A Staining Procedure to Determine the Extent of Bran Removal in Pearled Sorghum

J. F. SCHEURING and L. W. ROONEY,[1] Sorghum Breeding and Cereal Quality Lab, Soil and Crop Science Department, Texas A & M University, College Station, TX 77843

ABSTRACT

Cereal Chem. 56(6):545-548

The completeness of bran removal during milling is difficult to determine for many sorghum grains. Two dye systems that stain the germ, pericarp, and starchy endosperm of sorghum blue, green, and pink, respectively, are useful to determine the completeness of milling. The May-Grunwald solution (0.5% methylene blue and 0.5% eosin-Y in methanol) and 0.25% methylene blue and 0.75% eosin-Y in 70% ethanol are equally effective. Samples of pearled kernels from each of three varieties milled by three processes were stained. The exposed starchy endosperm, pericarp fragments, and germ tissue stained pink, green, and blue, respectively. The staining procedures were especially useful for sorghums with a white pericarp. The methods are simple and can be efficiently used in milling studies and in breeding programs to select for sorghums with improved milling properties.

Pearling is the removal of the bran layers from cereal grains. It can enhance the color, texture, and cooking quality of grain foods. Throughout Asia, pearling is a common practice in the food preparation of rice and barley. In India and some parts of Africa, sorghum and millet grains are pearled prior to the preparation of gruels, pastes, and baked products. In traditional African societies, debranning is accomplished by mortar and pestle pounding followed by winnowing. Pearling can be accomplished mechanically by abrasive tumbling action in a barley pearler or rice mill. The pilot sorghum milling schemes at Maiduguri, Nigeria (Spurgeon 1976), and Nairobi, Kenya (Kapasi-Kakama 1976), include debranning procedures prior to grinding.

The completeness of bran removal is difficult to ascertain visually in many sorghums, particularly those with white or yellow pericarps. By divising a simple May-Grunwald staining technique in which exposed endosperm of stained kernels is pink and adhering pericarp is blue, Tani et al (1952) were able to determine how effectively milled rice and pressed barley were pearled. This technique has been adopted by the Food Agency of Japan as a standard for testing the degree of rice milling (FAO 1972). The present study was undertaken to test the effectiveness of May-Grunwald solution and other alternative dyes in staining pearled sorghum kernels.

[1]Graduate research assistant and professor, respectively.

0009-0352/79/000088$3.00/0

MATERIALS AND METHODS

Grain Samples

Grain lots of Maldoni 35-1 (M-35-1), BTx398 ('Martin'), and BTx378 ('Redlan') were obtained from plots grown under similar conditions at College Station, TX, in 1977. The variety M-35-1 has large grain, intermediate hardness, and a thin pericarp. Martin and Redlan have red, medium thick pericarps, intermediate hardness, and medium-sized grain.

Staining

May-Grunwald dye described by Tani et al (1952) is 0.5% methylene blue and 0.5% eosin-Y in methanol. After equilibrating for 12 hr, the solution is decanted and diluted 3:1 in methanol. The actual concentration is unknown since some of the dye does not dissolve. Eight dye concentrations were made up in two solvents,

70% ethanol and distilled H_2O. Dye combinations included 0.5% methylene blue[2] and 0.5% eosin-Y; 0.33% methylene blue and 0.66% eosin-Y; 0.25% methylene blue and 0.75% eosin-Y; 0.2% methylene blue and 0.8% eosin-Y; and 3:1 dilutions of each of the four preceding combinations. Dye solutions were made and allowed to equilibrate for 24 hr prior to use.

Two-gram pearled grain samples of each of the three grain types were stained using a six-min procedure. The samples were immersed for 1 min in solvent, 1 min in dye, 1 min in each of three solvent rinses, and 1 min in deionized, distilled water. After staining, the grain was allowed to air dry on absorbent paper.

Staining Evaluation of Pearled Kernels

Ten-gram samples of M-35-1, Martin, and Redlan were pearled with a Strong-Scott barley pearler fitted with either a Carborundum wheel or wire brushes (Rooney and Sullins 1969). Pearling time for both procedures was kept at 30 sec. In addition, a 5-lb lot of Redlan was pearled in a Satake rice whitener, which was a continuous debranning system, equipped with a Carborundum wheel. The pearled kernels varied greatly in degree of pericarp removal.

Pearled grains were stained with 0.25% methylene blue and 0.75% eosin-Y in 70% ethanol. Pearled kernels were examined with light and a scanning electron microscope to evaluate the effectiveness of the staining technique and to determine where the pericarp tore away from the kernel.

