

/STRUCTURAL AND NUTRITIONAL CHARACTERISTICS OF
GRAIN SORGHUM THAT DIFFER IN ENDOSPERM TEXTURE/

by

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INTRODUCTION

Nutritional Evaluation

Chemical procedures have been used to identify and predict nutritional qualities of cereal grains. However, the chemical analyses have not consistently predicted the biological quality or nutrient availability in grain sorghum (Oswalt, 1973). Most of the information on the biological value of sorghum protein has been obtained from animal rather than human studies (Hoseney et al, 1981). Inconsistent results have been reported in the performance of ruminant and monogastric animals utilizing grain sorghum rations.

Superior performance of waxy corn compared to regular corn has been reported with ruminants (Braman et al, 1973) and swine (Jensen et al, 1973). Other investigations have shown no advantages of waxy compared to non-waxy for ruminants (McCormick and Farlin, 1974; Robinson et al, 1974) or swine (Cohen and Tanksley, 1973). Similar results have been shown with waxy sorghum (Hinders and Eng, 1970; McGinty and Riggs, 1968; Nishimata et al, 1969).

Utilizing steers in digestion and feeding trials, many workers (McCollough and Brent, 1972; McCollough, 1973; Sherrod et al, 1969; Brethour and Duttsman, 1965) found that waxy sorghum has improved nutritive value over nonwaxy sorghum grains. Similar results were reported with sheep (Nishimata et al,

1969; Sherrod and Albin, 1973). Waxy varieties produced equal gain on less feed than nonwaxy grains. Lichtenwalner et al, 1978 reported that ruminal digestibility as measured by nutrient disappearance or gas production, was significantly higher for the homozygous waxy grain than other genotypes. More soluble protein was encountered with the homozygous waxy grain. The genes that produce high amylose starch in corn, form starch that is highly resistant to enzymatic digestion, whereas those conditioned by the waxy gene are near the maximum in susceptibility to enzymatic hydrolysis (Sandsted et al, 1962). Walker and Lichtenwalner (1977) reported a 15% increase in the ruminal digestibility of the prolamines of waxy over nonwaxy grains. Sullins and Rooney (1974) and Sullins and Rooney (1975) suggested several possible reasons for the superiority of the waxy grains. They reported that the waxy grain is more susceptible to enzymatic hydrolysis because of the even distribution of protein throughout the grain kernel. In nonwaxy grain sorghum there is a greater concentration of protein in the peripheral endosperm than in other parts of the kernel.

Yellow floury endosperms show greater calorie and protein digestibility than those with white floury, white corneous or yellow corneous endosperms (Noland et al, 1977).

Most of the information on the nutritional quality of the protein of sorghum grain has been derived from studies with weanling rats as measured by weight gain and protein

efficiency ratio (PER). The interpretation of rat results for human nutrition evaluation has been discouraged because rats require more protein as a result of their faster growth and relative inactivity in test cages, and because PER measures only the quality (amino acid balance) of the protein and not the usefulness of a sample or type of grain as a food (Hoseney et al, 1981). For accurately measuring PER, other factors such as vitamins and minerals must not be limiting and protein content of the diet is generally adjusted to 10%.

The inferior nutritional quality of cereal grains in general, and sorghum in particular, has been related to the deficiency of the protein in certain essential amino acids. Biological studies have revealed that the first limiting amino acid is lysine (Pond et al, 1958; Bressani and Rios, 1962; Harden et al, 1976). Other limiting amino acids include threonine (Pond et al, 1958) and methionine (Waggle et al, 1966).

Comparing the nutritional quality of rice, corn, sorghum, wheat, barley, millet, rye, and oats, Howe et al (1965) found that albino rats gained more with a higher protein efficiency ratio (PER) when fed corn than when fed sorghum. The casein diet used as a check at 9.05% protein was two times better than corn and four times better than sorghum. Howe and Gilfillan (1970) found a significant improvement in the nutritional quality of a sorghum diet supplemented with lysine as compared to an unsupplemented diet. No significant results

were observed with lysine-supplemented rice, maize, wheat, or millet. It appeared that for the latter cereals, vitamins, minerals and possibly some other amino acids were limiting. In a second experiment, they found that addition of calcium carbonate at 1% of the diet produced significant weight gains in all the cereals tested except rice. They suggested that vitamins and minerals rather than lysine, are the limiting nutrients in cereal diets fed human infants, and that the deficiency of calcium and other nonprotein nutrients may contribute to the poor utilization of the protein in cereal grain. Essential amino acids concentrations, except for leucine and phenylalanine, were found in smaller proportion in a high protein sorghum than in a low protein one and, consequently, the low-protein diet displayed a superior biological value (Waggle et al, 1966). When isonitrogenous diets were used, rats fed the low-protein sorghum diet grew significantly faster. Shoup et al (1969) compared the nutritive value of milled sorghum grain products ranging in protein content from a white flour with 6.5% protein to a vitreous speckled product with 20.6% protein. The soft, low-protein flour was nutritionally better in terms of amino acid composition and growth of rats, than the vitreous high protein fraction. A bran-germ fraction scored better than the original grain and the vitreous fraction. Supplementation of the different fractions with lysine increased the growth rate and weight gain of rats while the PER of all the diets were similar.

Protein digestibility of six sorghum varieties and one commercial hybrid by weanling rats ranged from 71 to 75% (Ilori and Conrad, 1976). Daniel et al (1966) reported 70% sorghum protein digestibility of diets fed to 11-12 year old girls. Cohen and Tanksley (1973) studied the effect of different endosperm texture and starch type on sorghum protein digestibility in swine. The protein digestibility was less than 80% but was not significantly different for corneous or floury endosperm types. Waxy sorghum protein was slightly less digestible than that of non-waxy sorghum.

One serious nutritional implication of grain sorghum is its high leucine content (Axtell et al, 1972) which is responsible for the high incidence of pellagra in populations subsisting mainly on sorghum as their protein source (Srikantia, 1978; Gopalan, 1961; Deosthale et al, 1970). According to Deosthale et al (1970), any protein with a leucine/lysine ratio below 4.6 could be considered nutritionally safe as a major source of protein. High leucine levels were reported to interfere with niacin metabolism and increases the excretion of quinolinic acid (Gopalan and Srikantia, 1960). Rats fed sorghum diets high in leucine and adequate in tryptophan were reported to excrete more niacin and N'-methylnicotinamide than those fed wheat diets (Raghuramulu et al, 1965). For better nutrition, sorghum would need to provide correct and balanced levels of essential amino acids. Sidransky (1960) observed pathologic lesions in young rats force-fed for 3-7

days with purified diets devoid of a single essential amino acid. The lesions resembled symptoms described as present in kwashiorkor victims (a childhood disease mainly in the tropics and sub-tropics, occurring soon after weaning due to deficient quality of protein and characterized by edema, skin and hair changes, and impaired growth).

