Physical Changes in the Kernel During Reconstitution of Sorghum Grain[1]

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ABSTRACT

Reconstitution is a method of improving the feed efficiency of sorghum grain as feed for ruminants. The process of reconstitution consists of anaerobic storage of whole grain at 25 to 30% moisture for 21 days, followed by grinding and feeding. The purpose of this research was to study four different methods of processing grain in order to describe or elucidate the reasons for the improved performance of animals fed reconstituted grain over dry grain. The four processing methods used were: Dry-ground (D-G), ground-reconstituted (G-R), ground-reconstituted and ground (G-R-G), and reconstituted whole kernels and ground (R-G). Mean particle size distribution of the processed grain was smaller for the R-G and G-R-G treatments than the D-G and G-R treatments. Microscopic analyses indicated that the structure of the endosperm of reconstituted grain was modified, which facilitated the release of free starch granules and protein bodies and accounted for the high proportion of small particles in the ground grain. The release of starch and protein, combined with the decrease in particle size, probably account for the increased feed efficiency of the reconstituted grain. The probable cause of the modifications is enzymatic hydrolysis of protein, starch, and other carbohydrates that occurs during high-moisture storage especially in the grain which was not ground previous to reconstitution.

Sorghum grain is the major feed grain in the Southwest, and its efficient utilization by livestock is of major importance to this area. Grain is processed in

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modern feeding operations because greater feeding efficiency can be obtained with larger returns to the feeding enterprise. Some of the processing methods are dry grinding, dry rolling, steam rolling, steamflaking, popping, and reconstituting. Of these methods, dry grinding is the simplest and least expensive, but grain processed in this manner does not produce the optimum in rate and efficiency of gain (1).

In a recent review article, Riggs (2) reported that feeding reconstituted or early harvested high-moisture grain would significantly increase feed efficiency for cattle over that of dry ground grain. Reconstitution is the conditioning of grain by adding enough water to air dried (10 to 13% moisture) grain to raise the moisture level to approximately 25 to 30%. The grain is then stored in air-limiting structures for a period, usually 21 days, before it is ground and fed. The moist grain fed in whole form is unsatisfactory for finishing cattle; but when it is ground, it makes a fluffy, yeasty smelling, palatable feed which is efficiently utilized. Reconstitution to 25 to 30% moisture, followed by storage for at least 21 days and grinding prior to feeding, increased the digestibility of protein 16 to 22%, and the dry matter and organic matter 17 to 29% (3). Feeders are interested in grinding the grain prior to reconstitution. However, grain ground before reconstitution showed no improvement in feed efficiency over finely ground grain, whereas cattle on reconstituted-ground grain required 9% less feed per unit of gain on a shrunk-weight basis than those on finely ground grain (4).

Berry (1) evaluated four processing treatments of a commercial sorghum grain for feed efficiency through feedlot performance trials. The treatments were: Dry-ground (D-G), ground-reconstituted (G-R), ground-reconstituted-ground (G-R-G), and reconstituted-ground (R-G). The feeding trial consisted of four lots of seven steers fed for 140 days. Differences in the feedlot performance of the animals were evident among the four processing treatments, and results from the four methods could be divided into two categories: those from grain ground dry and those from grain ground after reconstitution. The G-R-G and R-G treatments had the greatest increase in feed efficiency when compared to the D-G treatment. The G-R treatment increased feed efficiency over the D-G treatment, but an additional 7 to 8% increase in feed efficiency was obtained by the G-R-G or R-G treatments. The data indicate that for maximum increase in feed efficiency, reconstituting the whole kernel, followed by grinding, is required.

The exact changes that occur in the kernel during reconstitution are not understood. Some investigators feel that these changes are similar to those which occur during the malting of barley, or, that the proteinaceous matrix is softened and starch granules swell during reconstitution. The objectives of this paper are: 1) to illustrate microscopically and explain the changes occurring at the subcellular level of the kernel during reconstitution, and 2) to determine the influence of the four processing treatments on the particle size of the processed grain and relate these changes to the reported differences in feed efficiency of steers fed processed grain (1).

METHODS AND MATERIALS

Processing Treatments

A commercial lot of sorghum grain was obtained from a local elevator and the following four processing treatments were applied to the grain:

dry-ground (D-G) .... The grain was ground dry.
ground-reconstituted (G-R) .... The grain was ground dry, then reconstituted.
ground-reconstituted-ground (G-R-G) ... The grain was ground dry, reconstituted, then reground.
reconstituted-ground (R-G) ...... The whole grain was reconstituted, then ground.

