USE OF SCANNING ELECTRON MICROSCOPY TO EVALUATE CEREAL GRAINS AND THEIR MILL FRACTIONS

by

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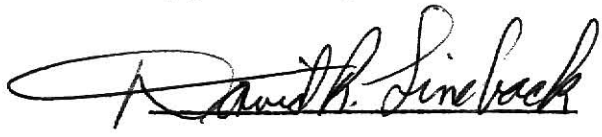

Major Professor

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INTRODUCTION

The application of the scanning electron microscope to cereal science has provided an excellent, relatively simple technique for viewing in detail a large number of samples. The high degree of resolution and the three-dimensional reproduction of the sample's structure combine to enhance the desirability of this instrument for viewing whole wheat kernels and their mill fractions.

Utilization of this instrument, since its beginning in the late 1960's, has been limited in the cereal area. Flour-dough transitions, kernel hardness and related structural characteristics, previously studied with light and transmission electron microscopy, have been examined. Although many studies using microscopy have been made on wheat kernels and their respective milled products, scanning electron microscopy has not been widely used in this area.

We examined structural characteristics of twenty varieties of wheat and the changes in structural characteristics during wheat kernel maturation. In addition, different streams from a flour mill and an air-classified flour were investigated by scanning electron microscopy.

This investigation was ($_A$) to determine whether wheat varieties differ in the thickness of their bran and ($_B$) to compare the sub-structures in the stream from a pilot flour mill and the fractions of an air-classified flour.

LITERATURE REVIEW

Bran Morphology

The bran layer is composed of the pericarp, seed coat, and aleurone layer.

The outer pericarp consists of the epidermis, hypodermis and thin-walled cells. The epidermis is a single layer of cells that forms the outer surface of the pericarp and covers the entire kernel. Located near the crease on both sides are numerous stomata extending to the apex of the kernel (7). In a normal kernel, the epidermal cells are very thin and dry. The kernel dehydrates from 70% moisture content at 11 days after half bloom to 12% or 15% by harvest time (31). As a result of drying, the outer cell layers tend to collapse.

Percival (24), using light microscopy, and Bradbury, MacMasters and Cull (6), using transmission electron microscopy, reported epidermal cell dimensions for wheat of 125-210 μ (length) and 25-30 μ (width). Those kernels were soaked and fixed using 70% ethanol, 5% glacial acetic acid and 5% formaldehyde. This treatment could have distorted the dimensions of the cell due to hydration and swelling during soaking and fixation.

At the apex of the kernel there exist modified epidermal cells that protrude from the surface, giving the appearance of hairs. The hairs taper upward from the base to the tip and may either be straight or curved. They vary in length

from 1 mm to 120 μ (22). These modified epidermal cells are hollow, however the diameter of the cell cavity does not exceed the thickness of the cell walls in most cases (7).

The hypodermal cells lie just below the epidermis and are similar to the epidermal cells. However, at the apex of the kernel, cellular structure becomes more oval and, in some cases, almost circular (7). The hypodermis may contain more than one layer of cells. It may be two to three cells thick with intermittent air spaces. Such a structure is found in the wheat variety Chiefkan (5).

The thin-walled cells are the last surface of the outer pericarp and separate easily from the inner pericarp. These cells are not a continuous layer, but exist in conglomerates. Mold mycelia exist between the outer and inner pericarp preventing contact. Open spaces between these layers are more numerous at the equator, or widest diameter of the kernel, than at the apex (7). Mold mycelia were present beneath the outer pericarp of all wheat seeds examined (11). Most of the mycelia were dormant. These mycelia provide inert matter that would prevent contact between the cell-layer surfaces in much the same way that surfaces are dusted to prevent adhesion.

The inner pericarp consists of intermediate cells, cross cells and tube cells. Intermediate cells are similar to thin-walled cells in that they exist as groups congregated at the apex and germ of the kernel. In both areas, the cells are somewhat flattened and irregularly shaped. These groups of inter-

mediate cells form a discontinuous layer surrounding the kernel. More than one layer of intermediate cells were observed near the germ (7, 24).

The cross cells are elongated and rather flat. Their axis runs perpendicular to the length of the kernel. As indicated earlier, considerable open space exists between the hypodermis and this layer. Therefore, in many cases, the cross cells lie directly beneath the hypodermis and are the main cell boundary of the inner pericarp (7). These cells lie closely together with little or no airspace between cell layers.

Depending upon where the cross cells were located in the kernel, different growth stresses and maturing factors occur that influence the shape of the cell. For example, near the center or equatorial region of the kernel, the cells appear longer and slightly flatter, whereas near the apex of the kernel or the crease, less stress evolved a cell that was shorter and thicker (24). Several investigators observed double cross cell layers near the apex crease (7). However, these were thought to be intermediate cells. Many investigators also fail to report intermediate cells, supposing them to be thin-cell remnants or outcasts of underdeveloped tube cells (13).

Tube cells lie perpendicular to the cross cells and parallel to the long axis of the kernel. They constitute the interior layer of the inner pericarp. Like the thin-walled cells and intermediate cells, they are located only in certain areas. They are present at the base of the kernel and par-

tially cover the germ. The tube cells extend from the base in a narrow band up the dorsal side of the kernel to the apex (7). They are separated by wide inter-cellular spaces and are attached to each other only where random cell protrusions touch. The walls are pitted and thinner than cross cells (24). Mycelia are not found at this depth in the pericarp, due primarily to the lack of penetration of the cross cell layer (11).

The seed coat is the next structural layer encountered between the inner pericarp and nucellar epidermis. The seed coat with the pigment strand forms a complete layer around the kernel. The seed coat is extremely impenetrable by water and is composed of approximately three layers (7). The outer layer is known as the outer cuticle. It is primarily this layer that prevents absorption of water. The color layer, next in the progression to the interior, is composed of two elongated layers of cells that overlay each other at nearly 45^{o} angles. These cells are compressed and are pigmented in hard red spring and hard red winter wheats. In white wheats, little or only slight pigmentation exists. The third layer consists of a much thinner inner cuticle. Both the outer and inner cuticle have been analyzed and were found to contain cutin, a waxy compound, and suberin, a corky substance. Suberin has been found only in certain varieties (22). A fourth extremely thin layer of hyaline material has been observed in the variety Pawnee. This layer contains both pigments and cellulose. It lies between the outer cuticle and the color layer (7).

Only in the micropylar area, over the protruding germ, is the seed coat modified to allow faster water absorption (6). The nucellar epidermis or hyaline layer is firmly attached to the seed coat and the aleurone layer. The cellular structure in this layer lacks definition. Due to expansion of the endosperm during growth and dehydration of the kernel during maturation, the nucellar layer is compressed to an extremely thin surface. The nucellar layer surrounds the entire kernel, except for an area surrounding the germ (24).

During kernel development, the embryo and endosperm drain the nucellar layer of nutrients, leaving compressed empty membranes that form the layer (7).

The aleurone cells comprise the last layer of the bran and border the surface layer of the endosperm. The layer is usually one cell in depth and completely surrounds the kernel except at areas over the germ and surrounding the crease, where it is modified. The modified layer that extends over most of the embryo is flattened to less than one-third of the depth found in aleurone cells surrounding the endosperm (8). The aleurone layer is non-existent over the projecting embryo tip.

In cross-sectional view, individual aleurone cells appear square to rectangular. They are tightly sealed between the nucellar epidermis and endosperm. The cell walls are extremely thick and are basically composed of cellulose. The walls of adjacent cells are cemented together by apparently noncellulosic material (8). The interior of these cells contain small granules embedded in a matrix.

Extraction, by aqueous media, of protein from aleurone cells resulted in the conclusion that the cell walls were not impermeable to the passage of protein out of the cell. However, during the extraction of protein from aleurone cells, scanning electron microscopy indicated that aleurone granules were not degraded or dissolved (37). This is contrary to the idea that aleurone granules were composed of protein.

Scanning Electron Microscopy of Whole Wheat Kernels and Mill Fractions

The scanning electron microscope (SEM) is utilized to examine surface phenomena. Transmission electron microscopy and light microscopy both use a source of energy that passes through the specimen. Mechanical manipulation and the chemical preservatives may combine to provide a distortion of the specimen. SEM produces a three-dimensional view of the object, similar to human vision (16). SEM is particularly applicable to cereal science. Cereal specimens, due to their low moisture content, can be viewed au naturel without costly and time-consuming preparative procedures.

SEM consists of a beam of electrons striking the surface of a specimen and detection of the secondary electrons that are produced (38). The electron beam is generated by passing high voltage through a tungsten filament. The beam then passes through a series of magnetic fields that produce an effect equivalent to a lens in a light microscope. The width of the beam at the sample surface is approximately 200 A^{O} (38). This small beam of electrons scanning across the

surface of a sample results in a picture that can be viewed on a cathode ray tube. As the beam sweeps across the specimen, the primary electrons created by the filament knock loose secondary electrons. The number of secondary electrons produced is dependent upon the angle at which the primary beam strikes the specimen. More electrons will be released from a direct collision rather than a glancing blow. This stream of electrons, which varies with the surface of the specimen, is electronically detected. The signal is transferred to a cathode ray tube whose scan is synchronized with the electron scan. Thus, line by line the image of the specimen is duplicated on the cathode ray tube. The picture presented by the cathode ray tube is approximately twice as clear as a television picture. This is because there are twice as many scan lines used to reproduce the features of the specimen (38).

Specimen preparation for SEM is extremely simple. Unlike conventional electron microscopy which requires exhaustive preparation, the wheat kernel can simply be fractured and mounted with a metallic paste. The stub, a metal platform 9 mm in diameter on which the specimen is mounted, and the specimen are coated with a layer of carbon approximately 80-100 A^{o} in depth and then with a layer of gold-palladium to about the same depth; both accomplished by vaporization <u>in vacuo</u> (26). This coating does not interfere with SEM resolution because the maximum resolving power for SEM is 100 A^{o}. Transmission electron microscopy can resolve images of 2 A^{o}, while the light microscope is limited to 2000 A^{o} (16).

One of the first applications of SEM to cereal science occurred when Aranyi and Hawrylewicz utilized this technique in an effort to gain a better understanding of flour-to-dough transitions (3). A hard red winter wheat and flour and an air-classified soft wheat flour were examined. The study was aimed at detecting possible changes in flour or dough specimens caused by a standard fixation-dehydration process required in preparation techniques for transmission electron microscopy. Two flour samples and one dough sample were fixed and post-fixed in buffered glutaraldehyde and osmium tetroxide solutions with subsequent stepwise dehydration in ethyl alcohol. These samples were not embedded in resin, nor were ultra-thin sections prepared with a microtome. Aranyi and Hawrylewicz found that the flour samples showed little if any change, but the dough sample was affected (3). The protein matrix connecting the starch granules seemed to have shrunk and pulled away from the larger starch granules. These investigators concluded that dough samples were sensitive to such treatment and SEM was desirable for this application.

Since this early period, SEM has become increasingly recognized as an investigative tool in biological assays. Pomeranz and associates have examined structural characteristics of a number of grains (oats, barley, wheat and buckwheat) with SEM (26, 27, 28, 29). The elimination of time-consuming preparative procedures in SEM and the capability of viewing an area one cm^2 has produced an instrument that, unlike the transmission electron microscope, can handle a large number of samples in

a relatively short period of time.

More recently, Hoseney and Seib analyzed with SEM the structural characteristics of three different wheat varieties representing three degrees of hardness (18). Their procedure consisted of fracturing the wheat kernels and examining the photomicrographs for fractures in the protein matrix or fractures in individual starch granules. Soft wheat samples fractured through the protein matrix, leaving starch granules intact. However, durum, an extremely hard wheat, fractured along a rough plane through starch granules and the surrounding protein matrix. Using SEM, Wrigley and Simmonds (34, 40) detected differing amounts of protein, shown to be water soluble, adhering to starch granules of both hard and soft wheats. Tests of the protein and starch isolated from either variety indicated that the hardness from either the starch or protein was similar. Therefore, Wrigley hypothesized that water-soluble material surrounding the starch granule is responsible for the cementing action between protein and starch, and thus is responsible for kernel hardness (40).

Moss and Stenvert (23) separated the bran from the endosperm and achieved a correlation between fiber (bran) and milling yield in soft wheats. Bran contains 35% to 50% fiber, while endosperm contains 0.5% (25). The correlation coefficient between milling yield and fiber for soft wheats was 0.88; for hard wheats, 0.29. The low correlation between fiber and milling quality in hard wheats is primarily due to kernel hardness. Fiber was derived from the bran utilizing the following process.

The wheat was milled in a Labconco mill to produce wheat meal of which 20% was retained on a 0.5-mm aperture sieve and 40% was retained on a 0.2-mm aperture sieve. Further processing consisted of boiling the wheat meal in 2% sodium lauryl sulfate containing 1.9% ethylenediaminetetraacetic acid and 2.7% sodium tetraborate. The meal was then washed twice with water and once with acetone. Utilizing SEM, the investigators were able to illustrate a clean separation between the aleurone layer and the endosperm using these detergents (23).

While milling yields varied from 59% to 72% for soft wheat, fiber calculated as a proportion of dry matter ranged in flour from 11.8% to 15.9%. Moss and Stenvert hypothesized that, if the bran contained 50% fiber, much of the variation in milling yields can be explained by variations in fiber (23). Thus, an increase in fiber can be directly correlated to an increase in bran surrounding the wheat kernel.

Characterization of the different streams in a mill flow has been accomplished by several different techniques. Some investigators (14, 15, 39) relied on proximate analyses, vitamin and amino acid surveys, mineral analyses and total energy content. These investigations represent a sizeable backlog of knowledge that are used to define the existing parameters of a mill fraction. However, these assays do not detect the continuing action of the mills to successively reduce the wheat kernel. Others (14, 15, 39) have extensively examined individual flours from hard and soft wheats, air-classified flours, and doughs, but characterization of the mill stream

and its fractions has not been totally accomplished. Visual examination of the mill stream utilizing SEM would be extremely applicable in illustrating the successive breakdown of the wheat kernel.