[2] The stock of methylene blue was Allied Chemical Cert. 44 and eosin-Y was Fisher Cert. EE-37. Mention of firm names or trade products does not imply that they are endorsed by Texas A & M University over other firms or similar products not mentioned.

RESULTS

The May-Grunwald solution in methanol (0.5% methylene blue and 0.5% eosin-Y in methanol) and the dye made up with 0.25% methylene blue and 0.75% eosin-Y in 70% ethanol were most acceptable for staining pearled sorghum kernels. Both dye solutions produced grains with sharp green/pink contrasts at pericarp/endosperm borders.

In Fig. 1, unstained, pearled kernels of M-35-1 sorghum are shown. The same kernels after staining are shown in Fig. 2. Grain of M-35-1 had a white pericarp. The unstained pearled kernels must be examined closely with the naked eye to determine completeness of pericarp removal. After staining, however, the green pericarp can be easily seen. The germ is blue-green and can be easily detected. The differences are dramatic and should permit relatively easy evaluation of completeness of bran removal.

Sorghum kernels with intact epicarp tissue did not stain, probably because of the cutin on the upper surface of the epicarp cells. The mesocarp, endocarp, and aleurone tissues stained various hues of green. The starchy endosperm stained pink. Starch granules did not stain. Therefore, the intensity of the pink color was related to the quantity of protein in the endosperm. The pink stain appeared most intense in the protein bodies.

Each of the three milling procedures produced milled products varying in completeness of pericarp and germ removal that was readily documented by the staining procedure. The barley pearler equipped with a Carborundum wheel removed most of the germ and all of the pericarp and aleurone layer. The pearled or milled kernels stained a brilliant pink. Relatively little green or blue staining material was present. Scanning electron microscopic examination of the pearled kernels confirmed that the surface was mainly starchy endosperm tissue (Fig. 3A, B) that was expected to

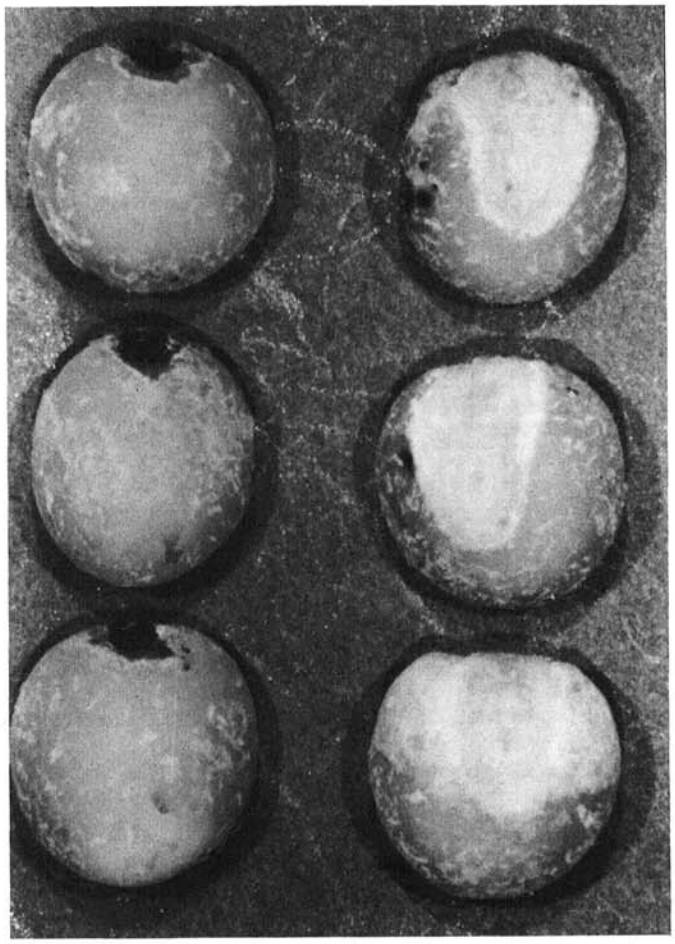

Fig. 1. Maldoni 35-1 sorghum kernels pearled by a Satake rice whitener. Dorsal and ventral sides of kernels are on the left and right, respectively. The black tips at the tops of kernels on the left are the remnants of the hilum.

