A MICRO METHOD FOR DETERMINING MOISTURE DISTRIBUTION IN WHEAT KERNELS, BASED ON IODINE STAINING[1]

H. L. SECKINGER, M. J. WOLF, AND R. J. DIMLER

ABSTRACT

A method is described for studying moisture distribution in wheat based on comparison microscopically of the starch-iodine color of isolated portions of endosperm with that of standards. Moisture content of a few endosperm cells was estimated to one percentage point over the range 12–21%. Application of the procedure to wheat during tempering showed that an increase in moisture could be detected in the center of the kernel after 2 hr. At 24 hr., moisture was evenly distributed throughout the endosperm. Sections cut from a single kernel after a 2-hr. tempering showed a moisture gradient of 4 percentage points over a distance of 1 mm.

Grain is conditioned primarily to obtain levels of moisture within the kernel that will improve its milling characteristics. To study the moisture distribution a method was required for determining moisture content of a few endosperm cells. Existing methods lack the degree of resolution needed for detailed examination of moisture distribution in the kernel; these methods are noted in the survey of literature on wheat conditioning by Bradbury *et al.* (1). Improved techniques now have been developed whereby moisture analyses can be carried out on microscopic portions of wheat endosperm.

Iodine staining for estimating moisture in wheat endosperm was first reported by Von Ugrimoff (2). The same technique was used by other workers (3–7) for investigating, grossly, the path of entrance and penetration of moisture into the grain of wheat. More recently, Schäfer (8) made some refinements and introduced a standard color chart for comparing stained endosperm. In most of these studies color was observed after wheat kernels were bisected and exposed to iodine vapor.

Another approach was investigated by Jones and Campbell (9), who dissected vitreous wheat and determined moisture on small pieces of endosperm by relating their density to moisture content.

The present method, which relates starch-iodine color to moisture content, involves techniques not previously reported in moisture distribution studies: (a) size of sample is approximately 10^{-4} mm.[3] (about 1,000 times smaller than for the density method, 9); (b) samples of

[1]Manuscript received June 25, 1962. Contribution from the Northern Regional Research Laboratory, Peoria, Illinois. This is a laboratory of the Northern Utilization Research and Development Division, Agricultural Research Service, U.S. Department of Agriculture. Reference to firm names or trade products in this publication is not intended to be a recommendation of that product by the U.S. Department of Agriculture over others not mentioned.

endosperm were isolated and stained with iodine in an oil medium; (c) standards of known moisture content were composed of material similar to the unknown; and (d) direct microscopic color comparisons were made with comparison eyepiece at 100× magnification.

Applicability of the procedure to research on moisture distribution was shown by measurement of moisture content in a few endosperm cells isolated from both partially and fully tempered wheat kernels. The improved staining procedure also permits a critical study of moisture entrance and penetration into the grain. Since this method depends on the starch-iodine color, it is directly applicable to other cereal grains.

Methods and Materials

Laboratory-prepared starch and flour from Ponca hard red winter (HRW) wheat was used for development of the method. Wichita HRW wheat was used for the conditioning experiments.

Equipment and Reagents. 1. Water bath, maintained at 30° ± 1.0°C.
2. Desiccators, 6-in., with distillation heads.
3. Culture slides, 78 by 28 by 6 mm. thick.
4. Split-field comparison eyepiece mounted on two microscopes, each at a total magnification of 100×.
5. Colorless mineral oil, light medicinal grade, saturated with iodine (1.5% at 25°C.).
6. Saturated salt solutions listed in Table I.

TABLE I
MOISTURE IN WHEAT ENDOSPERM EQUILIBRATED OVER SATURATED SALT SOLUTIONS

Saturated Salt Solutions, 25°C.	Moisture of Wheat Endosperm	Saturated Salt Solutions, 25°C.	Moisture of Wheat Endosperm
	%		%
K_2SO_4	26	KI	15
KNO_3	21	$NaCrO_4$	14
$ZnSO_4$	19	NaBr	13
KCl	18	K_2CO_3	12
KBr	17	$MgCl_2$	10
NaCl	16		

Comparison Eyepiece. The method for estimating the moisture content of starch or starchy endosperm of cereal grains is based on a simple color comparison in which samples of unknown moisture content are matched with iodine-stained endosperm standards. A comparison eyepiece was mounted on two similar microscopes fitted with 10× oculars and 10×, 16-mm. objectives which gave sufficient magnification and fields large enough for comparison. Each microscope had

an individual light source consisting of a 100-watt microscope lamp connected through a variable transformer for matching background illumination. Light of suitable quality was obtained by using a daylight filter in conjunction with a water cell containing a dilute solution of copper nitrate. Other lighting systems could be substituted for this one.