MATERIALS AND METHODS

Materials

Twenty different varieties of wheat, or the flours therefrom were examined. The varieties included Eagle harvested in 1970; Centurk harvested in 1971; Parker and Clarks Cream (hard white winter) harvested in 1972; Cloud, Gage, Hi Plains, Homestead, Kirwin and Lancer harvested in 1973; Chris (hard red spring), Sage, Scout, Trison and Triumph harvested in 1974. Unless designated differently, these wheats are hard red winter varieties, the predominant class found in the great plains.

Gaines (soft white winter), and Langdon (durum), varieties of unknown harvest dates were also included. Red Chief, harvested in 1975, and two Wichita samples, harvested in 1975 from the Kansas State University greenhouse, were supplied by Dr. Elmer Heyne, Department of Agronomy. A plot of Scout wheat was established to be monitored as the plants matured.

Twenty-three mill stream fractions from a Scout wheat milled on a pilot mill at Kansas State University in 1974 and an air-classified bakery flour were included in this study.

"Glucostat X4" reagent sets for enzymatic determination of D-glucose were obtained from Worthington Biochemical Corporation, Freehold, New Jersey. Bovine serum albumin and Triton X-100 were purchased from Sigma Chemical Company, St. Louis, Missouri. Folin-phenol reagent was obtained from Fisher Sci-

entific Company, Fair Lawn, New Jersey. Glucoamylase (1,4-glucan glucohydrolase, E.C. 3.2.1,3) was a commercial preparation (Diazyme-160, Miles Labs, Elkhart, Indiana). Solutions of glucoamylase were prepared by extracting the commercial powder with 0.05 M citrate buffer, pH 4.8.

Methods

Preparation of Whole Kernels and Mill Fractions for Scanning Electron Microscopy.

Twenty different wheat varieties were examined by SEM. To initiate a comparison of these different varieties required a standardized procedure that would result in uniform specimens. A sharp edge (razor blade) was used to fracture the kernel. The blade was placed at point A (Fig. 1) and pressure applied until cleavage resulted through an area between points B and C. A portion of the cross-sectioned kernel would expose two areas for viewing the bran-endosperm interface and structure. Observations were restricted to the two areas that are shaded in Fig. 1c. The kernel cross-sections were mounted for SEM on 9-mm stubs using silver conducting paste obtained from Pelco Industries, Tustin, California. The samples were coated *in vacuo* with 80 A^{o} of carbon then with about 100 A^{o} of gold-palladium. The samples were viewed in an ETEC Autoscan scanning electron microscope operating at 20-kv accelerating voltage. Images were photographed on Polaroid film (type 55P/N).

Observations of kernel cross-sections were normally

Figure 1. Illustration of the technique used to prepare grain samples for scanning electron microscopy: a. Point of fracture by sharp edge; b. Fractures of the kernel were contained within this area (B to C); c. Photo micrographs were taken of the kernel surface only in the shaded areas.

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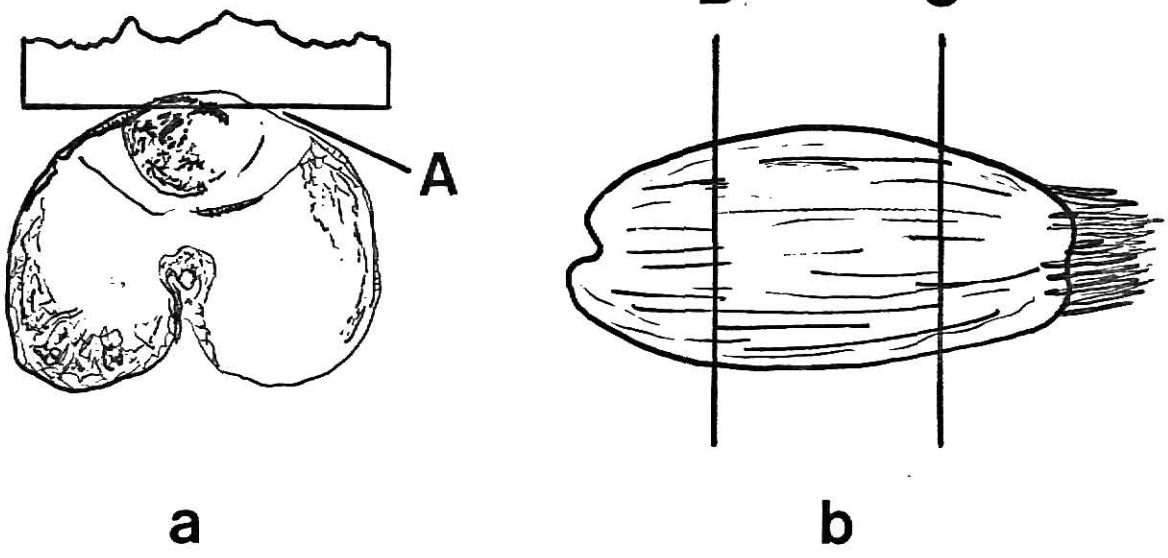

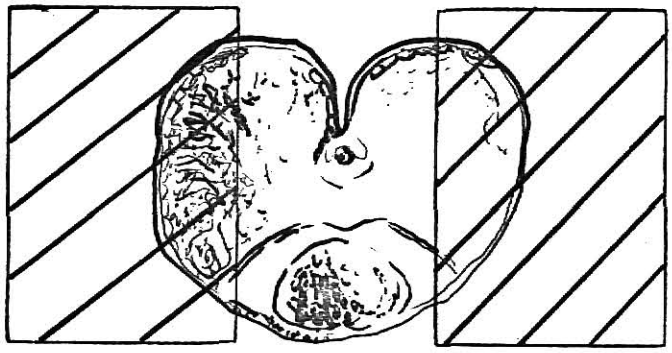

FIGURE 1

made at two magnifications, 400X and 1100X. The 400X magnification allowed examination of the outer and inner bran, the aleurone layer and a considerable portion of endosperm. This lower magnification produced perspective facilitating identification of different structural areas in the bran-endosperm interface. The 1100X magnification primarily was used to examine the bran and aleurone layers. Measurements of the bran and aleurone widths were recorded on a centimeter scale and converted to microns. A series of ten measurements per photomicrograph were recorded for each variety for three areas: pericarp, aleurone, and the total pericarp and aleurone width. These data were averaged, means were calculated and multiple range tests were conducted to determine whether a statistical difference in bran and aleurone layer widths existed between varieties.

Photomicrographs for the wheat-kernel maturity studies were obtained from cross-sectioned Scout wheat kernels. Individual Scout kernels were harvested beginning eleven days after half bloom.[1] The procedure for cross-sectioning the kernel was similar to the procedure outlined earlier for whole kernels (Fig. 1). However, the high moisture content in the immature kernels presented a charging[2] problem during SEM obser-

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[1]Half bloom is that stage of development of wheat where half of the plants have bloomed and fertilization is in progress.

[2]Charging on the SEM occurs at thin areas of the carbon gold-palladium coat producing illumination of the sample surface. Electrons are absorbed in the area causing an intense irradiation that blocks viewing.

vation. Therefore, an air-drying procedure was established that involved heating the kernels to 110°F at 5 pounds psi vacuum. The kernels were dried to 12% to 15% moisture content. Photomicrographs were taken at the same magnifications as those cited for examination of the whole kernel. The micrographs were examined for differences in structural characteristics as the kernels matured.

Since the mill stream reduced the bran and endosperm particles, no fracturing procedure was necessary. A stub was simply prepared by partially covering the surface with double sided adhesive tape and scattering the sample on the adhering surface. Excess sample was blown off to prevent caking and loss of the gold-palladium coating if excess sample should have dislodged while viewing. Photomicrographs were taken at 100X and 400X. Examination of mill stream composition entailed viewing a large area at an appropiate magnification. Lower magnification, 100X, provided a large viewing area plus ample clarity and definition of the different material in each mill stream.

Experimental Milling of Scout and Lancer Wheats. Two wheats were selected for milling based on their difference in pericarp width. A milling study was conducted to compare the amount of starch residue clinging to the bran. Adjustable roll settings were used to reduce the influence of the miller and the types of rolls (Fig. 2). Whole wheat kernels (100 g), cleaned and tempered to 16% moisture content, were ground on

Figure 2. Experimental mill flow used in the milling of Scout and Lancer wheats.

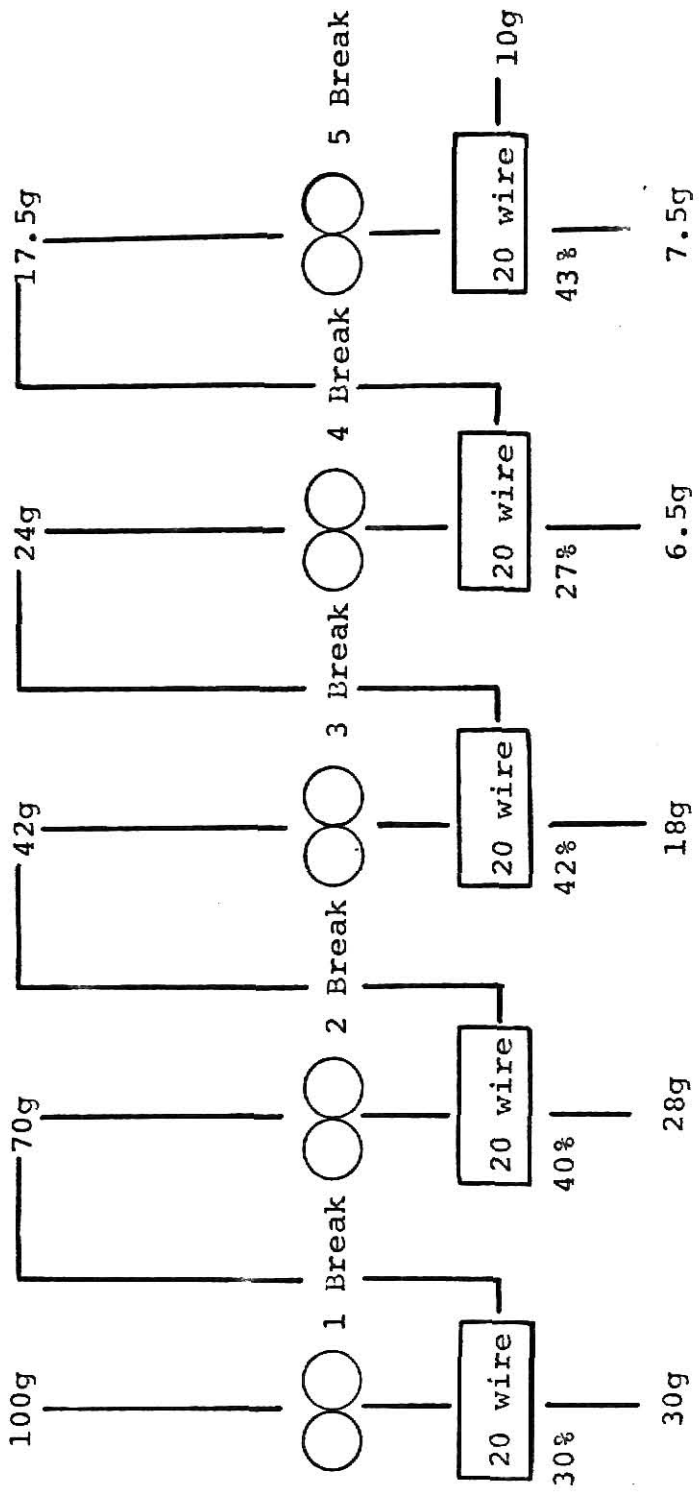
100g
1 Break
70g
20 wire
30%
30g
2 Break
42g
20 wire
40%
28g
3 Break
24g
20 wire
42%
18g
4 Break
17.5g
20 wire
27%
6.5g
5 Break
10g
20 wire
43%
7.5g

FIGURE 2

first break rolls to a size that yielded 30% thrus using a 20-wire sieve. The remaining 70 g were ground on second break rolls to produce 40% thrus using a 20-wire sieve. The overs of the second break weighed 42 g. Third break produced 42% thrus using a 20-wire sieve. The overs from the third break weighed 24 g. The 24 g of wheat meal went through fourth break and 27% of this mill stream were thrus. The overs of fourth break weighed 17.5 g. Fifth break was ground to permit 43% of this stock to pass through a 20-wire sieve. This final separation produced a sample size of 10 g of wheat meal.

Ross experimental mills were used to dislodge the endosperm from the bran. These mills were adjusted for each wheat variety to maintain the correct percentage through the 20-wire sieve. The bran samples were then ready to analyze for starch.

Determination of Starch Content. Glucoamylase was used to convert starch to D-glucose. The D-glucose released during enzymatic hydrolysis of starch was measured by the procedure described by Shetty et al. (32).

An appropiate amount of bran (approximately 2 g bran containing about 100 mg starch) was weighed into a 25-ml volumetric flask (containing a magnetic stirring bar of known volume). Dimethyl sulfoxide (DMSO), diluted to 90% by mixing nine volumes DMSO with one volume water, was heated with continuous stirring to 55°C and added to the bran to bring the contents of the flask to volume. After stirring for five min-

utes, the slurry was cooled to 37°C (water bath) which required about 10 min.

An aliquot (1.0 ml) of the gelatinized starch was removed and placed into a tube containing 150 mg of Diazyme 160 (3.25 mg of protein/ml) in 5.0 ml of 0.05 M acetate buffer, pH 4.8. Enzymatic hydrolysis was accomplished for 30 min at 37°C and D-glucose content in a 1.0 ml aliquot was measured using the method (Method A) for solutions containing 40 to 120 µg glucose/ml (32).

The hydrolyzed starch (1.0 ml), prewarmed to 37°C, was incubated for one hour at 37°C with 10.0 ml of "Glucostat" reagent that had also been warmed to 37°C. The reaction was terminated by adding 0.5 ml of 4 M hydrochloric acid. The solution was cooled to 25°C and the absorbance was measured at 400 nm using a Beckman DU spectrophotometer. Analyses were done in duplicate. A standard curve was prepared using 50-100 µg of D-glucose.