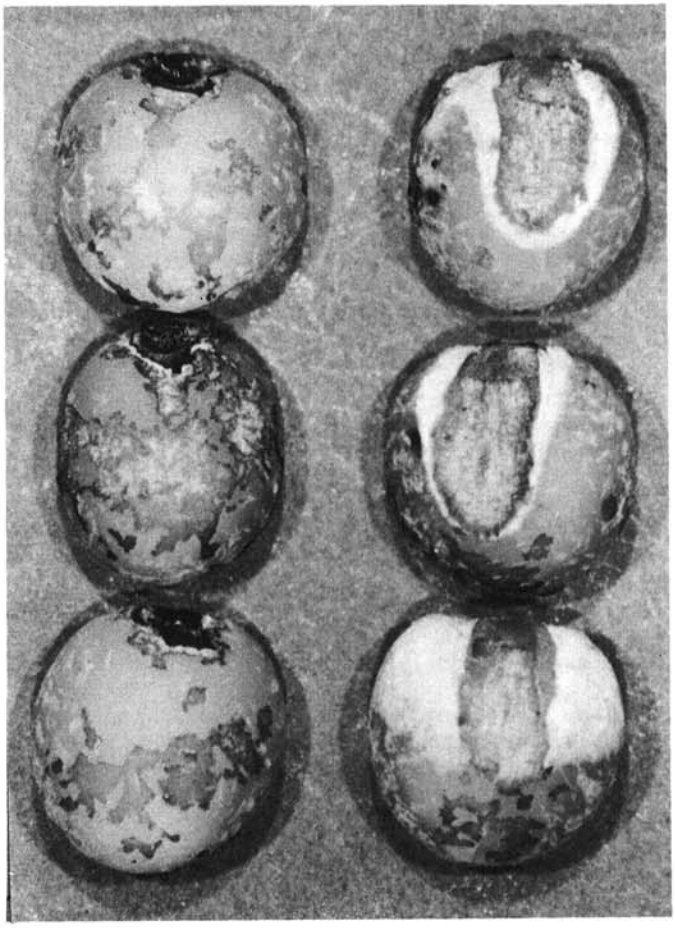

Fig. 2. Maldoni 35-1 sorghum kernels, shown in Fig. 1, after staining in May-Grunwald solution. Pericarp and germ tissue appear light gray.

