HISTOCHEMICAL CHARACTERIZATION OF WHEAT AND WHEAT PRODUCTS

II. Mapping of Protein Distribution in the Wheat Kernel[1]

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ABSTRACT

Topographical distribution of protein in wheat was followed on sections cut with a freezing microtome by means of the preferential adsorption of Xylidine Ponceau. The outer endosperm portions showed a higher dye-binding capacity than the central portions and the cheeks. Lowest protein concentration was in the pericarp; highest, in the aleurone layer and embryo.

Distribution of protein in the wheat kernel has attracted attention for many years. The recent separation of flour on a commercial basis to various products according to protein content has made protein distribution in the wheat kernel important.

Cobb (4) dissected individual kernels by hand and demonstrated a relatively sharp gradient in the protein content of the various endosperm zones. Morris et al. (13) dissected tempered wheat by using a dental drill and separated the endosperm into four fractions and the bran into two fractions. They found more protein in the peripheral endosperm zone than in the cheek, with the least in the central endosperm. The distribution of protein also has been studied in hand-dissected material by Hinton (9).

This report deals with the possibility of using a simple dye adsorption technique to map protein distribution in the wheat kernel. Various ways to demonstrate proteins by histochemical methods have been summarized by Serra (14), and more recently by Weiss et al. (17) and by Hopman (10). Burstone (3) discussed new approaches to the histochemical demonstration of proteins, based on condensation reactions in conjunction with coupling processes, or on oxidative deamination reactions. Although there are many tests for proteins, they are poorly adapted to histochemical work because they use strong acid or alkali which disintegrates cellular structure.

The method of Fraenkel and Cooper (6) to determine acidic and basic groups in proteins, based on the determination of unbound Orange G, was adapted by Udy (16) to estimate the protein content of ground wheat and flour, and by Bunyan (2) to evaluate organic nitrogen compounds in feed meals. Orange G gave yellow-brown

shades that were rather unsuitable for microscopic techniques. The
dye employed in this study, Xylidine Ponceau, was proposed by
Levine (12) to stain basic amino groups.

Materials and Methods

Twenty-nine basic and acidic dyes recommended in the literature
for protein staining were tested for localization and quantitative
evaluation of protein in the wheat endosperm. Best results were ob-
tained with Xylidine Ponceau 1 (5), Color Index 79, known also
under several other synonyms. The acid, mono azo dye, has adsorp-
tion maxima at about 5,380 Å and 4,990 Å. It is water-soluble but
has low solubility in 95% alcohol (about 0.2 g. in 100 ml.). Two series
of tests were made to estimate the possible use of the dye for staining
protein in wheat.

In one series, 67 samples of hard red winter wheat flour, milled
on a Buhler experimental mill, were tested. The protein range of the
flour samples was between 8.6 and 14.9%. The dye adsorption of the
tested flours was determined by the following procedure: 2 g. of flour
were suspended in 25 ml. of water containing 3 mg. of dye (using a
0.1% stock solution, diluted to the required strength) in a 125-ml.
rubber-stoppered Erlenmeyer flask and shaken for 1 hour. After the
material stood for 15 minutes, 15 ml. of the supernatant were trans-
ferred to a graduated tube and centrifuged until clear. (About 15
minutes at 2,000 r.p.m. (1,350 $\times$ g) was generally required.) The
clear supernatant was diluted 1:2 and the absorbance measured with
a Beckman model DU spectrophotometer within 4 hours from the
beginning of the test. The solution developed opalescence after pro-
longed standing. The absorbance of solutions containing known
amounts of dye obeyed the Beer-Lambert law in the range of concen-
trations used. Knowing the amount of unabsorbed dye permits the
amount of bound dye to be calculated by difference.

In the second series of tests the dye adsorption of the following
products was tested: a) commercial wheat starch; b) commercial
wheat gluten containing 60% protein; c) pulverized coarse wheat
bran; d) samples of commercial wheat germ, purified by removal of
foreign material under low magnification (24$\times$); e) wheat semolina;
and f) wheat flour prepared by grinding of the semolina.

The dye-binding procedure was essentially the same as in series 1,
except that the amount of tested material was reduced to 0.25 g. in
samples c to f.

Based on findings in these two series of experiments, determina-
tions of protein distribution were made on wheat sections 50 to 100

microns thick, prepared by the procedure of Grosh and Milner (8).