Buffers: The 0.5 M Tris-HCl buffer consisted of 61 g of Tris-(hydroxymethyl)-aminoethane dissolved in 80 ml of 5 M hydrochloric acid diluted to one liter with water. The Tris-HCl buffer was warmed to 37°C and the pH adjusted to 7.00. The acetate buffer, 0.05 M, contained 3 ml of glacial acetic acid and 4.1 g of anhydrous sodium acetate per liter adjusted to pH to 4.8.

Glucostat Reagent: Triton X-100 was mixed with ethanol (4:1 v/v) and 4.0 ml of this solution was added to one vial of

chromogen. The ethanolic solution of Triton X-100 should be injected into the chromogen vial before it is opened. The chromogen-surfactant mixture was then added to 375 ml of 0.5 M Tris-HCl buffer. Another 25 ml of Tris-HCl buffer was used to rinse the vial, bringing the combined volume of the solution to 400 ml. The glucostat reagent was stable for five days at 5°C.

Determination of Enzyme Activity. Specific activity of an enzyme is expressed as the units of enzyme activity per mg of protein. Smith (35) recorded 13.2 units activity per mg of protein for commercial grade Diazyme 160. The crude enzyme, before dialysis, had 3.6 mg of protein per ml. The protein content of solutions of Diazyme 160 used in this study was measured and the enzyme was assumed to have the specific activity reported by Smith (35). Protein was measured by the Lowry procedure (21) using bovine serum albumin as a standard.

Kernel Maturity Analysis. The wheat variety Scout was studied for a period of 27 days, beginning 11 days after half bloom. For this particular group of plants, half bloom was visually judged to have occurred on May 22, 1975. Approximately 50 plants were tagged to insure an adequate supply of kernels. The kernels were selected from the center of the head and harvested at 1:00 pm every third day. Once the kernels were harvested, moisture content and green weight were determined. The kernels, containing a relatively high mois-

ture content, were dried at 110°F at 5 pounds psi vacuum prior to examination by SEM.

Toluene distillation: Whole kernels (1 g) were fractured and placed in a 250-ml round-bottom flask with approximately 100 ml of toluene. The mixture was distilled and the water was collected in a Dean-Stark trap. A correction factor[3] was applied for water lost during the kernel-fracturing process and moisture content was calculated as percentage of total weight.

Green weight: A random sample of ten kernels was selected and their weight, without drying or further processing, was recorded in mg.

Analysis of Air-Classified Flour. Protein: Nitrogen was determined by the Kjeldahl procedure using AACC method 46-11 (1). Protein was calculated as N x 5.7.

Starch Damage: The amount of reducing sugar present was determined utilizing AACC Method 76-30A and AACC method 80-60 (1). This value was multiplied by Yamazaki's correction factor of 1.65 (1) to arrive at the total percentage

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[3]A correction factor was calculated for the amount of moisture lost during the process of fracturing the kernel. Several Scout samples were allowed to dry at 110°F for 24 hours at 5 pounds psi vacuum. Moisture was calculated as a percentage of weight. These were compared with Scout samples collected at the same time which were fractured and distilled. The difference between methods was calculated to be 15% of the total moisture. Correct moisture content is determined by multiplying the distilled moisture content by 1.15. This procedure applied only to the first few samples that contained a moisture content above 40%.

of damaged starch.

Particle Size Determination for Air-Classified Flour. A sub-sieve sizer from Fisher Scientific (Pittsburgh, Pennsylvania) was employed to record mean sizes within an effective range of 2 to 20 μ (12). The flour sample (1.44 g) was placed in a test cylinder with perforated pistons in either end. A standard pressure was applied to the test cylinder to pack the sample. The cylinder was locked into test position and the chart was adjusted to a porosity of 0.465. The range knob can be adjusted to either double read or direct read, depending upon the nature of the sample. The meniscus of the manometer rises and becomes stationary at a particular level. The average particle size of the sample can then be read directly from the chart. The nine air-classified samples were run in duplicate.

RESULTS AND DISCUSSION

Examination of Wheat Kernel Pericarp

The bran-endosperm interface was studied in twenty varieties of wheat. Fourteen of the twenty varieties were hard red winter wheats that were harvested in Kansas and Nebraska. The other six varieties of wheat consisted of two hard red winter, one durum, one hard white winter, one soft white winter, and one hard red spring wheats. SEM was utilized to provide a three-dimensional image of the interface that would be amenable to measurement and study.

Photomicrographs of the kernels were taken at primarily two magnifications: 400X (Fig. 3b) and 1100X (Fig. 3c). However, during the familiarization period with the SEM, several varieties were photographed at 80X (Fig. 3a) to identify structures. The data for bran width were obtained from the photomicrographs at 400X and 1100X and were analyzed by computer using an analysis of variance program. The average bran width for each variety and its standard deviation were calculated from the observations (Table I).

Differences between varieties were tested by examination with the least significant difference (LSD) multiple range test. The LSD value was calculated to be 3.267 for twenty varieties (Fig. 4) and 3.497 for fourteen varieties (Fig. 5). The LSD multiple range test on twenty varieties produced three statistically different groups. The varieties were separated

Figure 3. Photomicrographs of fractured wheat kernels at three magnifications used to study bran width: a. Scout variety, 80X; b. Trison variety, 400X; c. Sage variety, 1100X.

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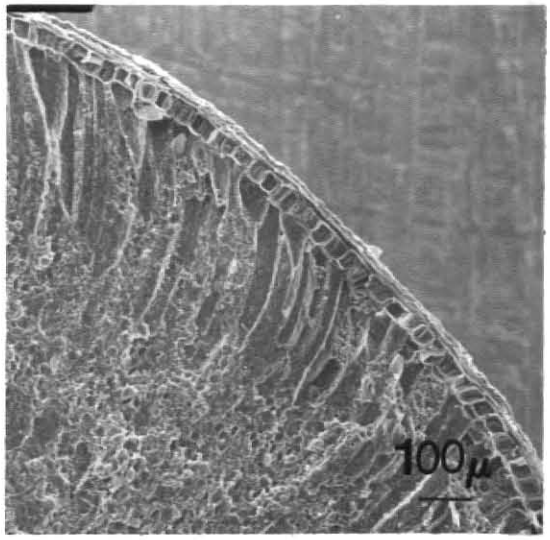

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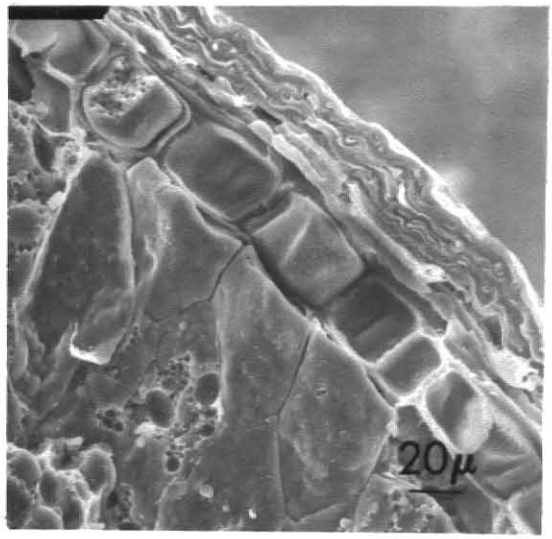

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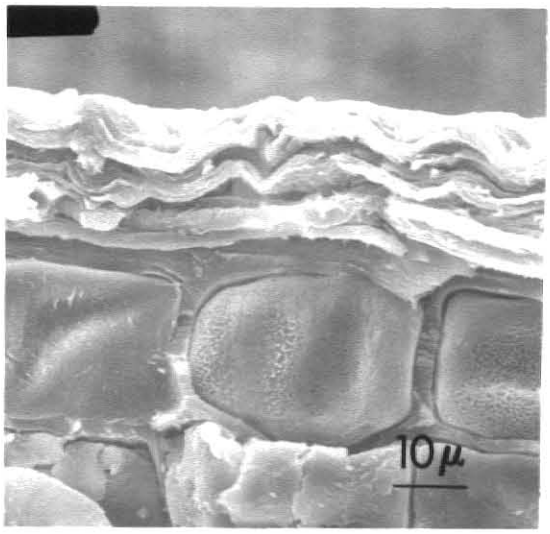

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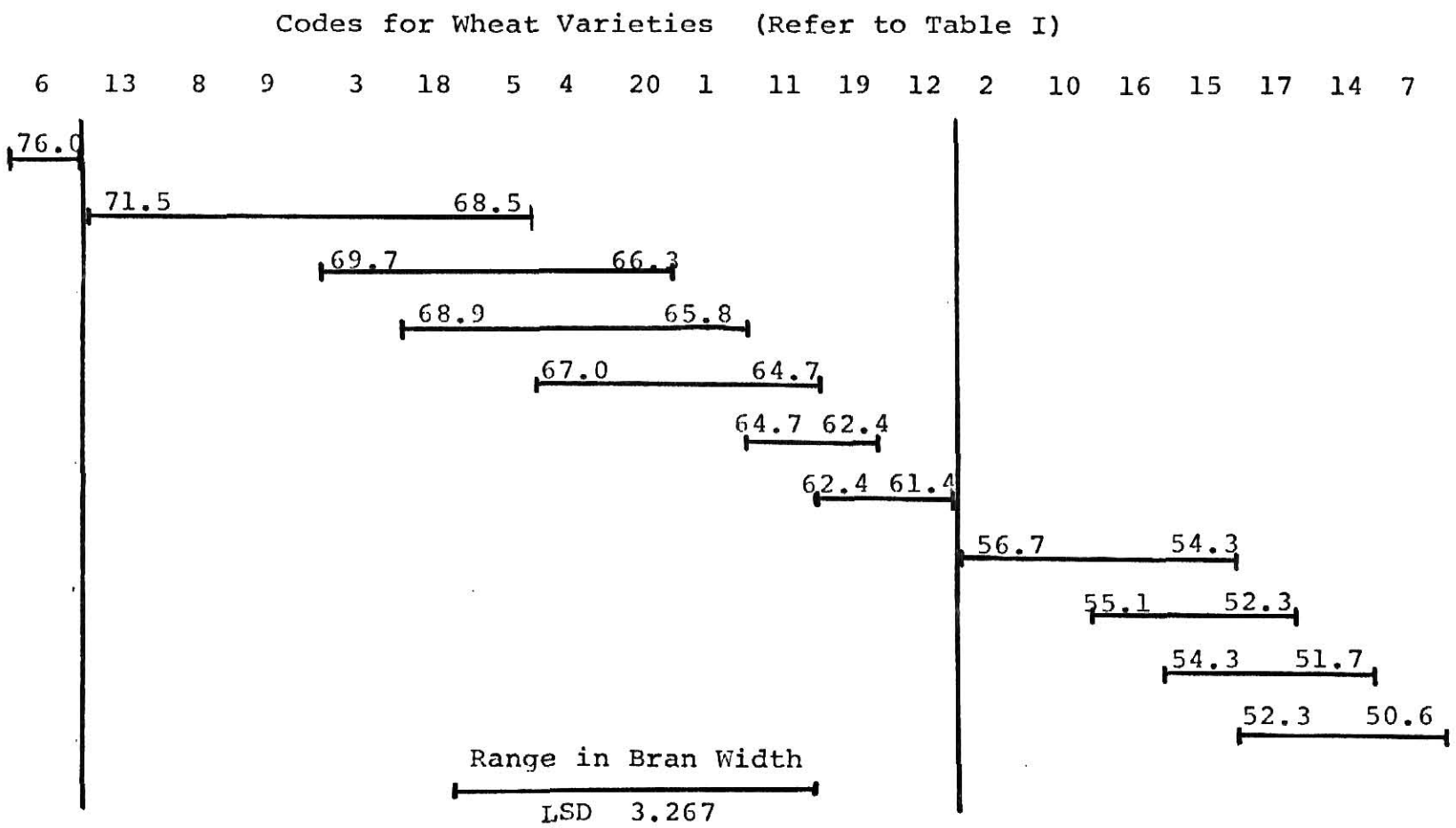
TABLE I

BRAN WIDTH OF TWENTY WHEAT VARIETIES

Code	Variety	Location	Means[a] (mm)	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	HiPlains	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	Redchief	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	Clarks 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 10 observations

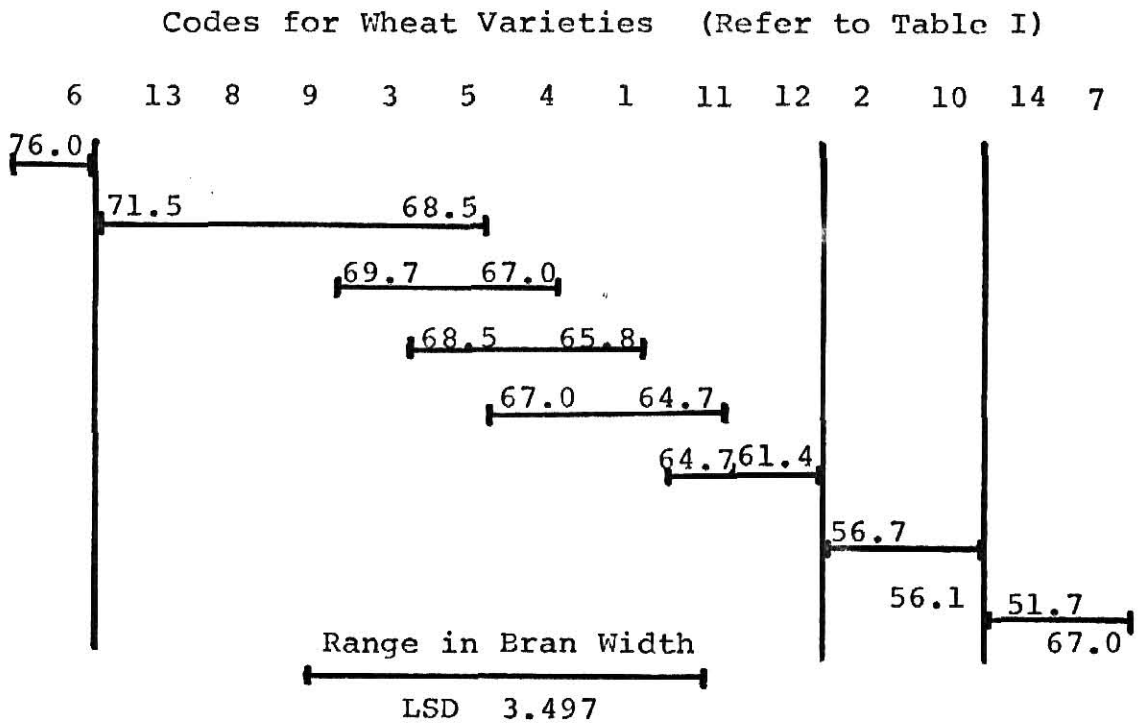
Figure 4. Multiple range test for 20 wheat varieties: Least significant difference (LSD value = 3.267), at .05 alpha level.