An apparent protein digestibility coefficient of 64-69% was reported for a sorghum/rice diet fed to 11-12 year old boys. A substantial reduction in nitrogen absorption and retention was observed when sorghum replaced 50% and 100% of the rice in the diet (Kurien et al, 1960). However, protein digestibility increased when sorghum content in diets of wheat and sorghum was increased. This finding suggests that human females utilize sorghum better than wheat as a nitrogen source (Obizoba et al, 1979).

The poor nutritional quality of cereal proteins for humans and monogastric animals is attributed to the deficiencies and incorrect balance of some essential amino acids. Normal corn is deficient in lysine and tryptophan (Frey, 1951; Wall and Paulis, 1978) while sorghum is deficient in lysine, threonine and sulfur-containing amino acids. The deficiency of lysine in cereal proteins is attributed to the high content of prolamine which may account for as much as 50 to 60% of the total endosperm nitrogen (Virupaksha and Sastry, 1968).

Germination

Many approaches have been suggested to improve the nutritional quality of sorghum and corn endosperm proteins. One is through direct supplementation with deficient amino acids (Daniel et al, 1966; Bornstein and Lipstein, 1971; Fernandez et al, 1974). Others are supplementation with legumes that are high in lysine content (Desai et al, 1970; Pushpamma and Devi, 1979); genetic improvement (Mertz et al, 1964; Nelson et al, 1965; Singh and Axtell, 1973); or by germination of the grains (Wang and Fields, 1978; Wu and Wall, 1980).

Germination of the seeds will allow the biochemical processes to convert the poor quality prolamine of the storage proteins to a more usable protein for both humans and animals. Encouraging results have been reported in corn (Tsai et al, 1975) and sorghum (Wang and Fields, 1978; Wu and Wall, 1980; Chavan et al, 1981; Aisien and Ghosh, 1978).

Ellis (1975) studied the relationship between germination and sorghum endosperm type and texture. Endosperm types included normal, waxy, yellow and yellow waxy. Texture varied from essentially all corneous to all floury. Lines with normal endosperm had higher germination than those with waxy or yellow endosperm. Those with corneous endosperm texture had higher germination and emergence than those with intermediate or floury texture. Similar results were reported earlier by Thasher and Dungan (1928) in corn. The vigorous germination of the flinty (corneous) endosperm was attributed

to a more rapid mobilization of starch reserves from the corneous than from the floury endosperm (Dungan, 1927).

One of the most important changes that takes place in the germinating seed is the breakdown of storage proteins in the endosperm and the synthesis of new protein in the developing new plant. Ingle et al (1964) reported a linear decline in nitrogen content in the endosperm from 24-120 hours after germination; however, there was a several-fold rise in nitrogen content of the embryo during that time. Wu and Wall (1980) found a large increase in the lysine-rich fragment (albumin) and a large decrease in kafirin and cross-linked kafirin (both low in lysine). They observed an increase in protein concentration of the germinated seeds. They attributed that to dry matter loss. There was no increase in the absolute amount of protein per kernel.

Generally, there is an increase in the relative nutritive value of germinated sorghum grain over the ungerminated one. The concentration of lysine, tryptophan (Tsai et al, 1975; Wang and Fields, 1978) and methionine (Wang and Fields, 1978) increase as a result of germination. After 10 days lysine content increased in normal sorghum from 2.2 to 3.2 g/16 g nitrogen; in high lysine sorghum lysine increased from 3.0 g to 7.8 g/16 g nitrogen in 7 days (Wu and Wall, 1980). Wang and Field (1978) found an increase in the relative nutritive value of germinated sorghum from 55.5% to 78.3% and for corn from 66.8% to 99.5%. The highest relative nutritive

nutritive value for corn was accomplished after 4 days of germination at 25°C. or 3 days at 35°C; for sorghum it was 5 days at 25°C. or 3 days at 35°C. The highest relative nutritive value achieved by germination was 78.3% for sorghum and 99.5% for corn.

The nutritive value of sorghum can also be increased by fermentation. Au and Field (1981) reported an increase in lysine and methionine levels from 9.1 to 25.68 mg/g nitrogen and from 11.25 to 32.20 mg/g nitrogen, respectively, following fermentation.

Germination also reduces the concentration of tannin in grain sorghum. Chavan et al (1981) germinated high and low tannin sorghum grains for 120 hours and noticed a reduction in tannin content by 73% and 20%, respectively.

Kernel Structure

The sorghum kernel is approximately 4.0 mm long, 3.5 mm wide and 2.5 mm thick and weighs 8-50 mg (average 28 mg), (Rooney and Clark, 1968). The kernel is a caryopsis and is composed of three main parts--pericarp, endosperm and the germ or embryo. The pericarp, the original ovary wall, is surrounded by a waxy cuticle and is composed of an epicarp or epidermis which is the outermost layer, followed by a hypoderm which is not always differentiated from the epicarp, mesocarp (middle layer), and endocarp (inner layer) (Hoseney et al, 1974). The epicarp consists of elongated rectangular cells which may be pigmented; the mesocarp cells are thin-

walled and may contain small starch granules (characteristic of grain sorghum). The endocarp consists of cross cells at right angle to the kernel axis, and tube cells parallel to the axis (Rooney and Sullins, 1976). Some sorghum varieties have a layer of pigmented cells immediately below the endocarp and above the aleurone, which is a characteristic of bird-resistant varieties (Rooney and Clark, 1968; Hosene et al, 1974).

