Following the 21 day reconstitution period, the grain was ground through a laboratory Wiley Mill using a 20 mesh screen. Dry

ice was used to facilitate grinding of the moist grain.

Micronization

In preparing the micronized grain, cleaned grain was heated by gas fired infrared generators as it passed along a reciprocating steel table. As the grain came off of the table it was passed through rollers to produce a flaked appearing product. Heating time and temperature were adjusted to produce a final product density of approximately 335g/ liter. Processed samples were then ground through a 20 mesh screen in a laboratory Wiley mill.

Purified, Isolated Starch

Purified, isolated starch was obtained from each whole, unprocessed grain using a modification of the laboratory wet milling procedure of Norris and Rooney (1970). The wet milling procedure was done in triplicate on each variety in the 1973 crop and in duplicate on the 1974 crop.

A 300g quantity of air dried grain was subjected to a two-phase steeping. Phase I consisted of steeping the grain in 2 ℓ beakers 40 hr at 50° C in 1.5 ℓ of a solution containing 0.05% SO₂ and 1.5% lactic acid. Phase I solution was replaced by Phase II solution which contained 0.10%SO₂ and 0.5% lactic acid. The grain was then steeped 8 hr at 50° C in the Phase II solution. Steep solutions are shown in Appendix Table XVI. At the end of the 8 hrs the Phase II steep was drained and the grain was refrigerated overnight and warmed to room temperature prior to milling.

The function of steeping is to soften the protein matrix and endosperm cell walls for easy rupture to release the starch during milling. If the protein matrix is not thoroughly softened, starch granules may not be released. Sulfur dioxide was first used in steeps to prevent the growth of putrefactive organisms. The SO₂ is necessary for maximum starch yields in that, it causes the protein matrix to swell, become globular and finally disperse to yield loosely held starch granules that are easily released in the wet milling process. The lactic acid is used to prevent the growth of undesirable organisms by the lowered pH which also stimulates the growth of lactic acid producing bacteria. Lactic acid also aids in softening of the cell walls (Watson, 1967).

The decanted steep solutions were evaporated to approximately 50 ml using a rotary flash evaporator. Evaporated samples were then lypholyzed to obtain an estimate of the dry weight of steep solubles.

Steeped grain was then ground in 2 parts in approximately 375 ml of distilled water in a Waring Blender. The blades of the blender were reversed and the speed was controlled by a voltage regulator set at 85 volts to minimize starch granule damage. Corn samples were ground for 1 minute and sorghum samples were ground l_{2}^{1} minutes to free the starch granules.

The slurry was then sieved over U.S. number 80, 230, and 325 mesh sieves. Distilled water in a wash bottle was used to wash starch and gluten from the material remaining on the sieves.

The material on the No. 80 sieve was primarily bran and germ. The No. 230 sieve, with openings of $63 \ge 63\mu$, retained mainly peripheral endosperm cells and the No. 325 sieve (44 \ge 44 μ) retained primarily coarse gluten particles (Watson, 1955).

The slurry of starch and gluten (approximately 6%) passing through the sieves was separated on a starch table. In tabling, the denser starch settles on the table while the lighter gluten flows off the end

of the table. The aluminum trough-like table was 10.2 cm wide and 266.7 cm long. Pitch of the table was 2.54 cm for its entire length. The starch was washed with distilled water while it was on the table, let dry to a solid cake, removed and dried in a 40° C oven for 24 hr to approximately 7% moisture. The starch was then weighed and placed in storage bottles.

The water and gluten that washed off the table was centrifuged at 2500 rpm for 15 min to facilitate collection of the gluten fraction. Bran and germ, peripheral endosperm cells and combined gluten fractions were dried at 100° C for 24 hr before being weighed and placed in sample bottles for storage. Yields of starch, bran and germ, peripheral endosperm cells and combined gluten were calculated as % of initial weight of the grain sample on a 100% dry matter basis.

Evaluation of Processed Grains

In Vitro Dry Matter Disappearance

In vitro dry matter disappearance of the processed grains (untreated, micronized and reconstituted) was determined by modified procedures of Schneider (1971). The basic technique is given in Table III.

TABLE III

IN VITRO TECHNIQUE

Element	Level
Grain Dry Matter	0.4g
Artificial Saliva	22.0 ml
Rumen Inoculum	8.0 ml
Temperature Time of Incubation	39 ⁰ C 6 hr, 24 hr.

Prior to inoculation with fermentation media, all grain samples, regardless of processing treatment, were prepared in the following manner:

Sufficient quantities of grain samples were weighed to obtain
 4g of sample dry matter.

2) Grain samples were weighed to the nearest milligram into dried, preweighed numbered 50 ml centrifuge tubes.

The artificial saliva used is given in Table IV.

TABLE IV

COMPOSITION OF ARTIFICIAL SALIVA

Ingredient	g/liter of Distilled Water
NaHCO ₃	9.8
NaHPO4 · 12 H2O	9.3
KCl	0.57
NaCl	0.47
MgSO ₄ ·7 H ₂ O	0.12
CaCl ₂	0.04

Two liter quantities of the artificial saliva were mixed and warmed to 39° C prior to mixing with rumen inoculum.

While the artificial saliva was being warmed, a quantity of rumen fluid was recovered from a fistulated steer on a ration of 84% grain.

The rumen samples were obtained by using a vacuum pump and placed into a warmed thermos jug and immediately taken to the laboratory where it was filtered through four and then six layers of cheesecloth. This was 'accomplished as quickly as possible to minimize bacterial loss. Seven hundred twenty-seven milliliters of the rumen inoculum were then mixed with the 2 ℓ of warmed artificial saliva and CO₂ was bubbled through the mixed media until all sample tubes were inoculated. Temperature of the media was maintained at 39° C and solids kept in suspension by a heated magnetic stir plate. The substrate containing tubes were suspended in a pulsating water bath which was thermostatically controlled at 39 $^{
m O}$ C and 30 ml of the buffered inoculum was added to each tube. Following inoculation, the unfilled portion of each tube was outgased with CO2 and stopped with a #6 stopper. All stoppers had a 2 mm hole drilled through them to allow gas to escape. The samples were incubated for 6 and 24 Three preweighed tubes containing 30 ml of the buffered inoculum hr. only were incubated at the same times to obtain an average of dry matter constituents after fermentation not attributable to the grain samples. Following incubation, both the "blanks" and grain containing tubes were centrifuged at 2500 rpm for 15 min. Supernatant solution was decanted off and tubes were placed in a drying oven at 80° C for 48 hours. The tubes containing undigested dry matter were removed from the oven and cooled in dessicators and again weighed.

Percent dry matter disappearance was calculated by dividing dry undigested grain weight by the original grain sample dry weight, to obtain % feed remaining. Disappearance or % digested was obtained by subtracting this value from 100.

In Vitro Gas Production

<u>In vitro</u> gas production of the same processed grains was measured hourly for 6 hr and at 12 hours. Procedures used were those of Galyean (1975). Samples of 0.4g of dry matter of the ground grains were incubated in a 39[°] C water bath with 10 ml of a 0.1% amyloglucosidase solution and .25g of commercial bakers yeast. Gas production was measured in an inverted buret recovery system. Gas production is reported as milliliters of gas produced per gram of sample dry matter.

Evaluations of Isolated Starch

In Vitro Dry Matter Disappearance

In vitro dry matter disappearance of isolated starch from each variety was determined at 6 and 24 hr incubation periods using a slightly modified version of the previous procedure. In vitros were conducted using 0.2g of starch dry matter to approximate equivalent starch content in the fermentation tubes. Urea was added at the rate of 20 mg per tube to facilitate approximately equal nitrogen levels as that of the processed grains. After incubation, tubes were filtered instead of centrifuged to minimize sample loss by decanting after centrifugation. The contents of the tubes were transferred quantitatively to weighed No. 541 filter paper in a buchner funnel and filtered by vacuum. Filter paper and its contents were dried at 100° C for 24 hours.

In Vitro Gas Production

In vitro gas production of raw isolated starch was measured with 0.2g of starch dry matter. The 0.2g quantity was used to approximate

equal starch concentration in the digestion media. Procedures were those of the previously described technique.

In Vitro Enzymatic Evaluations of Starch

In vitro enzymatic digestion was performed on purified isolated starch samples of each variety to evaluate susceptability of the starches to alpha-amylase enzymatic attack. The enzyme used was crystallized <u>B</u>. <u>Subtilis</u> alpha-amylase (($1 \div 4$)-alpha-D-glucan 4 glucanohydrolase)3.2.1.1. Starch samples were prepared for evaluation by two methods; soluble starch and raw, isolated starch.

Alpha-Amylase Digestion of Soluble Starch. When raw, isolated starch is solubilized, the soluble component contains the amylose component or fraction of starch (Langlois and Wagoner, 1967). Raw, isolated starch obtained by wet milling was weighed into 150 ml beakers in 100 mg quantities (except for the Dwarf Redlan variety of which 500 mg was used in the 1973 crop.) One hundred milligrams was used with all varieties in the 1974 crop except Dwarf Redlan, 1126, 1122-2 and 1133-2 of which 800 mg was used. One hundred milliliters of .01 M acetate buffer (pH 6.0) was added and the mixing solution was brought to boil on a heated magnetic stir plate. After the suspension came to boil it was centrifuged in 50 ml centrifuge tubes at 2500 rpm for 15 min. An insoluble opalescent pellet was formed in the bottom of the tube and the solubilized amylose cr soluble starch was in the supernatant. The supernatant was decanted into clean 150 ml beakers and refrigerated overnight. One milliliter of an iodine solution was added to triplicate tubes containing 1.0 ml of the starch solution and distilled water was added to a total volume of 8.0 ml. Iodine solution preparation is shown in Appendix Table XVII. A spectrophotometer was used to determine the absorbance at 620 nm.

Amylose or soluble starch content of the samples was calculated by extrapolating from a standard curve. A standard curve was prepared daily using 100 mg of Soluble Starch (according to Litner) solubilized in the same manner as the sorghum samples. Aliquots of 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 and 1.0 ml in triplicate tubes were used to establish the curve. The volume of each tube was brought to 1.0 ml with acetate buffer. Blanks were prepared with 1.0 ml of acetate buffer. One milliliter of iodine solution and 6.0 ml of distilled water were added to each tube, mixed well, and absorbance determined at 620 nm on a light spectrophotometer. Absorbance was plotted against milligrams of soluble starch or amylose per milliliter by least squares regression.

All samples were then diluted to an amylose content equal to the lowest sample soluble starch value with acetate buffer. One milliliter of adjusted soluble starch of each variety was added to 6 tubes. Three tubes were used as controls and three tubes were incubated with 100 μ 1 of <u>B</u>. <u>Subtilis</u> alpha-amylase (0.5 mg/ml of acetate buffer) for 5 min in a 30[°] C circulating water bath. After incubation all tubes were subjected to the Nelsons test. Reducing carbohydrate content was obtained by extrapolating absorbance values from a glucose standard curve. The amount of glucose equivalents liberated by alpha-amylase was calculated by subtracting the values of control tubes from the enzyme digested tubes.

The glucose standard curve was prepared with a .05 mM glucose solution. Aliquots of 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, 1.6, 1.8 and 2.0 ml were used to establish the curve. The volume of the first 4 aliquots

was brought to 1.0 ml with acetate buffer. Blanks were prepared with 1.0 ml of acetate buffer. The Nelsons reagent was prepared fresh daily by mixing 50 ml of reagent A with 2.0 ml of reagent B. One milliliter of the Nelsons reagent was added to each tube and marbles were placed on the tops of the tubes and boiled in a steam bath for 20 min. The tubes were then cooled in tap water and 1.0 ml of arsenomolybdate reagent was added to each tube, mixed, and allowed to stand a few minutes. Distilled water was then added to a final volume of 10 ml, mixed well, and absorbance determined at 510 nm. Absorbance values at 510 nm were plotted against micromoles of glucose added by least squares regression. Reagents of the Nelsons test are shown in the Appendix (Table XVIII).

Alpha-Amylase Digestion of Raw, Isolated Starch. The second method of enzymatic digestion was conducted with whole raw starch (as contrasted to just the soluble portion) obtained by the wet milling procedure. Isolated starch was weighed into 125 ml erlenmeyer flasks in 50, 100, 200, 400, 800 and 1200 mg quantities in duplicate in the 1973 crop and 100, 400, 800 and 1200 mg quantities in the 1974 crop. Fifty milliliters of acetate buffer was added to each flask. One flask served as a control while the other had 100 μ l of alpha-amylase solution (5 mg/ml acetate buffer). All flasks were incubated 1.5 hr at 39^o C on a gyroshaker.

After incubation, a 10 ml aliquot was filtered by vacuum through a Millipore apparatus equipped with Watman #50 filter paper. One milliliter of the filtered solution was added to three test tubes and the Nelsons test for reducing sugars was performed on all tubes and the amount of glucose equivalent liberated was calculated as described pre-

viously.