GROUP I	GROUP II			GROUP III	
6	13	18	1	2	17
	8	5	11	10	14
	9	4	19	16	7
	3	20	12	15	

FIGURE 4

Figure 5. Multiple range test for 14 wheat varieties: Least significant difference (LSD value = 3.497), at .05 alpha level.



GROUP I	GROUP II			GROUP III	GROUP IV
6	13	3	1	2	14
	8	5	11	10	7
	9	4	12		

FIGURE 5

into groups when LSD ranges were not overlapping. The first group consisted of Lancer which had an extremely thick bran. The second group consisted of Triumph, HiPlains, Homestead, Eagle, Langdon, Cloud, Kirwin, Chris, Parker, Sage, Gaines and Trison varieties. Those wheats, excluding Langdon, Chris and Gaines are hard red winter varieties. HiPlains, Homestead, Cloud and Kirwin are varieties that have been recently developed to withstand severe environmental conditions and produce greater yields. The third group consisted of Centurk, Scout, two samples of Wichita, Clarks Cream, Red Chief and Gage. Wichita and Red Chief are older hard red winter varieties that have been replaced by newer varieties. Scout is still the variety most widely grown in Kansas. Analysis of the LSD multiple range test for fourteen varieties illustrated a significant difference exists between Scout and Red Chief (Fig. 5). This produced an altered Group III consisting of Scout and Centurk, and a Group IV consisting of Red Chief and Gage.

Statistical analysis of the wheat kernels differentiated the twenty varieties into three groups based on bran widths. A milling test and subsequent starch analysis were performed to determine whether bran width influenced the forced separation of bran and endosperm during milling.

Scout and Lancer, varieties of wheat exhibiting extremes in bran width, were logical choices as samples for milling tests. Scout represents a thin bran width (mean 56.10 mm, Table I) while Lancer represents a thick bran width (mean 76.00

mm, Table I). The milling procedure involved adjustable roll settings that produced a certain percentage of thrus. This procedure reduced the effects of different types of rolls and different millers. A wheat sample of 500 g produced 50 g of bran using the Ross mills, resulting in an extraction rate of 90%. The process of adjusting the rolls to the correct break releases required approximately 500 to 700 g of wheat. Therefore, 1000 to 1200 g of cleaned, tempered wheat were used for the milling process.

Two grams of the milled bran samples were treated with DMSO to gelatinize the starch. An aliquot (1.0 ml) was subjected to glucoamylase digestion to determine the amount of glucose present. The quantity of starch released from treating Scout bran with glucoamylase was 74 µg per 20 mg of bran. Lancer bran produced 62.5 µg per 20 mg of bran (Fig. 6). These results indicate that Lancer bran probably contained less starch.

Based on these results, thicker bran appeared to separate from the endosperm, providing more usable endosperm. The relationship between bran width and separation of the bran and endosperm is tentative because (A) the completeness of starch extraction and hydrolysis was not measured, and (B) the fixed break release milling procedure might have forced excess starch with less bran (Scout) to maintain the fixed break release.

Figure 6. Hydrolysis of starch by glucoamylase (Diazyme 160) at 37°C in 0.05 M acetate buffer, pH 4.8: □, D-glucose standard; △, D-glucose from Lancer wheat meal hydrolyzed by glucoamylase; ○, D-glucose from Scout wheat meal hydrolyzed by glucoamylase.

HYDROLYSIS OF STARCH BY GLUCOAMYLASE

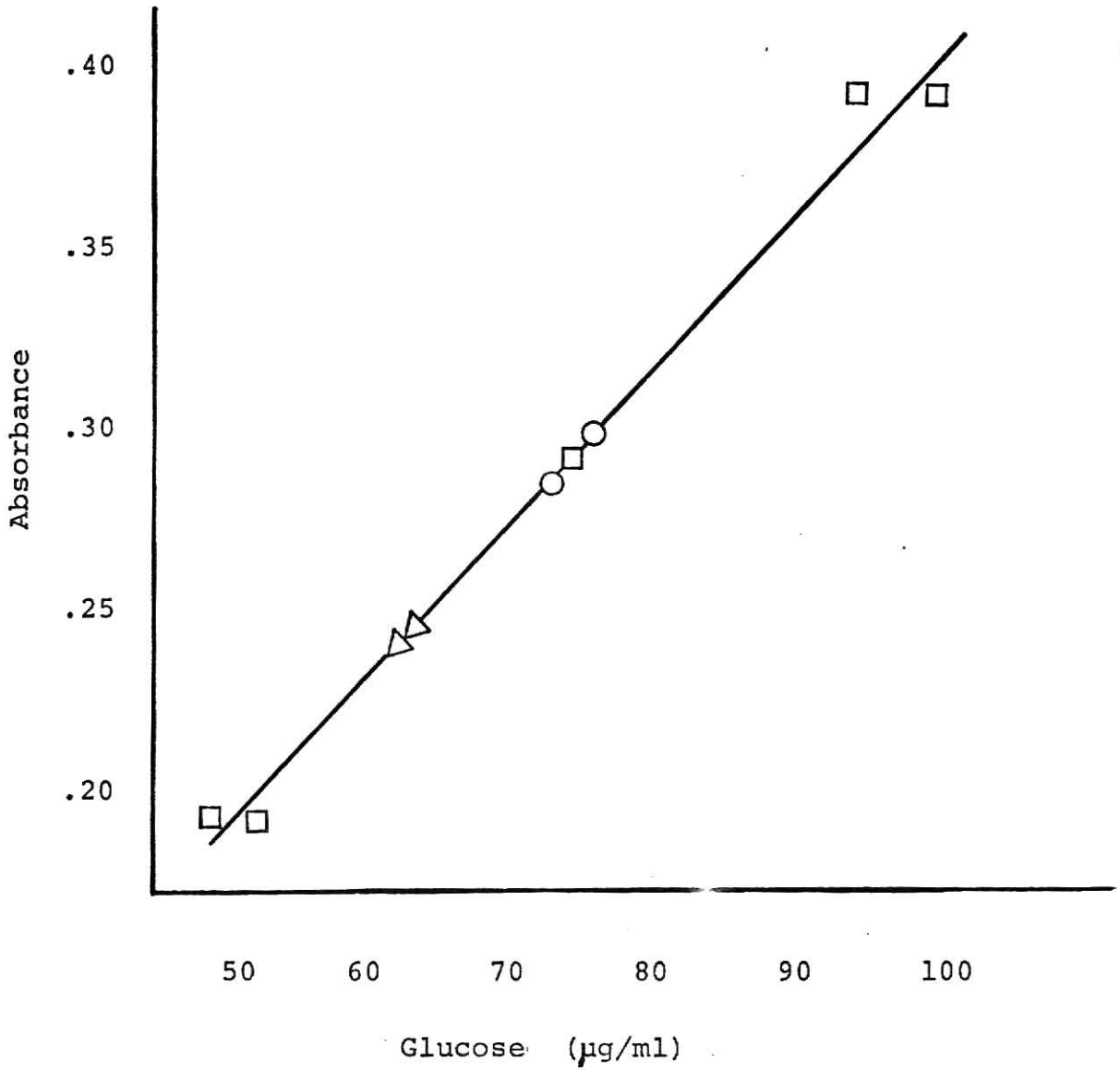


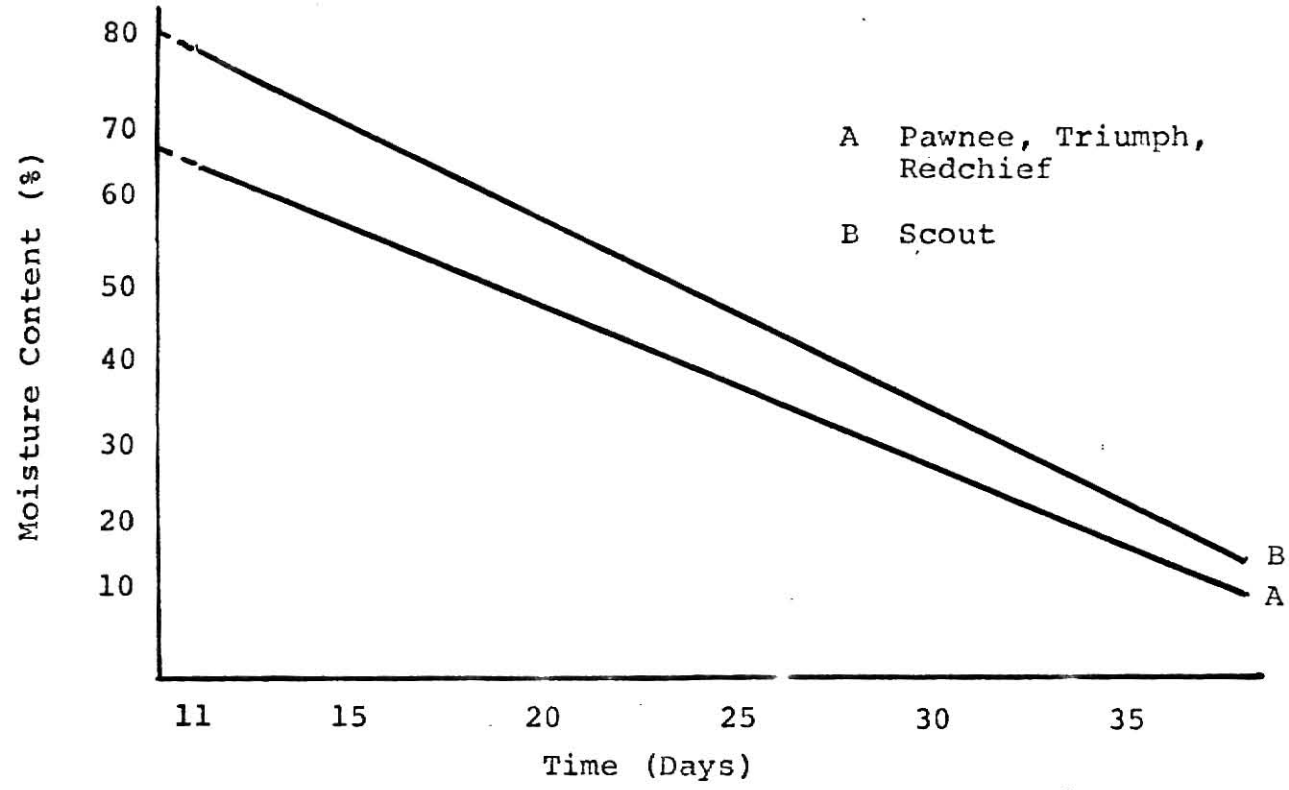
FIGURE 6

Utilization of the Scanning Electron Microscope to Define Structural Development within the Maturing Wheat Kernel

SEM was used to study structural development in kernels of Scout wheat for a period of 27 days beginning 11 days after half bloom. A regression analysis (Fig. 7) was utilized to compare moisture content and green weight of the Scout wheat with the moisture contents and green weights of Pawnee, Triumph and Red Chief varieties reported by Scott (32) in 1953 and 1954. Those varieties were examined for climatological differences that restricted or enhanced yields in different areas of Kansas. Scott's data were used to insure that the small Scout sample used achieved a normal growth pattern. The regression analysis indicated that at 11 days after half bloom, Scout had 12% more moisture than the other three varieties. However, as the kernels reached maturity, moisture contents of the four varieties dropped to 10 to 15%. The regression analysis showed a high correlation between the green weights among the four varieties. The regression lines crossed on the twenty-second day, indicating that Scout kernels were losing water faster than endosperm was being produced.

Preservation of kernel cell structure and dimensions was necessary during the drying procedure to prepare the specimen for SEM observation. Three different techniques were tested. One method, freeze-drying, resulted in a collapse of the endosperm and several bran layers, leaving a thin fragile layer of the outer pericarp that outlined the kernel dimensions. Secondly, transpiration and evaporation of kernel moisture were

Figure 7. Regression analysis of moisture content and green weight between Scout, Pawnee, Triumph and Red Chief varieties.