The endosperm is located under the pericarp and is composed of a single-celled aleurone layer, peripheral endosperm, corneous endosperm, and floury endosperm (Hosene et al, 1981; Rooney and Clark, 1968). The peripheral endosperm is located beneath the aleurone and consists of 2-6 endosperm cells and contains small starch granules embedded in a dense proteinaceous matrix. Hydrolysis of the starch by enzymes and digestive fluids is slowed by the high concentration of protein bodies and protein matrix (Rooney and Sullins, 1976). The corneous endosperm is located beneath the peripheral endosperm and has a continuous interface between the starch and protein. The starch-protein bond is strong enough that some starch granules break rather than pull apart from the matrix when the kernel is fractured (Rooney and Sullins, 1976). The floury endosperm has loosely packed cells and the starch granules are spherical and covered with a thin layer of protein. The protein bodies and matrix are present, but the matrix is not continuous and consists of relatively thin

bodies have been found (Miller, 1958; Inglett, 1972). The floury endosperm is soft and readily susceptible to enzyme attack. The floury endosperm has loosely packed cells with relatively large intergranular air spaces. The opaque appearance of the soft endosperm (floury) is caused by the air pockets upon light diffraction. The hard endosperm (corneous) is translucent because it is tightly packed with no air pockets (Hoseney et al, 1974; Rooney and Sullins, 1976). But not all hard endosperms are translucent and not all soft endosperms are necessarily opaque! The texture of the endosperm is influenced by heredity and environment. Whether a certain variety is classified as floury or corneous depends on the ratio of floury to corneous endosperm within the kernel. Rooney and Sullins (1973) reported that floury sorghum varieties often contain a brown pericarp and thick pigmented testa. Bidwell et al (1922) estimated the amount of corneous endosperm in Feterita, dwarf milo, and dawn kafir as 61%, 55%, and 49%, respectively; floury endosperms were 25%, 29%, and 35%, respectively. Corneous endosperms had more protein than the floury.

The germ (embryo) comprises about 7.8 to 12.1% of the sorghum kernel (Watson, et al, 1955). The germ is attached firmly in the kernel and is more difficult to remove during dry or wet-milling than is corn germ (Watson et al, 1955; Hubbard et al, 1950). The germ consists of a large scutellum, embryonic axis, plumule and primary root.

Composition

Heredity, climate, cultural practices and soil were found responsible for the variation of protein content in sorghum grains (Waggle et al, 1967; Miller et al, 1964). Protein content of the grain ranges from 6.6% to 16% (Miller, 1958). Virupaksha and Sastry (1968) reported 8.61% to 18.21% protein in genetic varieties and 8.55% to 16.48% in hybrid varieties. Sorghum grain proteins are classified into prolamine (kafirin), glutelin, globulin and albumin (Osborne and Mendel, 1914). The ratio of prolamine to glutelin is reported to be higher in the corneous than in the floury endosperm of the kernel (Virupaksha and Sastry, 1968). Skoch et al (1970) extracted 26 to 40% of the nitrogen from sorghum meal using a sequential scheme of solvents similar to that of Osborne and Mendel. The prolamine was found to be less soluble in 70% ethanol than was the prolamine (zein) of corn. Kafirin yields were 29.9% at 65° C. compared to only 15% at 25° C. Jones and Beckwith (1970) showed that at room temperature aqueous 60% tertiary butyl alcohol could extract as much kafirin from defatted sorghum as 70% ethanol could at 60° C. Other solvents such as 8M urea, 0.1M sodium dodecyl sulfate are also suitable to extract kafirin (Skoch et al, 1970). The second largest protein fraction of sorghum is glutelin. It has a high molecular weight because of the presence of disulfide bonds. Glutelins are insoluble in neutral solvents, but are solubilized when the disulfide bonds are broken with reducing agents such as 2-mercaptoethanol. Jambunathan and

Mertz (1973) extracted 55% of the total sorghum protein with 2-mercaptoethanol. Albumin is soluble in H₂O at pH 6.0, and globulin is soluble in 0.5M NaCl.

Different proteins are composed of different combinations of amino acids. Amino acids which cannot be synthesized in vivo are known as "essential" or "indispensable" and must be provided by the food eaten. As in most cereals, sorghum is deficient in lysine, the amino acid that occurs in greatest quantity in newly-formed tissue proteins. Therefore, adequate lysine is critical for growth of children (Oke, 1976). Besides lysine, sorghum proteins are low in threonine, methionine, arginine, histidine, glycine and tyrosine (Wall and Blessin, 1969; Virupaksha and Sastry, 1968). Sorghum is high in glutamic acid (glutamine), proline and aspartic acid (asparagine) (Rooney, 1973). The albumin and glutelin fractions contain relatively higher amounts of lysine, tryptophan and threonine than kafirin. However, kafirin is high in glutamine, leucine and nonpolar amino acids such as proline, valine and alanine (Wall and Paulis, 1978). Kafirin and glutamic acid are positively related to total protein content of the grain (Rooney, 1973). On the other hand, lysine content is negatively related to total protein (Virupaksha and Sastry, 1968; Shoup et al, 1970; Hosoney et al, 1981). The lysine concentration in grain sorghum is higher in grains harvested early due to frost damage (Shoup et al, 1970); and lower towards

the end of ripening (Gupta and Gupta, 1974). Higher lysine levels were also reported in the bran fraction (Shoup et al, 1969). The bran-germ fraction contains approximately four times as much lysine and two times as much arginine and glycine as the endosperm protein; whereas the glutamic acid, proline, alanine, leucine and tyrosine contents are half that of the endosperm protein (Shoup et al, 1969). The significantly higher levels of lysine in the floury over the corneous endosperm (Shoup et al, 1969) does not rule out the possibility of a corneous endosperm with a high lysine content (Rooney and Sullins, 1973). In general, an opaque floury endosperm is indicative of a protein high in lysine. Nanda and Rao (1975a) found the following significant negative correlations: protein with yield, seed size, and grain-hardness; lysine with leucine, isoleucine, and cystine; leucine with methionine; carotene with protein, methionine, and cystine. The following significant positive correlations were observed between: grain hardness with seed size, and lysine; B-carotene with lysine, threonine, isoleucine and leucine.