Amylose-Amylopectin Ratios of Isolated Starch

Amylose-amylopectin ratios of the isolated starches were determined by modifications of procedures of McCready and Hassid (1943). One hundred milligrams of dry powdered sample was introduced into 100 ml volumetric flasks, wetted with 1.0 ml ethanol and 10 ml of distilled water. The sample was dissolved by adding 2.0 ml of 10% NaOH and heating on a steam bath, shaking occasionally, until a clear solution was formed (about 1.5 hr.). The flasks were then cooled and diluted to the 100 ml mark with distilled water. A 5.0 ml portion of the alkaline starch solution was introduced into a 500 ml volumetric flask and about 100 ml of distilled water was added and slightly acidified with 3 drops of 6N The contents were well mixed by shaking the flask. Five milli-HC1. liters of iodine solution (0.2% iodine by mixing 20g of KI in 1000 ml water) was added and the flask filled to the 500 ml mark. The color developed immediately and absorbance values were obtained at 643 nm. The 643 nm was determined as the peak for the color by scanning the spectrum. Amylose-amylopectin ratios were extrapolated from a standard curve. The standard curve was calculated using amylose-amylopectin ratios of 0:100, 10:90, 30:70, 50:50, 70:30, 90:10 and 100:0 which were prepared in the same manner as the starch samples.

Scanning Electron Microscopy

Scanning electron microscopy was used to observe endosperm structure, starch granule structure and starch hydrolysis.

Samples to be examined were mounted on aluminum stubs with colloidal

silver. Samples were then coated with 200Å of gold palladium and observed with a Joel JSM 35 Scanning Electron Microscope at an accelerating voltage of 25 Kv. Polaroid PN55 film was used to photograph the scanned image. Photomicrographs were taken at 480X, 2000X and 10,000X magnifications. The electron microscope was purchased by Oklahoma State University for research purposes. Courtesy of the Physiological Sciences Department made the use of the elctron microscope possible for this study.

Statistical Analysis

Data obtained from the gas production and in vitro dry matter digestibility studies of processed grains were subjected to statistical analysis procedures for randomized block designs with a factorial arrangement of treatments. In vitro starch gas production, starch IVDMD, amylose content, alpha-amylase soluble starch and whole starch digestibility data were subjected to statistical analysis procedures for randomized block design. Berry size and wet milling yields were analyzed as completely randomized designs. Standard errors of treatment means were derived from the residual mean square entry of the analysis of variance. Significance among treatment means was determined by a LSD protected by a significant preliminary F test. Differences between variety means within endosperm type were tested with an LSD protected by a significant F test Comparisons were made between processing techniques within an endosperm type when interaction values were significant (P < .05). When interaction was significant, conclusions about processing methods have to be qualified by endosperm types. Therefore, comparisons were made between processing methods within an endosperm type to see if all endosperm types responded the same to processing.

CHAPTER IV

RESULTS AND DISCUSSION

1973 Crop

The seven grains grown in the 1973 crop year were grouped by endosperm type into the five endosperm types represented. The grains were grouped as follows: Corn = Pioneer 3306 and Pioneer 3149; White-Bird Resistant = Darset; Waxy = Dwarf Redlan; Hetero-yellow = OK612; and White-normal = Soft Endo and Redlan Normal. The grains were grouped in this manner because of the large number of varieties available today and varieties of a common endosperm type should be more alike than varieties of different endosperm types. If comparisons are made by variety, the findings of these studies applies only to the varieties that were used; whereas, when comparisons are made by endosperm type, the application of the data from these studies may possibly be expanded to a larger number of varieties if endosperm type is known.

Physical and Chemical Composition

The composition of the grains are given in Table V. Crude protein, ether extract and ash percentages on a dry matter basis (respectively) were as follows: corn (9.56, 5.36, 1.70); white-BR (12.8, 3.28, 1.36); waxy (13.12, 1.27, 1.90); hetero-yellow (12.90, 2.00, 1.19); and whitenormal (14.26, 1.30, 2.14).

TABLE V

а

WHOLE	GRAIN	COMPOSITION	-	1973	CROP	

Endosperm Type	Protein ^b	Ether Extract	Ash
		- % -	
Corn	9.56	5.36	1.70
White-BR	12.80	3.28	1.36
Waxy	13.12	1.27	1.90
Hetero-yellow	12.90	2.00	1.19
White-normal	14.26	1.30	2.14

^aDry Matter Basis.

^bKjeldahl nitrogen x 6.25.

Relative berry size, as determined by the weight of 100 kernels, is given in Table VI. As expected, the weight of corn kernels (30.00g) was much heavier (P < .05) than sorghum kernels. Hetero-yellow grain was significantly (P < .05) heavier (3.42g) than all other sorghums, while waxy and white-normal grains (2.68 and 2.38g, respectively) were not significantly different (P < .05). The white-BR grain (1.92g) was the lightest (P < .05) of all endosperm types.

In Vitro Gas Production of Processed Grains

Six-hour <u>in vitro</u> gas production of the processed grains, utilizing amyloglucosidase enzyme and yeast to measure starch availability, is illustrated in Figure 1. Gas production of all reconstituted endosperm types was higher (P < .05) than when the grains were dry ground. Dry

TABLE VI

RELATIVE BERRY SIZE - 1973 CROP

Endosperm Type	Wt. of 100 Kernels (g)	Determinations
Corn	30.00 ^a	6
White-BR	1.92 ^d	3
Waxy	2.68 ^C	3
Hetero-yellow	3.42 ^b	3
White-normal	2.38 ^C	6

a,b,c,d $_{\rm Means}$ in the column with different superscripts are significantly different (P < .05).

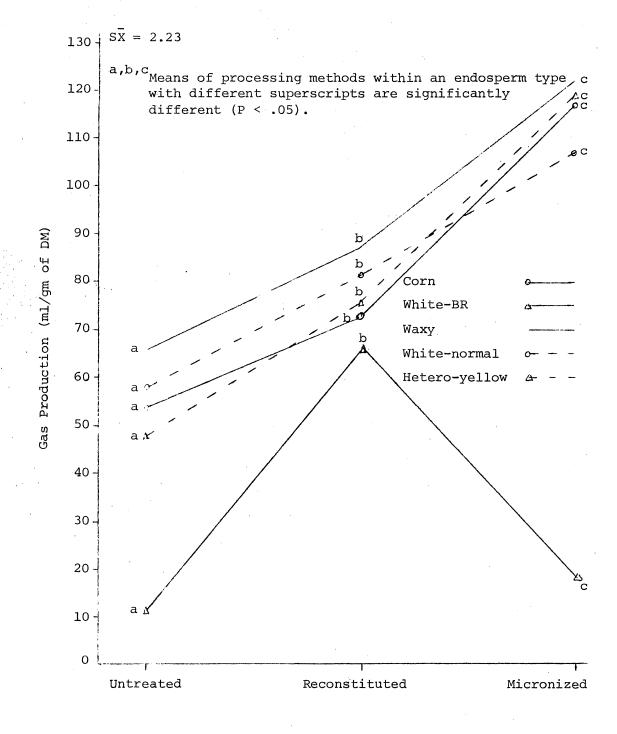


Figure 1. Six Hour Gas Production of Processed Grains (1973 Crop)

ground corn and the white-BR, waxy, white-normal and hetero-yellow sorghum endosperm types produced 54.1, 11.9, 65.9, 58.3 and 48.0 ml of gas per gram of dry matter, respectively, which was significantly less (P < .05) than reconstituted grains which produced 72.9, 66.1, 87.0, 81.8 and 75.6 ml, respectively.

Six hour gas production of micronized corn and the white-BR, waxy, hetero-yellow and white-normal sorghum endosperm types (117.9, 18.8, 121.9, 119.4 and 107.6 ml, respectively) was significantly (P < .05) more than in the dry ground form (54.1, 11.2, 65.9, 48.0 and 58.3 ml, respectively). Although micronization caused a significant increase in gas production in all endosperm types, it is interesting to note that gas production of corn, waxy, hetero-yellow and white-normal endosperm types approximately doubled while gas production of the white-BR type was only increased by 40%. Moreover, the gas production of this type was much lower than the other types.

On the average, micronized corn, waxy, hetero-yellow and whitenormal endosperm types produced 37.3 ml more gas than when these endosperm types were reconstituted. The white-BR type produced significantly (P < .05) less gas when micronized (18.8 ml) than when reconstituted (66.1 ml).

Significant (P < .05) differences within the corn endosperm type were observed as Pioneer 3306 corn produced more gas in 6 hours when reconstituted and micronized than the Pioneer 3149 (6.0 and 10.5 ml, respectively). Within the white-normal endosperm type differences were observed in 6 hr gas production when Redlan produced 17.0 ml more gas when micronized than Soft Endo, and Soft Endo produced 14.3 ml more gas than Redlan in the dry ground form.

Twelve hour gas production data (Figure 2) shows the same trends as

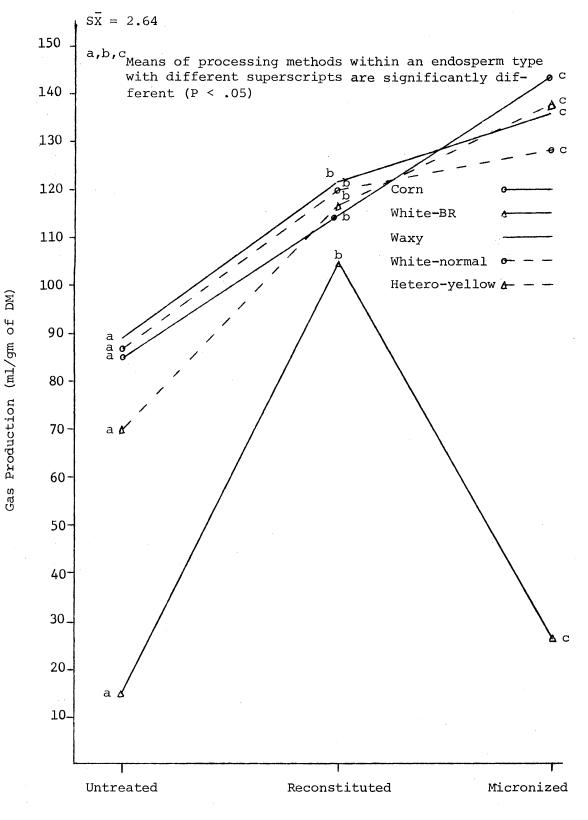


Figure 2. Twelve Hour Gas Production of Processed Grains (1973 Crop)

in the 6 hour gas production data. Corn, white-BR, waxy, hetero-yellow, and white-normal endosperm types produced significantly (P < .05) more gas (30.2, 89.5, 32.9, 45.6 and 34.1 ml, respectively) when reconstituted than when dry ground. Micronizing also increased (P < .05) gas production of all endosperm types compared to dry ground grains. Corn, waxy, hetero-yellow and white-normal grains produced, on the average, 54.1 ml and white-BR 10.4 ml more gas per gram of dry matter when micronized. Micronized corn, waxy, hetero-yellow and white normal types (143.9, 136.5, 137.8 and 129.0 ml, respectively) produced significantly (P < .05) more gas than these grains in the reconstituted form (115.6, 121.8, 115.9 and 120.4 ml, respectively). However, the 12 hr. gas production of the white-BR micronized grain was significantly (P < .05) less than when reconstituted (26.1 vs. 105.2 ml).

Differences within the white-normal endosperm type were observed as Soft Endo produced 18.2 ml more gas than Redlan in the dry ground form. Whereas, Redlan produced 9.8 ml more than Soft Endo when the grains were micronized.

The trend for increased starch availability (as measured by ml of gas produced per gram of dry matter) of the micronized treatments is in agreement with studies of Schake et al. (1970), Sullins and Rooney (1974), Croka and Wagner (1975) and McNeill et al. (1975). In contrast, McNeill (1970) observed micronized grain sorghum producing less gas per gram of dry matter than untreated, reconstituted and steam flaked grain of the same variety. Endosperm type, however, was not reported in most studies.

The low level of gas production exhibited by the dry ground white-BR endosperm type at both incubation times is in agreement with several

authors (Samford et al., 1970, 1971; McCollough and Brent, 1972; Saba et al., 1972) which observed low gas production values with several bird resistant varieties. The higher level of gas production for the dry ground waxy endosperm types is in agreement with studies of Sherrod et al. (1969), and Nishimuta et al. (1969).

This gas production data suggests that endosperm types with a lower starch availability (i.e., white-BR) respond more favorably to reconstitution while endosperm types with better starch availability (i.e., corn, waxy, white-normal and hetero-yellow) respond most favorably to micronization.

In Vitro Dry Matter Digestibility of Processed

Grains

Six hour <u>in vitro</u> dry matter digestibility (Figure 3) of corn, waxy, white-normal and hetero-yellow endosperm types in the dry ground form (15.9, 15.0, 14.4 and 11.8%, respectively) was not significantly (P < .05) different than these endosperm types when reconstituted (17.2, 15.1, 16.4 and 16.2%, respectively). However, the 6 hr digestibility of the white-BR endosperm type when reconstituted was significantly (P < .05) more digestible than when dry ground (14.9 vs. 9.3%). Corn, waxy and hetero-yellow endosperm types in the micronized form (20.9, 24.9 and 21.4%, respectively) were more (P < .05) digestible than when dry ground (15.9, 15.0 and 11.8%, respectively). However, the increase in digestibility of the white-BR and white-normal endosperm types (2.9 and 1.5%, respectively) when micronized, was not significant (P < .05).