The reconstitution in every case was conducted by raising the moisture content to 30% by addition of water. The whole grain or ground samples were stored in airtight structures for 21 days. The material was ground through a hammer mill with screen apertures of 0.635 cm. (.25 in.) diameter.

Particle Size Distribution

Particle Size Index (PSI) was determined by placing 300 g. of ground material from a processing treatment over a nest of U.S. Standard sieves. Prior to sieving, the samples were dried in a forced-air oven for 24 hr. at about 50°C. If not dried, the moist material would not pass through the sieves. The sieving method was that described by Florence (5), with some modifications. Two additional screens (Nos. 12 and 100) were included with the original nest of sieves. Two replications of each treatment were sieved for 5 min. on a Tyler Rotap. The overs of each sieve were weighed and expressed as a percentage of the sample, and used in PSI calculations. PSI was calculated by multiplying the percent overs of a sieve by a factor assigned to that sieve. The sum of the products for all the sieves and pan fraction gave a total PSI as presented in Table I. This index places more weight on fractions with smaller particle size; therefore, the smaller the index, the larger the average particle size, and conversely.

TABLE I. CALCULATION OF PARTICLE SIZE INDEX

U.S. Standard Sieve Number	Size of Sieve Opening	Overs of Sieve, %	Factor	Product
12	1.68 mm.	A	1	A X 1
20	841 μ	B	2	B X 2
30	595 μ	C	3	C X 3
40	420 μ	D	4	D X 4
70	210 μ	E	5	E X 5
100	149 μ	F	6	F X 6
				Particle Size Index

Laboratory Reconstitution

A 1,000-g. sample of the commercial sorghum grain was used for microscopic study. The sample was divided into two equal parts. One subsample was stored dry, and the other was reconstituted to 30% moisture and stored in airtight plastic bags for 21 days.

Sections for Light Microscopy

At the termination of the 21-day storage period, kernels from both reconstituted and dry samples were killed and fixed in formaldehyde, acetic acid, and alcohol (8:1:1). The two samples were then taken through the dioxane dehydration series of Sass (6), as modified by Clark.[3] The modification was an extension of time from 12 to 24 hr. for each step in the dehydration series. Next they were embedded in paraffin and longitudinal sections were cut 12 μ thick with a rotary microtome. The sections were then stained with safranin-O and fast green for examination with the light microscope. Sections were prepared from both samples after storage of 2, 4, 6, 8, and 10 months.

Microscopy and Photography Equipment

A Baush & Lomb Steromicroscope series B was used for low-magnitude observations of particle-size fractions. An American Optical Microstar 10 polarizing microscope was used for microscopic study and photomicrography. The camera system employed for all photography was an Exakta 1000.

Statistical Analysis

Statistical analyses were conducted by methods described by Steel and Torrie (7).

RESULTS AND DISCUSSION

Particle Size of Grain Processed by the Four Treatments

Visual examination of grain from the four processing treatments indicated considerable differences in the degree of fineness (Fig. 1). Grain from the D-G and

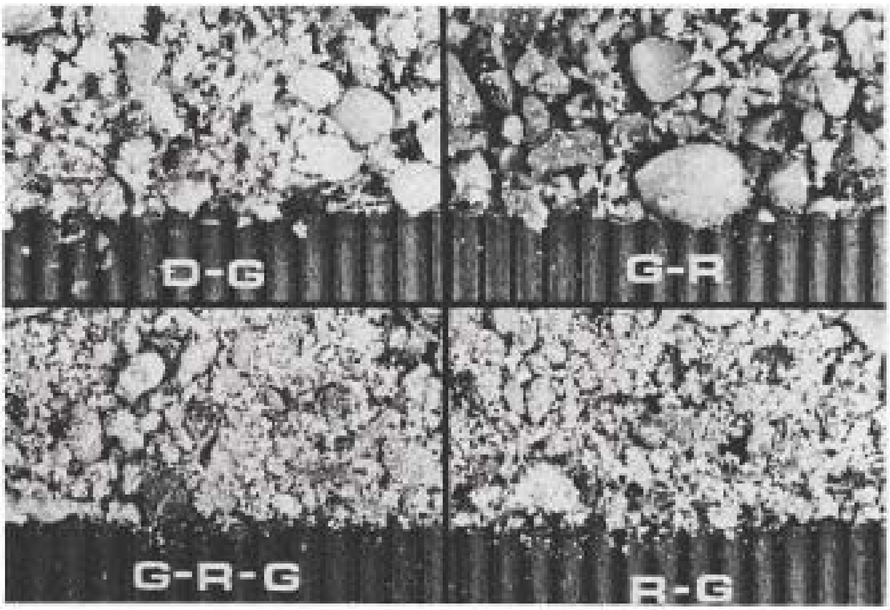


Fig. 1. Sorghum grain subjected to four processing treatments: D-G, dry-ground; G-R, ground-reconstituted; G-R-G, ground-reconstituted-ground; and R-G, reconstituted-ground. (Magnification, 6X.)