In all cereal grains, including sorghum, the starch content is negatively correlated to protein. Carbohydrates including starch, cellulose, pentosans, and simple sugars comprise about 80 to 85% of the sorghum kernel (Rooney and Clark, 1968). Generally sorghum and corn starches have the same properties, small differences in the swelling power and

starch paste viscosity. Varietal differences within corn and sorghum are greater than the differences between the two crops (Horan and Heider, 1946). Normal sorghum starch contains 20-28% of the straight-chain amylose and 72 to 80% of the branched-chain amylopectin (Rooney and Sullins, 1976; Deatherage et al, 1955). Waxy sorghum starch is 100% amylopectin and has a higher amylograph paste viscosity than waxy corn starch. Gelatinization temperature range of sorghum and corn starch is 67 to 77° C and 62 to 72° C, respectively (Watson and Moffet, 1959). Waxy sorghums have less starch but more oil and protein than nonwaxy grains (MacMasters and Hilbert, 1944). The grain has about 1 to 2% total sugars, made up of about 0.85% sucrose, 0.09% glucose, 0.09% fructose and 0.11% raffinose (Nordin, 1969). There are 2.5 to 5.21% pentosans in the grain of which 22% is found in the pericarp, 7.5% in the germ and only 1% in the endosperm (Rooney and Sullins, 1976). Positive correlations were found between pentosan content and total grits yield (Norris and Rooney, 1970).

The sorghum grain contains approximately 3 to 4% ether extract, part of which is wax (Watson and Moffet, 1959). The oil is produced in large quantities as one of the products of wet-milling. The oil is composed of a number of fatty acids of which linolenic is found in largest concentration (Kummerow et al, 1947). Other fatty acids include oleic, palmitic, stearic, myristic and hexadecenoic acids, respectively, in decreasing order (Kummerow et al, 1947).

The whole grain sorghum was reported to contain 2.66 to 3.37% free lipids and 0.14 to 0.28% bound lipids (Rooney and Clark, 1968). Kummerow (1946) found that sorghum oil was less saturated than corn oil and contained more oleic and stearic acids, and less linoleic, myristic and palmitic acids than corn oil.

The general objectives of this study were: to evaluate the nutritional quality of six sorghum cultivars that differ in endosperm type and texture on the basis of amino acid content and rat performance; to study the germination process as a simple method to improve the nutritional quality of the grain; and whether the endosperm type or texture could affect germination of grain sorghum.

MATERIALS AND METHODS

Samples SR-788, SR-712, SR-778, SR-1429 and SR-919 were supplied by Seed Research Associates, Inc., Scott City, KS. The bulk red sorghum was obtained from the KSU feed mill.

Diet Composition

Sorghum grain samples used for this study ranged in texture from corneous to flour endosperm and included two waxy cultivars. Endosperm texture was based on microscopical examinations of half kernels. Grain samples were cleaned of hulls and dirt and ground to pass a 36 wire sieve.

Protein content of the six grain samples ranged from 7.86% to 12.48%. Sample SR-919 had the highest protein content (12.5%). The ration for this group was formulated on the basis of 92% sorghum, no casein was added. Protein content for the other rations was brought up to 12.5% by adding calculated amounts of casein. Added casein ranged from zero (SR-919) to 5.11 g/100g (bulk sorghum) as in Table 1.

Lysine, methionine (plus half cystine), and tryptophan contents of the grains were below maintenance levels. The three deficient amino acid levels were upgraded to the recommended level by adding calculated amounts to the rations. The protein content in each amino acid added was also calculated. All the rations were made isonitrogenous. The overall sources of protein in the rations are presented in Table 2. In addition 2% vitamin premix (Table 3), 2% mineral

premix (Table 4) and 2% corn oil were added to each ration. Composition of all rations is shown in Table 1.

Rat Feeding

This experiment was performed in the Animal Nutrition Lab., Dept. of Biochemistry, KSU.

Twenty-four male weanling rats, with similar initial weights were used for the nutritional evaluation of six sorghum grain diets. The rats were arranged in groups of four so as to make their average weights close to their overall mean weight. The rats were housed at random in individual wire-mesh cages and kept under controlled environmental conditions.

Experimental diets and water were given to each rat ad libitum for 21 days. Feed cups were designed and attached to the cages to reduce feed spillage. Spilled feeds were weighed and subtracted from the amount of ration consumed. Feed cups were checked every day and water changed on alternate days.

Individual rat weight gains and feed consumption were measured at the end of each seven-day period. Feed conversion ratio was determined as grams of feed consumed per gram of weight gained.

Amino Acids

Amino acid composition of whole grains, untreated and those sprouted for 5 days, was determined with D-300 amino

Table 2. Protein Sources of the Diet

% Protein	SR-788	SR-712	SR-778	SR-1429	SR-919	Bulk Sorghum
Supplied by Sorghum	11.80	9.61	11.87	11.67	12.48	7.42
Supplied by Casein	0.68	2.87	0.61	0.81	0.00	5.06
-by added Lysine	0.68	0.502	0.646	0.636	0.706	0.331
Trypt.	0.083	0.061	0.074	0.074	0.083	0.041
Meth.	0.198	0.194	0.228	0.217	0.178	0.163
% in the Ration	13.44	13.24	13.43	13.41	13.45	13.02

Table 3. Vitamin Premix

	g/Kg Diet
Vitamin A (10,000 I.U./g)	0.500
Vitamin B (10,000 I.U./g)	0.067
Vitamin E (25% supply)	0.160
Menadione	0.007
Choline Chloride	1.00
Niacin	0.020
Ca Pantothenate	0.008
Riboflavin	0.003
Pyridoxine Hcl	0.007
Thiamin Hcl	0.004
Folic Acid	0.0005
Vitamin B ₁₂ (0.1% preparation)	0.101
Biotin	0.00015
	2.02
Glucose	18.00
Total	20.02 g/Kg

Table 4. Salt and Mineral Mix

	g/kg diet
NaCl	3.0
KCl	2.0
P + Ca (mono & Di CaPO ₄)	12.2
CaCO ₃	2.5
Trace Mineral Mix *	0.3
Total	20.00

* Contained in ppm ., Mn 10, Fe 10, Ca 14, Cu 1, Zn 5, I 0.3, and Co 0.1.

acid analyzer (Dionex Corporation). A single column accelerated system was used. The samples were hydrolysed with p-toluenesulfonic acid for 31 hours at 100°C. Amino acid content of the grains was expressed on dry-weight basis and 100% protein recovery as grams amino acid per 100 grams protein.

Protein content of the samples was determined by the micro-kjeldahl procedure (AACC 1976) with a conversion factor of 6.25.

Germination

About 20 grams samples were placed in one liter flasks and washed 4 times with distilled water stirring and shaking each time to remove dirt and husk. The grains were then soaked in 700 ml distilled water for 24 hours at 25°C. Water was changed 3 times during that period. Soaking prior to germination proved to give a more uniform germination (Wang and Fields, 1978). To retard mold growth, 300 ml of 0.2% formaldehyde was then added for 40 minutes. Formaldehyde was found effective and has minimum, if any, deleterious effects on the grains (Wu and Wall, 1980). The grains were then washed 3 times and let stand in distilled water for another 20 min.