The sections were fixed overnight in 95% alcohol, kept for 1 minute in a 0.1% aqueous dye solution, washed in three changes of water, dehydrated by two changes of alcohol, mounted, and observed with the unaided eye or under low-power magnification. Photomicrographs were made with Kodachrome film.

The choice of the fixative should be governed by the chemical groups which serve for the visualization of the protein and the histochemical method employed (1). Alcohol has been found to be a rather unreactive fixative that causes the fewest changes in the structure of the protein and in available basic groups (15).

Results and Discussion

The correlation coefficient between the protein (as determined by the Kjeldahl method) and dye adsorption was +0.721 (significant at the 0.1% level) for the 67 experimentally milled flours.

The conditions under which the experimentally milled flours were tested for dye adsorption are not considered to be optimal for quantitative protein evaluation. Such a determination would involve use of more highly concentrated dye solutions. Because such solutions (i.e., 1%) gave poor contrast on stained wheat sections, a more dilute dye solution was employed.

Dye adsorption, under the described test condition, was 1.3 mg. per gram of wheat gluten, compared with 0.1 mg. per gram of wheat starch, confirming that the dye is bound mainly by endosperm proteins.

Figures 1 and 2 appear at the end of Part IV, page 129.

In tests made on transverse and longitudinal wheat sections, clear differentiation in protein content was obtained in samples of both hard and soft wheats; mapping was more pronounced in hard wheat. The stained transverse section of wheat (Fig. 1) showed great differences in protein concentration between outer and inner endosperm, but no zone differentiation as postulated by Cobb. No demarcation line existed between zones of protein-rich and protein-poor endosperm tissues; thus the over-all picture is that of a gradual change in protein content. Figure 1 is representative of the wheat examined. In practically all cases the more deeply stained areas of the wheat endosperm corresponded to "glassy" areas of the wheat kernel.

The more detailed picture of a longitudinal section taken under low magnification (Fig. 2) shows the low level of protein in the pericarp, the high dye adsorption of the germ, and a distinctly high protein content in the aleurone layer.

The results confirm previous findings regarding protein distribution

in the wheat kernel and data on wheat hardness in various layers recently reported (11). Hinton (9) found the following concentration of protein in dissected wheat; pericarp, 4.4%; aleurone layer, 19.7%; endosperm, 13.7%, 8.8%, and 6.2% respectively (from outer to inner endosperm); embryo, 33.3%; and scutellum, 26.7%. The dye-binding capacity of Xylidine Ponceau is correlated with these protein values for various tissues.

The dye-staining technique cannot replace chemical tests, as it supplies only information of a qualitative nature. Though acidic dyes give more consistent and intense staining when applied in buffered form (low pH solutions), solutions employed were unbuffered, because a buffer might affect the dye uptake in various parts of the tested tissue (12).

Despite limitations of the test, it fulfills the prerequisite of a histological procedure (7): The microtome sections are prepared so that no significant alteration takes place in the position of the constituents being investigated; the reagent is fairly specific for the tissue constituent; there is little diffusion of the constituent tested or of the reaction product; and the reaction product is capable of being visualized.

The simplicity and rapidity of the test, as well as the results obtained from testing flour, point to the possibility of employing the procedure as a screening aid in air-classification of flour.

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Fig. 1, page 111. Stained transverse sections of wheat, showing differences in protein content between outer and inner endosperm. Microscope not used, but magnification of approximately three times normal size obtained by use of extension tubes on camera.
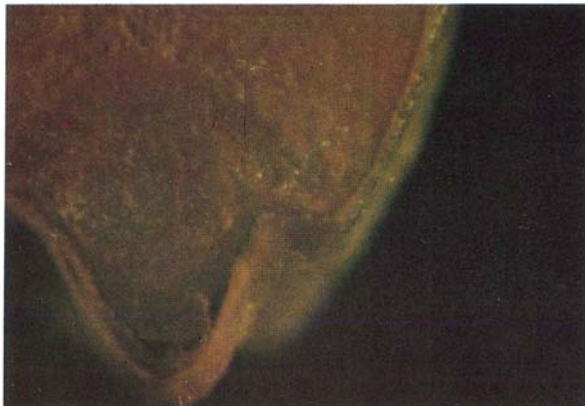

Fig. 2, page 111. Stained longitudinal section of germ and adjacent layers of wheat kernel showing areas of high protein concentration in the embryo and aleurone layer and the low protein content of the pericarp. (20×)