Moisture Content (%)
80
70
60
50
40
30
20
10
A Pawnee, Triumph, Redchief
B Scout
B
A
11
15
20
25
30
35
Time (Days)

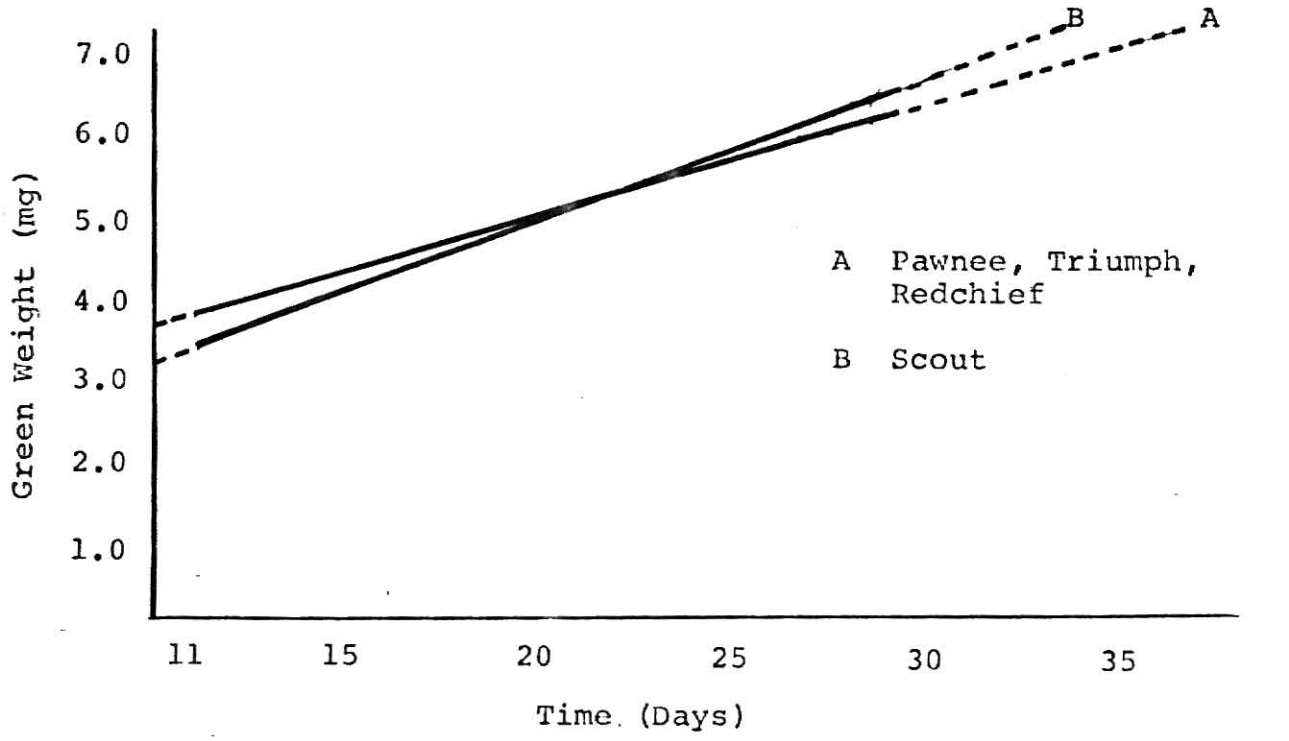

Green Weight (mg)
7.0
6.0
5.0
4.0
3.0
2.0
1.0
B
A
A Pawnee, Triumph, Redchief
B Scout
11
15
20
25
30
35
Time (Days)

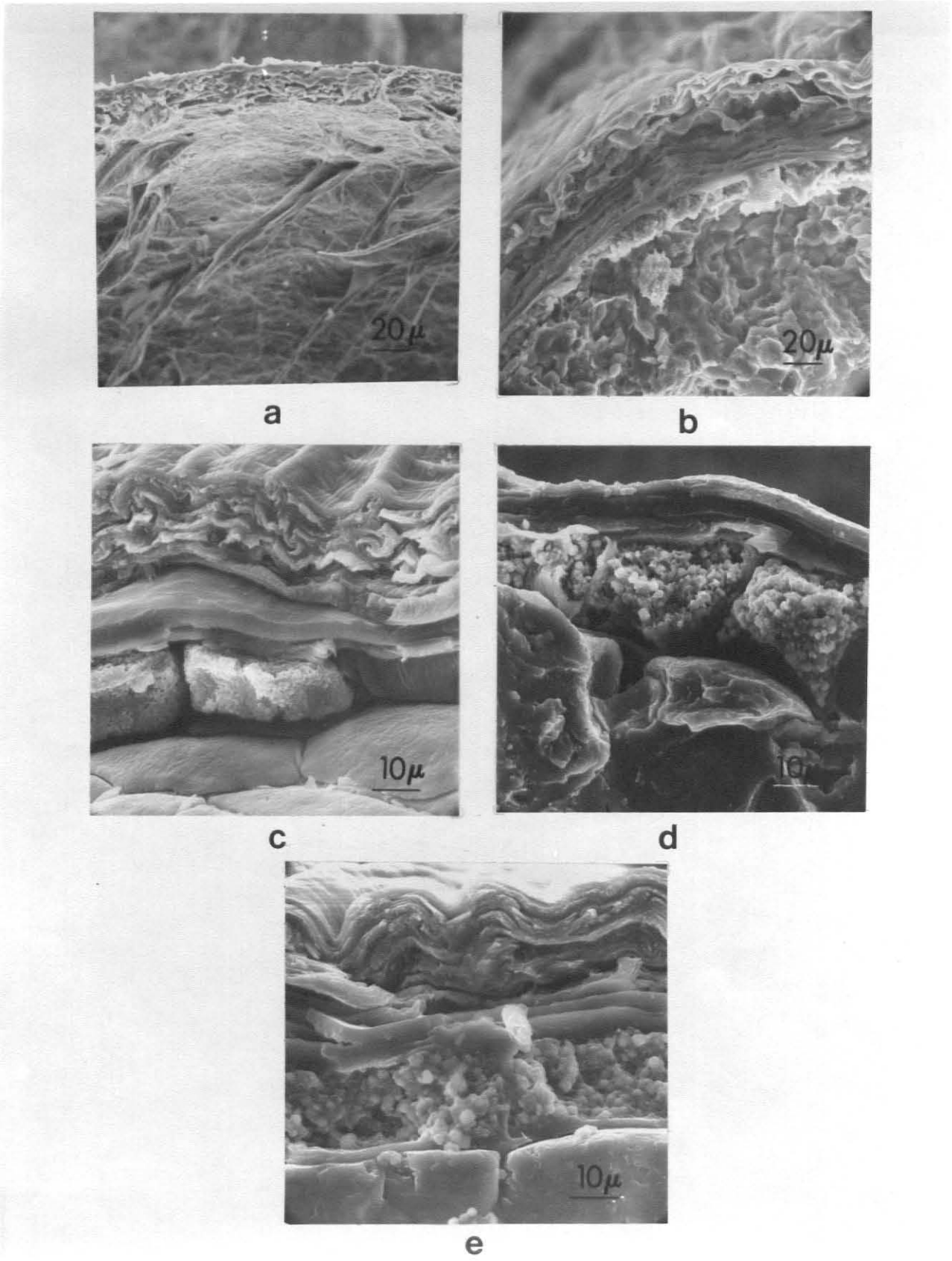
FIGURE 7

allowed to occur for 48 to 72 hours resulting in enzymatic action that rendered the bran layers of the immature kernels undefinable. Thirdly, the method that was utilized dried the kernels at 110°F with 5 pounds psi vacuum until a moisture content of 12 to 15% was reached.

Kernels were examined by SEM at specified intervals for variations in structure and morphology. The first photomicrographs of the maturing kernels (Fig. 8a) were taken 11 days after half bloom. The most notable characteristic at that time was the absence of the aleurone layer. The layers of the inner and outer pericarp appeared to be present, but were not well defined. The endosperm lacked cellular definition. After the kernel was air dried, the endosperm appeared to have separated from the layers of bran, leaving a hollow cavity between the bran and the endosperm (Fig. 8a).

Fourteen days after half bloom the outer bran layers appeared to contain small granules approximately 10 μ in diameter (Fig. 8b). The aleurone layer seemed to contain small aleurone granules, but lacked cellular definition at this stage of growth. The lack of cellular definition gave the appearance that the aleurone granules formed a continuous ring surrounding the endosperm. Due to the fracture through the parenchyma cells, large starch granules were evident. Small starch granules (5 to 15 μ) were not recognizable, due either to the abundance of protein matrix or to the high moisture content of the endosperm 14 days after half bloom.

Figure 8. Scanning electron photomicrographs of Scout wheat kernels: a. 11 days (after half bloom); b. 14 days; c. 17 days; d. 20 days; e. 23 days; f. 26 days; g. 29 days; h. 32 days; i. 35 days; j. 38 days.


20μ
a
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b
10μ
c
d
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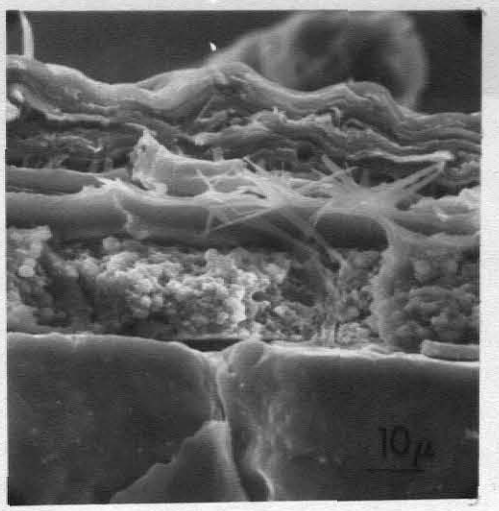
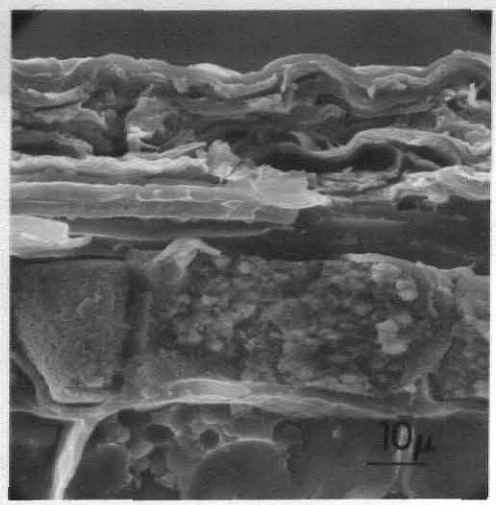
e



f



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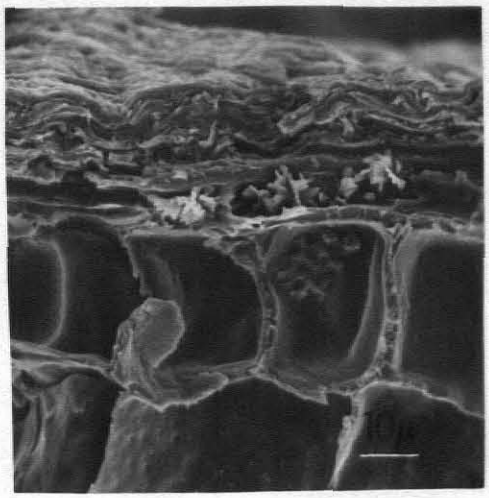

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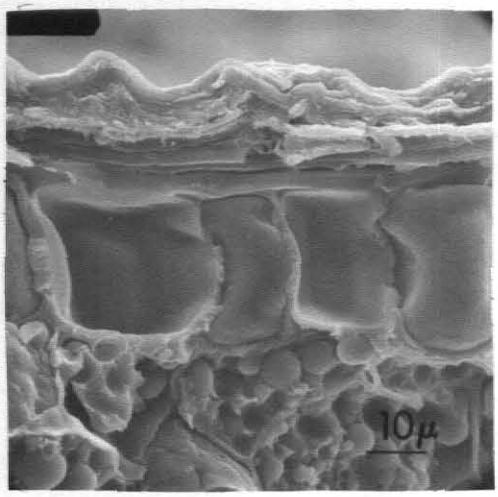

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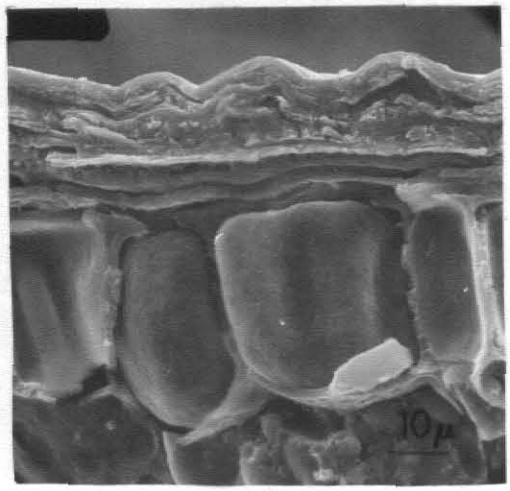


j

Approximately 17 days after half bloom the aleurone layer had formed rigid cells (Fig. 8c). The aleurone granules were tentatively identified by their porous, cake-like appearance. The outer pericarp separated from the inner pericarp; both the pericarp and aleurone layers separated from the large parenchyma cells that compose the endosperm. The outer pericarp appeared to be folding and collapsing. Retention of moisture by the endosperm and aleurone layer was contrasted with the dry crumpled appearance of the pericarp. The seed coat, containing the substances suberin and cutin, separates the pericarp and aleurone layer. The parenchyma cells of the endosperm contained an ample quantity of starch granules and protein matrix causing an inflated appearance.

Twenty days after half bloom the aleurone granules were easily recognizable as round nodules in thick cellular capsules (Fig. 8d). The outer and inner pericarp layers were blurred, which rendered some layers unrecognizable. The bran did not appear to have folded as extensively compared to the 17-day series. External moisture had been received during this time interval. The endosperm had fractured between parenchyma cells. Proteinacous material appeared to be concentrated in the exterior of the parenchyma cells.

The bran layer had collapsed and folded dramatically 23 days after half bloom (Fig. 8e). A separation had occurred between the inner and outer pericarp. The form and lack of folding in the aleurone layer was an indication that suberin

and cutin in the seed coat were limiting moisture evaportion. The aleurone granules were still underdeveloped, but the spherical nodules were more apparent in their matrix material. The endosperm had fractured between the parenchyma cells. At that point, it was still difficult to recognize starch granules under the protein matrix on the exterior of a parenchyma cell.

The bran continued to dry at 26 and 29 days after half bloom (Figs. 8f and g) which led to increased folds in the outer pericarp. Continued separation of the pericarp from the seed coat and aleurone layer was apparent. The aleurone layer was very pronounced. The exterior matrix material of the aleurone cells appeared very porous and gave no indication of the aleurone granules that existed on the interior. The endosperm had a moisture content of approximately 35 to 45% (Fig. 7). Starch granules of 15 and 50 μ were visible. Protein matrix within the fractured parenchyma cell seemed to have sufficient strength leaving indentations of starch granules that had separated when the kernel was fractured. At this point, the matrix did not appear to have developed the hardness associated with certain varieties at lower moisture contents where starch granules were broken when endosperm cells were fractured (18).