One hundred sound kernels were placed between two moistened filter papers and placed in a petri dish. The petri dishes were covered, sealed with Parafilm and wrapped with aluminum foil. Germination was carried for 1 to 5 days at 25°C. This experiment was done in duplicates.

Percent germination and kernel weight loss (after drying) were calculated and a sample was taken for viewing with scanning electron microscope. The rest of the kernels were dried in an air oven at 50°C, ground in a Wig-L-Bug (Crescent Dental Mfg., Chicago, IL) and stored at 4°C for protein and amino acid analysis.

Scanning Electron Microscopy

Effects of germination on the structure of different sorghum kernels were studied by scanning electron microscopy. Samples which had been germinated for 1 to 5 days were first freeze dried and individual kernels were fractured at the center with a blunt blade to break rather than to have a smooth cut. Half kernels were mounted on aluminum stubs with silver paste, coated with gold-palladium and observed under an autoscan ETEC electron microscope. Photographs were taken at an accelerating voltage of 20 KV.

RESULTS AND DISCUSSION

Amino Acid

Data listed in Table 5 show amino acid composition of six sorghum varieties. Whole grain hydrolyzates were used for analysis. The values are expressed as grams of amino acid per 100 grams of protein. Protein content of the samples varied from a low of 7.86% (bulk sorghum) to a high of 12.48% (SR-919). The corneous varieties (SR-778, SR-1429, SR-919) showed higher protein contents compared to the other samples.

All samples had low concentrations of the amino acids, lysine, methionine, threonine, arginine, glycine, isoleucine, cystine and higher levels of glutamic acid, aspartic acid, leucine, alanine and proline. These values are in general agreement with what was reported for sorghum (Virupaksha and Sastry, 1968; Wall and Blessin, 1969; and Rooney, 1973).

The bulk sorghum with the lowest protein content, had higher amounts of lysine, methionine, arginine, histidine and lower levels of glutamic acid, aspartic acid, alanine and leucine. This may indicate as suggested in the literature (Virupaksha and Sastry, 1968) that the lower the quantity of sorghum protein within a sample, the better the nutritional quality of that protein.

Table 5. Amino Acid Analysis (g/100 protein)*

Amino Acid	SR-788	SR-712	SR-778	SR-1429	SR-919	Bulk Sorghum
1 Aspartic Acid	6.51	7.18	7.06	7.12	6.68	6.14
2 Threonine	2.91	3.10	2.96	2.72	2.85	3.06
3 Serine	4.67	4.79	4.65	4.49	4.60	4.69
4 Glutamic Acid	20.84	20.04	20.46	20.64	21.45	19.15
5 Proline	8.16	7.78	8.21	7.83	7.25	8.20
6 Glycine	2.76	2.96	2.80	2.54	3.11	3.07
7 Alanine	11.20	11.16	10.90	10.83	11.01	10.02
8 Half Cystine	1.19	1.24	0.92	0.89	1.56	1.48
9 Valine	3.77	3.89	3.57	3.37	4.04	3.86
10 Methionine	1.66	1.59	1.34	1.50	1.80	2.07
11 Isoleucine	2.74	2.69	2.80	3.15	2.95	2.92
12 Leucine	14.50	13.77	14.20	14.24	13.42	12.68
13 Tyrosine	4.23	4.14	4.31	4.25	4.01	4.42
14 Phenylalanine	5.25	4.97	5.62	5.29	4.81	5.43
15 Histidine	3.33	3.72	3.39	3.12	2.81	3.92
16 Lysine	1.26	1.59	1.56	1.54	1.38	2.03
17 Ammonia	2.50	2.58	2.48	3.34	3.16	2.44
18 Arginine	2.51	2.82	2.79	3.14	3.11	4.41
% Protein	11.89	9.92	11.95	11.77	12.48	7.86

* Except tryptophan

Table 6 shows the essential amino acid content, except tryptophan. Their estimated biological value in terms of chemical score compared to the provisional standard for humans established by WHO (1973) are presented in Table 7. The most seriously limiting amino acid in all the samples is lysine with only 23 to 37% of the requirement. This fact becomes of great importance in parts of Africa and Asia where people are subsisting on sorghum as a main source of food. The data presented in Table 7 also show that all the samples are deficient in isoleucine, threonine, methionine, and valine. Therefore, supplementing the sorghum flour with cotton seed, peanuts, soybeans and other pulses that are known to have high concentrations of lysine and sulfur-containing amino acids will significantly improve the nutritional quality of sorghum. Leucine is present in very high concentrations in all samples. The high leucine content in sorghum has been implicated as a possible cause of pellagra in areas where sorghum is used as a staple food (Deosthale et al., 1970).

Rat Feeding Experiment

The rat weight gains are presented in Table 8. Statistical analysis of the data showed that only the bulk sorghum was significantly different than SR-919 in terms of weight gains. All the other means were not significantly different at 0.05 level of significance. The best weight was produced

Table 6. Selected Essential Amino Acid Content (g/100 g protein)

Amino Acid	<u>Variety</u>					Bulk Sorghum	WHO*
	SR-788	SR-712	SR-778	SR-1429	SR-919		
Lysine	1.26	1.59	1.56	1.54	1.38	2.03	5.5
Threonine	2.91	3.10	2.95	2.72	2.85	3.06	4.0
Meth. + Cys/2	2.85	2.83	2.26	2.39	3.36	3.55	3.5
Leucine	14.50	13.77	14.20	14.24	13.42	12.68	7.0
Isoleucine	2.74	2.69	2.80	3.15	2.95	2.92	4.0
Valine	3.77	3.89	3.57	3.37	4.04	3.86	5.0
Phe + Tyr	9.48	9.11	9.93	9.54	8.82	9.85	6.0

*Standard for Humans Established by WHO.

