STEEPING STUDIES WITH CORN ENDOSPERM SECTIONS[1]

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ABSTRACT

A technique is presented for studying the effect of variables in the steeping medium on the release of starch granules from corn endosperm. Corn kernels are softened in a water-saturated atmosphere and 10-micron sections are cut from the horny endosperm. The sections are suspended with gentle agitation in dilute solutions of potassium metabisulfite at pH 4 and 52° or 60°C. Sections are removed at intervals during a 6-hour steeping period and stained with iodine. Extent of starch release is measured photometrically and plotted against steeping time. This technique measures directly the tenacity with which the protein matrix holds the starch granules.

In the absence of sulfur dioxide, no starch was released from the sections. Maximum rate and extent of starch release were obtained from corn samples steeped in solutions containing 0.15 to 0.4% sulfur dioxide. Corn dried from 32% to 12% moisture in air at 120°F. (49°C.) gave normal starch release, but corn dried at 200°F. (93°C.) gave very little starch release under any steeping conditions. Corn dried at 180°F. (82°C.) and steeped at 52°C. (optimum commercial steeping temperature) with 0.2% sulfur dioxide, released two-thirds as much starch as the corn dried at 120°F. (49°C.).

In the manufacture of corn starch, the corn is first softened by steeping for 40–48 hours at about 50°C. in water containing 0.1 to 0.2% sulfur dioxide. The corn is then ground and processed for separation of starch, gluten, germ, and hulls. It is sometimes desirable to determine, in the laboratory, steeping conditions that will give optimum starch recovery for a given lot or type of corn. This is usually done by simulating commercial steeping and milling in order to determine the yield and quality of starch and other fractions (2,4,8). Since the steeping conditions affect specific component structures of cells and tissues, microscopic evaluation of sections of corn kernels should give the desired information directly.

Cox, MacMasters, and Hilbert (1) cut microtome sections of steeped corn kernels and microscopically examined the condition of the endosperm. They found that the extent of swelling of the protein matrix, particularly when sulfur dioxide was present, was directly related to the ease of milling and starch separation. Other workers (5,6) have employed the sectioning procedure to determine the degree of steeping by visually examining the extent of starch loss from sections. Steeping whole kernels for this purpose has the disadvantage that one cannot know the exact composition of steeping medium in direct contact with endosperm inside the kernel. It is known that

[1]Manuscript received May 5, 1960. Presented at the 45th annual meeting, Chicago, Ill., May 1960.

both the external steeping medium and the water phase inside the kernels continually change in pH and sulfur dioxide content for a number of hours after steeping is started[2].

To steep endosperm under accurately known conditions, thin sections of endosperm were cut from water-softened corn kernels and then steeped in a large excess of medium. Extent of steeping was determined by measuring the increased transmission of light through the section resulting from dislodgement of starch granules. Horny endosperm was used because its starch granules are embedded in relatively thick protein matrix and it therefore presents the greatest difficulty in starch extraction. Section thickness was set at 10μ, because starch granules in the horny endosperm average about 10μ in diameter but the endosperm cells have dimensions of 50 by 100μ (10). These sections, therefore, could offer little resistance to passage of water or chemicals into their interiors. The starch granules were retained in the sections only by the strength of the protein matrix, because in a 10μ section no endosperm cell could remain unopened.

With this technique we have studied the effects of sulfur dioxide concentration and steeping temperature upon the rate and extent of starch release from horny endosperms of several varieties of corn and of corn that was artificially dried.