Preparation of Standards. Wheat endosperm of known moisture content was obtained by equilibrating it at 25°C. in closed chambers containing saturated salt solutions. The relative humidity in the chambers ranged from 33 to 97%. Moisture of the equilibrated samples was determined by the vacuum-oven method (4 hr., 100°C.). Table I lists the salt solutions and moisture content of equilibrated endosperm to the nearest percent.

Six-inch desiccators with distillation heads provided access to the equilibrated sample without removal of the lid. The hole in the top of the distillation head was covered with a piece of rubber dam.

To facilitate handling, pieces of wheat endosperm were placed on the frosted surface of small glass squares made from microscope slides. The sample was ground with a glass rod on the frosted surface to release almost all the starch granules. The glass containing the sample was then placed in the well of a culture slide and put into one of the relative humidity chambers for equilibration (at least 24 hr.).

After equilibration, the well of the slide containing the endosperm was nearly filled with iodine-oil stain from a long pipet inserted through a slit in the rubber dam. After the slide was removed and before a cover glass completely sealed the well, additional iodine-oil was added until all air was expelled from the well (Fig. 1).

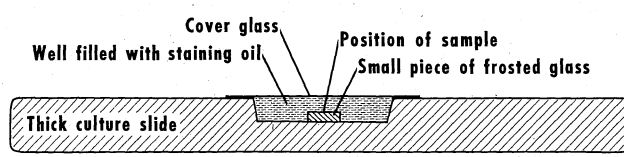

Fig. 1. Culture slide assembly for staining endosperm samples.

The sealed slides were placed in a shallow copper pan which was covered and placed on a 30°C. water bath for 16 to 18 hr. A difference of an hour or two in the staining period had little effect on the color of the starch. After staining, the small pieces of glass with the endosperm were carefully removed from the well of the slide and placed on a standard microscope slide for observation.

A complete series of standards containing 13 to 21% moisture was run simultaneously; in addition, several runs were made to check the

reproducibility of the colors. The starch-iodine color was fairly stable; therefore, for routine work one set of standards could be used several days.

Sampling Procedure. To prevent exchange of moisture between the exposed endosperm and the surrounding atmosphere, tempered wheat kernels must be covered with at least 1/8 in. of mineral oil. All subsequent sampling operations were conducted under oil. In many cases, freehand sections were cut under a dissecting microscope and a few endosperm cells isolated from specific areas. A more refined technique used special attachments for a rotary microtome (to be described elsewhere) to cut thin sections of wheat under oil. Moisture can be localized by isolating small areas of endosperm. The smaller the area isolated, the better the resolution. Areas as small as 60 μ square were isolated from 30-μ sections, such samples being about 1,000 times smaller than previously reported (9). The limit of resolution depends on the skill of the analyst.

Since mineral oil does not prevent migration of moisture within the endosperm, tempering periods include all elapsed time between addition of water and isolation of a sample.

Small pieces of dissected material were transferred to the well of a hanging drop slide which contained a small square of frosted glass and oil saturated with iodine. After the sample was ground with a small glass rod, the well was sealed with a cover glass, and the sample stained for 16 to 18 hr. as described.

After staining, samples were compared with standards of known moisture. Areas of equivalent particle concentration were best suited for accurate comparisons.

Application of Method

Preliminary studies on partially and fully tempered kernels were carried out on a sample of Wichita wheat with an initial moisture content of 11%. Ten grams of wheat were immersed in water and then rubbed on a cotton towel to remove free water. The kernels were tempered in closed bottles at room temperature (25°–28°C.). A few cells from the central cheek area of several kernels were removed for moisture analysis. At early tempering periods moisture varied from kernel to kernel between a low of 13% and a high of 18% (Table II). At 6 hr., the range of moisture was only 2%, and at 24 hr. the sample was equilibrated.

To check the completeness of equilibration in wheat kernels at 24 hr. of tempering, four separate samples of wheat tempered to 13, 15, 16.5, and 18% moisture were used. Seven kernels from each sam-

TABLE II

MOISTURE IN ENDOSPERM TAKEN FROM CENTRAL CHEEK AREA OF TEMPERED WHEAT KERNELS

HOURS OF TEMPERING	NO. OF KERNELS TESTED	MOISTURE KERNEL TO KERNEL
		%
2	12	13–17
5	5	15–18
6	5	15–17
24	4	17

ple were bisected, and the central cheek area of each was analyzed for moisture. In each case, the moisture content found by color comparisons agreed with the moisture content of the tempered grain as determined by oven method. There was no variation in moisture from kernel to kernel within each of the tempered samples. Further tests of endosperm from brush, germ, dorsal, and peripheral areas of single kernels showed that the moisture was evenly distributed throughout. Both vitreous and mealy-textured areas of equilibrated kernels showed the same moisture content.