Micronized corn, waxy and hetero-yellow endosperm types (20.9, 24.9 and 21.4%, respectively) were more (P < .05) digestible than when recon-

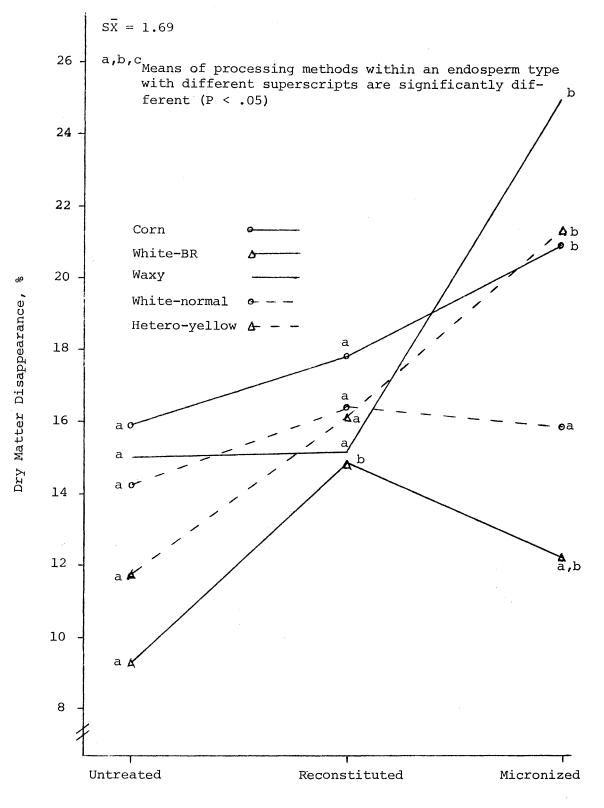


Figure 3. Six Hour <u>In Vitro</u> Dry Matter Disappearance of Processed Grains (1973 Crop)

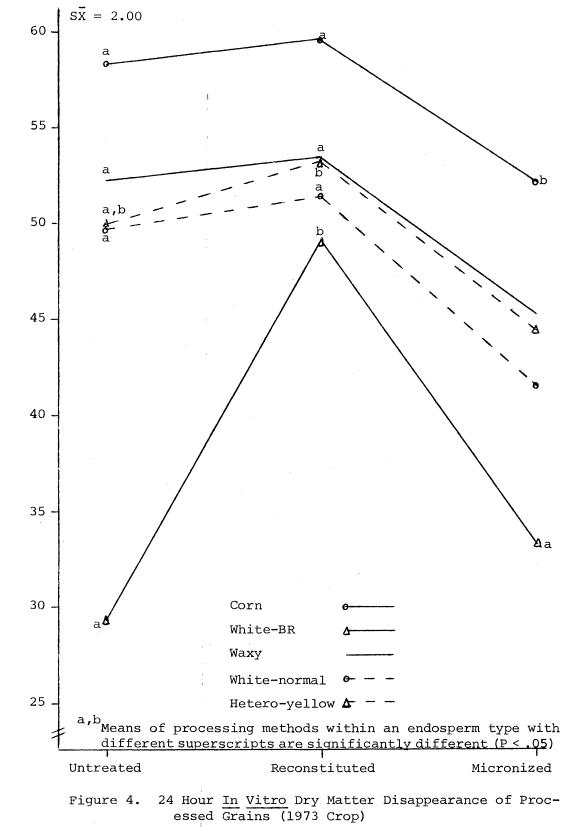
stituted (17.2, 15.1 and 16.2%, respectively). Differences were not significant (P < .05) between the micronized and reconstituted forms of white-BR and white-normal endosperm types (1.7 and .5%, respectively). The only difference observed within endosperm type was observed when reconstituted Redlan was 5.8% more digestible than reconstituted SoftEndo.

At the 24 hr incubation time (Figure 4), white-BR was the only endosperm type that exhibited an significant (P < .05) difference between dry grinding and reconstituting (29.1 and 49.2%, respectively). All other endosperm types studied showed an average improvement in IVDMD due to reconstituting of only 1.9%.

Twenty-four hour IVDMD of micronized corn, waxy and white-normal endosperm types (52.2, 45.2 and 41.6%, respectively) was significantly (P < .05) lower than these endosperm types in the dry ground form (58.3, 52.4 and 49.7%, respectively). Differences between hetero-yellow dry ground (49.8%) and micronized (44.8%) forms were not significantly different (P < .05). The only improvement in <u>in vitro</u> digestibility due to micronization was that exhibited by the white-BR (4.0%), though not significant (P < .05). IVDMD of all endosperm types in the micronized form were significantly (P < .05) lower than in the reconstituted form (7.5, 16.0, 8.1, 9.8 and 8.5%, respectively, for corn, white-BR, waxy, white-normal and hetero-yellow endosperm types). The only significant (P < .05) difference within endosperm type occurred when reconstituted Redlan (18.8%) was more digestible than reconstituted Soft Endo (13.0%).

Wet Milling

The laboratory wet milling procedure was done in triplicate with raw unprocessed grain from each variety. Data presented is on a dry



%

Dry Matter Disappearance,

matter basis.

<u>Yield Characteristics</u>. Wet milling compositional characteristics of the grains are presented in Table VII. Starch yield of corn (64.9%) was significantly (P < .05) higher than a-1 sorghum endosperm types. Waxy (59.5%) and hetero-yellow (58.9%) endosperm types, though not significantly different (P > .05), were higher (P < .05) in starch content than white-BR (54.9%) and white-normal (54.8%). Bran and germ content of corn, waxy and hetero-yellow types were 15.0, 16.0 and 16.4%, respectively, and not significantly different (P > .05). White-normal endosperm grain which had the lowest starch yield also produced the greatest amount of bran and germ (18.4%) which was not significantly (P > .05) different than the white-BR type (17.7%).

The peripheral endosperm yield of corn (1.1%) was significantly (P < .05) lower than all sorghum endosperm types. White-BR, heteroyellow and white-normal endosperm types (4.4, 5.1 and 5.1%, respectively), though not significantly different (P > .05), yielded more (P < .05) peripheral endosperm cells than the waxy type (3.0%).

Gluten yield was highest in the white-BR endosperm type (12.2%) but was not significantly different (P < .05) than white-normal (ll.6\%). Corn yielded the least (P < .05) gluten (7.7\%) with waxy and heteroyellow types being intermediate (l0.9 and l0.6¢, respectively). No significant differences were observed within endosperm type in any of the wet milling products.

This wet milling data shows that as starch content decreases, wet milling by products (bran and germ, peripheral endosperm cells and gluten) increase. It is also interesting to note that corn yielded, on the average, 7.9% more starch than the sorghums; perhaps the lower

TABLE	VII
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WET MILLING COMPOSITIONAL CHARACTERISTICS OF GRAINS^A (1973 CROP)

Endosperm Type	Starch	Bran and Germ	PEC ^B	Gluten	No. of Determinations
			- % -		
Corn	64.9 ^a	15.0 ^a	1.1 ^a	7.7 ^a	4
Waxy	59.5 ^b	16.0 ^{a,b}	3.00 ^b	10.9 ^{b,c}	3
White-BR	54.9 ^C	17.7 ^{b,c}	4.4 [°]	12.2 ^d	2
Hetero-yellow	58.9 ^b	16.4 ^{a,b}	5.1 ^C	10.6 ^b	3
White-normal	54.8 ^C	18.4 [°]	5.1 ^C	11.6 ^{c,d}	6
sx	0.8	0.8	. 4	.4	

A Dry Matter Basis.

B Peripheral Endosperm Cells.

a,b,c,d_{Means} in a column with different superscripts are significantly different (P < .05).

amounts of starch obtained from the sorghum grains is a partial explanation for the lower feeding value of sorghum grains.

This data is in agreement with that of Watson et al. (1955) who found starch recovery and purification from grain sorghum are more difficult than from corn. Difficulties occur because the sorghum kernel has a larger portion of corneous endosperm and a layer of dense cells rich in protein at the periphery of the endosperm just inside the aleurone layer. Sorghum grain gluten particles were more dense than corn gluten and difficulty was encountered with sorghum gluten settling on the table with the starch. Corn kernels usually contain a homologous but less extensive layer than sorghum grain.

Protein Content of Wet Milling Fractions. The protein content of wet milling fractions is given in Table VIII. The protein content of corn starch (.30%) is the same as that reported by Watson (1967). The protein content of the sorghum starches (.31, .23, .26 and .36%, respectively for white-BR, waxy, hetero-yellow and white-normal types) was low, but quite variable. The protein content of the bran and germ of corn (9.9%) was considerably lower than sorghum bran and germ (avg. 16.8%). On the average, protein content of the peripheral endosperm cell fraction was 5.4% higher in sorghum grains than corn. This increased protein content of the peripheral endosperm cell fraction, which represents the protein matricies surrounding the starch granules, may be related to the solubility of the protein matrix and the ease in which starch granules are released.

Several relationships appear to exist between protein content of the various fractions and wet milling yield characteristics which have been studied by Norris and Rooney (1970). These researchers observed that as peripheral endosperm cell content of sorghum grain increased

TABLE VIII

Peripheral Endosperm Bran and Cells Endosperm Type Starch Germ Gluten જ ક્ર ષ્ઠ ૪ Corn .30 9.90 18.76 47.69 37.63 White-BR .31 16.41 23.80 Waxy 17.12 27.32 51.61 .23 Hetero-yellow 46.32 .36 16.48 20.42 25.15 49.06 White Normal .36 17.20

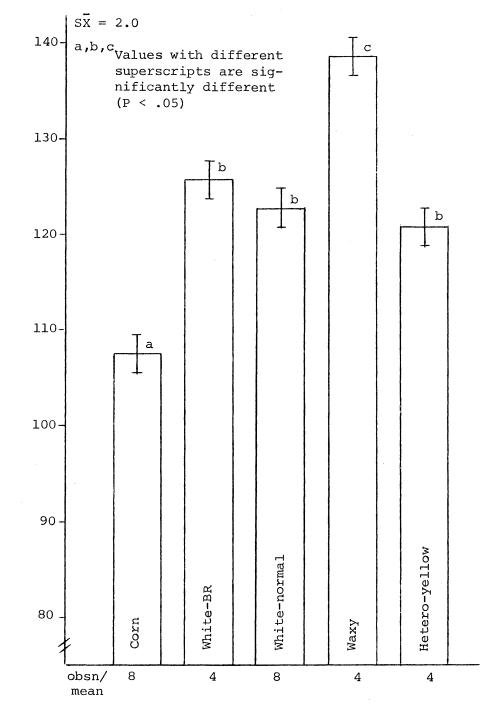
PROTEIN CONTENT^a OF WET MILLING FRACTIONS (1973 CROP)

^aDry Matter Basis.

protein content of the starch also increased, all endosperm types, except waxy, responded in a similar manner in this study. The protein content in the isolated starch was very low in all cases, being only .23 - .36% protein. The slightly lower protein content of waxy type starch (.23%), however, is in agreement with work of Sullins and Rooney (1974) where it was found that the protein matrix in waxy endosperm type sorghum grains were more susceptable to solubilization and had less peripheral endosperm and amorphous protein than normal sorghum grains which permits the starch granule to be released easier and in a purer form. Norris and Rooney (1970) also observed that protein content of the starch is indicative of the relative ease of wet milling potential.

Gas Production of Raw Isolated Starches

Six hour <u>in vitro</u> gas production (Figure 5) of the isolated starches was used to evaluate the susceptibility of isolated starch to amyloglucosidase enzymatic attack. Gas production of the waxy endosperm type was significantly (P < .05) higher than all other endosperm types producing 138.8 ml of gas per gram of starch dry matter. Corn starch (107.5 ml) produced significantly (P < .05) less gas than all other endosperm types. White-BR, white-normal and hetero-yellow endosperm types (125.8, 122.8 and 120.7 ml, respectively) were not significantly (P > .05) different and were intermediate to corn and waxy endosperm types. Differences within endosperm types were not significant (P > .05). The higher gas production of the waxy type is in agreement with studies of Sullins and Rooney (1974). It is interesting to note that all of the sorghum starches were more susceptable to amyloglucosidase attack than was corn starch. This data suggests that perhaps chemical and/or physi-



Endosperm Type

Figure 5. Six Hour Gas Production of Raw, Isolated Starches (1973 Crop)

Gas Production (ml/gm of DM)

cal properties of the protein matrix surrounding the starch granules, not the starch, are responsible for the major differences in grain digestibility.

IVDMD of Isolated Starch

Six hour <u>in vitro</u> digestibilities of isolated starch are presented in Figure 6. Though differences were not significant (P > .05), trends do exist. Corn and waxy starches (25.7 and 25.8%, respectively) were slightly more digestible than white-normal and hetero-yellow starches (24.5 and 23.9%, respectively). White-BR tended to be digested the least (22.3%) digestible at the 6 hour incubation period.