Materials and Methods

Corn Samples. The sources of corn samples used in this work are given in Table I. The first two yellow-dent hybrid corns are typical of those found in commercial trade channels and are used as reference grains in this laboratory. One of them, CPC-50, is of average

TABLE I
IDENTIFICATION OF CORN SAMPLES

CPC Number	Type	Location Grown	Drying Conditions	Protein
				% d.b.
50	Yellow dent hybrid	Justice, Ill.	104°F. (40°C.)	9.3
3	Yellow dent hybrid	Clinton, Ill.	104°F. (40°C.)	10.5
80	Yellow flint variety	Argentina	Field-dried	12.1
11	High-protein yellow dent hybrid[a]	Argo, Ill.	Field-dried	12.6
65	Yellow dent hybrid[b]	Urbana, Ill.	120°F. (49°C.)	11.7
62	Yellow dent hybrid[b]	Urbana, Ill.	180°F. (82°C.)	11.8
78	Yellow dent hybrid[b]	Urbana, Ill.	200°F. (93°C.)	11.7

[a] Experimental hybrid derived from Illinois high-protein strain by Funk Brothers Seed Co., Bloomington, Illinois.
[b] Picked at 32% moisture, dried to 11% moisture in air at 40% relative humidity and the indicated temperature. From the 1952 crop. (See reference 3.)

[2] Watson, S. A., and Hirata, Y., unpublished data.

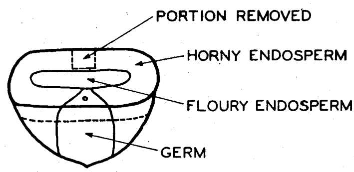

Fig. 1. Sketch of decrowned corn kernel showing approximate location of endosperm cube removed for sectioning.

hardness and protein content and the other one, CPC-3, is harder and of higher protein content than average dent corn. Argentine flint corn and the high-protein hybrid corn were included because of expected differences in endosperm properties. Three samples of yellow dent corn artificially dried by the Agricultural Experiment Station, University of Illinois, were included to study the steeping properties of heat-damaged corn.

Preparation of Sections. Eight intact kernels of each sample were allowed to sorb moisture from a saturated atmosphere for 24 to 48 hours. A cube of horny endosperm measuring about 3 mm. on each side was cut from the abgerminal side of each kernel as shown in Fig. 1. Sections of 10μ thickness were cut from each cube on a freezing stage mounted on a Leitz rotary microtome. About five sections were cut and discarded to avoid the aleurone and peripheral endosperm layers; the next five sections, after cutting, were held in water to await steeping. Holding time in water was never more than 2 hours and this did not influence results.

Steeping. The steeping vessels were 2-oz. screw-cap bottles. Twenty-five milliliters of the steeping medium were put in the bottle with five intact sections from a single kernel. Air was displaced with nitrogen to minimize oxidation of sulfur dioxide and the consequent drop in pH of the medium. Steeping media were solutions of potassium metabisulfite in distilled water at concentrations equivalent to 0.05 to 0.4% sulfur dioxide as determined by iodine titration. Initial pH of the potassium metabisulfite solution was 4.0 and it did not drop below 3.6 in any experiment. Bottles containing the sections, fastened in a reciprocating shaker immersed in a constant-temperature water bath, were shaken continuously at a rate of 80 (2-in.) strokes a minute. Temperature of most experiments was 52°C., but several runs were made at 60°C. Eight bottles each containing five sections from a single kernel were used in each experiment. Occasionally sections were discarded if they curled, fragmented, or produced results completely out of line, but data presented include readings from no less than six kernels.

Photometric Estimation of Starch Release. To score sections for starch release, one section from each of the eight kernels was removed at time intervals of 0.5, 1, 2, 4, and 6 hours. Each section was then placed on a microscope slide and covered with a drop of dilute iodine-potassium iodide solution (about 0.005*N* I_2). After several minutes, when the starch granules had stained dark blue, the iodine solution was replaced with glycerine and the section covered with a glass slip. The section was centered and focused under a 32-mm. (6×) objective lens of the microscope equipped with a 20× ocular lens. Amount of light transmitted was measured with a photocell-galvanometer combination. Light intensity was adjusted by positioning the substage condenser to give a reading of 10 ma. through the glass slide with the section moved out of the field. Readings were recorded as percent light transmitted by the sections.