Single-kernel analysis of moisture distribution was also performed on wheat tempered for short periods. Kernels were immersed in distilled water for 3 min., rubbed with a towel, then tempered in a closed bottle for 2 hr. A single kernel was cut into 40-μ cross-sections starting at the brush end. Another kernel was sectioned starting at the endosperm immediately above the germ. Every fifth section was divided into center and peripheral area for moisture analysis.

The germ end, as expected, had higher moisture than the brush end (Table III). The moisture content was higher than the method

TABLE III

MOISTURE IN WHEAT ENDOSPERM AFTER 2 HOURS OF TEMPERING

FIRST KERNEL			SECOND KERNEL		
Distance from Top of Germ	Center of Cheek	Peripheral Area	Distance from Brush End	Center of Cheek	Peripheral Area
μ	%	%	μ	%	%
0	21+	21+	0	17	15
200	21+	21+	200	15	17
400	19	19	400	15	17
600	18	18	600	14	16
1,000	19	19	1,000	13	17

could detect and is recorded as 21%+ in the table. The peripheral area of the cheek at the brush end varied in moisture from 15 to 17%. Only the central cheek area of the brush end was consistent with nor-

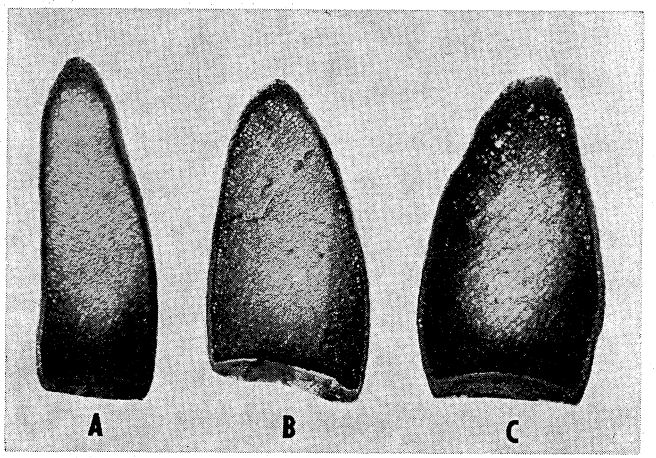

Fig. 2. Thirty-micron wheat sections stained for 10 min. with iodine in oil, as seen by transmitted light (Approx. 7×). The dark areas indicate a high moisture level at 2 hr. of tempering after a 5-min. steeping period.

mal diffusion patterns shown in Fig 2, A. The moisture gradient over the 1-mm. distance was 4% and ranged from 13 to 17%.

Besides making quantitative moisture determinations on a few endosperm cells, one can apply the iodine-oil stain to sections of wheat to determine moisture patterns qualitatively. Because oil limits the transfer of moisture to the atmosphere, a more critical examination of thin sections can be made than is possible with the iodine vapor method. In Fig. 2, A, B, and C are photomicrographs of wheat sections taken with transmitted light and show areas of high moisture which occur in different kernels during early stages of tempering. The kernels were immersed in water for 5 min., then placed in a closed container for 2 hr. Thirty-micron sections were cut from the back of the kernel immediately under the aleurone layer. Sections were cut under oil and stained for 10 min. with oil saturated with iodine. The tissue at the bottom of the section is part of the germ. The dark area, stained blue, indicates high moisture content.

With intact kernels, the first sign of moisture in the endosperm was detected at the tip next to the germ. In Fig. 2, A and B, little or no moisture penetrated the seed coat in 2 hr., and the preferential path was through or around the germ rather than through the seed coat. In Fig. 2, C, the seed coat was damaged, resulting in penetration of moisture. Figure 3 is a highly magnified section of Fig. 2, C. Some starch granules remained unstained in the area of high moisture, but the reason for this is not apparent.