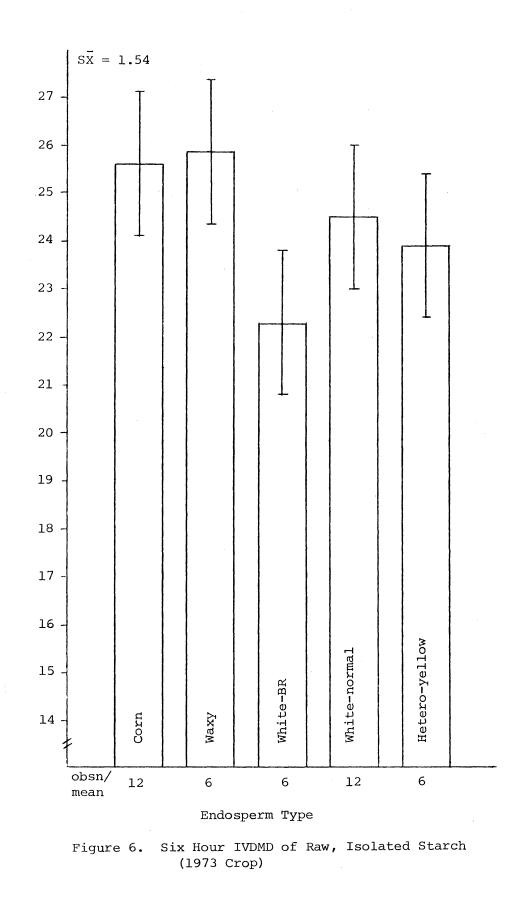
Differences in isolated starch digestibility at the 24 hour incubation time (Figure 7) were not significantly (P > .05) different. Waxy starch tended to be the most digestible (60.8%) while other endosperm types were very similar (avg. 57.8%). Differences between varieties within endosperm types at both 6 hr and 24 hr were not significant (P < .05). This data also suggests that the starch alone is not responsible for differences in digestibility of the native grain.

Amylose-Amylopectin Ratios of Isolated Starch

Amylose-amylopectin ratios of isolated starches (Table IX) were not significantly (P > .05) different in the corn, white-BR, white-normal and hetero-yellow starches (18.2, 18.0, 17.9 and 19.4% amylose, respectively). These percentages of amylose of normal type endosperms are slightly lower than the common range of 20-30% amylose. The waxy type starch contained the least (P < .05) amylose (2.5%) of the starches studied which is in agreement with studies of Rooney and Clark (1968)

Dry Matter Disappearance,

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Dry Matter Disappearance,

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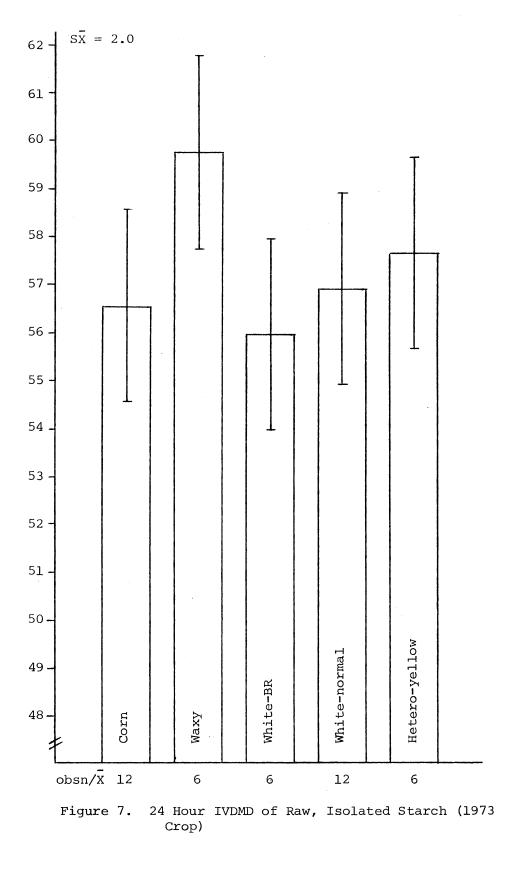


TABLE IX

		Determinations	
Endosperm Type	Amylose	Amylopectin	Per Mean
Corn	18.25 ^a	81.75	4
White-BR	18.04 ^a	81.96	2
Waxy	2.46 ^b	97.54	2
Hetero-yellow	19.44 ^a	80.56	2
White-normal	17.91 ^a	82.09	4
sx	1.02		

AMYLOSE-AMYLOPECTIN RATIOS OF ISOLATED STARCH (1973 CROP)

 $^{\rm a,b}_{\rm Means}$ which do not have the same superscript are significantly different (P < .05).

and Nishimuta et al. (1969) when waxy varieties were reported to contain nearly 100% amylopectin. Amylose content of varieties within endosperm types were not different (P > .05).

Enzymatic Digestion of Isolated Starch

Native starch is insoluble in water because of its high degree of intermolecular organization. With heating (up to boiling with water) the amylose fraction leaches out of the starch leaving the insoluble amylopectin portion of the starch molecule (Langlois and Wagner, 1967). Alpha-amylase enzymatic digestion studies were conducted with the soluble (amylose) starch portion (lst study) and whole raw isolated starch (2nd study).

Since digestion of starches by alpha-amylase yields primarily glucose units, the Nelsons Test was employed to evaluate the amount of glucose equivalents present. The Nelsons test was used since it is both sensitive and reproducible in determining reducing sugars. In these assays, the sugar, or sample in this case, is heated with an alkaline copper reagent resulting in the formation of cuprous oxide. The copper is then oxidized by arsenomolybdate which is a deep blue in its reduced form. The amount of reduced arsenomolybdate is proportional to the amount of carbohydrate present.

Enzymatic Digestion of Soluble Starch (Amylose). Soluble starch from the waxy endosperm type (.136 µmoles of glucose equivalents/ml/min) was more than 4 times (P < .05) more susceptable to alpha amylase attack (Figure 8) compared to all other starches (.030, .032, .033 and .031 µmoles, respectively for corn, white-BR, white-normal and hetero-yellow starch). Digestion of corn, white-BR, white-normal and hetero-yellow

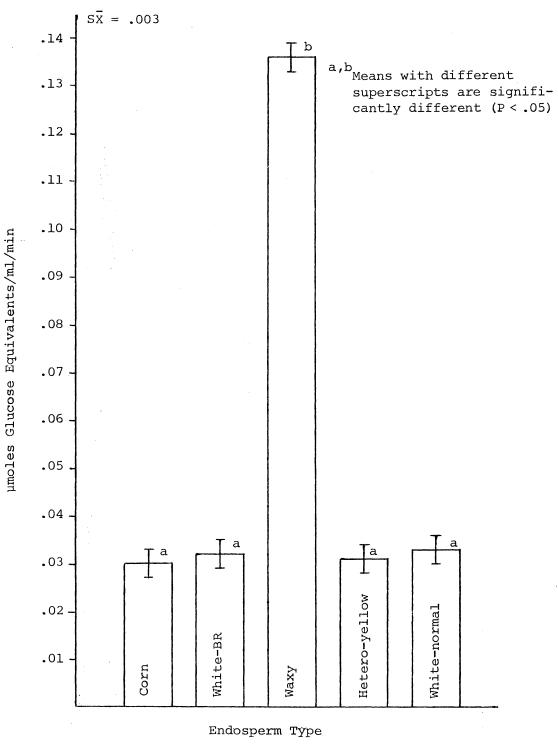
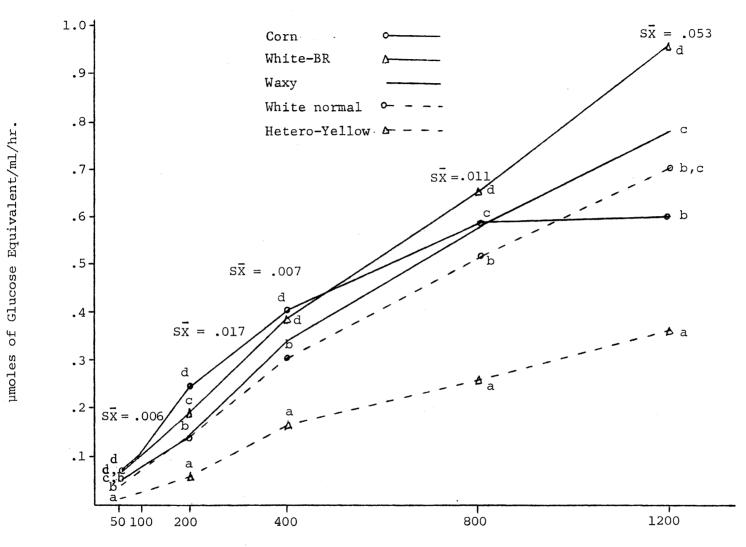


Figure 8. Alpha-Amylase Digestion of Soluble Starch (Amylose) (1973 Crop)

starches were not significantly (P > .05) different. Differences between varieties within the corn and white normal endosperm types were not significant (P > .05).

Enzymatic Digestion of Raw Whole Starch. Whole starch digestion at 6 concentrations is shown in Figure 9. At lower starch concentrations (50, 100, 200 and 400 mg/50 ml) the white-BR starch liberated more glucose equivalents than other endosperm types (.069, .107, .248 and .404 μ moles, respectively). Hetero-yellow starch was significantly (P < .05) less digestible than all others at 50, 200, 400, 800 and 1200 mg (.014, .030, .066, .261 and .360 µmoles, respectively). Starch digestion by alpha-amylase was not significantly (P < .05) different at the 100 mg level. At the 800 mg level waxy starch (.652 µmole) was digested more than other starches. White-BR and corn starch liberated more (P < .05) glucose equivalents (.588 and .577 µmoles, respectively) than white normal (.518 µmoles) and hetero-yellow (.261 µmoles) starches. At the 1200 mg concentration waxy starch (.958 µmoles) liberated more glucose equivalents than all other varieties. Corn and white-normal starches (.780 and .709 μ moles), though not significantly different (P < .05), were more extensively digested than white-BR (.600 µmoles) and heteroyellow (.360 µmoles) starches. The difference between white-normal and white-BR digestion at the 1200 mg level was not significant (P < .05). Differences within endosperm type were observed in the corn endosperm type at the 800 mg level where Pioneer 3306 liberated more (P < .05) glucose equivalents than Pioneer 3149. Significant differences (P < .05) were observed within the white-normal endosperm type at starch concentration of 400 and 800 mg. Redlan starch was more susceptable to alphaamylase enzymatic attack than Soft Endo starch at both levels.



Starch Concentration (mg/50 ml Acetate Buffer)

Figure 9. Alpha-Amylase Digestion of Raw, Isolated Starch (1973 Crop)

The depressed response at the 800 and 1200 mg levels of the white-BR starch is similar to observations of Davis and Harbers (1974) in their work with bird resistant sorghum starch. These researchers said that this response is characteristic of substrate inhibition. The lack of accurate estimates of enzymatic concentrations <u>in vivo</u> precludes direct interpretation of these findings for practical nutrition, but this data does provide evidence that at increased starch intake levels the type of starch may influence digestibility of the starch in the ration.

The results of these studies suggest that grains of different endosperm types respond differently when processed by different methods (reconstitution and micronization). Endosperm types that exhibit a low starch availability in the dry ground form responded most favorably to reconstitution while endosperm types that exhibit better starch availability in the dry ground form responded most favorably to micronization (as documented by the gas production data).

These studies also agree with work of McNeill et al. (1975) who stated that the effects of processing methods upon solubility of the protein matrix in the endosperm seems to be the major factor affecting digestibility and efficiency of utilization. Solubility of the protein matrix may also depend on the protein content and/or composition of the grain.

Sorghum grain has generally been considered lower in feeding value than corn. Evidence from the starch digestion studies suggest that the lower feeding value is not due to the starch alone, but could be partially explained by less available starch as indicated in the wet milling study. The higher protein content and possibly protein composition

of most sorghum grains may render it less digestible and therefore, lower in feeding value.

These studies found sorghum grain endosperm types to be quite variable. Digestibility studies show the waxy endosperm type to be more digestible than normal (non-waxy) endosperm types. The increased protein solubility and increased susceptability to enzymatic attack of waxy endosperm types (Sullins and Rooney, 1974) appears to be responsible for most differences.

1974 Crop

The ten grains grown in the 1974 crop year were grouped by endosperm type into the five endosperm types represented. The grains were grouped as follows: Corn = Pioneer Corn and Northrup King Corn; White-Bird Resistant = Darset; Waxy = Dwarf Redlan, 1126, 1122-2 and 1133-2; Hetero-yellow = OK 612; and White-normal = Soft Endo and Redlan Normal. Endosperm classification was the same as that used in the 1973 crop.

Physical and Chemical Composition

The composition of the grains are given in Table X. Crude protein, ether extract and ash contents (%, DM basis), respectively, were as follows: Corn (9.4, 6.72, 1.73); White-BR (12.02, 4.24, 2.18); Waxy (13.54, 5.08, 2.78); Hetero-yellow (11.61, 4.04, 1.98); and White-Normal (11.48, 5.48, 2.25).