[3]Clark, L. E. Personal communication.

G-R treatments was similar in appearance. The D-G grain contained large pieces of endosperm, bran with considerable amounts of endosperm still adhering, and small amounts of free starch and protein. The G-R grain, for all practical purposes, was the same as the D-G. Both the G-R-G and R-G treatments were similar and much finer in particle size than the D-G and G-R treatments. The G-R-G and R-G treatments gave bran fragments relatively free of endosperm and considerable amounts of free starch and protein.

TABLE II. PSI AND OVERS OF SIEVES USED TO CALCULATE PSI FOR THE GRAIN FROM FOUR PROCESSING TREATMENTS

U.S. Standard Sieve Number	D-G %	G-R %	G-R-G %	R-G %
12	11.8	15.7	0.5	2.4
20	41.2	43.2	13.6	16.4
30	17.0	16.5	14.2	11.0
40	8.6	10.1	11.8	7.6
70	9.4	8.8	16.3	13.6
100	9.6	3.3	18.9	22.3
Pan	2.2	2.3	24.7	24.6
PSI	300.3[b]	271.8[a]	485.2[c]	485.7[c]

[a,b,c] Means followed by different superscripts are significantly different at the 95% level. Analysis by Duncans Multiple Range Test.

The PSI of the treated grain was determined to confirm the preliminary visual observations (Table II). As expected, the G-R-G and R-G treatments gave the highest PSI, which meant they had the finest particle size. The overs of the sieves indicate that the G-R-G and R-G treatments gave a high proportion of material on the No. 70, No. 100, and pan. These are the fractions which are high in small endosperm fragments and free starch granules. Each of these fractions is presented in Figs. 2 and 3. These photographs are representative of the particles composing the various fractions. The overs of a given sieve varied in quantity, but did not vary significantly in the type of particles. The quantity of free starch granules was significantly higher for the G-R-G and R-G. The three finest fractions (No. 70, No. 100, and pan) of each treatment were combined, wetted and sieved over Nos. 230 and 325 U.S. Standard sieves. The material passing through the No. 325 was centrifuged and its starch recovered. The G-R-G and R-G yielded 10% more starch than the D-G and G-R. The R-G treatment tended to cause a slightly higher proportion of fine particles (overs of No. 100) than the G-R-G. This slight difference may be a consequence of changes which occur in the reconstituted kernel prior to grinding.

The G-R treatment gave the smallest PSI; hence, it had the largest particle size, but it was only slightly larger than that of the D-G treatment. The G-R had a larger particle size than the D-G, probably because the smaller particles of the G-R, when wetted during reconstitution and dried prior to sieving, adhered to the larger particles. Thus, the amount of fine particles was reduced. This would account for the lower PSI. The decrease in percentage overs of the Nos. 70 and 100 sieves (Table II) support this hypothesis.

