NOTE ON MEASURING THICKNESS OF WHEAT BRAN BY SCANNING ELECTRON MICROSCOPY[1]

D. R. LINEBACK,[2] W. E. CASHMAN,[3] R. C. HOSENEY, and A. B. WARD,[4] Department of Grain Science and Industry, Kansas State University, Manhattan, KS 66506

Cereal Chem. 55(3): 415-419

Milling wheat involves removing the outer bran, which is valuable as roughage but indigestible by humans, to produce flour. The thickness of the bran is of interest because of its potential effect on milling behavior and flour yield. Using microscopic techniques, Bates (1) found differences in the combined thickness of the bran and aleurone layers among five wheat varieties. Shellenberger and Morgenson (2,3) observed slight differences in the bran thickness of four varieties of hard red winter wheat, although these differences were not related to flour yield. Crewe and Jones (4) microscopically measured the thickness of the bran, both in dry and wet sections, from nine wheats. For all types of wheat examined, the total bran thickness at ordinary moisture content (13–14%) was in the range of 50–77 μm, with a mean of 67 μm; no appreciable difference between varieties was observed. Larkin *et al.* (5) microscopically measured the thickness of the outer four layers of bran from seven wheat varieties and found differences between varieties. The mean thickness of bran sections from transections of the seven varieties ranged from 51.9 to 65.0 μm. Differences in bran thickness did not correlate with the general milling behavior of the varieties.

Moss and Stenvert (6) separated the fibrous outer layers (bran) from the endosperm of wheats of different varieties using the neutral detergent method of fiber determination. Measurement of the separated fiber then gave an indication of milling yield. A highly significant inverse relationship (r, −0.88) was found between flour yield and fiber (bran) among soft wheats, but the relationship (r, −0.29 or −0.57) for hard wheats was much lower than that for soft wheats.

A factor that must be considered in interpreting the results of microscopic studies on sections of wheat is the amount of swelling that occurs during the preparation of the sample in an aqueous medium. Crewe and Jones (4) found that with water-mounted sections, considerable variation in bran thickness occurred between and within individual grains. Larkin *et al.* (5) observed increases in bran thickness and aleurone thickness in sections that were stained and mounted in Congo red solution compared with sections mounted in water; the increases were within the limits of error of the measurements. Such swelling, or increases in bran thickness, do not become a problem with scanning electron microscopy (SEM), since fixation, staining, or aqueous mounting of the specimen is not required. SEM has provided an excellent, relatively simple technique for viewing detailed structures of cereal grains. Pomeranz (7) recently

[1]Contribution No. 978-j, Department of Grain Science and Industry, Kansas Agricultural Experiment Station, Manhattan, KS 66506.

[2]Present address: Department of Food Science, 111 Borland Laboratory, The Pennsylvania State University, University Park, PA 16802.

[3]Present address: Durkee Foods Division, SCM Corporation, 16651 Sprague Rd., Strongsville, OH 44136.

[4]Professor, graduate research assistant, professor, and professor, respectively, Department of Grain Science and Industry, Kansas State University.

0009-0352/78/000046$03.00/0

reviewed this subject thoroughly. Using SEM, we measured the thickness of bran from several wheat varieties to determine whether bran thickness differs between varieties.

MATERIAL AND METHODS

We examined 19 varieties (20 samples) of wheat, including Eagle, harvested in 1970; Centurk, harvested in 1971; Parker and Clark's Cream (hard white winter), harvested in 1972; Cloud, Gage, Hi Plains, Homestead, Kirwin, and Lancer, harvested in 1973; Chris (hard red spring), Sage, Scott, Trison, and Triumph, harvested in 1974; Red Chief and two Wichita samples (from the Kansas State University greenhouse), harvested in 1975 and supplied by Dr. Elmer Heyne, Department of Agronomy; and Gaines (soft white winter) and Langdon (durum), with unknown harvest dates. Unless otherwise designated, these wheats are hard red winter varieties, the predominant class in the Great Plains.

Preparing Whole Kernels for SEM

Wheat kernels were fractured transversely with a razor blade. The razor blade does not cut through the endosperm, but causes it to fracture. Kernel cross sections were mounted on 9-mm stubs with silver conducting paste (Pelco Industries, Tustin, CA), coated *in vacuo* with carbon and then with gold-palladium alloy, and viewed at a 90-degree angle in an ETEC Autoscan SEM operating at 20-kv accelerating voltage. Photographs were made on Polaroid film, type 55 P/N.