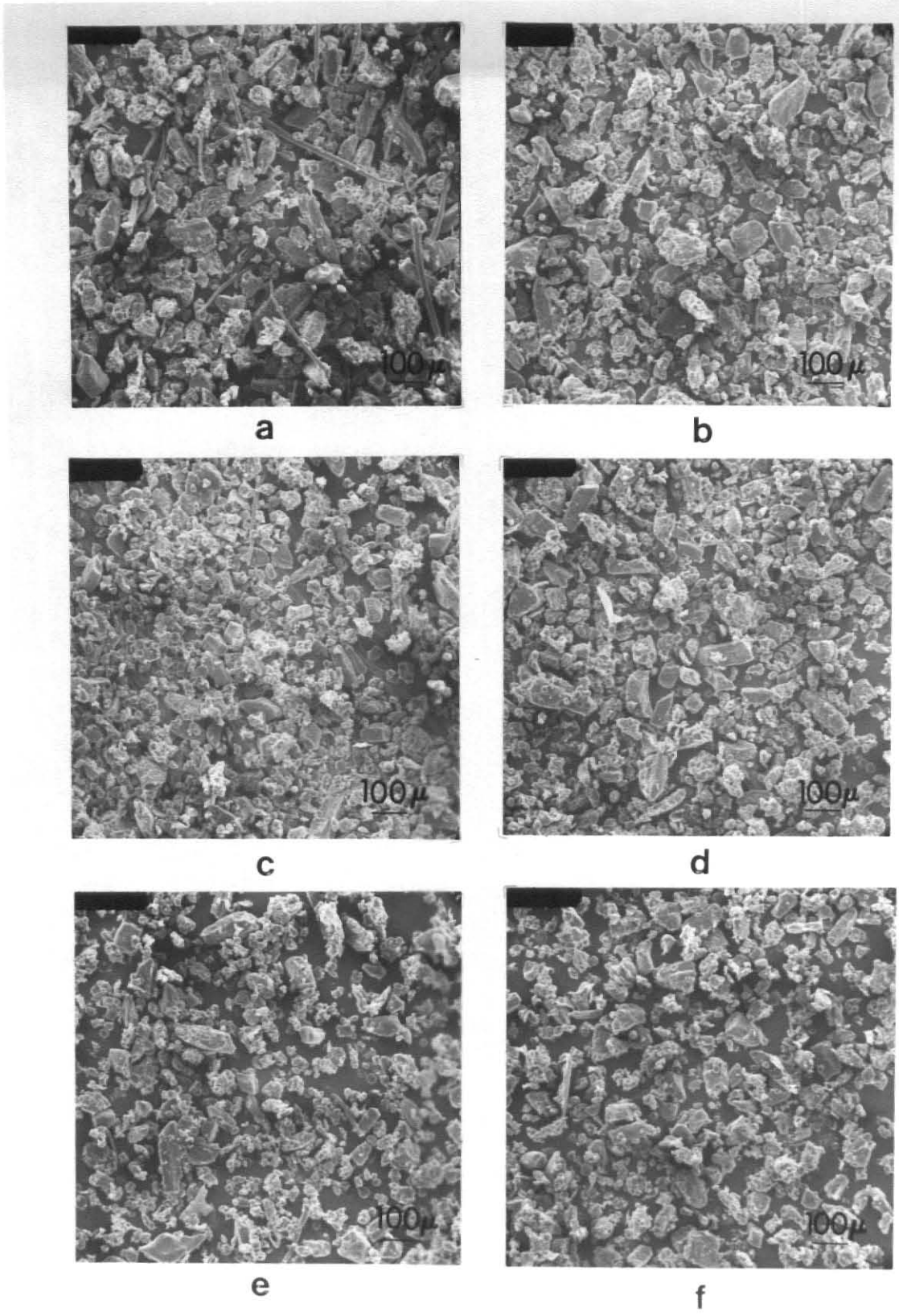
The last series of samples were collected prior to harvest (32, 35, and 38 days after half bloom). Little change in structural characteristics of the bran-endosperm interface occurred between 29 (Fig. 8g) and 38 days (Fig. 8j). The outer

bran continued to dehydrate and separate from the seed coat. Internal moisture declined during the six day period to 10 to 15% (Fig. 7). The aleurone bodies were round and clearly defined in the matrix material. The wheat plant had increased its energy reservoir by producing starch granules in the endosperm. The granules ranged in size from 5 to 50 μ. With the loss of water, the protein material had strengthened to the point where parenchyma cells were fractured when the kernel was fractured.

Examination of Mill Stream Components

The roller-milling process is divided into two major functions: breaking the kernel and reducing the endosperm. The breaking products are illustrated in Figure 9. The kernel is fractured by pressure and shear imparted by the rolls. The first fracture of the cleaned, tempered wheat kernels resulted in a range of particle sizes (Fig. 9a). Brush hairs from the kernel apex are also present. The pre-break fraction was sieved and portions sent to first break and third break redust, to the number one purifier or separated as finished product flour (Fig. 10). Figure 9 illustrates the break release components that are mixed to produce the finished product - flour. Bran particles are absent or particularly difficult to identify. The flour fractions in Figure 9a - 9d were collected from the thrus of a 12XX sieve. Fourth break and fifth break used 9XX and 8XX sieves, respectively, largely resulting in the absence of larger endosperm particles in Fig-

Figure 9. Scanning electron photomicrographs of a 23-fraction mill stream obtained from Scout (74-425) wheat variety: a. Pre-break; b. First break; c. Second break; d. Third break; e. Fourth break; f. Fifth break; g. Fine sizings top; h. Fine sizings bottom; i. Coarse sizings top; j. Coarse sizings bottom; k. First mids top; l. First mids bottom; m. Second mids top; n. Second mids bottom; o. Second quality; p. First tailings; q. Third mids; r. Fourth mids; s. First, second, third break redust; t. Fifth mids; u. Sixth mids; v. Bran and shorts duster; w. Suction.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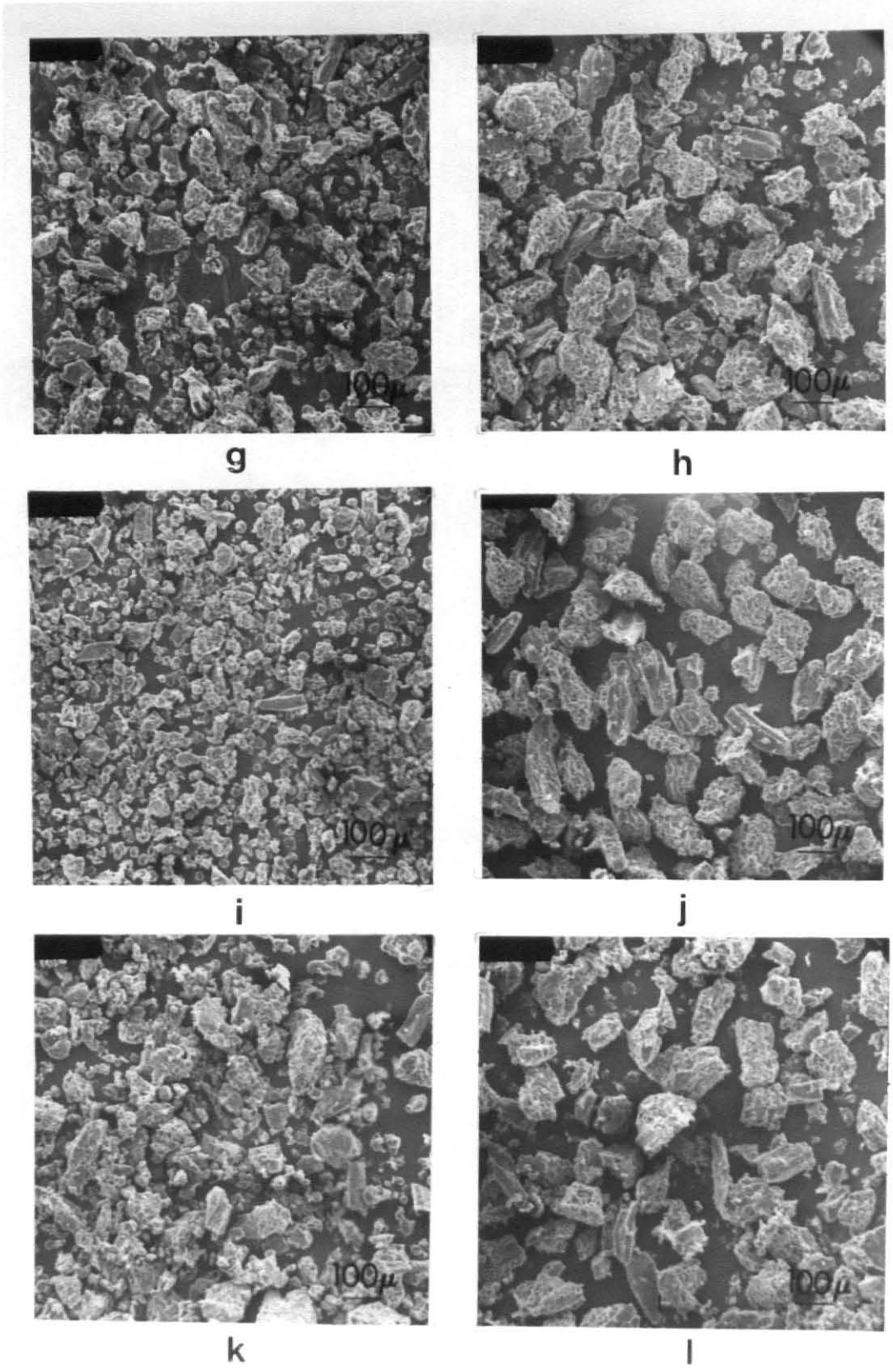
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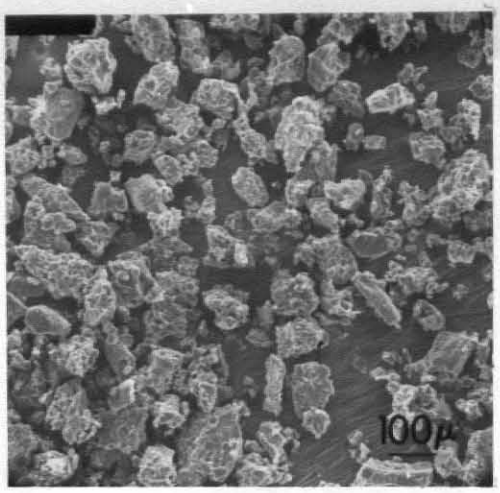

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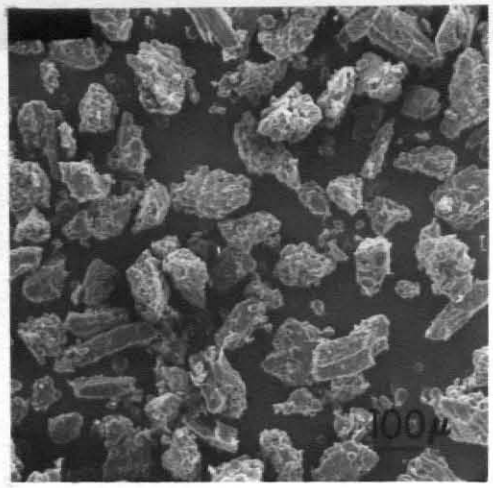

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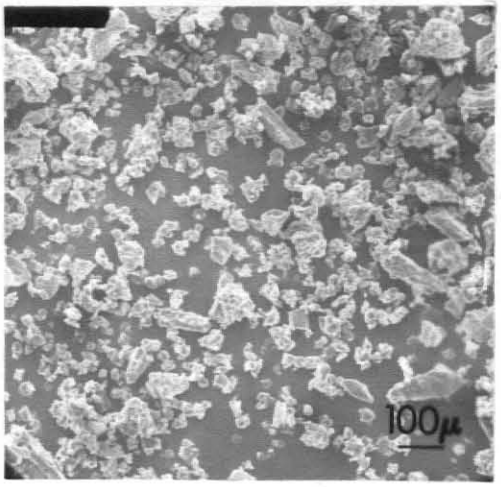

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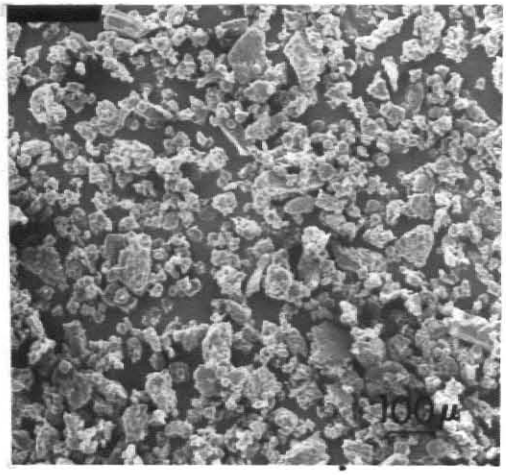

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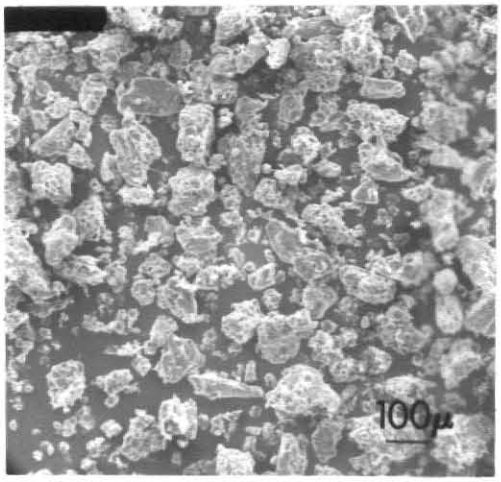

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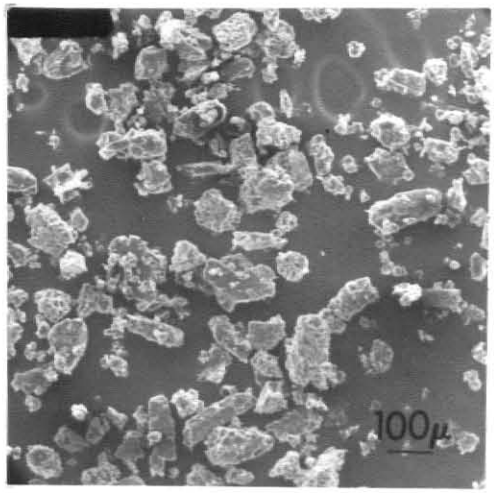