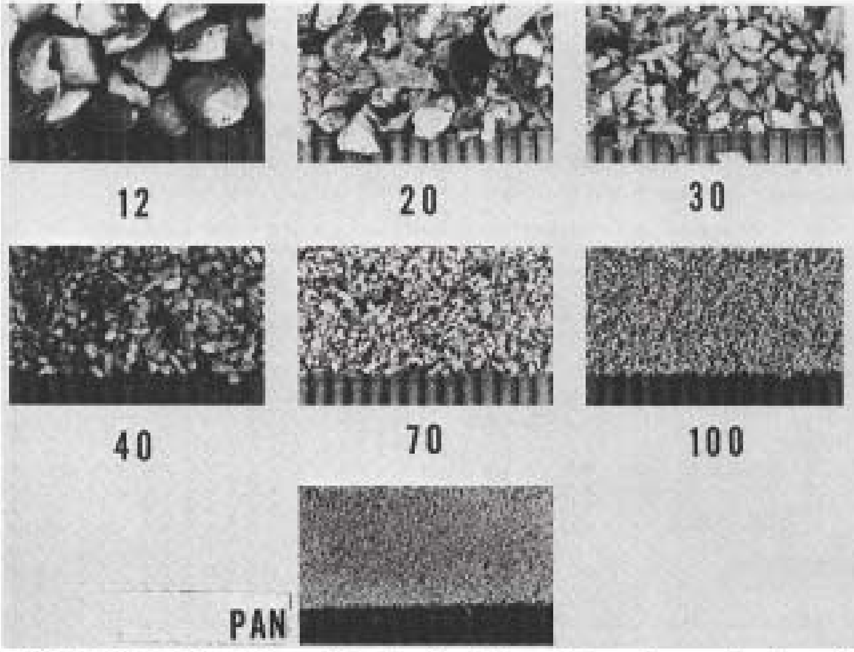
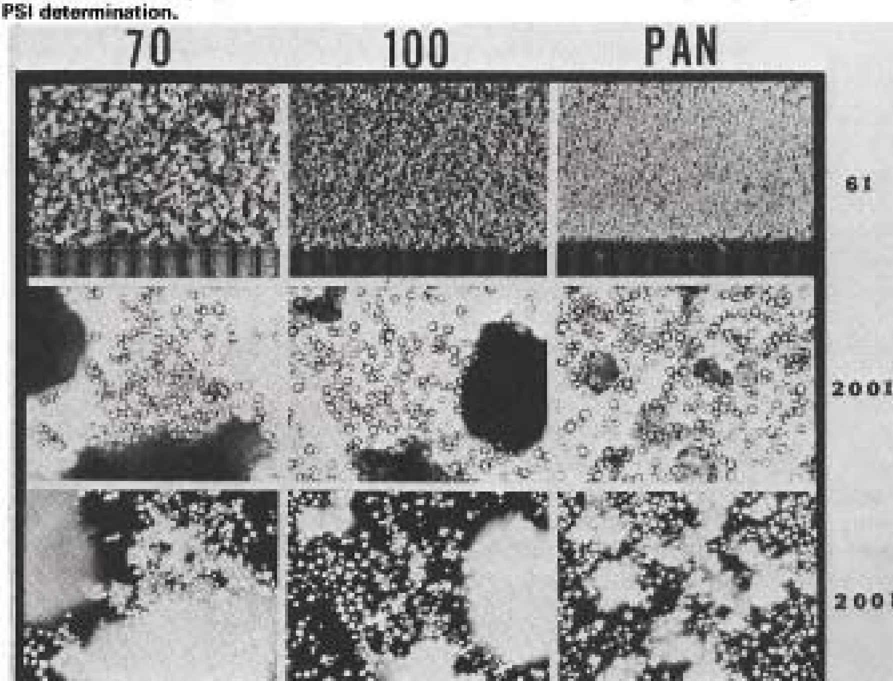


Fig. 2. Comparison of the relative particle sizes of sorghum grain from the seven fractions of the PSI determination. Overs of sieves Nos. 12, 20, 30, 40, 70, 100, and pan. (Magnification, 6X.)

Fig. 3. Photomicrographs of the three finer fractions (overs of Nos. 70, 100, and pan) of the PSI determination.



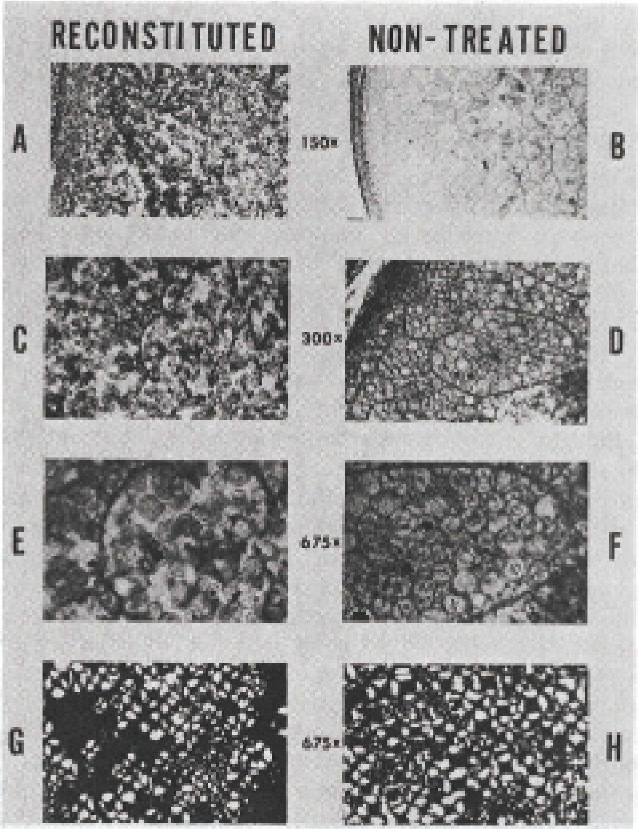


Fig. 4. Differences in organization and subcellar structure of the endosperm of reconstituted and nontreated sorghum grain. Photomicrographs G and G are sections of E and F photographed under plane polarized light.