Although the iodine-staining method can estimate moisture in

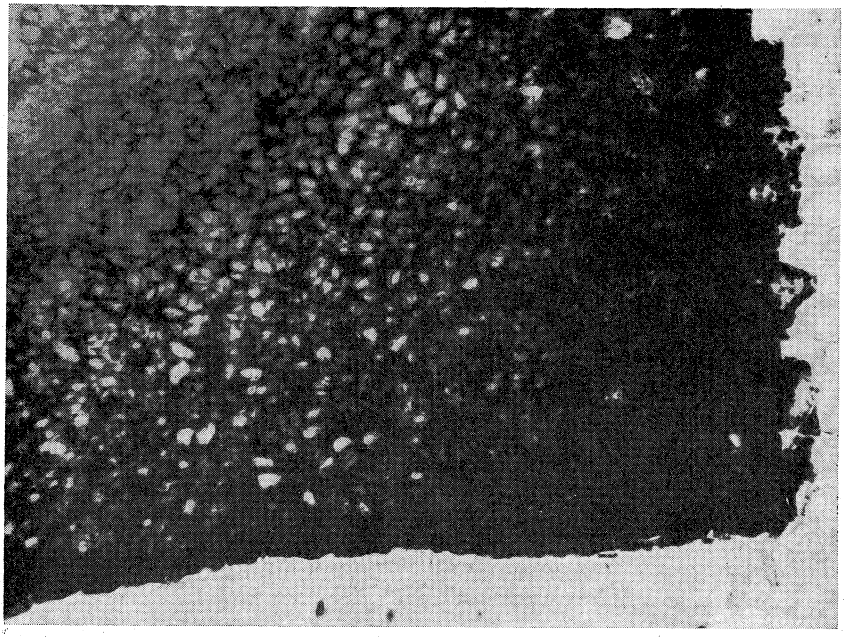

Fig. 3. Magnification of lower right corner of section in Fig. 2c in which gradual decrease in staining indicates a decrease in moisture content. (Approx. 66×)

starch, additional methods are necessary to determine moisture in the protein and cell walls of the endosperm. Determination of refractive index with an interference microscope provides a method potentially useful for estimating moisture in these cellular constituents. Thus far, this approach has been used only on wheat starch where the refractive index was shown to vary inversely with the starch moisture content over the range 12 to 21% moisture (10). Preliminary results by Wolf *et al.* (10) showed that the two methods – iodine staining and measurement of refractive indices – are in satisfactory agreement as to the moisture content of starch granules isolated from tempered wheat kernels.

Further work will be necessary to correlate moisture distribution and milling behavior.

Literature Cited

1. Bradbury, Dorothy, Hubbard, J. E., MacMasters, Majel M., and Senti, F. R. Conditioning wheat for milling. A survey of the literature, pp. 83–101. U.S. Dept. Agr., Misc. Pub. 824 (May 1960).
2. Von Ugrimoff, A. Einige Versuche über das Eindringen des Wassers in Weizenkörner. Mühle **70:** 603–604; 625–627 (1933).
3. Buré, J. Le cheminement de l'eau a l'intérieur des grains au cours du conditionnement et du séchage. Meun. Franc. No. 39: 26–32 (1949).

4. GROSH, G. M., and MILNER, M. The penetration of moisture into the wheat kernel. Northwest. Miller **254** (15): Sec. 2, 1a, 19a, 21a, 22a (Oct. 11, 1955). *Also in* Milling Prod. **20** (10): 1, 21, 23, 24 (Oct. 1955).
5. HALTMEIER, O. Water penetration in the wheat kernel. [*Translation from* Mühle **70** (45): 1165–1170 (1933) *by Clinton L. Brooke.*] Natl. Am. Miller **62** (5): 35–38 (1934).
6. HINTON, J. J. C. Resistance of the testa to entry of water into the wheat kernel. Cereal Chem. **32:** 296–306 (1955).
7. JONES, C. R. Observations on the rate of penetration of water into the wheat grain. Milling (Liverpool) **113** (4): 80–82, 84, 86 (1949). *Also* Northwest. Miller **239** (11): Sec. 2, 3a–8a (1949).
8. SCHÄFER, W., and ALTROGGE, L. Wissenschaft und Praxis der Getreidekonditionierung. Verlag Moritz Schäfer, Detmold, Germany, pp. 75–76 and color chart (1960).
9. JONES, C. R., and CAMPBELL, J. D. Micro-determination of endosperm density as a means of mapping moisture distribution in wheat grains. Cereal Chem. **30:** 177–189 (1953).
10. WOLF, M. J., RUGGLES, VERONICA J., and MACMASTERS, MAJEL M. Refractive indices of wheat starch granules at various moisture levels determined with an interference microscope. Biochim. Biophys. Acta **57:** 135–142 (1962).