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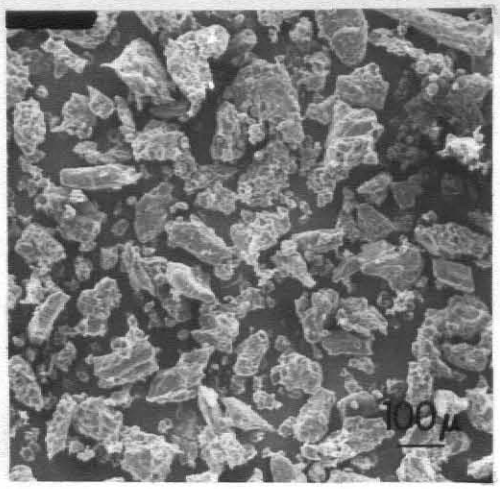

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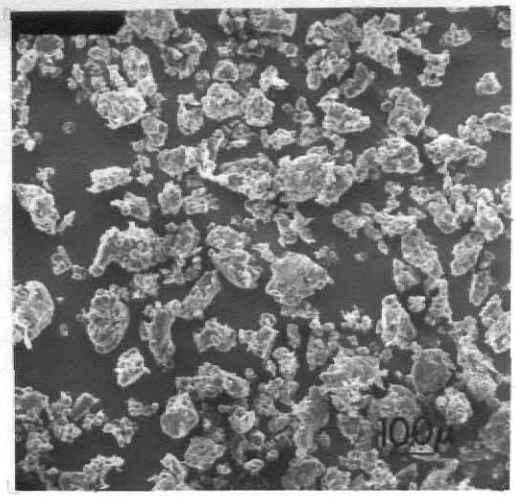

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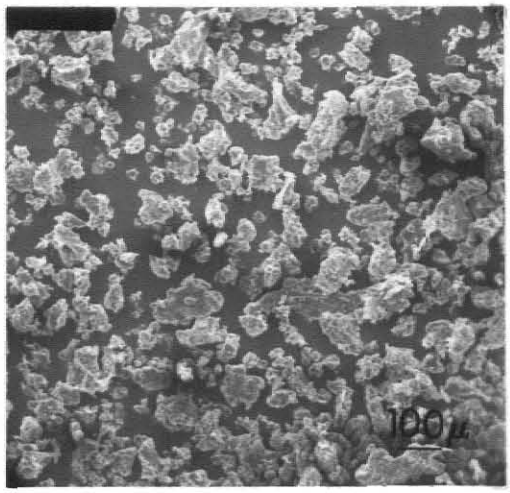

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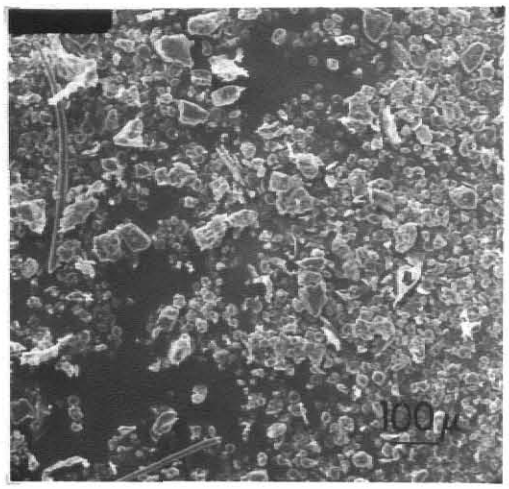
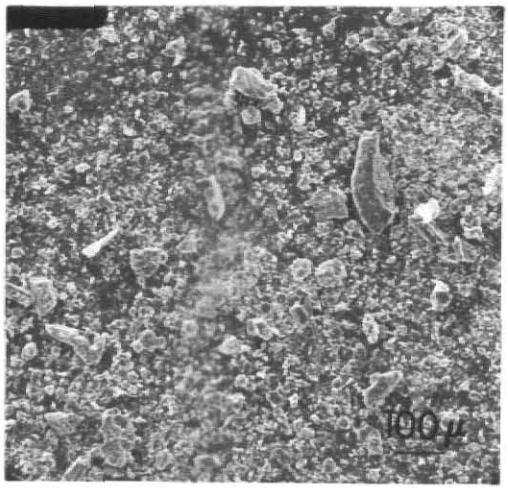
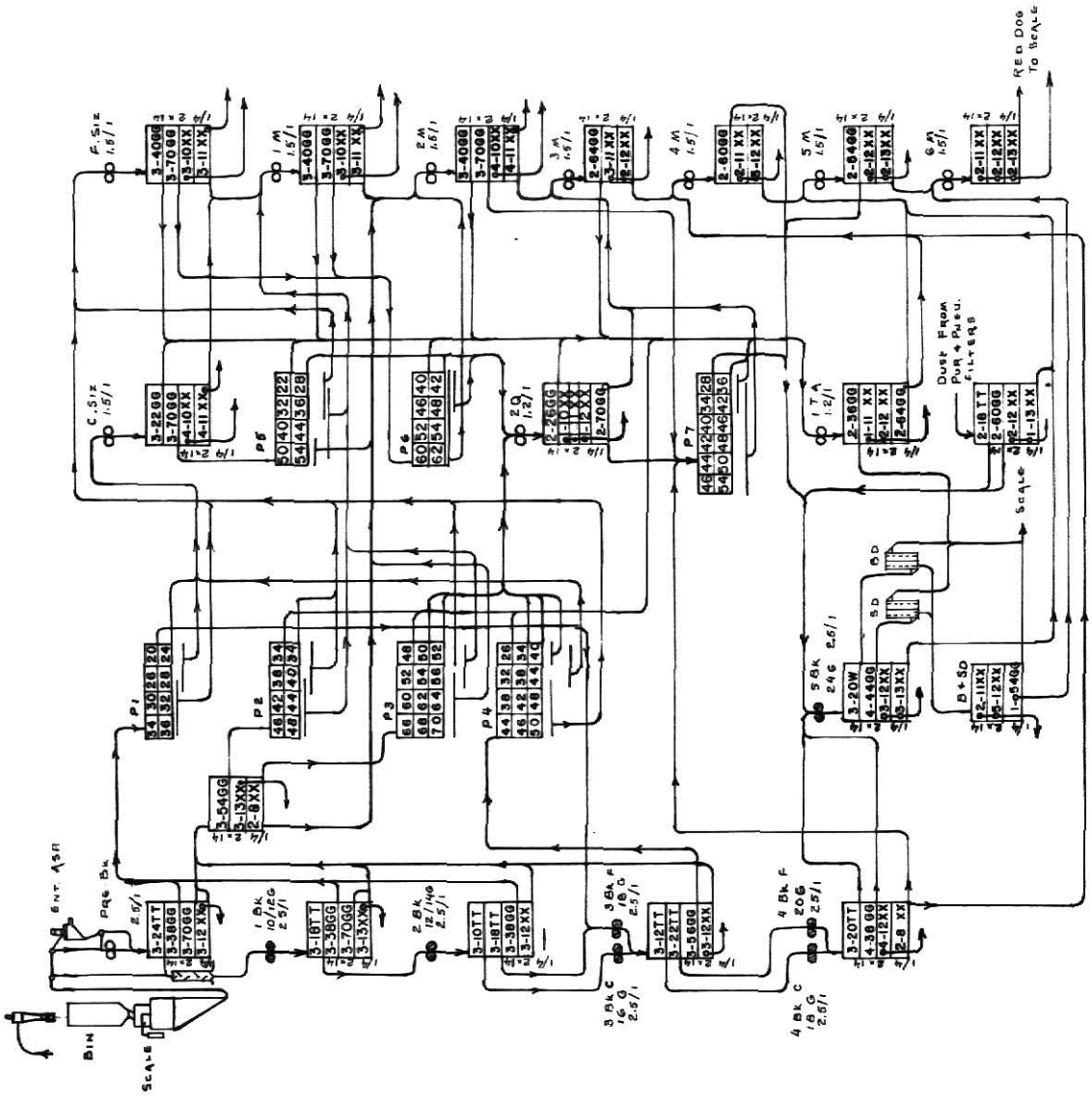


v



w

Figure 10. Hard wheat flow utilized to mill samples at the Kansas State University pilot mill. Scout variety (74-425) was milled under these conditions.



KANSAS STATE UNIVERSITY'S PILOT FLOUR MILL FLOW

ure 9e and 9f. First, second and third break redust flour (Fig. 9s) were the thrus of a 9XX. The majority of starch granules appeared to be contained within large endosperm particles.

The reduction process is performed with rolls similar in appearance to break rolls except that they are smooth. They impart no shear since they are usually driven at equal speeds. Endosperm particles would easily flatten, if reduced to flour abruptly. Therefore, a series of rolls (sizings and first through second mids) reduce endosperm particles to an intermediate size (middlings). Those flour fractions from reduction rolls are shown in Figure 9g - 9n. Endosperm particles in Figure 9g, 9i, 9k, and 9m are the "throughs" of a 10XX sieve. These flour fractions represent four samples: fine sizings top, coarse sizings top, first mids top, second mids top. These samples are characterized by extremes in particle size, ranging from individual starch granules to conglomerates of starch granules and protein. The counterparts of these mill fractions consist of: fine sizings bottom, coarse sizings bottom, first mids bottom and second mids bottom. Those endosperm particles shown in Figure 9h, 9j, 9l, and 9n are the throughs of an 11XX sieve. Larger conglomerates of starch granules and protein matrix are present in the photomicrographs with virtually complete absence of individual starch granules or fragments of protein matrix.

Flour fractions obtained from the second quality rolls,

first tailing rolls, third mid rolls, fourth mid rolls, fifth mid rolls and sixth mid rolls after sieving are shown in Figure 9o - 9r and 9t - 9u, respectively. The action of those rolls reduces the size of the middling particles. The second and third mid-flour fractions supply the bulk of the mill flour (Fig. 10). Further reductions were an attempt to improve the extraction rate. Too large a yield from the middling rolls beyond fourth mids will result in flour discoloration, due primarily to an increased amount of bran in the flour. The overs of the 54 gg sieve on five mids go to the bran and shorts duster (Fig. 10) and are shown in Figure 9v. An abundance of flaked bran and broken brush hairs are evident. In an effort to improve the profitability of the mill, the suction air is sifted. That mill fraction contains an assortment of flour components and dust. Sifter collections of the pneumatic system are shown in Figure 9w.

SEM examination of the 23 fractions of the mill stream provided a qualitative approach to evaluate the kernel substructures that characterize the mill fractions. Recognition of particular mill stream components - brush hairs, large endosperm particles, abundance of bran - enabled identification, or at least approximate placement, of that fraction in the mill stream.

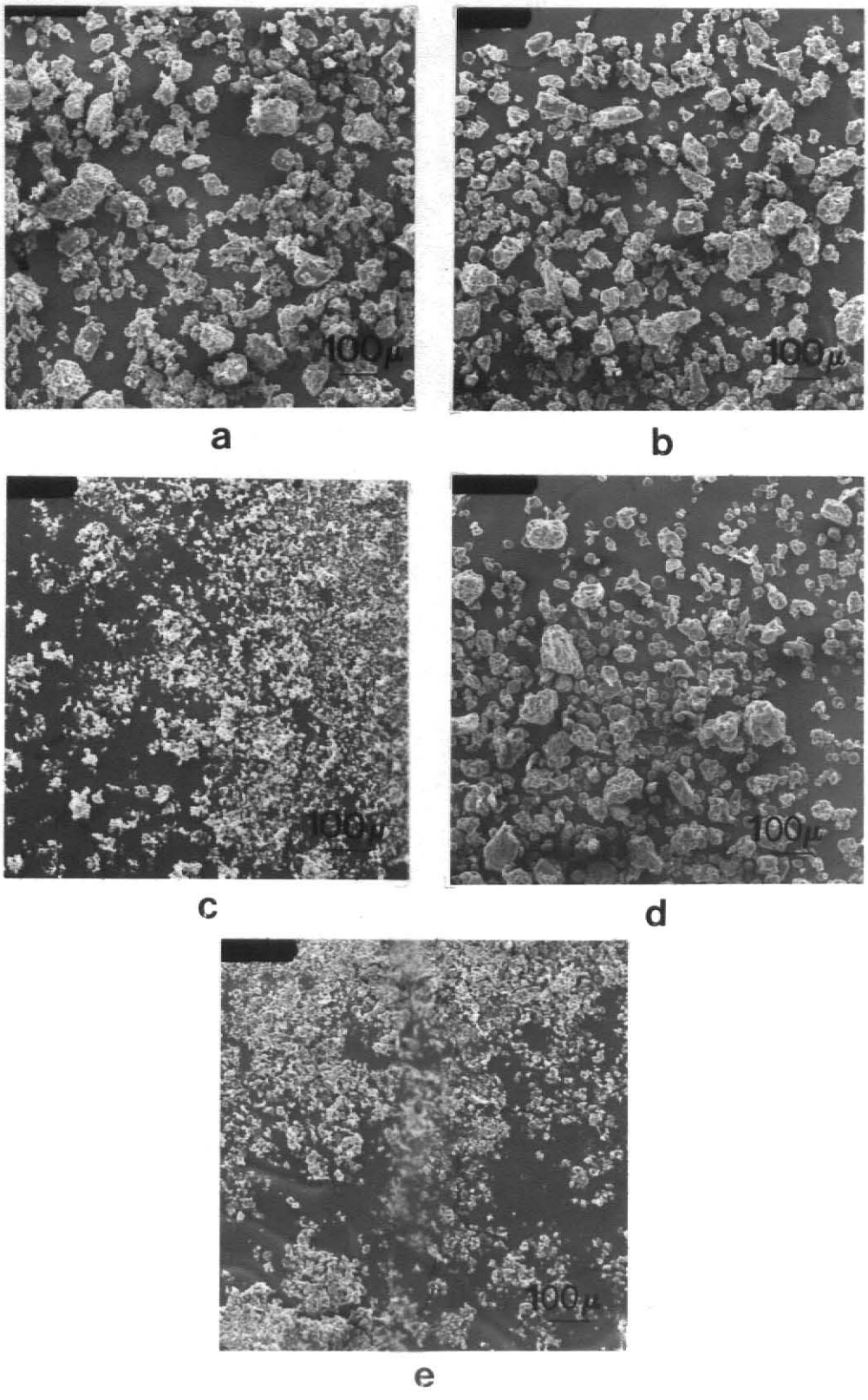
Composition of Air-Classified Flour

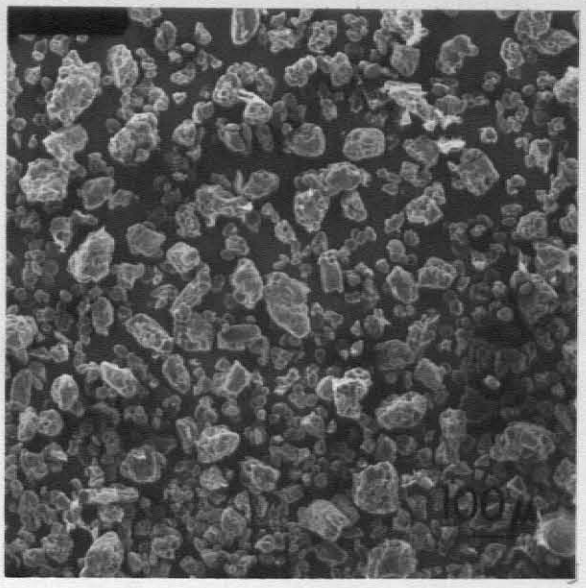
SEM was used on air-classified bakery flour to visually assess the effect of concentrating protein and its relationship