Relative berry size (wt. of 100 kernels) is shown in Table XI. As expected, the corn (33.16g) was much heavier (P < .05) than sorghum kernels. Waxy grain (2.60g) was significantly heavier (P < .05) than all other sorghum grains. White-BR, hetero-yellow and white-normal

TABLE	Х
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WHOLE GRAIN COMPOSITION^a - 1974 CROP

	8		
Endosperm Type	Protein	Ether Extract	Ash
Corn	9.40	6.72	1.73
White-BR	12.02	. 4.24	2.18
Waxy	13.54	5.08	2.78
Hetero-yellow	11.61	4.04	1.98
White-normal	11.48	5.48	2.25

^aDry Matter Basis.

TABLE XI

RELATIVE BERRY SIZE - 1974 CROP

Endosperm Type	Wt. of 100 Kernels	Determinations
Corn	33.16 ^a	6
White-BR	2.20 [°]	3
Waxy	2.60 ^b	12
Hetero-yellow	2.32 [°]	3
White-normal	2.32 [°]	6

a,b,c $_{\rm Means}$ in the column with different superscripts are significantly different (P < .05).

grains were not significantly (P > .05) different (2.20, 2.32 and 2.32g, respectively).

In Vitro Gas Production of Processed Grains

Six-hour <u>in vitro</u> gas production of the processed grains, utilizing amyloglucosidase enzyme and yeast to evaluate starch availability, is illustrated in Figure 10. In contrast to the 1973 crop 6 hr gas production data, corn, white-normal and hetero-yellow endosperm types in the dry ground form (82.9, 89.8 and 83.4 ml, respectively) produced more (P < .05) gas than when reconstituted (60.1, 71.2 and 69.1 ml, respectively). Differences were not significant (P > .05) between the dry ground and reconstituted waxy endosperm type. The white-BR endosperm type was the only grain that produced more (P < .05) gas when reconstituted (56.8 ml) than dry ground (48.4 ml).

Micronized forms of corn, waxy, white-normal, white-BR and heteroyellow endosperm types produced (35.3, 34.4, 13.5, 50.9 and 35.0 ml, respectively) significantly (P < .05) more gas than these endosperm types in the dry ground form. Micronized corn, waxy, white-normal, white-BR and hetero-yellow endosperm types (118.2, 117.3, 103.3, 99.3 and 118.4 ml, respectively) produced significantly more gas (P < .05) than these endosperm types when reconstituted (60.1, 80.9, 71.2, 56.8 and 69.1 ml, respectively).

Significant differences (P < .05) within endosperm types were observed in corn wherein NK corn produced 8.7 ml more gas than Pioneer corn in the dry ground form. Untreated waxy 1122 and Dwarf varieties produced (P < .05) more gas than 1126 and 1133 when untreated and when reconstituted, the 1122 variety produced less (P < .05) gas than the other

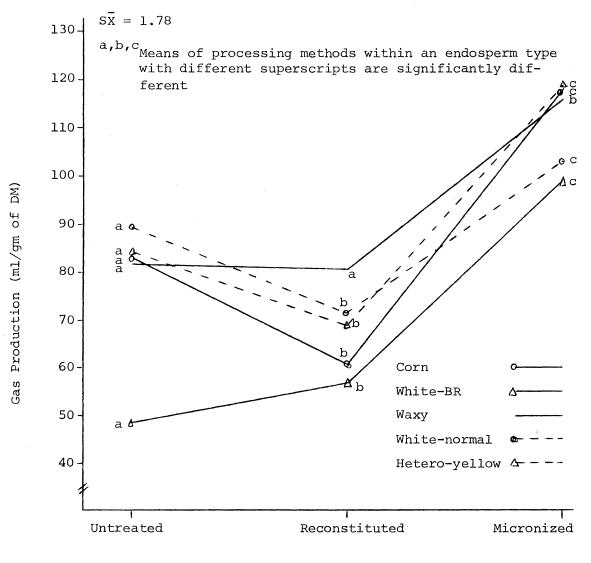


Figure 10. Six Hour Gas Production of Processed Grains (1974 Crop)

three waxy varieties. When micronized, the 1133 variety produced less (P < .05) than the other waxy varieties. Differences were also observed within the white-normal endosperm type with the untreated and micronized treatments when dry ground Soft Endo produced more (P < .05) gas than Redlan Normal, but when micronized Redlan-Normal produced more gas (P < .05) than the Soft Endo variety.

Twelve hour gas production (Figure 11) of dry ground corn (108.5 ml) was significantly higher (P < .05) than the reconstituted corn (96.9 ml). White-normal and hetero-yellow untreated grains were not significantly different (P > .05) from these grains when reconstituted (110.6 and 101.4 vs. 109.1 and 107.4 ml, respectively. Reconstitution of waxy and white-BR varieties (115.3 and 92.6 ml) yielded higher (P < .05) gas production than untreated waxy and white-BR grain (100.6 and 68.8 ml, respectively). Micronized corn, waxy, white-normal, white-BR and heteroyellow endosperm types (143.0, 133.0, 125.2, 122.6 and 140.7 nl, respectively) produced more (P < .05) gas than untreated grains (108.5, 100.6, 58.8 and 101.4 ml, respectively). Micronized corn, waxy, white-normal, white-BR and hetero-yellow grains produced (46.1, 17.7, 16.1, 30.0 and 33.3 ml, respectively) more gas than the reconstituted grains.

Differences within the white-normal endosperm type were observed as Soft Endo produced 15.9 ml more gas than Redlan Normal when the grains were dry ground. Within the waxy endosperm type, Dwarf-Redlan grain produced more gas (P < .05) than the other three waxy varieties in both the reconstituted and micronized processed grains.

The trend for increased starch availability of the micronized grains is in agreement with studies Schake et al. (1970), Sullins and Rooney (1974), Croka and Wagner (1975) and McNeill et al. (1975).

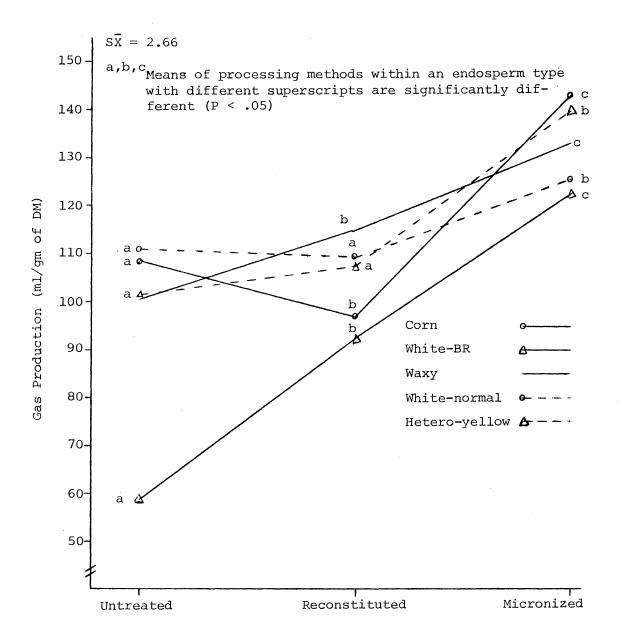


Figure 11. Twelve Hour Gas Production of Processed Grains (1974 Crop)

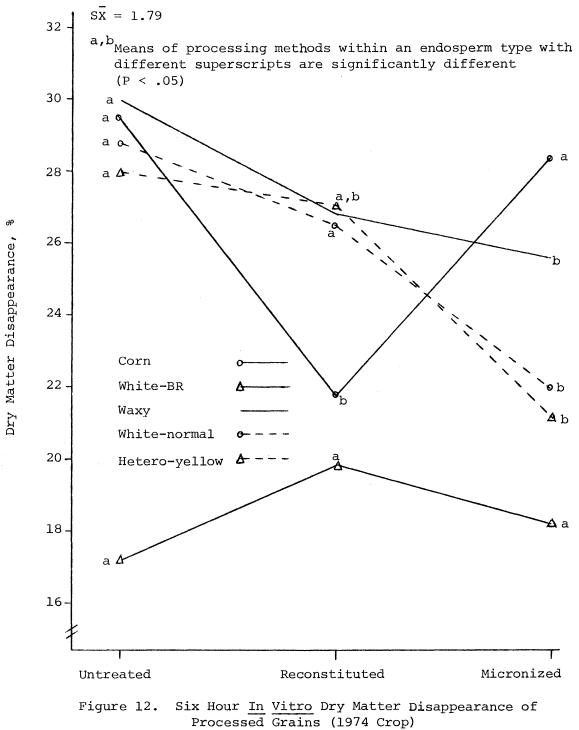
The relative starch availability of the untreated corn, waxy, whitenormal and hetero-yellow endosperm types compared to the reconstituted grains is in contrast to observations in the 1973 crop and the work of numerous researchers. Reconstituted and micronized gas production values were very similar in both crop years, but the ground grains produced much more gas in the 1974 crop year.

The response of the micronized white-BR endosperm type is in contrast to 1973 data. Possible explanations are that in the 1974 white-BR grain micronization was more extensive, in that more of the starch was gelatinized, which would increase susceptability of the starch to enzymatic attack. Also, chemical and/or physical make up of the protein and/or starch may be different in the 1974 crop because of the better moisture conditions during the growing season.

In Vitro Dry Matter Digestibility of Processed

Grains

Six-hour <u>in vitro</u> dry matter digestibility (Figure 12) of whitenormal, white-BR and hetero-yellow sorghum endosperm types when untreated (28.9, 17.2 and 28.0%, respectively) was not significantly different (P > .05) than when reconstituted (25.9, 19.8 and 26.9%, respectively). Reconstituted corn and waxy types (21.7 and 21.9%) were significantly less (P < .05) digestible than these grains when dry ground (29.3 and 30.0%, respectively, for corn and waxy types). The response of micronized corn, waxy, white-normal and hetero-yellow endosperm types are in contrast to data of the 1973 crop and studies by Hinders and Freeman (1969), Hinman and Johnson (1973), McNeill et al. (1974) and Croka and Wagner (1974). Digestibility values of dry ground grains are about



twice that of the 1973 values while micronized grains were about the same. No logical explanation can be provided for such a response.

Six-hour IVDMD of untreated waxy, white-normal and hetero-yellow endosperm types (30.0, 28.9 and 28.0%, respectively) was significantly (P < .05) higher than these grains in the micronized form (25.5, 22.0 and 21.1%, respectively). Micronized and untreated corn digestibilities were very similar (29.3 and 28.7%, respectively) as were white-BR sorghum digestibilities (17.2 and 18.3%, respectively, for the untreated and micronized grain).

Differences between reconstituted and micronized grains were quite variable and did not follow trends set in the 1973 crop. Micronized white-normal and hetero-yellow endosperm types were significantly less digestible (3.9 and 5.8%, respectively) than reconstituted white-normal and hetero-yellow endosperm types. Waxy and white-BR endosperm types were slightly less digestible in the micronized form than the reconstituted form, but were not significantly different (P > .05). The sixhour IVDMD of micronized corn (28.7%) was significantly higher (P < .05) than reconstituted corn (21.7%). The only difference observed within endosperm type was that 1133 waxy variety was less digestible (P < .05) than 1122, 1126 and Dwarf Redlan varieties when micronized.

At the 24 hr IVDMD (Table XII) incubation period, variety by processing method interaction was not significant (P > .05). That is, all endosperm types responded in a similar manner to each processing method; therefore, comparisons can be made between endosperm types and between processing methods.

All endosperms when dry ground or when micronized were significantly (P < .05) less digestible than when reconstituted. Digestibilities

TABLE XII

24 HOUR IN VITRO DRY MATTER DISAPPEARANCE OF PROCESSED GRAINS (1974 CROP)

Endosperm	· · · · · · · · · · · · · · · · · · ·	20 20		Endosperm	Determination	
Type	Untreated	Reconstituted	Micronized	Mean	Per Mean	sx
Corn	61.6	66.8	64.9	64.4 ^a	8	1.2
White-BR	48.8	55.4	46.7	50.3 ^d	4	2.6
Waxy	54.7	60.1	52.0	55.6 ^b	16	1.3
White-normal	52.5	55.9	50.3	52.9 ^C	8	1.8
Hetero-yellow	55.0	60.3	53.5	56.3 ^b	4	2.6
Mean	54.5 ^A	59.7 ^B	53.5 ^A			
sīx	1.0	1.0	1.0			

 A,B Means in a row with different superscripts are significantly different (P < .05).

a,b,c,d_{Means} in a column with different superscripts are significantly different (P < .05).

of each endosperm type were not significantly different (P > .05) when dry ground compared to micronized treatments.

Regardless of processing method, corn was more (P < .05) digestible than all sorghum endosperm types. Hetero-yellow and waxy endosperm types were not significantly different (P > .05) but were more (P < .05) digestible than the white-BR endosperm type. The white-normal endosperm type was intermediate to waxy and white-BR endosperm types and not significantly different from either type.