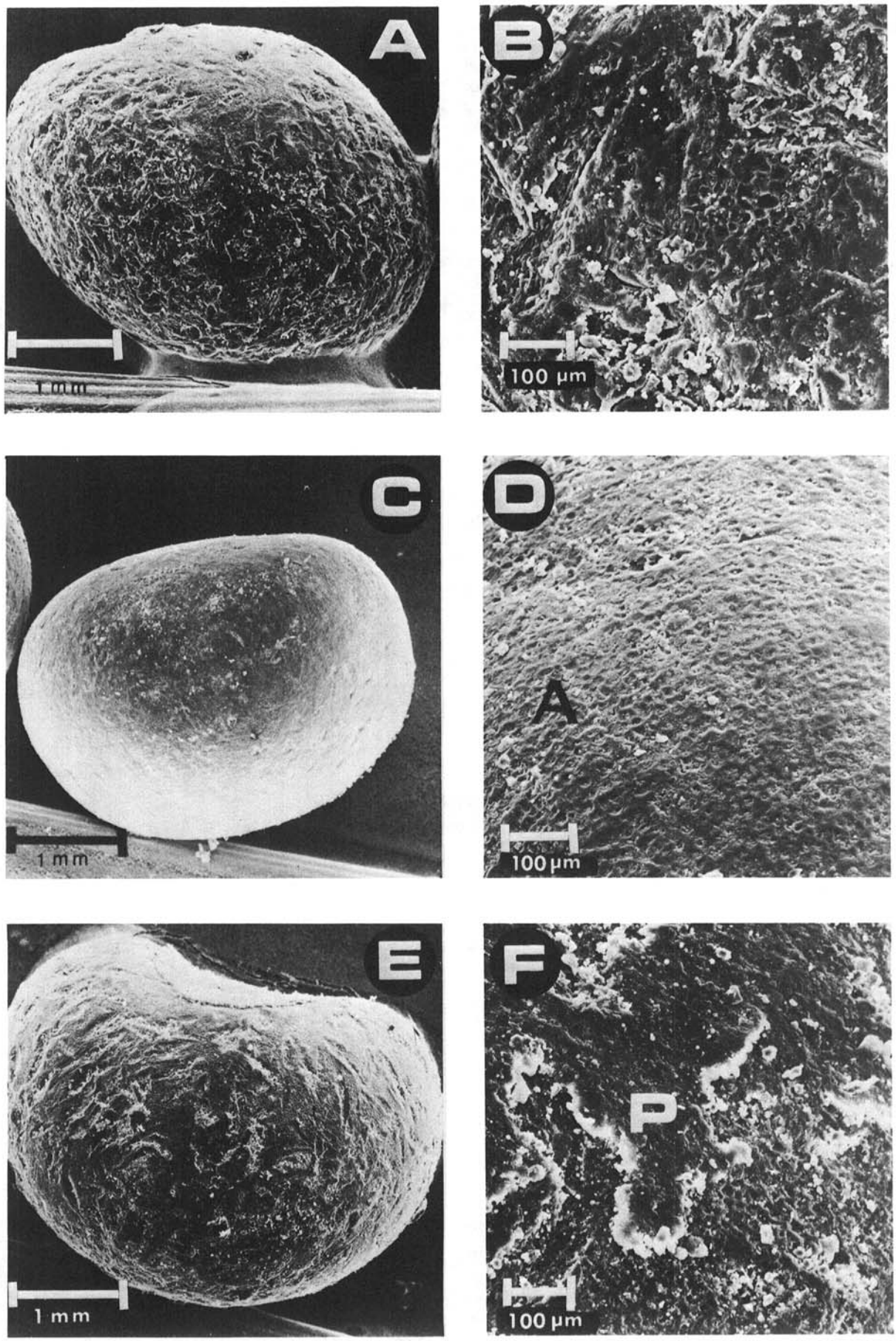

Fig. 3. Scanning electron micrographs of Martin sorghum kernels pearled with a barley pearler (**A, B**), a barley pearler fitted with wire brushes (**C, D**), and a Satake rice whitener (**E, F**). **A** = aleurone cell wall remnants; **P** = pericarp remnants.

stain pink. In Fig. 3B, the rough surface topography of the pearled kernel is shown.

The grains milled with a wire brush had good germ removal, but the exposed endosperm stained a dull, light pink. Scanning electron microscopy (Fig. 3C,D) confirmed that the surface was composed of aleurone anticlinal and inner periclinal walls. The surface of the kernel was smooth and uniform. The aleurone cell walls masked the pink color of the protein of the starchy endosperm so the net effect was a light pink color.

The Satake rice whitener gave nonuniformly pearled kernels with considerable pericarp and germ tissue remaining on the kernel as illustrated in Fig. 3E,F. Bright pink spots were observed on the kernel that corresponded to areas where the pearler had penetrated into the starchy endosperm, which was easily confirmed by the SEM data. The same conclusions were reached when milled samples of M-35-1 and BTx378 were examined.

DISCUSSION

The May-Grunwald methanol dye, which has been recommended for staining milled rice, was found adequate for staining pearled sorghum samples. In addition, 70% ethanol solution with 0.25% methylene blue and 0.75% eosin-Y also differentiated adequately between pericarp, germ, and endosperm in all samples from the three varieties and three pearling procedures in this study. The method using ethanol as the solvent was less expensive. In addition, ethanol may be more easily obtained than methanol. Since both methods give equal results, the method used can be based on economy and convenience.

The staining procedure for 2-g grain samples is a sequence of 1-min immersions in the following solutions: solvent, dye, three rinses in solvent, and a final rinse in deionized, distilled water. This staining procedure has several advantages: It is rapid (6 min/2-g sample), wet kernels can be evaluated without drying, and the dye is stable for at least 12 weeks at 6° C.

The stain technique can be used as an aid to determine differences in the completeness of pericarp and germ removal of sorghum caused by variation in milling procedures or by differences among sorghum varieties. It could be used in breeding programs to determine relative differences in ease of pericarp removal among sorghum lines and in village milling tests of new cultivars.

LITERATURE CITED

FOOD AND AGRICULTURE ORGANIZATION. 1972. Degree of milling of rice. Sub-group on rice grading and standardization. Intergovernmental group on rice. FAO: Rome. FAO Doc. CCP:RI/GS C.R.S. 1.

KAPASI-KAKAMA, J. 1976. Some characteristics which influence the yield and quality of pearled sorghum grain, p. 21. Proc. Symp. Sorghum and Millets for Human Foods. Intern. Assoc. Cereal Chem. Symp., Vienna.

ROONEY, L. W., and SULLINS, R. D. 1969. A laboratory method for milling small samples of sorghum grain. Cereal Chem. 46:486.

SPURGEON, D. 1976. Hidden Harvest: A Systems Approach to Post-harvest Technology, p. 36. Intern. Dev. Res. Centre, Ottawa.

TANI, T., CHIKUBU, S., and SHIKANO, T. 1952. A new May-Grunwald method of staining for grain. Part I. Application to pressed barley and milled rice. Rep. Food Res. Inst. (Japan) 6:75.

[Received March 12, 1979. Accepted May 30, 1979]