Several attempts were made to calibrate the transmission readings in terms of actual starch released. Individual sections and steepwater from sections were analyzed for starch by a microcolorimetric iodine method. Results by this method correlated positively with the transmission method results, but the sensitivity of the iodine method was too low to validate completely the other method. A more positive check on the results by the transmission method was provided by running a blank section from which all starch had been removed. Cox, MacMasters, and Hilbert (1) found that, when a section is covered with 70% sulfuric acid for 1 minute, all starch granules are released but protein matrix is not altered. This procedure was applied to sections of the corn samples employed in this work. These values are considered to be maximum transmission values obtainable by steeping and are therefore recorded on the graphs for comparison with readings of steeped sections. Maximum transmission values of all steeped sections were below the values for the sulfuric acid-treated sections. This was due to occasional islands of starch granules that still remained after the 6-hour shaking period. Since most curves were still ascending at the sixth hour, these pieces of endosperm should eventually have been shaken loose.

Sections shaken in distilled water for 6 to 24 hours at 52°C. gave zero light transmission. Several sections treated with alkaline detergent to remove all starch and protein, leaving only the cell-wall network, gave 97.5% transmission. The transmission value of sulfuric acid-destarched sections was about 90% for ordinary dent corn but was only 65% for the high-protein corn.

Results and Discussion

When light-transmission values of steeped sections of individual kernels were plotted against time of steeping, families of curves were obtained as shown in Figs. 2 and 3. The dent corn used in the drying studies, CPC-65, showed rather good agreement among individual kernels, but the reference dent corn, CPC-3, exhibited considerable variation among kernels (Fig. 3). Other samples fell between these extremes. Curves shown in Figs. 4 to 8 are constructed from the average transmission values for sections of the eight kernels at each time interval. Use of a larger number of kernels in each experiment was impractical, so statistical evaluation was used as an aid in data interpretation. The data were subjected to analysis of variance, and least significant difference (LSD) values at the 5% level were calculated. It is assumed that variances of each group of eight samples are similar, since the kernels were taken from the same populations. Differences between any two points smaller than the LSD are not significantly different at the 5% level.

The results obtained with horny endosperm sections from the two samples of ordinary yellow dent corn are given in Figs. 4 and 5. The sample, used for Fig. 4 (CPC-50) is representative of average commercial corn. The greatest rate and extent of starch release were ob-

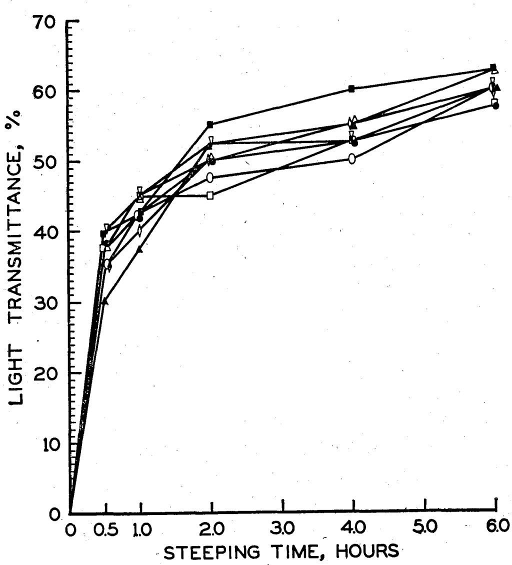

Fig. 2. Reproducibility of starch release from 10-μ sections of horny endosperm of eight kernels of yellow dent corn, CPC-65.

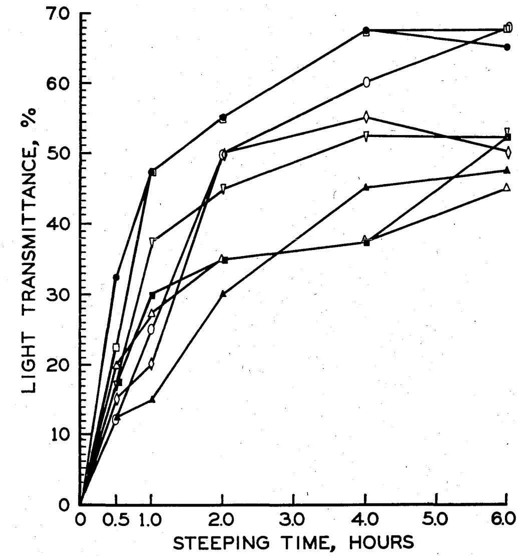

Fig. 3. Reproducibility of starch release from 10-μ sections of horny endosperm of eight kernels of yellow dent corn, CPC-3.