Differences (P < .05) were observed within the waxy endosperm type when the 1133 was not as digestible as Dwarf Redlan, 1126 and 1122 varieties in the untreated and reconstituted form. In the white normal endosperm type, Redlan Normal was significantly less (P < .05) digestible than Soft Endo when the grains were micronized.

The 24 hr. IVDMD results follow the same trends as in the 1973 crop, except for the white-BR endosperm type. This data suggests that reconstituted grain is more digestible than either dry ground or micronized grain regardless of endosperm type. It appears that environmental conditions during the growing season play an important role in the digestibility and potential for possible improvement by various processing methods. The 24 hr IVDMD values show the grain in the 1974 crop to be more digestible in the untreated form; perhaps, as stated previously, grains with lower digestibilities or starch availabilities have the greatest potential to be improved by various processing techniques. Maybe the lower digestibilities in the untreated grain in 1973 crop allowed more improvement to be made by processing while in the 1974 crop the grains were more digestible in the untreated form and did not show as much or any improvement by processing.

The laboratory wet milling procedure was done in duplicate with raw unprocessed grain from each variety. Data presented is on a dry matter basis.

<u>Yield Characteristics</u>. Wet milling compositional characteristics of the grains are presented in Table XIII. Starch yield of corn (70.3%) was significantly (P < .05) higher than all sorghum endosperm types. Hetero-yellow (61.6%), white-normal (60.0%), waxy (55.6%) and white-BR (53.2%) starch yields were all significantly different (P < .05) from each other.

Bran and germ yield of waxy and white-BR were 20.3 and 20.1%, respectively, and not significantly different (P > .05), but these two types were higher (P < .05) in bran and germ content than other endosperm types tested. White-normal (18.3%) bran and germ content were significantly higher (P < .05) than that of hetero-yellow (15.8%). Corn yielded the least (P < .05) bran and germ of all grains tested (14.4%).

The peripheral endosperm cell content of white-BR (4.0%) grain was significantly higher (P < .05) than all other grains. Corn, which contained the most starch, had the lowest peripheral endosperm cell content (P < .05) of all grains tested. The peripheral endosperm cell content of white-normal, waxy and hetero-yellow endosperm types (3.5, 3.2 and 2.2%, respectively) were all significantly different (P < .05).

Gluten yield was highest in the white-BR type (15.8%) and significantly different (P < .05) than other grains. As in bran and germ yield and peripheral endosperm content, corn contained (6.4%) significantly less (P < .05) gluten than the sorghum grains. Hetero-yellow and white-

TABLE	XIII
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WET MILLING COMPOSITIONAL CHARACTERISTICS OF GRAINS^A - (1974 CROP)

		8			
	Starch	Bran and Germ	Gluten	PEC ^B	No. of Determinations
Corn	70.3 ^a	14.4 ^a	6.4 ^a	1.0 ^a	4
Waxy	55.6 ^b	20.3 ^d	14.5 [°]	3.2 ^C	8
White-BR	53.2 ^C	20.1 ^d	15.8 ^d	4.0 ^e	2
Hetero-yellow	61.6 ^d	15.8 ^b	13.2 ^b	2.2 ^b	2
White normal	60.0 ^e	18.3 [°]	12.9 ^b	3.5 ^d	4
sx	.4	.1	.3	.1	

a,b,c,d,e_{Means} within a column with different superscripts are significantly different (P < .05).

A Dry Matter Basis.

^BPeripheral endosperm cells.

normal grains contained 13.2 and 12.9% gluten, respectively, which were not significantly different (P > .05) but were lower (P < .05) than waxy types (14.5%).

Differences within endosperm type were noted as 1126 yielded less starch and Dwarf Redlan yielded more (P < .05) starch than the 1122 and 1133 varieties. The 1122 and Dwarf varieties also yield more (P < .05) bran and germ than the other waxy varieties. The 1133 variety yielded less (P < .05) peripheral endosperm cells and more gluten than 1126,1122 and Dwarf Redlan varieties. Within the white-normal endosperm type, the only difference (P < .05) observed was that the Redlan variety contained less bran and germ than Soft Endo.

This wet milling data, as in the 1973 crop milling, demonstrates that as starch content decreases, wet milling by-products (bran and germ, peripheral endosperm cells and gluten) increase. Corn, on the average, yielded 12.7% more starch than the sorghums, perhaps the lower amounts of starch obtained from the sorghum grains or the higher amounts of gluten and peripheral cells, which make up the protein matricies, are a partial explanation for the lower feeding value of sorghum grains.

This data is in agreement with that of the 1973 crop and Watson et al. (1955) who found starch recovery and purification from grain sorghum is more difficult than from corn and that corn starch is generally lower in protein content than sorghum starch.

Protein Content of Wet Milling Fractions. The protein content in the isolated starch (Table XIV) was very low in all cases, being only .22 to .28% protein. The protein content of corn bran and germ (9.79%) was considerably lower than sorghum bran and germ (avg. 17.9%). On the average, protein content of the peripheral endosperm cell fraction was

TABLE IV

Endosperm Type	Starch	Bran and Germ	Peripheral Endosperm Cells	Gluten
	8	ę	8	8
Corn	.22	9.79	21.51	43.71
White-BR	.28	18.76	30.28	32.25
Waxy	.24	19.04	25.48	40.36
Hetero-yellow	.26	16.50	26.52	40.01
White normal	.28	17.48	25.87	42.15

PROTEIN CONTENT OF WET MILLING FRACTIONS a (1974 CROP)

a Dry Matter Basis.

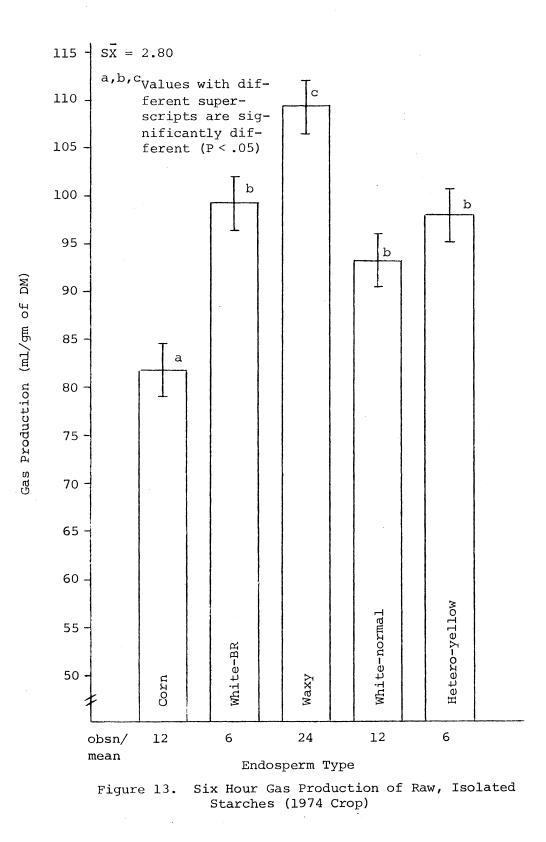
5.5% higher in sorghum grains than corn. This higher protein content of the peripheral endosperm cell fraction (makes up protein matrices) may be related to the solubility of the protein matrix and the ease in which starch granules are released.

The trends and relationships observed in the 1973 crop study and work of Norris and Rooney (1970) were the same as in this data. Most evident, as peripheral endosperm cell content of gluten of sorghum grain increased protein content of the starch also increased.

Gas Production of Raw, Isolated Starch

Six hour <u>in vitro</u> gas production (Figure 13) of the raw, isolated starches was used to evaluate the susceptability of isolated starch to amyloglucosidase enzymatic attack. Gas production of the waxy type starch (109.3 ml) was significantly higher (P < .05) than all other starches. Corn starch (81.9 ml) produced significantly less (P < .05) gas than all sorghum starches. White-BR, white-normal and hetero-yellow starches produced 99.1, 93.3 and 98.0 ml of gas, respectively, and were not significantly different (P > .05). No significant differences (P > .05) were observed between varieties within the various endosperm types.

The much higher gas production of the waxy type starch is in agreement with the 1973 crop study and work of Sullins and Rooney (1974). As in the 1973 crop study, sorghum grain starches were more susceptable to amyloglucosidase attack than was corn starch. This data suggests that the starch type alone is not responsible for the differnce in corn and sorghum grain digestibility.



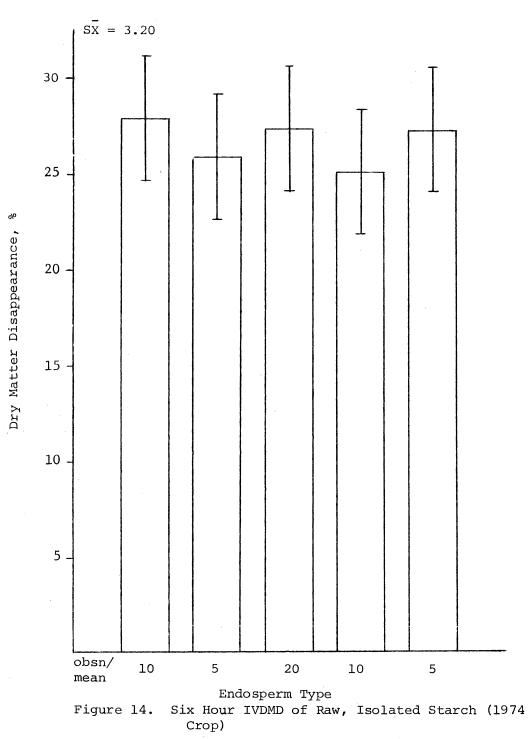
IVDMD of Raw, Isolated Starch

Six hour <u>in vitro</u> digestibilities of raw, isolated starch are presented in Figure 14. Though differences were not significant (P > .05), there was a trend for corn, waxy and hetero-yellow starch to be slightly more digestible (27.8, 27.4 and 27.2%, respectively) than white-BR and white-normal starches (25.8 and 25.1%, respectively).

Differences in isolated starch digestibility at the 24 hour incubation period (Figure 15) were not significantly different (P > .05). Waxy starch tended to be the most digestible (73.1%), corn (69.1%) and white-normal (68.2%) intermediate and white-BR and hetero-yellow starch slightly less digestible (65.1 and 64.0%, respectively). Differences between varieties within endosperm types at both 6 and 24 hr were not significant (P > .05). This data is in agreement with that of the 1973 crop study and suggests that the starch alone is not responsible for differences in digestibility of the grain.

Amylose-Amylopectin Ratios of Isolated Starch

Amylose-amylopectin ratios of isolated starches (Table XV) were not significantly different (P > .05) in the corn, white-BR, white-normal and hetero-yellow starches (21.2, 20.5, 21.1 and 19.4% amylose, respectively). The percentages of amylose in the normal endosperm types are somewhat low but usually within the common range of 20-30% amylose. The waxy starch contained the least (P < .05) amylose (3.7%) of the starches studied which is in agreement with the 1973 crop study and studies of Rooney and Clark (1968) and Nishimuta et al. (1969). Amylose content of varieties within endosperm type were not significantly different (P > .05).



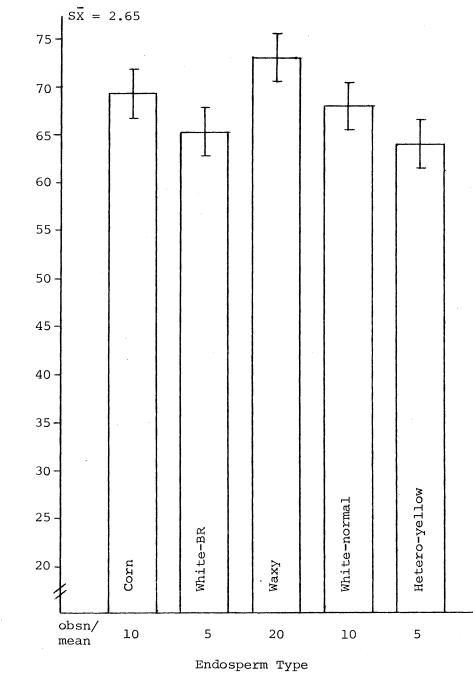


Figure 15. 24 Hour IVDM of Raw, Isolated Starch (1974 Crop)

Dry Matter Disappearance, %

TABLE XV

		8		
Endosperm Type	Amylose	Amylopectin	Determinations	
Corn	21.19 ^a	78.81	4	
White-BR	20.54 ^a	79.46	2	
Waxy	3.69 ^b	96.31	8	
Hetero-yellow	21.10 ^a	78.9	2	
White-normal	19.35 ^a	80.65	4	
sx	.54			

AMYLOSE AMYLOPECTIN RATIOS OF ISOLATED STARCH (1974 CROP)

 $^{\rm a,b}_{\rm Means}$ which do not have the same superscript are significantly different (P < .05).

Enzymatic Digestion of Soluble Starch (Amylose). Soluble starch from the waxy endosperm type (.261 µmoles) was more than 10 times (P < .05) more susceptable to alpha-amylase attack (Figure 16) compared to corn, white-BR, white-normal and hetero-yellow (.020, .018, .026 and .020 µmoles, respectively). Digestion of corn, white-normal and heteroyellow soluble starch was not significantly different. Differences between varieties within the waxy endosperm type were significant (P < .05). The 1133 soluble starch was least digestible with 1126 and 1122 not significantly different (P > .05). Dwarf Redlan was the most digestible (P < .05) of the waxy types.