Table 7. Nutritional Quality of Different Sorghum Samples Based on Their Chemical Scores*

Amino Acid	Variety					Bulk Sorghum
	SR-788	SR-712	SR-778	SR-1429	SR-919	
Lysine	23	29	28	28	25	37
Threonine	73	78	74	68	71	77
Methionine + Cyst/2	81	81	65	68	96	101
Leucine	207	197	203	203	192	181
Isoleucine	69	67	70	79	74	73
Valine	75	78	71	67	81	77
Phenylalanine + Tyrosine	158	152	166	159	147	164

*
$$\frac{\text{Amino acid present (g/100 g protein)}}{\text{Amino acid recommended by WHO (g/100 g protein)}} \times 100$$

by the bulk sorghum followed by SR-788, SR-1429, SR-778, SR-712 and SR-919 respectively.

Sample SR-712 (white waxy) had slight pigmentation due to field damage. This might explain the lower weight gains of this sample compared to SR-788 which was also a white waxy variety.

The amount of feed consumed and feed conversion ratio are shown in Tables 9 and 10. The feed ratio is calculated as grams of feed consumed per gram of weight gain. The lower the ratio, the higher the nutritional quality of the feed. There was no significant difference between samples in terms of feed conversion ratio or amount of feed consumed. However, the best feed conversion ratio was obtained with SR-788 (waxy) and the bulk sorghum (normal), both with intermediate endosperm texture. Samples with corneous endosperm resulted in high feed conversion ratios. Within the corneous endosperm types, there was a trend towards higher feed ratio with the increase in corneous endosperm. The protein efficiency ratios (PER) followed closely the feed conversion ratios and increased with the increase in corneous endosperm as shown in Table 10.

If we compare the bulk sorghum and SR-919 in terms of protein sources of the diet, Table 11, one might think that the superiority of the bulk sorghum was due to the addition of relatively large amounts of casein. However, SR-712 had the next highest amount of casein added and still ranked second worst in terms of weight gain. On the other hand, SR-788 had the 3rd lowest amount of casein added, but ranked second best

Table 8. Comparison of Rat Weight Gain

Diet	7 Days	Weight Gain (g)	
		14 Days	21 Days*
SR-788	47.5	106	171.7 ab
SR-712	49.0	103	160.8 ab
SR-778	45.5	100.5	164.3 ab
SR-1429	48.5	102.5	166.0 ab
SR-919	40.0	85.0	149.5 b
Bulk Sorghum	55.3	115.8	181.3 a

*Values followed by the same letter(s) are not significantly different at 0.05 level of significance.

Table 9. Weekly Feed Consumption of Rats

Diet	Feed Consumed (g)		
	7 Days	14 Days	21 Days*
SR-788	113.8	239.3	393 a
SR-712	114.5	242	387.5 a
SR-778	112.8	240	393.8 a
SR-1429	122.3	264.8	420.5 a
SR-919	113	227	384 a
Bulk Sorghum	125.8	267.5	431.3 a

*Values followed by the same letter are not significantly different at 0.05 level of significance.

Table 10. Feed Consumed, Weight Gain, Protein Efficiency Ratio (PER) and Feed Conversion Ratio after Twenty-One Days

Diet	Weight Gain (g)	Feed Consumed (g)	Feed Conversion Ratio ^a	PER
SR-788	171.67	393.00	2.29	3.25
SR-712	160.75	387.50	2.41	3.13
SR-778	164.25	393.75	2.40	3.11
SR-1429	166.00	420.50	2.53	2.94
SR-919	149.5	384.00	2.57	2.90
Bulk Sorghum	181.25	431.25	2.38	3.23

a Grams of feed consumed per one gram of weight gained.

Table 11. Effect of Protein Sources on Rat Performance

	<u>Diet</u>					Bulk Sorghum
	SR-788	SR-712	SR-778	SR-1429	SR-919	
% Protein Supplied by Sorghum	11.80	9.61	11.87	11.67	12.48	7.42
% Protein Supplied by Casein	0.68	2.87	0.61	0.81	0.00	5.06
% Protein Supplied by Amino Acids	0.961	0.757	0.948	0.927	0.967	0.535
Weight Gain	171.67	160.75	164.25	166.00	149.50	181.25
Feed Conversion Ratio	2.29	2.41	2.40	2.53	2.57	2.38

in weight gains and the best in terms of feed conversion ratio. Therefore, under this experiment's conditions, there was no apparent relationship between the amount of added casein and rat weight gains. This might be expected since all diets were made isonitrogenous, and the individual amino acids, lysine, tryptophan, and methionine were added to bring them to the recommended allowances for rat growth.

The amount of sorghum in the diet seems not to correlate to rat weight gains except for the bulk sorghum having the highest weight gain and least amount of sorghum in the diet.

All grain sorghum samples used in this study were from low-tannin varieties. The commercial bulk sorghum had relatively more tannin (0.68 mg/100 mg) than the other samples but is still considered a low-tannin sorghum. The study shows that lower amounts of tannin in the diet does not significantly affect the growth rate of rats when the diets are supplemented to optimum protein, vitamins and minerals levels and adequate amino acid balance. The bulk sorghum which had relatively high amounts of tannin, gave the best weight gain. Palatability of this sample was not affected since the average feed consumption for this group was higher (Table 12).

Evaluation of endosperm type and texture on the basis of rat performances showed that sorghum grains with waxy endosperm type and intermediate texture and those with normal type and intermediate texture scored better in terms of weight gain and feed conversion ratio than grains with normal type and corneous texture (Table 13). The endosperm texture seemed to

Table 12. Effect of Tannins on Rat Performance

Variety	Weight Gain (g)	Feed Consumed (g)	Feed Ratio	Tannin (mg/100 mg) d.b.
SR-788	171.7	383.0	2.29	0.09
SR-712	160.8	387.5	2.41	0.11
SR-778	164.3	393.8	2.40	0.20
SR-1429	166.0	420.5	2.53	0.11
SR-919	149.5	384.0	2.57	0.17
Bulk Sorghum	181.3	431.3	2.38	0.68

Table 13. Effect of Kernel Characteristics on Rat Performance

Sample Designation	Pericarp Color	Testa	Endosperm Type	Endosperm Texture	Tannin (mg/100 mg)	Weight Gain(g)	Feed Consumed(g)	Feed Conversion Ratio
SR-788	White	Absent	Waxy	Intermediate*	0.09	171.7	393.0	2.29
SR-712	White	Absent	Waxy	Intermediate	0.11	160.8	387.5	2.41
SR-778	White	Absent	Normal	Corneous	0.20	164.3	393.8	2.40
SR-1429	White	Absent	Normal	Corneous	0.11	166.0	420.5	2.53
SR-919	White	Absent	Normal	Corneous	0.17	149.5	384.0	2.57
Bulk Sorghum	Red	Absent	Normal	Intermediate	0.68	181.3	431.3	2.38

* Relatively more floury

affect the rat performance. Samples SR-778, SR-1429, and SR-919 all have corneous endosperms and gave relatively poor results. In addition, sample SR-919 was more corneous than SR-1429 and SR-1429 more corneous than SR-778 and the feed ratios were higher (lower nutritional quality) with the increase in corneous endosperm.