Measuring Bran Thickness

Bran (pericarp plus seed coat or coats plus nucellus plus aleurone) thickness was measured from the photographs on a centimeter scale and converted to

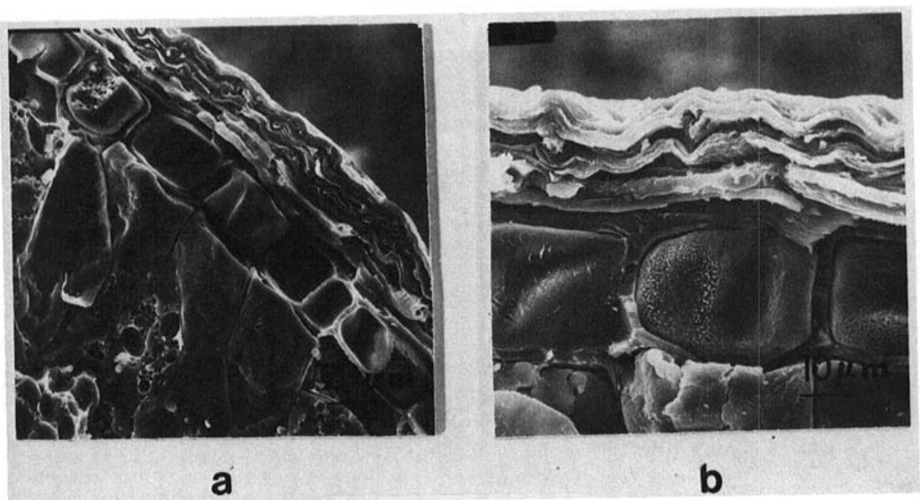

Fig. 1. Electron micrographs of fractured wheat kernels at magnifications used to study bran width. a) Trison variety, b) Sage variety.

micrometers. Some measurements were made from the image displayed on the cathode-ray tube. Measurements were made on the sides of the kernel; ten measurements per kernel were recorded for each of five kernels. Measurements were made at 400× or 1,100× magnification. Data were averaged, means calculated, and multiple-range tests conducted to determine whether statistical differences in bran thickness existed among varieties.

RESULTS AND DISCUSSION

Electron micrographs of fractured kernels from two wheat varieties at magnifications used for measuring bran widths are shown in Fig. 1. The average bran width for each variety and the standard deviation are shown in Table I. Average bran width ranges from 76.00 μm in Lancer wheat to 51.70 μm in Red Chief. Measurements for the two samples of Wichita wheat had excellent agreement. These values are in good agreement with those that Shellenberger and Morgenson (2,3), Crewe and Jones (4), and Larkin *et al.* (5) reported. The difference, however, between the bran thickness for Red Chief that Shellenberger and Morgenson (2,3) reported and the value that we observed is significant.

Differences among varieties were tested with the least significant difference (LSD) multiple-range test. The LSD was calculated as 3.267 for 19 varieties (Fig. 2) and 3.497 for 14 hard red winter wheat varieties (Fig. 3), excluding the two greenhouse-grown Wichita samples. The LSD multiple-range test on 19 varieties produced three statistically different groups formed by separating the varieties

TABLE I
Bran Width of Nineteen Wheat Varieties

Code	Variety	Where Grown	Means[a] (μm)	Variances	Standard Deviations
1	Parker	Kansas	65.80	13.733	3.705
2	Centurk	Kansas	56.70	12.455	3.529
3	Eagle	Kansas	69.70	21.822	4.671
4	Kirwin	Nebraska	67.00	12.888	3.590
5	Cloud	Kansas	68.50	10.277	3.205
6	Lancer	Nebraska	76.00	15.333	3.915
7	Gage	Kansas	50.60	10.266	3.204
8	Hi Plains	Nebraska	71.20	5.955	2.440
9	Homestead	Nebraska	71.10	20.766	4.557
10	Scout	Kansas	56.10	8.100	2.846
11	Sage	Kansas	64.70	26.011	5.100
12	Trison	Kansas	61.40	14.266	3.777
13	Triumph	Kansas	71.50	19.611	4.428
14	Red Chief	Kansas	51.70	21.344	4.598
15	Wichita	Kansas	54.30	7.122	2.668
16	Wichita	Kansas	55.10	10.100	3.178
17	Clark's Cream	Unknown	52.30	10.455	3.283
18	Langdon	Unknown	68.90	9.433	3.071
19	Gaines	Unknown	62.40	6.044	2.485
20	Chris	Nebraska	66.30	11.788	3.433

[a]Means of 50 observations.

into groups whose LSD ranges did not overlap. The first group consisted of Lancer, which had a thick bran. The second group contained Triumph, Hi Plains, Homestead, Eagle, Langdon, Cloud, Kirwin, Chris, Parker, Sage, Gaines, and Trison varieties. The third group, with the thinnest bran layers, consisted of Centurk, Scout, Wichita, Clark's Cream, Red Chief, and Gage.