Enzymatic Digestion of Raw, Whole Starch. Whole starch digestion at four starch concentrations is illustrated in Figure 17. At the 100 and 400 mg concentrations white-BR starch (.211 and .830 µmoles) was significantly (P < .05) more digestible than all other starches. Waxy starch (.141 and .515 µmoles, respectively, at 100 and 400 mg) was more digestible (P < .05) than corn, white-normal and hetero-yellow starch at low starch concentrations. Hetero-yellow starch (.084 and 0.282 µmoles) was the least (P < .05) susceptable to enzymatic attack. The difference between white-normal (.101 and .372 µmoles) and corn starch (.118 and .392 µmoles) was not significant (P > .05) at both concentrations, 100 and 400 mg, respectively.

At the 800 mg level white-BR, waxy, corn, white-normal and heteroyellow starch liberated 1.564, 944, .790, .696 and .588 µmoles of glucose equivalents, respectively. These means are all significantly different from each other at the .05 level.

At a starch concentration of 1200 mg/50 ml the white-BR starch

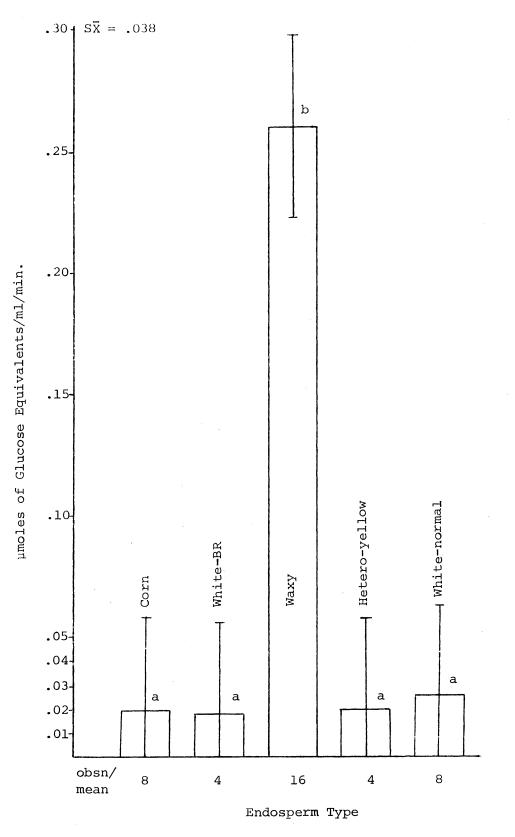
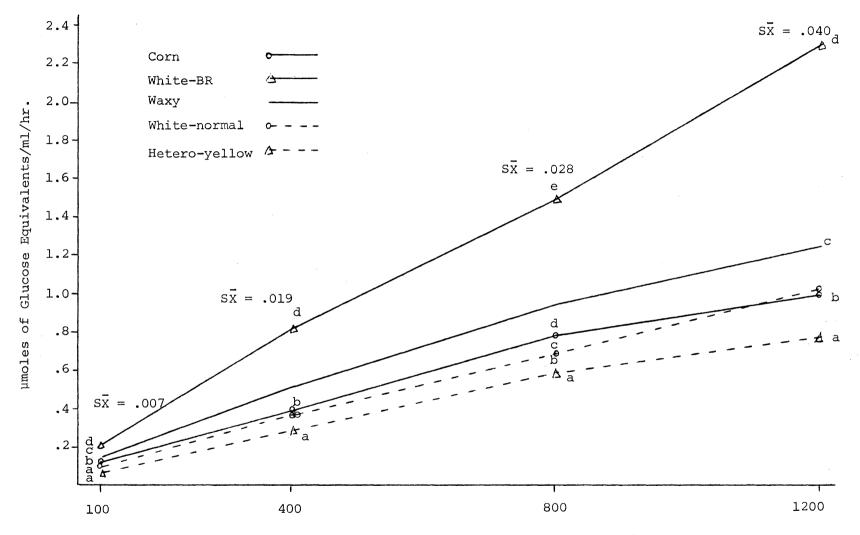


Figure 16. Alpha-Amylase Digestion of Soluble Starch (Amylose) (1974 Crop)



Starch Concentration (mg/50 ml Acetate Buffer)

Figure 17. Alpha-Amylase Digestion of Raw, Isolated Starch (1974 Crop)

(2.310 µmoles) liberated approximately twice as many glucose equivalents (P < .05) as any other starch tested. Waxy starch (1.254 µmoles) was significantly more digestible than corn, white-normal and hetero-yellow starches. The hetero-yellow was the least digestible (P < .05) of all starches. Glucose equivalents liberated by corn and white-normal starches (1.068 and 1.001 µmoles) was not significantly different (P > .05). Differences within the corn endosperm type were observed as Pioneer liberated more glucose equivalents than Northrup King corn at the 400 mg level. In the waxy endosperm type, 1126 starch was more digestible than other waxy starches at all concentrations and Dwarf Redlan starch was consistently lower than other waxy varieties. At the 100, 400 and 800 mg levels Soft Endo starch consistently liberated more glucose equivalents than Redlan Normal.

The response of the white-BR starch is in contrast to observations in the 1973 starch study and studies of Davis and Harbers (1974). It appears that environmental conditions during the growing season may have an affect upon the susceptability of the starch to alpha-amylase enzymatic attack. Reasons for such varied responses of the white-BR variety are unknown.

Scanning Electron Microscopy

Raw, isolated starch granules of corn (Pioneer 3149) hetero-yellow, white-normal and waxy endosperm sorghum types at 2000X magnification are shown in Figures 18, 19, 20 and 21, respectively. Corn starch granules (Figure 18) were irregular in shape with many polygonial shaped granules. The sharp corners or squared appearance of the granules were only observed in corn starch. Starch granules of the hetero-yellow sorghum type (OK

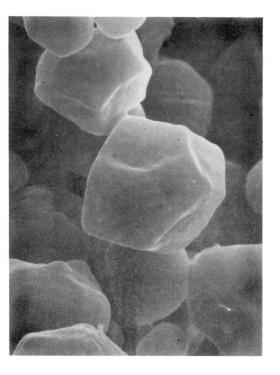


Figure 18. Isolated Corn Starch (2000X)

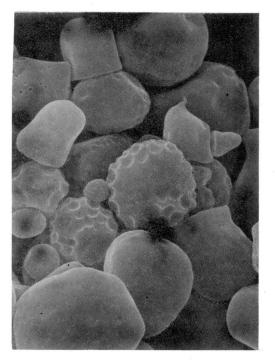


Figure 19. Isolated Hetero-Yellow Sorghum Starch (2000X)

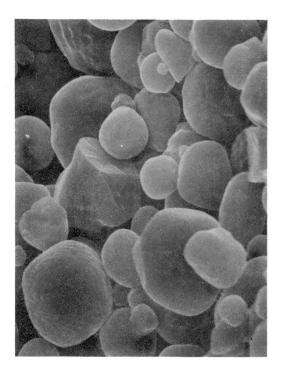


Figure 20. Isolated White-Normal Sorghum Starch (2000X)

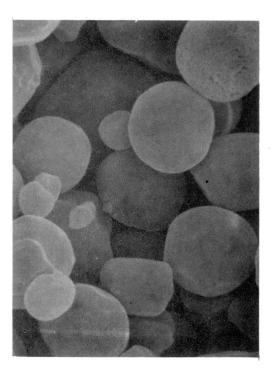


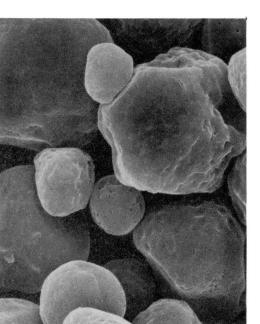
Figure 21. Isolated Waxy Sorghum Starch (2000X)

612) were more spherical with several indented granules (Figure 19). Starch granules with the dimpled surface results from shrinkage of the protein matrix during seed maturation (water loss) which forces small protein bodies into the surface of the granule. During the wet milling procedure the protein was removed leaving the indentations in the starch granule surface (Hoseney et al., 1974).

Starch granules of white-normal (Redlan normal) are very irregular in shape and size (Figure 20). Most starch granules were oval shaped with many relatively small granules present. The waxy (1133-2) starch granules were relatively smooth and round (Figure 21). The small holes observed in some granules were presumed to be caused by partial digestion of a bacterial contaminant.

Figures 22a, 22b, 23a and 23b depict raw, isolated waxy (Dwarf Redlan) and white-normal (Soft Endo) sorghum starch before and after 1½ hour incubation with alpha-amylase enzyme at 2000X magnification. The raw starch (Figures 23a and 24a) prior to incubation shows the granule surfaces to be relatively smooth. After enzyme digestion, waxy starch (Figure 22b) granules showed many small holes covering the entire surface of all granules. The enzyme digested white normal starch (Figure 23b) granules show fewer, but larger, holes in the surface, and some granules showed no evidence of degradation. These observations tend to agree with the findings of previous studies in that waxy starches were more digestible than white normal starches.

Micronized white-BR (Darset) sorghum starch is shown in Figure 24 at 480X magnification. Many starch granules escaped gelatinization and retained their granular structure while gelatinized starch forms thin sheets. Micronized corn starch (Pioneer 3306) as illustrated in Figure



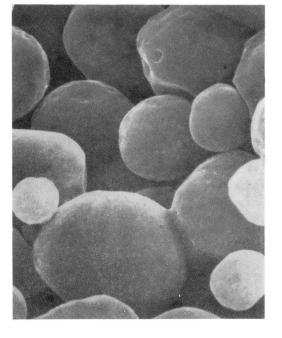


Figure 22a. Isolated Waxy Starch (2000X)

Figure 22b. Isolated Waxy Starch After Alpha-Amylase Digestion (2000X)

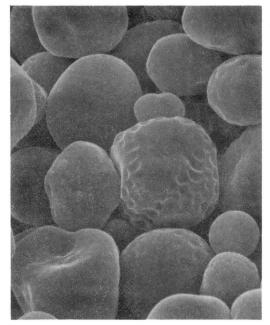


Figure 23a.

Isolated White-Normal Starch (2000X)

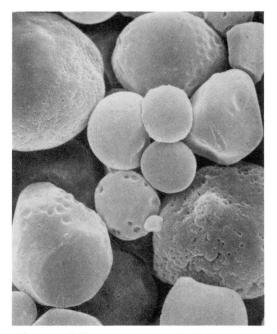
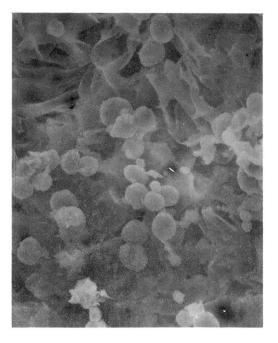


Figure 23b. Isolated White-Normal Starch After Alpha-Amylase Digestion (2000X)

25 shows the endosperm to be more crowded or packed, presumably due to rolling after the micronization process. Entirely gelatinized waxy starch (Dwarf Redlan) is shown in Figure 26. No starch granules are evident and the endosperm takes the appearance of thin lacey sheets. Figure 27 shows waxy starch (1126) that is not entirely gelatinized; granule structure is breaking down but the thin sheets have not been formed.

Peripheral endosperm of a raw, untreated corn kernel (Pioneer 3306) is shown in Figure 28. The peripheral endosperm is that near the outside of the kernel endosperm. The peripheral endosperm is a dense area with starch granules covered with a thick layer of protein white the floury endosperm contains loosely arranged starch granules with relatively little protein present (Figure 29). After reconstitution the protein matrix is hardly noticeable; the starch granules are nearly free of protein matrix (Figure 30). This softening and easy separation of protein matrix from starch granules is in agreement with similar work of McNeill et al. (1975). Figure 31 depicts the globulation of the protein matrix in the floury endosperm of reconstituted corn (Pioneer 3306). Differences between the peripheral and floury endosperm are very evident in these micrographs.