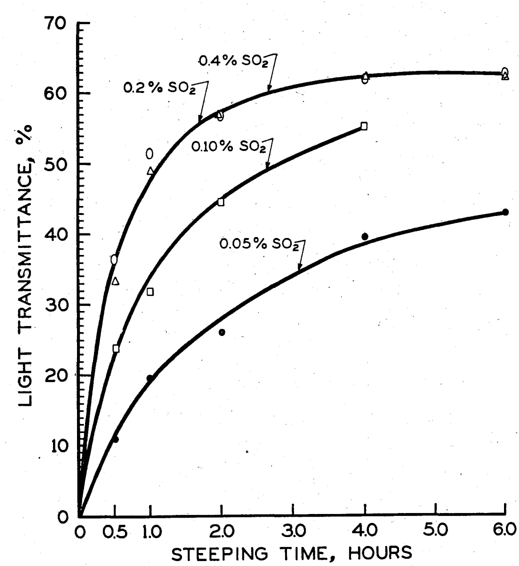

Fig. 4. Average starch-release rate from 10-μ sections of horny endosperm of yellow dent corn, CPC-50. LSD (5% level) = 6.8%. Starch-free transmittance = 91%. Steep temperature, 52°C.

tained with 0.2% sulfur dioxide. Although a run was not made using 0.15% sulfur dioxide, it is probable that the curve would have been

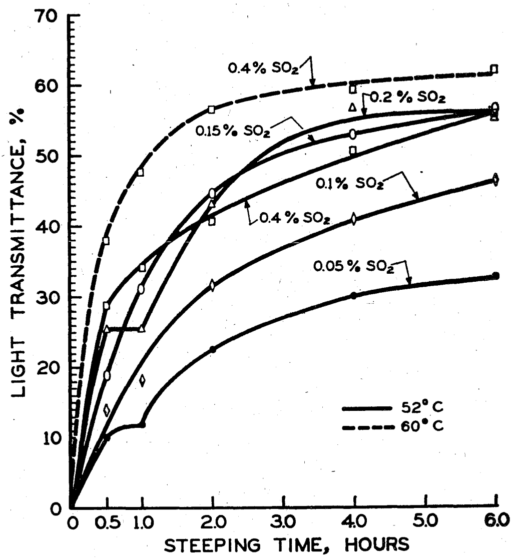

Fig. 5. Average starch-release rates from 10-μ sections of horny endosperm of yellow dent corn, CPC-3. LSD (5% level) = 1.3%. Starch-free transmittance = 87.5%. Steep temperatures: 52°C., solid lines; 60°C., broken lines.

similar to the curve for 0.20% sulfur dioxide since this was the case in Fig. 5. Comparison of these two curves indicates that CPC-3 horny endosperm is somewhat more resistant to the action of sulfur dioxide in releasing starch than CPC-50. This confirms previous data that CPC-3 has a harder kernel (7). Rate and extent of starch release are lower for CPC-3 than for CPC-50 at equivalent sulfur dioxide concentrations. Highest rate and greatest extent of starch release for CPC-3 were obtained on steeping at 60°C. with 0.4% sulfur dioxide, but a 0.2% sulfur dioxide concentration probably would have given a similar curve. No evidence of starch damage, as judged by loss of birefringence, was observed in those sections steeped at 60°C.