to starch damage. The initial flour sample (Fig. 12, 74-3001) is shown in Figure 11a. The flour contained endosperm particles ranging in size from 10 to 100 μ. The average particle size was 17.75 μ. The protein content was 10.45%. Starch damage was determined to be 10.9%. Figure 12b represents the first separation of sample 73-3001. Starch damage was reduced from 10.89% to 7.26% (Fig. 11). This was illustrated by the absence of small starch granules and endosperm particles in Figure 12b. The total protein content decreased slightly to 10.10%. A larger reduction in protein content was predicted due to separation of small endosperm particles from sample 74-3002 (Fig. 12b). The separated material (3% of initial flour) was examined in Figure 12c. Protein content was extremely high (17.5%) while starch damage was only slightly greater than in the initial flour. This fraction is particularly important since the major goal of air-classification is to produce flours that differ markedly in protein content from an ordinary wheat.

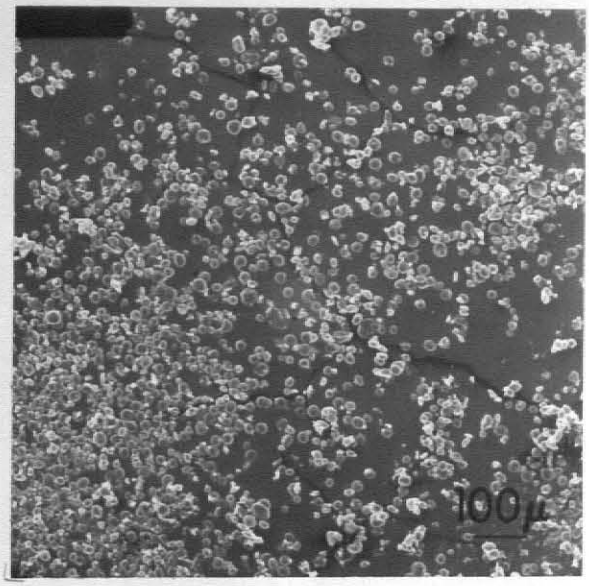
Figure 12d contained primarily large endosperm particles (mean size 16.50 μ). The protein content was lower than the fraction in Figure 12c. A correlation existed between smaller particles and increasing protein content. Figure 12g and 12i support this conclusion. For Figure 12d, 12f and 12h, the opposite was true; as the particle size increased to 24.75 μ (Fig. 12h), the protein content also increased from 9.30% (Fig. 12d) to 10.70% (Fig. 12h). Surprisingly, starch damage

Figure 11. Scanning electron photomicrographs of an air-classified hard red winter wheat bakery flour: a. 74-3001; b. 74-3002; c. 74-3003; d. 74-3004; e. 74-3005; f. 74-3006; g. 74-3007; h. 74-3008; i. 74-3009.

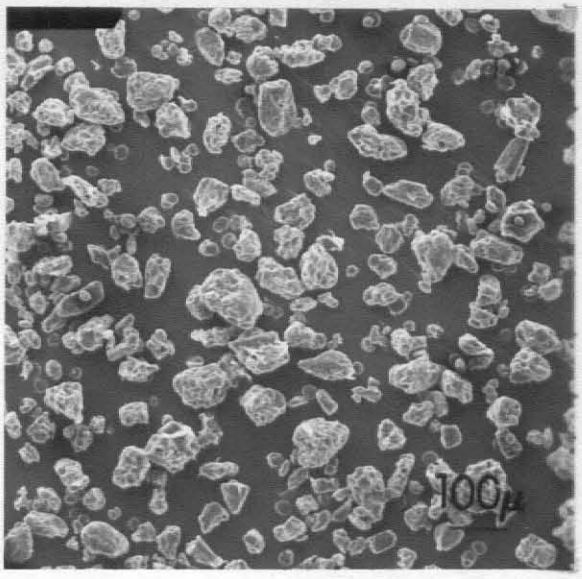

100µ
a
100µ
b
100µ
c
100µ
d
100µ
e



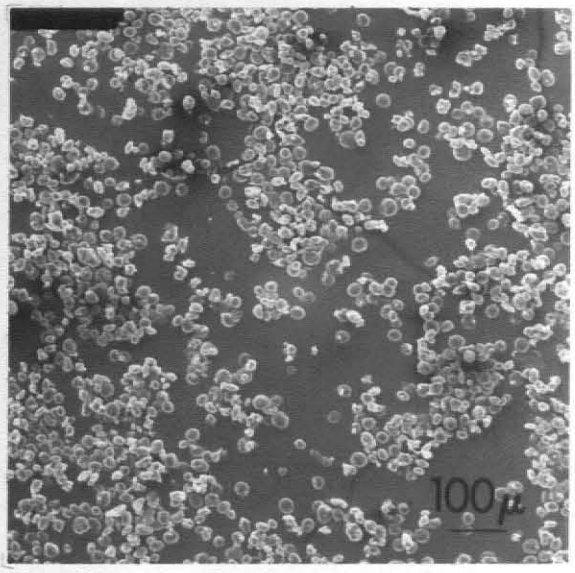
f



g



h



i

Figure 12. Air-classification flow and data sheet of a hard red winter wheat bakery flour.

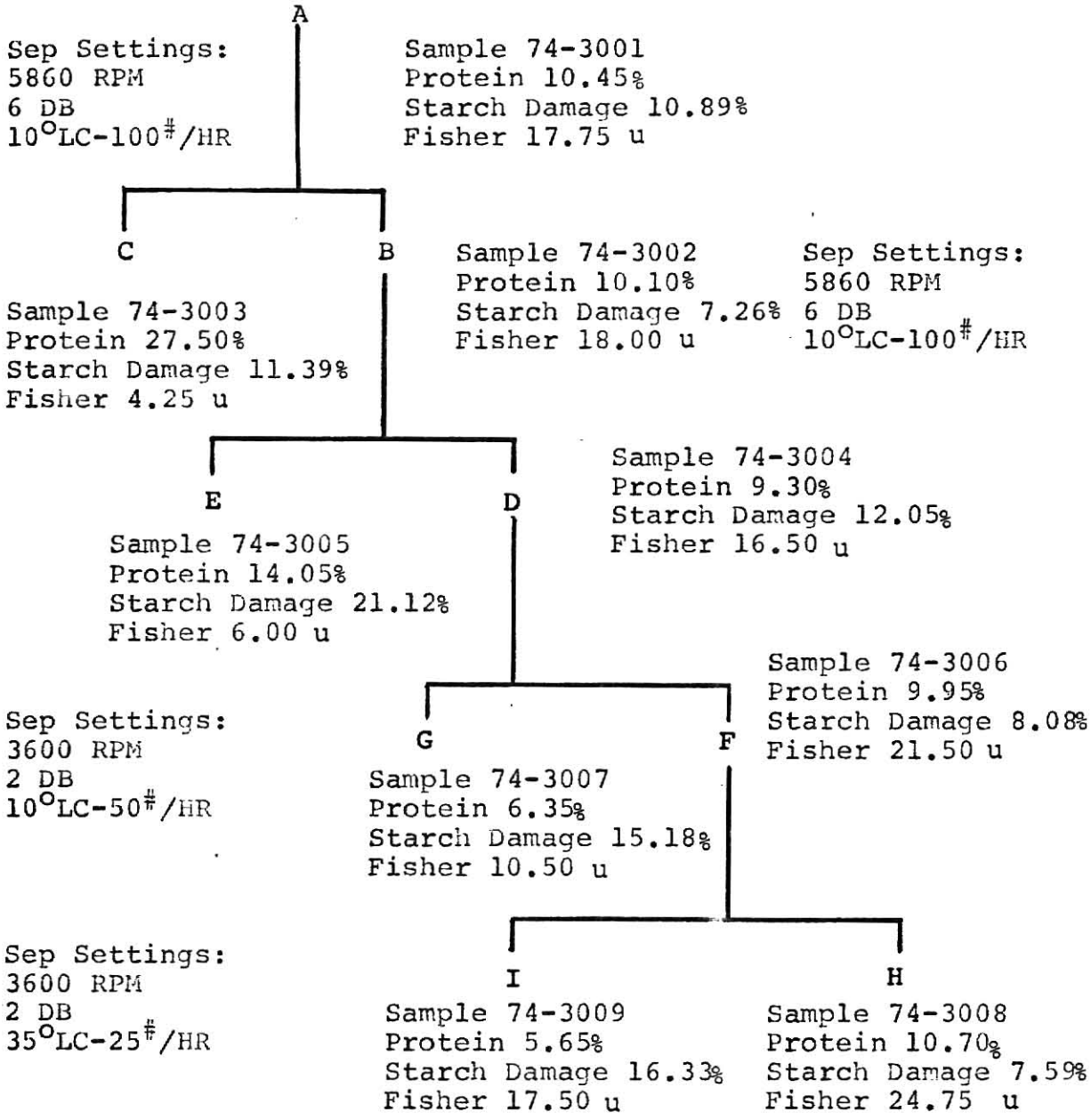

A
Sep Settings:
5860 RPM
6 DB
10°LC-100#/HR
Sample 74-3001
Protein 10.45%
Starch Damage 10.89%
Fisher 17.75 u
C
B
Sample 74-3002
Protein 10.10%
Starch Damage 7.26%
Fisher 18.00 u
Sep Settings:
5860 RPM
6 DB
10°LC-100#/HR
Sample 74-3003
Protein 27.50%
Starch Damage 11.39%
Fisher 4.25 u
E
D
Sample 74-3004
Protein 9.30%
Starch Damage 12.05%
Fisher 16.50 u
Sample 74-3005
Protein 14.05%
Starch Damage 21.12%
Fisher 6.00 u
Sep Settings:
3600 RPM
2 DB
10°LC-50#/HR
G
F
Sample 74-3006
Protein 9.95%
Starch Damage 8.08%
Fisher 21.50 u
Sample 74-3007
Protein 6.35%
Starch Damage 15.18%
Fisher 10.50 u
Sep Settings:
3600 RPM
2 DB
35°LC-25#/HR
I
H
Sample 74-3009
Protein 5.65%
Starch Damage 16.33%
Fisher 17.50 u
Sample 74-3008
Protein 10.70%
Starch Damage 7.59%
Fisher 24.75 u

FIGURE 12

decreased significantly as particle size increased (Fig. 12d, 12f, and 12h). Kent (19) has shown that sub-aleurone endosperm particles, which are high in protein, tend to resist reduction in the milling process and, when air-classified tend to collect in the coarse fractions. Therefore, an increase in protein above that content in sample 74-3008 (Fig. 12h) is not unusual.

The fraction in Figure 12e contained a relatively high protein content (14.05%), a starch damage of 21.12%, and an average particle size of 6 μ. The separation resulted in accumulating free protein and detached starch granules that had sustained damage. Further separations indicated that the majority of damaged starch granules were removed at this size separation.

The relationship between protein content and starch damage of individual air-separated fractions must be evaluated by examination of the types of flour particles present. Examination of samples (Fig. 12c, 12e and 12h) substantiates this conclusion.

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USE OF SCANNING ELECTRON MICROSCOPY TO EVALUATE CEREAL GRAINS AND THEIR MILL FRACTIONS

by

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B.S., Kansas State University, 1974

AN ABSTRACT OF A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

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Department of Grain Science and Industry

KANSAS STATE UNIVERSITY
Manhattan, Kansas

1976

Twenty varieties of wheat were examined with the scanning electron microscope. Bran widths were analyzed to determine if a significant difference existed between the varieties studied. Two varieties were investigated to determine if differing bran widths influenced the separation between bran and endosperm during milling. The samples, Scout and Lancer, were experimentally milled and the amount of starch remaining with the bran was determined. Measurement of glucose released upon hydrolysis indicated Lancer bran probably contained less starch and achieved more complete separation from the endosperm. Structural characteristics of the bran-endosperm interface were observed in Scout wheat during kernel maturation from a period 11 days after half bloom until 38 days after half bloom. Mill stream fractions from Scout wheat were studied by scanning electron microscopy. The sub-structures in the mill stream were examined in an effort to characterize the nature of the various mill fractions. Components in mill fractions ranged from large bran endosperm conglomerates and kernel hairs to smaller protein starch particles and starch granules. An air-classified flour was evaluated on the basis of protein content, starch damage, particle size and scanning electron microscopic observation of the air-separated fractions. Each fraction was compared to assess the effects of concentrating protein and its relationship to starch damage.