It appears that the corneous endosperm has an inferior nutritional quality. The starch granules in the corneous endosperm are surrounded by a layer of protein matrix and the starch-protein bond is strong. On the other hand, the protein matrix in the floury endosperm is very thin and not continuous. It appears that the digestion of the starch of the corneous endosperm by rats is delayed due to the presence of the protein matrix. On the other hand, the floury endosperm having a discontinuous protein matrix will be more friable and the particle size will be small, having a larger surface area and, therefore, more easily digested.

Germination

Percentage germination of the different sorghum grain samples are presented in Table 14. The waxy varieties, SR-788 and SR-712 had low germination. After 5 days of germination, more than 50% of those varieties did not germinate. Maximum germination was attained after three days.

Varieties with normal endosperm type and corneous texture, SR-1429, SR-919 or normal endosperm type and intermediate texture bulk sorghum, had significantly higher germination than the waxy ones. By the end of 5-day germination about 90% of the kernels sprouted.

Table 14. Germination Percentage and
Kernel Weight-Loss

Day	<u>Germination (%)</u>				Bulk Sorghum
	SR-788	SR-712	SR-1429	SR-919	
1	36	21	85	75	83
2	39	39	86	83	83
3	48	39	90	86	85
4	48	30	88	84	86
5	43	35	90	90	89
	<u>Weight Loss (%)</u>				
1	14.3	14.1	13.6	8.1	21.9
3	16.7	18.3	13.8	17.6	23.5
5	17.5	19.1	16.4	17.4	26.9

Table 15. Change in Protein Content of Sorghum as a Result of Germination (Percent Dry Basis)

Treatment	<u>% Protein</u>				Bulk Sorghum
	SR-788	SR-712	SR-1429	SR-919	
None	11.89	9.92	11.77	12.48	7.86
1-Day Germination	12.41	10.48	12.52	12.72	8.95
3-Day Germination	12.63	10.74	12.91	12.87	9.23
5-Day Germination	12.85	11.24	13.28	13.10	9.28
% Protein Increase*	8.1%	13.3%	12.8%	5.0%	18.1%

*% protein increase over control

Table 16. Effect of Germination on Selected Essential Amino Acid Contents (g/100 g protein)

		A				Bulk Sorghum
		Amino Acid				
		SR-788	SR-712	SR-1429	SR-919	
Ile	Control	2.74	2.69	3.15	2.19	2.92
	5-day germination	3.51	3.43	3.95	3.80	3.56
	% change	+28.1%	+27.5%	+25.4%	+28.8%	+21.9%
Leu	Control	14.50	13.77	14.24	13.42	12.68
	5-day germination	12.73	12.86	12.10	10.93	11.17
	% change	-12.2%	-6.6%	-15.0%	-18.6%	-11.9%
Lys	Control	1.26	1.59	1.54	1.38	2.03
	5-day germination	1.77	1.98	2.25	2.60	2.50
	% change	+40.5%	+24.5%	+46.1%	+88.4%	+23.2%
Met + Cys/2	Control	2.85	2.83	2.39	3.36	3.55
	5-day germination	3.08	3.36	2.79	4.22	4.05
	% change	+8.1%	+18.7%	+16.7%	+25.6%	+14.1%
Phe + Tyr	Control	9.48	9.11	9.54	8.82	9.85
	5-day germination	10.19	10.10	9.88	9.60	9.70
	% change	+7.5%	+10.9%	+3.6%	+8.8%	-1.5%
Thr	Control	2.91	3.10	2.72	2.85	3.06
	5-day germination	3.01	3.20	2.83	3.00	3.13
	% change	+3.4%	+3.2%	+4.0%	+5.3%	+2.3%
Val	Control	3.77	3.89	3.37	4.04	3.86
	5-day germination	3.90	4.10	3.60	4.36	4.10
	% change	+3.4%	+5.4%	+6.8%	+7.9%	+6.2%

Weight loss was determined for all the samples (Table 14). Weight-loss increased with germination time. There was no correlation between percent germination and weight loss because the waxy varieties with the low germination percentages had comparable weight losses with other samples having higher germinations.

Protein content of all samples increased during germination (Table 15). Protein content increased with germination time. The apparent protein increase of the germinating seed is because of the dry matter loss.

Data listed in Table 16 show the change in essential amino acid content (except tryptophan) of the germinated seeds. The control samples (no sprouting) had lysine as the most limiting amino acid (Table 7). After five days of germination we noticed a considerable increase in lysine content. The largest increase in lysine was 88% compared to the control for SR-919. Higher increments in lysine was reported by Wang and Fields (1978). In our experiment the sprouted grains were not analyzed separately. Each 100 kernels of sprouted and unsprouted grains were ground together and a sample was taken for analysis. From economical point of view it will be costly, on a large scale production, to separate the unsprouted grains.

Other than lysine, higher amounts of isoleucine, methionine + half cystine and phenylalanine + tyrosine, were found. The bulk red sorghum showed slight decrease in phenylalanine +

tyrosine. All samples had slight increase in threonine and valine. Leucine content was lower than in the controls. The corneous varieties, SR-1429 and SR-919, had the highest reduction in leucine and most increase in lysine, valine, and threonine.

Germination apparently improved the nutritional value of sorghum grain protein, especially for the corneous varieties. The corneous endosperm contained more protein bodies which are high in prolamine (kafirin) and low in lysine. It appears that sprouting modified the prolamines of the protein bodies to a more nutritious form for the seedling.

Scanning Electron Microscopy

The effect of germination on the structure of the endosperm under the aleurone layer was studied by scanning electron microscopy (SEM). Fig. 1, top and bottom show sections of untreated and treated waxy grain (SR-788) respectively. The untreated kernel has protein bodies scattered throughout the endosperm. The starch granules are loosely packed. Some starch granules are polygonal while others are spherical in shape. The starch granules are smaller in size under the aleurone and increasingly larger towards the center of the kernel. The germinated kernel shows an increase in the size of the aleurone cells. The protein bodies are fewer in number than in the control. Plenty of indentations are observed on the surface of the starch granules due to the removal of protein bodies. Fragments of protein matrix are present between some starch granules.

