CHANGES IN SOME PROPERTIES OF THE ALEURONE CELL LAYER CAUSED BY STEAM-CONDITIONING[1]

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ABSTRACT

Dispersibility of the protein of the aleurone cell layer was significantly and progressively decreased by conditioning temperatures of 60°C. and greater. Intensity of staining of the aleurone cell layer of Ottawa hard red winter wheat with aniline blue, fast green, methyl green, and acridine red, respectively, was found to be visibly increased by steam-conditioning to 60°C., and further increased as the conditioning temperature was raised to 70°C. Protein dispersibility was more sensitive than staining as an indication of changes occurring in the aleurone cell layer as the result of steam-conditioning.

Steam-conditioning of wheat for milling has been reported by Pence (1) to decrease greatly the holding time required; others have reported steam-conditioning to improve millability (2–7). Altrogge (3) and El-Gindy and Schäfer (8,9) stated that steam-conditioning coagulates the cell contents of the aleurone cell layer, and a concurrent change in the ability of the material to stain with neutral red has been demonstrated (8,9). El-Gindy and Schäfer (8) considered the change in staining ability to result from formation of a lipoid-protein complex.

The present study was undertaken to extend current knowledge concerning changes in staining properties and in the physical nature of the protein of the aleurone cell layer that are brought about by steam-conditioning. This is part of a broad study being pursued on conditioning of wheat.

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Materials and Methods

Ottawa variety hard red winter wheat, harvested in 1961 at the Kansas State Agricultural Experiment Station Farm, was used for the study. The grain was first passed through the cleaning house of the University pilot flour mill. The cleaned grain was found, by analysis, to contain 12% moisture, and, on a 14% moisture basis, 11.6% protein and 1.74% ash.

Methods 44-15, 46-10, and 08-01, respectively, in *Cereal Laboratory Methods* (10) were used to determine moisture, protein, and ash contents of the wheat.

Preliminary work was done on kernels immersed in boiling water for 4 to 10 min., to assure heating of the aleurone cell layer at least equivalent to that attained during steam-conditioning.

Tempering was to a final moisture content of 15.5%. Control samples were conditioned by adding the calculated amount of water to the wheat in a rotating metal drum. Steam-conditioning was carried out in a Miag laboratory conditioner. The details are described elsewhere (11).

The method described by El-Gindy and Schäfer (9) was used for staining with neutral red.

When Congo red or eosin was used as stain, the sections were mounted in the stain solution.

The most successful staining was obtained by heat-treating the kernels either by immersion in boiling water or by steam-conditioning, soaking the treated kernels for 4 days in distilled water in a refrigerator, sectioning the soaked kernels at 30 μ on a freezing microtome, and staining. The sections then were placed in a 0.5% solution of the stain for 30 sec., washed in distilled water for 1 min., and mounted in glycerol. This method was used for staining with aniline blue, fast green, methyl green, acridine red, chlorazol black, Bismarck brown Y, safranine Y, and a combination of fast green and methyl green. The same method was used for staining with combinations of fast green and aniline blue, and aniline blue and safranine Y, except that a 15-sec. staining time was used.

Dispersibility of the protein of the aleurone cell layer was determined as follows:

The wheat was milled and the bran was passed through a Paradox bran finisher three times, sieved on a No. 44 wire screen on a Smico laboratory sifter, and the overs passed through a Mikro-pulverizer equipped with a 0.02-in. screen.

Starch in the purified bran was determined by the method of Earle and Milner (12), except that uranyl acetate was used in place of stannic

chloride (13). As starch content of the prepared bran was found to be statistically constant at around 5%, it could be assumed that the amount of contaminating endosperm was approximately constant. Further, preliminary studies showed that the dispersibility (17 to 19%) of the protein in the starchy endosperm was practically unaffected by any of the conditioning treatments used. It was therefore concluded that any changes in dispersibility of protein of the prepared bran must be definitely related to changes in the properties of the protein in the aleurone cell layer.