Flint corn is so named because the kernel appears to be very hard, owing to a larger proportion of horny endosperm than is found in dent corn. The floury portion of flint corn endosperm is entirely encased in horny endosperm. The data in Fig. 6 show that 0.2% sulfur dioxide effects starch release from Argentine flint horny endosperm sections comparable to that obtained with sections of the reference yellow dent corn, CPC-50 (Fig. 4). At sulfur dioxide concentrations below 0.2%, the Argentine flint endosperm gave lower rate and extent of starch release than the dent corns. This suggests that its horny endosperm contains thicker strands of protein matrix that require a

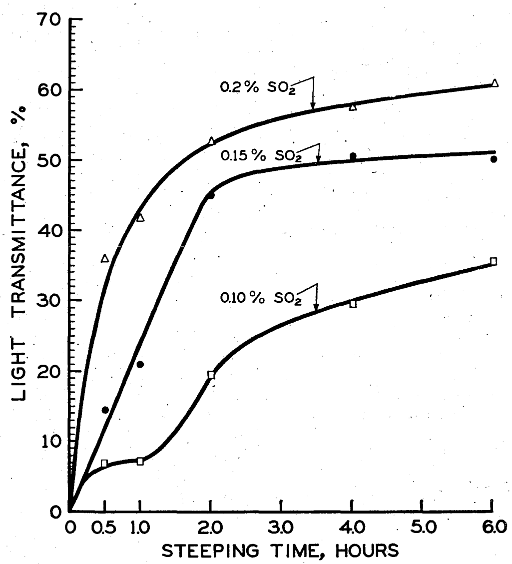

Fig. 6. Average starch-release rates from 10-μ sections of horny endosperm of Argentine flint corn, CPC-80. LSD (5% level) = 3.1%. Steep temperature 52°C. Starch-free transmittance, 86%.

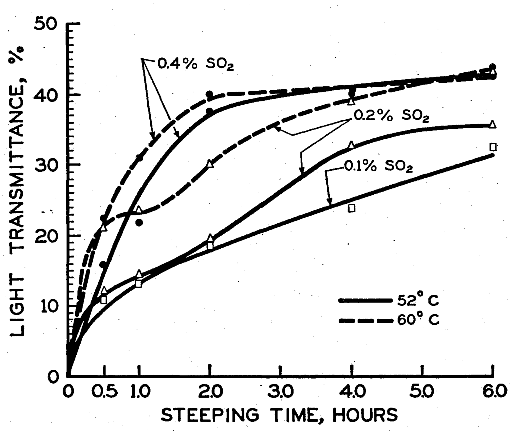

Fig. 7. Average starch-release rates from 10-μ sections of horny endosperm of high-protein hybrid dent corn, CPC-11. LSD (5% level) = 2.7%. Steep temperatures: 52°C., solid line; 60°C., broken line. Starch-free transmittance = 65%.

higher concentration of sulfur dioxide for maximum starch release.

The starch release from Argentine flint endosperm at 0.10% sulfur dioxide shows a pronounced lag between 0.5 and 1 hour (Fig. 6). This might be explained as release of loosely held granules during the first half-hour of steeping. These granules may have been some that were cut with the microtome knife and hence were not entirely encased in protein matrix. For the granules entirely encased in protein matrix, there may be an induction period of about 1 hour for the low concentration of sulfur dioxide to react with the protein to an extent sufficient to weaken it so the starch granules will be released. There is no direct evidence that this is the correct explanation, but this same trend can be seen in the other two curves in Fig. 6 and on some curves in the other figures.

Figure 7 gives the results obtained with horny endosperm sections of high-protein yellow dent corn. A much restricted starch-release picture was obtained with this corn at all sulfur dioxide levels as compared with ordinary dent corn. One parent of this hybrid is the Illinois high-protein strain of corn (9), which carries the trait of a horny endosperm of high density due to thick protein matrix strands. The greatest rate and extent of starch release were obtained with 0.4% sulfur dioxide at 52° and 60°C. and with 0.2% sulfur dioxide at 60°C. However, with the latter, the rate up to 2 hours was lower than at 0.4% sulfur dioxide because of a lag at 1 hour similar to that obtained with the flint corn. The higher temperature apparently enhanced the action of sulfur dioxide at the 0.2% level.