Fig. 1. Scanning electron photomicrographs of waxy sorghum (SR-788). Control (top) and germinated for 5 days (bottom).

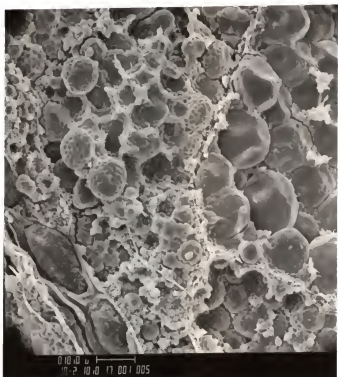
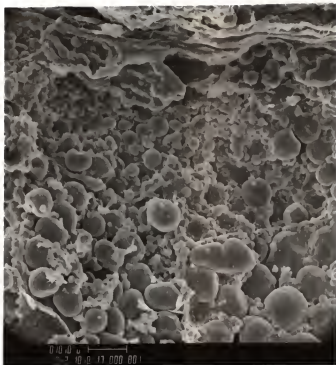


Fig. 2. Scanning electron photomicrographs of corneous sorghum (SR-919). Control (top) and germinated for 5 days (bottom).

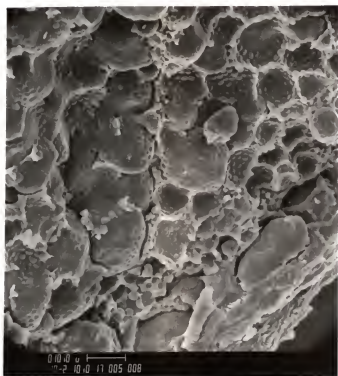
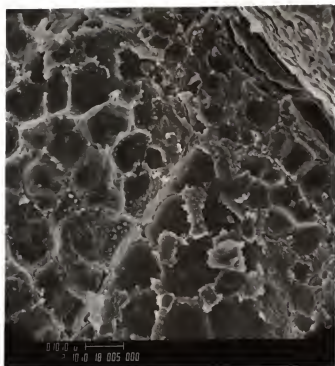
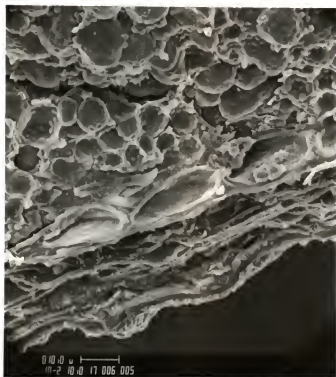
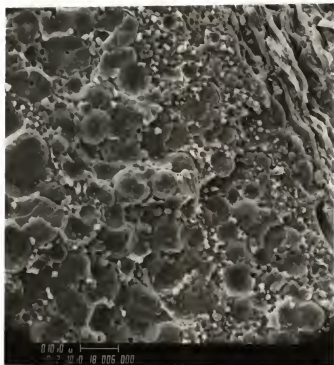


Fig. 3. Scanning electron photomicrographs of grain sorghum (bulk). Control (top) and germinated for 5 days (bottom).



SEM photos of the corneous variety, SR-919, are illustrated in Fig. 2. The starch granules of the untreated kernel are polygonal in shape and very tightly packed with no air spaces in contrast to that of the waxy variety, SR-788. A continuous protein matrix is present between the starch granules. Numerous protein bodies are embedded in the matrix protein. On the other hand the treated kernel showed reduction in the amount of protein bodies, leaving behind numerous indentations on the surface of the starch granules. Part of the protein matrix had been dissolved and as a result some air spaces are present between the starch granules. The germinated kernels appear softer and more easily fractured. than the control due to the weakening of the bond that glue the granules together.

Photographs of the bulk sorghum, with normal endosperm type and intermediate texture are shown in Fig. 3. The protein bodies are more concentrated in the peripheral endosperm. In the sprouted kernel most of these protein bodies have been removed.

The modification of the subaleurone endosperm was more intense with the corneous variety, SR-919, than the other samples. Germination modify the endosperm of grain sorghum by converting the protein bodies (high in kafirin) into a more nutritious form. The quantitative increments in the amount of lysine by germination support this fact.

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STRUCTURAL AND NUTRITIONAL CHARACTERISTICS OF
GRAIN SORGHUM THAT DIFFER IN ENDOSPERM TEXTURE

by

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ABSTRACT

Six sorghum cultivars that varied in their endosperm characteristics were evaluated structurally (SEM) and nutritionally (amino acid composition and rat weight gains). Effect of germination on the nutritional quality of the grains was also studied.

Protein content of the grains ranged from 7.0 to 12.5%. Low concentrations of the amino acids lysine, methionine, threonine, arginine, glycine, isoleucine, cystine and high levels of glutamic, aspartic, leucine, alanine and proline were found. The low-protein cultivars were found to have a higher level of lysine, methionine, arginine and histidine. The first limiting amino acid in all the samples, in terms of chemical score, was lysine with only 23% to 37% of the requirement.

A significantly higher rat weight gain was obtained with a commercial bulk sorghum (normal endosperm type and intermediate texture) than with the other samples. Other cultivars did not result in different weight gains. No significant differences were found among the diets in terms of feed conversion ratios. The best feed conversion ratio was obtained with SR-788 (waxy) and the bulk sorghum, both with intermediate endosperm texture. The study showed that low levels of tannin in the diet do not significantly affect rat growth when the sorghum diets are supplemented with vitamins, minerals and critical amino acids calculated to be at the required levels. It appears that

the nutritional quality of sorghum grain becomes lower with increase in the corneous endosperm of the kernel.

The waxy sorghum cultivars had poor germination (35% to 43%) compared to the others (90%). Kernel weight-loss and protein content increased with germination time. Germination induced a higher concentration of lysine over the control (88% lysine for SR-919). Leucine level decreased in all samples as a result of germination. Germination, apparently improved the nutritive value of sorghum grains, especially the corneous cultivars. It appears that germination modifies the prolamine (kafirin) of the protein bodies to a more nutritious form of protein.

Scanning electron microscopy showed that the germinated kernels had fewer protein bodies than the control. Modification of the subaleurone endosperm during germination was more intense in the corneous varieties.

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