Peripheral endosperm of raw, untreated waxy (Dwarf Redlan) grain (Figure 32) is very hard and dense. When the kernel was split the starch granules were fractured and split leaving a flat surface. When the grain was reconstituted (Figure 33) the protein matrix formed globules, and starch granules are readily identified. The floury endosperm of whitenormal (Redlan Normal) sorghum grain (Figure 34) is much denser, and starch granules have a heavier protein matrix compared to corn (Figure



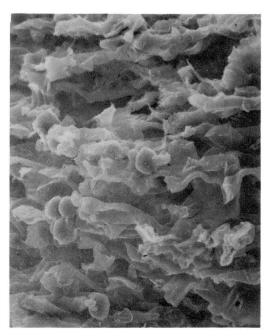


Figure 24. Micronized White-BR Grain Sorghum (480X)

Figure 25. Micronized Corn (480X)

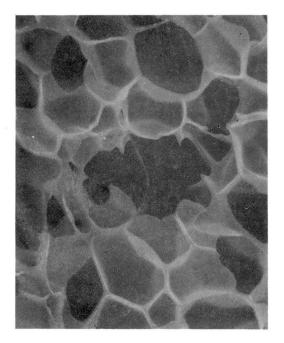
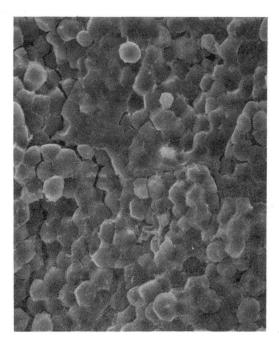




Figure 26. Micronized Waxy Grain Figure 27. Micronized Waxy Grain Sorghum (480X)

Sorghum (2000X)



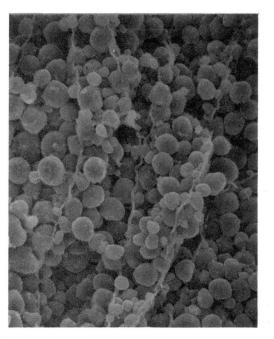
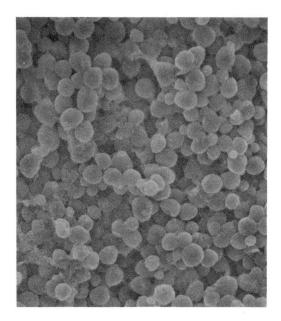


Figure 28. Peripheral Endosperm of a Raw Split Corn Kernel (480X)

Figure 29. Floury Endosperm of a Raw Split Corn Kernel (480X)



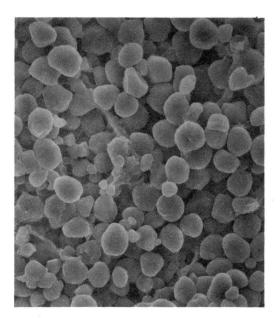


Figure 30. Peripheral Endosperm of a Split Reconstituted Corn

Kernel (480X)

Figure 31. Floury Endosperm of a Split Reconstituted Corn Kernel (480X)

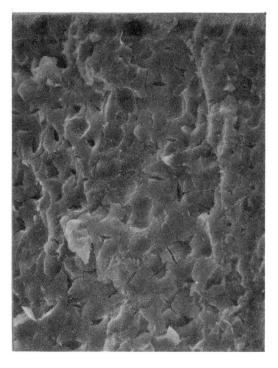


Figure 32. Peripheral Endosperm of Raw Split Waxy Grain Sorghum (480X)

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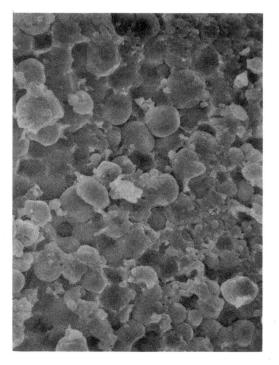


Figure 33. Peripheral Endosperm of Reconstituted Split Waxy Grain Sorghum (480X)

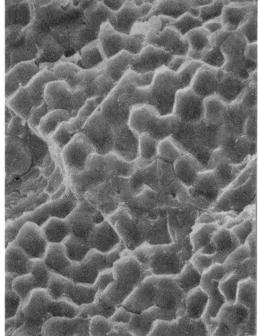


Figure 34. Floury Endosperm of Raw Split White-Normal Grain Sorghum (480X)

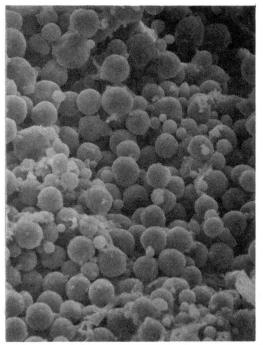


Figure 35. Floury Endosperm of Reconstituted Split White-normal Grain Sorghum (480X)

31). But when contrasted to peripheral endosperm of sorghum grain (Figure 32), the floury endosperm contains less protein or a softer protein matrix (Figure 34) which allows starch granule structure to be exhibited. Floury white-normal (Redlan Normal) endosperm when reconstituted (Figure 35) shows starch granules as being clean, smooth and round with little evidence of the protein matrix.

An enlarged view of raw, untreated peripheral endosperm of heteroyellow (OK 612) sorghum grain displaying the dense protein matrix is shown in Figure 36a. When contrasted to the peripheral endosperm of the same grain when reconstituted (Figure 36b) starch granules are more distinct and globules of hydrated protein are very evident. Many starch granules exhibit the dimpled appearance.

The surface of a split sorghum kernel shown in Figure 37 shows the difference between the peripheral and floury endosperm in raw, untreated white-BR (Darset) grain. In this particular variety the majority of the endosperm was hard, flinty peripheral endosperm. The floury endosperm constitutes the soft spongy oval area in the center of the kernel. The relative proportions of floury and peripheral endosperm varies with endosperm type (Hoseney et al., 1974). The division between the floury and the peripheral endosperm is shown in Figure 38. The peripheral endosperm is the denser area with starch granules surrounded by a heavy protein matrix while starch granules are more distinct with more intergranular air space and very little matrix protein present.

With these limited number of visual observations direct interpretations to nutritional characteristics is questionable; however, visual appraisal does show structural characteristics which may affect grain digestibility. Structure of the raw starch granule itself apparently

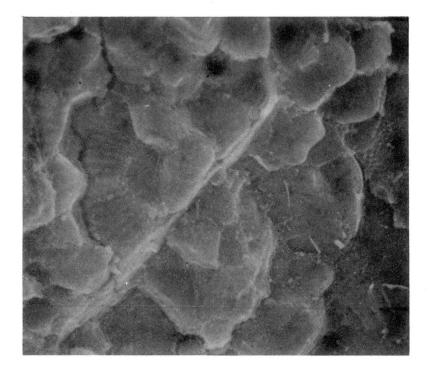


Figure 36a. Peripheral Endosperm of Raw Split Hetero-yellow Grain Sorghum

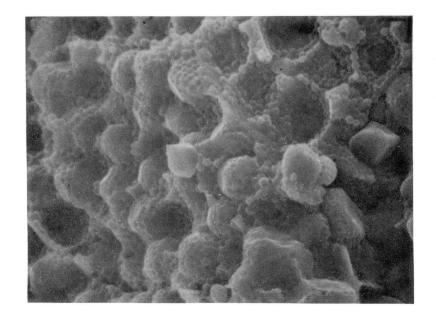


Figure 36b. Peripheral Endosperm of Reconstituted Split Hetero-yellow Grain Sorghum

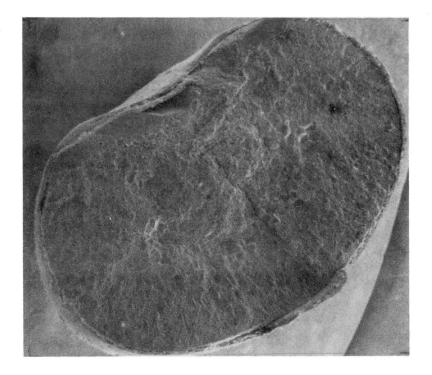


Figure 37. Surface of a Split Raw White-BR Sorghum Kernel (20X)

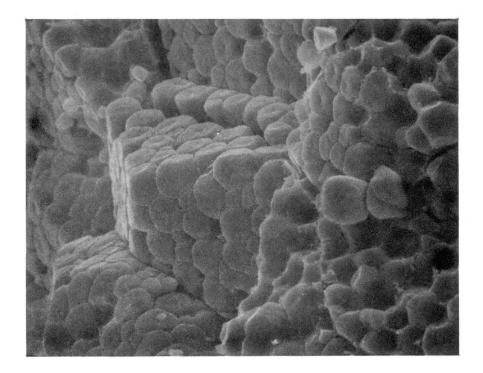


Figure 38. Endosperm of Split Raw Waxy Sorghum Kernel (480X)

has very little effect upon starch digestibility as indicated by the gas production and <u>in vitro</u> dry matter disappearance data. Photomicrographs, of the processed grains depict vividly the effects of micronization and reconstitution. The mode of action in the reconstitution process appears to be primarily an altering of the protein matrix leaving the starch granules more susceptable to enzymatic and bacterial attack. No evident starch granule alteration occurs during the reconstitution process. Improvements in digestibility of micronized grain appears to be the result of primarily alteration of the starch granules (gelatinization).

CHAPTER V

SUMMARY

A series of laboratory evaluations were conducted to determine the effects of processing (dry ground, reconstituted and micronized) on corn and sorghum of different endosperm types and to investigate properties associated with increased starch availability. Studies were also conducted to investigate starch characteristics of corn and sorghum grain and the influence of endosperm type on them.

In general, starch availability was greater in the micronized grains for all endosperm types except the white-BR type. Gas production data for both crop years suggest that starch availability of the white-BR type is improved more by reconstitution than other endosperm types. The six hour <u>in vitro</u> dry matter disappearance values indicate more readily available starch was present when the grains were micronized; however, starch digestibility, as measured by 24 hour <u>in vitro</u> dry matter disappearance, tended to favor reconstitution of the grains. Gas production and <u>in vitro</u> dry matter disappearance data of both crop years tested suggest that endosperm types with low starch availability or grain digestibility (white-BR) in the untreated or dry ground form show the greatest improvement when reconstituted. Endosperm types with better starch availability or digestibility (corn, waxy, white-normal and hetero-yellow types) in the dry ground form show the greatest improvement when the grains were micronized.

When raw, isolated starches were evaluated by gas production studies to determine susceptibility to amyloglucosidase enzymatic attack, all sorghum starches were more susceptable than corn starch, and waxy starch proved more susceptable than all other sorghum starches. <u>In vitro</u> dry matter digestibilities suggested there was no difference in starch susceptability to rumen microbial digestion, although waxy starch was slightly more digestible than other starches.

Environmental conditions during the growing season may play a very important role in the susceptibility of certain starches to alpha-amylase enzymatic attack. The susceptibility of raw, isolated starch to alphaamylase enzymatic attack was quite variable from year to year; however, hetero-yellow type starch was consistently less susceptable to enzymatic attack than other starches. White-BR starch responded very differently in the two crop years and reasons for such responses are not clear. Alpha-amylase digestion studies of raw, isolated starch indicate some sorghum starches, waxy in particular, are more susceptible to enzymatic attack than corn starch.

From the starch digestibility studies, it appears that differences in the digestibilities of untreated or dry ground normal (non waxy) grains are probably not attributable to the starch itself. The type or composition of the protein in the kernels appears to be primarily responsible for the major differences in untreated grain digestibility. Since starch does not appear to be altered during reconstitution, it is reasonable to assume that the increased digestibility is caused primarily by alterations of the protein in the grain endosperm. Research should be conducted to ascertain the composition, type and/or structure of the proteints in cereal grains and the effects of processing methods thereon.

Plant breeding efforts should also be directed in developing varieties that would be higher in feeding value.

Wet milling data showed that corn yielded more starch than the sorghum grains. Sorghum grains yielded considerably more gluten and peripheral endosperm cells than corn.

Visual appraisal, made possible by the scanning electron microscope, indicates little difference in starch granule structure among sorghums, but corn starch differs from sorghum starch. It appears that reconstitution improves grain digestibility by altering the protein matrix surrounding the starch granules, while micronization improves grain digestibility by altering (gelatinizing) the starch granules. The affect of heat treating, such as micronization, on the protein in the kernel is not known and studies should be conducted to investigate the effects of processing methods on the endosperm protein.

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APPENDIXES

TABLE XVI

WET MILLING STEEP SOLUTIONS

	Ingredient	Amount/3000 ml
Steep	I	
	Na25205	2.226 g
	Lactic Acid (20%)	225 ml
	Distilled Water	2775 ml
Steep	II	
	Na ₂ S ₂ O ₅	4.4600 g
	Lactic Acid (20%)	75 ml
	Distilled Water	2925 ml

TABLE XVII

IODINE SOLUTION FOR SOLUBLE STARCH ASSAY

Ingredient	Amount
KI	4.15 g
I ₂	.888 g
HCl (.05N)	1000 ml
Dilute 7X with .05N HCl for assay	

TABLE XVIII

REAGENTS OF THE NELSONS TE	REAGENTS	OF	THE	NELSONS	TES
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Ingredient	Amount
Nelsons Reagent A	
Anhydrons Na ₂ CO ₃	12.5 g
KNaC4H4O6·4H2O	12.5 g
NaHCO3	10.0 g
Anhydrous Na ₂ SO ₄	100.0 g
Distilled Water	500.0 ml
Nelsons Reagent B	
CuSO ₄ ·5H ₂ O	15.0 g
Conc. H ₂ SO ₄	1 to 2 drops
Distilled Water	100 ml
Arsenomolybdate Reagent	
$\binom{(NH_4)_6}{MO_7} \binom{MO_7}{24} \cdot \binom{4H_2O_7}{24}$	25.0 g
Conc. H_{2} SO $\frac{2}{4}$	21.0 ml
$Na_2H A_5O_4 \cdot 7H_2O_2$	3.0 g
Distilled Water	475 ml
Incubate at 37 ⁰ C for 24 hr.	

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