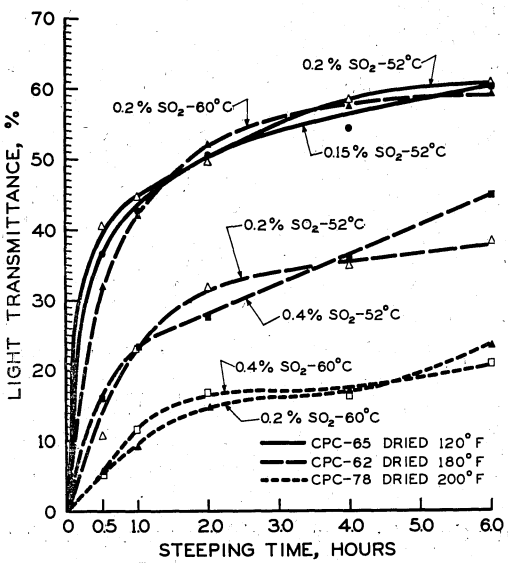

Fig. 8. Average starch-release rates from 10-μ sections of horny endosperm of yellow dent corn dried at three temperatures; CPC-65 dried at 120°F. (solid line); CPC-62 dried at 180°F. (broken line); CPC-78 dried at 200°F. (dashed line). Steep temperatures 52° and 60°C. LSD (5% level) = 2.5%. Starch-free transmittance = 88%.

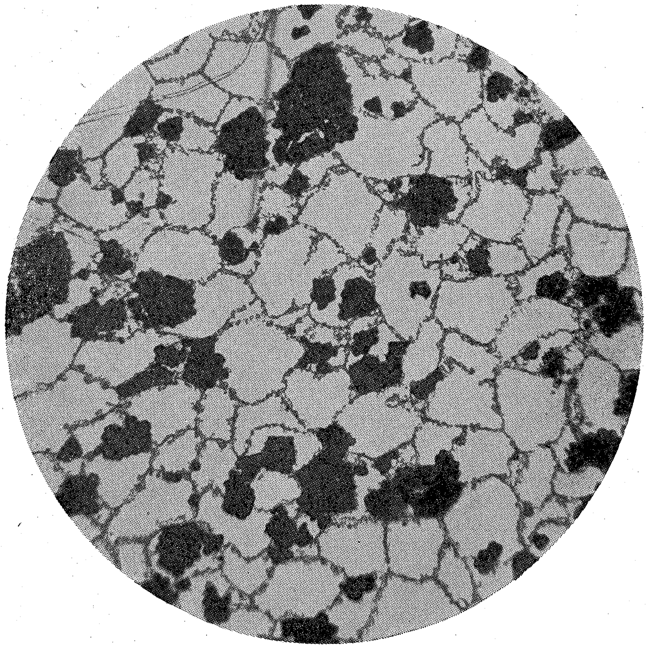

Fig. 9. Section of horny endosperm of dent corn, CPC-62, dried at 120°F. (49°C.) after 4 hours of steeping at 0.15% SO_2, 52°C. Light transmission, 65%. Mag. 44×.

Effect of Conditions of Artificial Drying. The use of excessive temperatures in the artificial drying of corn causes decreased yield and quality of starch in the wet-milling process (2,3). The swelling of protein matrix observed by Cox, MacMasters, and Hilbert (1) with endosperm sections of normal corn steeped in sulfur dioxide was not observed by them with corn dried at high temperatures.