Therefore, 2 g. of prepared bran was placed in a 125-ml. Erlenmeyer flask and 40 ml. of 0.5*M* $MgSO_4$ solution was added. A few crystals of thymol were also added, to inhibit microorganic growth. The mixture was shaken for 7 hr. on an Eberbach automatic shaker. (Preliminary studies had indicated this period gave maximum dispersion.) The mixture was then filtered on Whatman No. 4 filter paper. Protein (N × 5.7) was determined on a 20-ml. aliquot of the filtrate and on a 1-g. sample of the bran. Moisture was determined on the bran.

The following formula was used to calculate the percentage of protein dispersed from the bran:

$$\frac{\frac{P_1}{S_1} \times \frac{40}{20}}{\frac{P_2}{S_2}} \times 100 = \text{percent protein dispersed}$$

when P_1 = protein content of 20 ml. of filtrate, in percent;
P_2 = protein content of bran, in percent;
S_1 = weight of bran used for dispersion, in g.; and
S_2 = weight of bran used for protein determination, in g.

Results and Discussion

Four stains — aniline blue, fast green, methyl green, and acridine red — and two combinations of stains — fast green and aniline blue, and fast green and methyl green — were found, in addition to neutral red which was used by El-Gindy and Schäfer (8,9), all of which stained the aleurone cell layer differently before and after immersion of the kernel in boiling water. In each case, the staining was deeper after the heat-treatment.

No difference in staining with Congo red, sodium nitroprusside, eosin, chlorazol black, Bismarck brown Y, safranine Y or a combination of aniline blue and safranine Y was effected by heat-treatment.

After it was established that staining of the aleurone cell layer could be changed in intensity by heat-treatment of the kernel, fast

green or aniline blue was used for the remainder of the study.

Depth of staining of the aleurone cell layer was visually the same for the control samples, conditioned at room temperature, and for samples steam-conditioned to a temperature of 47°C. Slightly darker staining was evident in kernels steam-conditioned to 60° or 65°C., and still darker staining was apparent in kernels steam-conditioned to 70°C. or to a higher temperature. A barely visible difference was found between the intensity of color of the aleurone cell layer after steam-conditioning at 60° or 65°C. and that after steam-conditioning at 70° to 87°C.

From the results, it appears evident that some change in the aleurone cell contents of the wheat studied is initiated by heat in the temperature range between 47° and 60°C. and that this change is still in progress between 60° and 70°C. Any further change that may occur at conditioning temperatures of 75°C., or greater, is not microscopically detectable under the conditions used in this study.

Dispersibility of the protein of the aleurone cell layer was significantly affected by steam-conditioning. The results are shown graphically in Fig. 1. Each point is the mean of eight replicates.

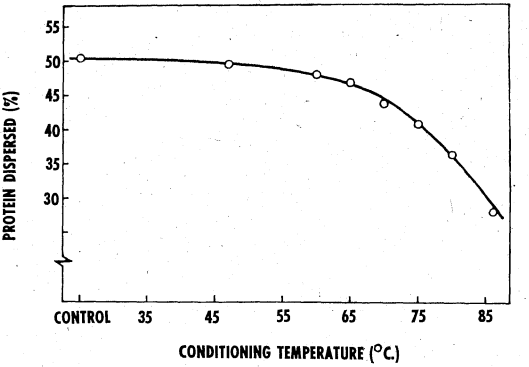

Fig. 1. Effect of steam-conditioning on dispersibility of protein of aleurone cell layer.

There was no statistically significant difference between dispersibility of protein in the control and in the samples steam-conditioned at 47°C. Significant differences were found ($\alpha = 0.05$), however, in dispersibility of the protein of the aleurone cell layer between samples steam-conditioned at 47°, 60°, 65°, 70°, 75°, 80°, and 87°C.

Protein dispersibility evidently is more sensitive than staining as an indication of changes brought about in the aleurone cell layer by steam-conditioning.

Further study is needed to determine the temperature at which the dispersibility of the protein is first affected. On the basis of present data, however, it seems that denaturation of the protein of the aleurone cell layer may be a major change brought about by steam-conditioning. The present study did not touch upon the possibility of the formation of a lipoid-protein complex, as suggested by El-Gindy and Schäfer (8).

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