The LSD multiple-range test showed that among the hard red winter varieties, with the two greenhouse-grown Wichita samples omitted, Scout and Red Chief

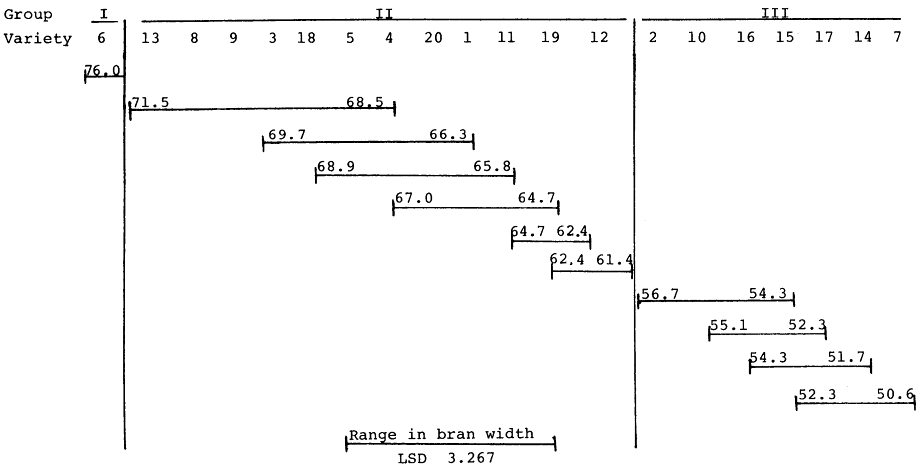

Fig. 2. Multiple-range test for 19 wheat varieties. Least significant difference (LSD value, 3.267) at 0.05 alpha level. See Table I for codes for wheat varieties.

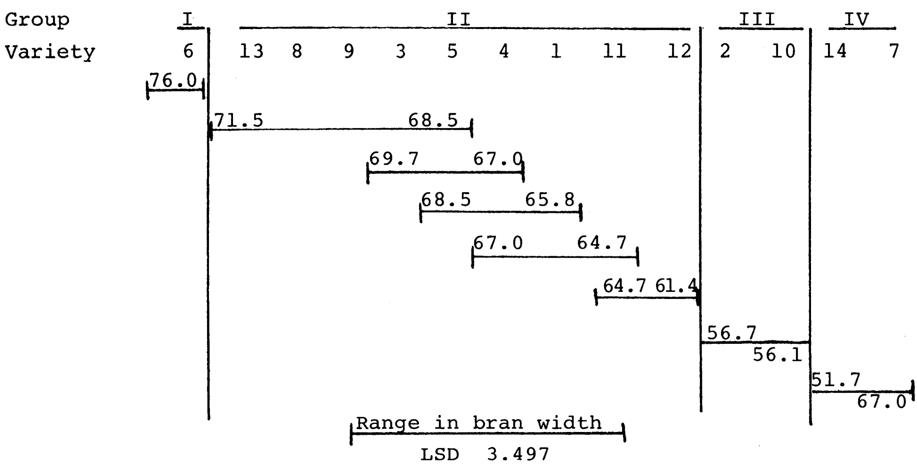

Fig. 3. Multiple-range test for 14 wheat varieties. Least significant difference (LSD value, 3.497) at 0.05 alpha level. See Table I for codes for wheat varieties.

differed significantly. That produced a group III (Fig. 3), consisting of Scout and Centurk, altered from that in Fig. 2, and a group IV (Fig. 3), consisting of Red Chief and Gage.

The evidence clearly establishes that the thickness of bran layers differs in some wheat varieties as Bates (1), Shellenberger and Morgenson (2,3), and Larkin *et al.* (5) previously reported. SEM provides a simple, effective way to study structural details of cereal grains without some of the problems inherent in light microscopy of grain sections. Additional milling tests should be performed to establish whether correlations exist between bran thickness and flour yield, cleanness of endosperm separation, and ease of milling.

Literature Cited

1. BATES, J. C. Varietal differences in anatomy of cross section of wheat grain. Bot. Gaz. 104: 490 (1943).
2. SHELLENBERGER, J. A., and MORGENSON, J. B. Comparative thickness of bran layers of hard red winter wheat. Am. Miller 77(8): 37 (1949).
3. SHELLENBERGER, J. A., and MORGENSON, J. B. Bran thickness and flour yield of four varieties of wheat. Am. Miller 78(8): 29 (1950).
4. CREWE, J., and JONES, C. R. The thickness of wheat bran. Cereal Chem. 28: 40 (1951).
5. LARKIN, R. A., MacMASTERS, M. M., WOLF, M. J., and RIST, C. E. Studies on the relation of bran thickness to millability of some Pacific northwest wheats. Cereal Chem. 28: 247 (1951).
6. MOSS, H. J., and STENVERT, N. L. The determination and properties of the fibrous covering of the wheat grain. Aust. J. Agr.. Res. 22: 547 (1971).
7. POMERANZ, Y. Scanning electron microscopy in food science and technology. Adv. Food Res. 22: 206 (1976).

[Received August 10, 1977. Accepted January 24, 1978]