The data in Fig. 8 confirm this effect, using our technique with artificially dried corn. The corn was dried from 32 to 12% moisture with air at 40% relative humidity and at temperatures of 120°, 180°, and 200°F. (49°, 82°, and 93°C.). These samples were dried in a farm-scale batch dryer (2) and are part of the same lots used by MacMasters *et al.* (3) from the 1952 crop year. The corn dried at 120°F. (49°C.) gave data (Fig. 8) similar to the reference dent corn dried at 104°F. (40°C.) (Fig. 5). The endosperm sections of corn dried at 180°F. (82°C.) released about two-thirds as much starch as the control when steeped at 52°C. Steeping at 60°C. with 0.2% sulfur dioxide, however, apparently overcame the damaging effect of the drying at this temperature. Corn dried at 200°F. (93°C.) gave only one-third the extent of starch release obtained with the control

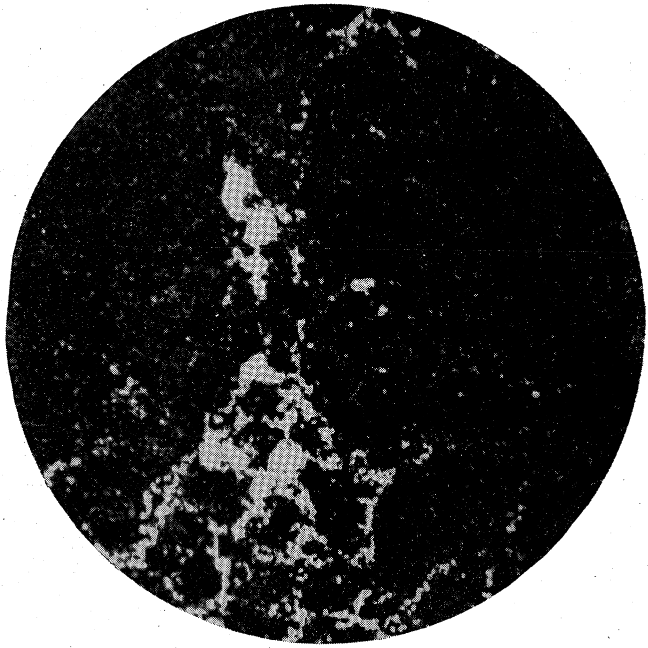

Fig. 10. Section of horny endosperm of dent corn, CPC-78, dried at 200°F. (93°C.) after 6 hours of steeping at 0.4% SO_2, 60°C. Light transmission 15%. Mag. 35×.

even with steeping at 60°C. with either 0.2 or 0.4% sulfur dioxide. The transmission of 15% recorded for these sections was due to loss of pieces of intact endosperm, and it is doubtful that individual starch granules were released from protein matrix. Cracking and crazing of endosperm in the samples dried at the two higher temperatures made the preparation of intact sections very difficult.

The inability to release starch from corn dried at high temperature is dramatically illustrated in Figs. 9 and 10. The first photograph shows the normal starch release obtained with the corn dried at 120°F. (49°C.). The second shows the almost complete retention of starch in the section of the corn dried at 200°F. (93°C.) even though it was steeped under drastic conditions.

It is evident that the use of a temperature of 200°F. (93°C.) for artificially drying corn damages endosperm by irreversibly locking the starch granules into the protein matrix. This observation explains the inability of the wet-miller to extract starch from horny endosperm. Starch obtained from such badly damaged corn comes only from the floury endosperm portion of the kernel and carries a high protein content due to pieces of protein that adhere to starch granules. The horny endosperm cannot be ground into pieces small enough to pass through the screens used for fiber removal and therefore must pass into the by-product cattle feed. Starch in feed has about one-sixth the monetary value that it has as purified starch.

The use of a 60°C. steeping temperature gave normal starch release from the corn dried at 180°F. (82°C.). However, the countercurrent manner used with batteries of commercial corn steeps makes impossible the use of a temperature above 55°C. or a sulfur dioxide concentration higher than about 0.2%; the delicate balance of lactic acid fermentation necessary for optimum steeping would be destroyed.

It is obvious that the full commercial value of corn cannot be realized by processors unless farmers and receiving terminal elevator operators adhere strictly to moderate drying conditions.

Acknowledgment

The co-operation of J. H. Ramser, Department of Agricultural Engineering, and G. H. Dungan, Department of Agronomy (retired), of the University of Illinois for supplying samples of artificially dried corn is gratefully acknowledged.

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