Reconstitution has a major effect on the structure of the kernel at the subcellular level. Sorghum grain, with no treatment applied to it, has a high degree of organization of its components at the subcellular level (Fig. 4B). In contrast, the reconstituted grain, Fig. 4A, shows a distinct lack of organization and a general disruption of the endosperm, especially in the peripheral area. Fig. 4C of the reconstituted and 4D of the nontreated grain are typical endosperm cells from approximately the same location within the kernel. At greater magnification, the endosperm cell of the nontreated grain (Fig. 4F) illustrated the orderly orientation of starch granules surrounded by the protein matrix within the intact cell. In contrast, the photomicrograph of the reconstituted sample (Fig. 4E) illustrates the general disruption of the endosperm cell. During reconstitution the protein matrix is also partially disrupted, causing a release of free starch granules and protein bodies. The starch granules and protein bodies were consistently observed at a magnification of 860X, but with the equipment available a photomicrograph illustrating this was unobtainable. Photomicrographs (Figs. 4G and 4H) show sections (Figs. 4E and 4F) undergoing birefringence. Examination of these sections under plane polarized light better illustrates the lack of organization of the reconstituted sample as compared to the high degree of organization of the nontreated sample. Birefringence of the starch granules was not influenced by reconstitution.

This high degree of disorganization is probably caused by enzymatic activity and is probably similar to that occurring during malting. For instance, during malting, the barley embryo immediately imbibed water and released gibberellin-like hormones that migrated to the aleurone layer and stimulated the release of enzymes responsible for hydrolysis (8). The principle enzymes released during malting of barley are β-glucanases for cell wall degradation, α- and β-amylases for starch solubilization, and proteases for protein hydrolysis. The enzymatic breakdown probably begins with that material nearest the aleurone layer and moves inward into the kernel. The material adjacent to the aleurone layer is the peripheral endosperm, thought to be relatively indigestible to the ruminant animal, which is composed of very small starch granules embedded in a proteinaceous network (9). We observed that this area appears to show the highest degree of modification. A disruption in this area prior to ingestion would greatly facilitate the microbial digestion process of the animal and give better utilization of the grain. The degree of disruption appears to be reduced toward the center of the kernel.

No additional breakdown of the endosperm sections from reconstituted grain that had been stored under airtight conditions for 2, 4, 6, 8 and 10 months was noted. These findings tended to support the idea of McGinty et al. (10) that the reaction stopped prior to 21 days after reconstitution. Since storage is under anaerobic conditions, there is enough oxygen in the system to initiate germination but not enough to sustain it for the development of the radicle and coleoptile. The autolysis process, being dependent on oxygen to continue the breakdown, thus subsides when the oxygen level is depleted.

The results from the feedlot performance trial indicated very little difference in the feeding efficiency of the G-R-G treatment and the R-G treatment (1). PSI indicated no significant difference in the two treatments. The reason for this close relationship of the two treatments is that in the processing of G-R-G, the grain was ground first. Dry grinding of the whole kernel breaks it into an array of particles ranging from small to quite large. Since the grain is not in whole form, there is probably a marked reduction in autolytic breakdown. Girdling the aleurone layer of barley prior to steeping was shown to inhibit degradation of the endosperm because the hormone-like substance was unable to move from the embryo to the aleurone layer to activate the necessary enzymes. Therefore, grinding sorghum grain prior to reconstituting destroys the necessary enzymatic pathways (11) and inhibits much of the autolytic process. However, when water is added to the ground sample for reconstitution, some of the proteolytic and amylolytic enzymes present in these particles are activated—which would cause a limited modification of the protein matrix and other structural components of the individual particle. On the other hand, addition of water to the ground sample and storage for 21 days allows the water enough time to penetrate into the particles and soften them prior to grinding. Up to this point there is no difference in processing between the G-R and G-R-G treatments, yet there is a difference in the feedlot performance and PSI between the two treatments. Therefore, the increase of 7.24% in feed efficiency (1) and a difference of the mean particle size must be due to the additional grinding of the G-R-G treatment after reconstitution.

CONCLUSION

This study indicates that an actual breakdown occurs within the kernel during the reconstitution process. Modification of the kernel structure in the peripheral endosperm area is especially significant because it is usually hard to disrupt by normal grinding techniques. Therefore, grinding the reconstituted kernel causes more complete breakdown of the endosperm, which accounts for part of the increased feed efficiency of the reconstituted grain.

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