HISTOCHEMICAL CHARACTERIZATION OF WHEAT AND WHEAT PRODUCTS

III. Use of Methyl Green in Estimating Flour Extraction Rate[1]

Y. POMERANZ AND J. A. SHELLENBERGER

ABSTRACT

The quantity of bran in flour may be determined by its high adsorption of methyl green. Flour is shaken with a dilute solution of methyl green, and the dye adsorbed is calculated from the absorbance of the centrifuged supernatant solution.

The estimates of bran in flour, as determined by the proposed method, are highly correlated with the ash content or the color grade of the flour. Staining of flour with methyl green has also been shown useful as an aid in detection of insect fragments, especially in high-extraction flours.

The objectives of milling are to separate endosperm from bran and germ and subsequently to reduce endosperm particles to flour. The efficiency of separation can be judged by several empirical, indirect methods based on measuring one constituent that is concentrated to a larger extent in bran or germ than in endosperm. The most widely accepted chemical measures of flour purity are crude fiber content and mineral or ash content. Although the composition of the branny layers permits basing measurements of flour refinement on determinations of other constituents, such as water-soluble vitamins, phosphorus, and pentosan content, the distribution of those constituents in individual bran layers is not uniform. Nor does relative concentration of the constituents in certain bran layers follow a definite pattern (5,6,7,13). The ash determination is the most widely accepted criterion of flour extraction and efficiency of bran separation. The method is simple, can be performed fairly rapidly, is

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reproducible, and is well suited for routine determinations. Major objections to the ash test are that mineral-matter content of flour may be affected by factors unrelated to flour extraction, such as variations in the mineral content of the wheat. The ash test also is unsatisfactory in flour fortified by calcium carbonate and in high-extraction flour. Ash content does not always reflect correct quantities of bran, because mineral constituents are higher in the aleurone layer than in the fibrous coatings. Hinton (7) found the gradient in ash content from outer to inner layers of the endosperm to vary in different wheats; more ash was present in the aleurone layer than in the inner endosperm; small portions were found in the fibrous pericarp and testa. While ash determinations are justified in highly refined flour, their use as a yardstick of efficient milling of dark flours may exclude the highly nutritious aleurone layer from flour and replace it with indigestible fibrous bran that has a deleterious effect on baking quality. Hence, a simple and rapid method to express flour grade, irrespective of mineral-matter determination (12), is needed.

The Kent-Jones and Martin Flour Color Grader measures light reflected by a flour slurry (9). The instrument uses a filter with maximum transmittance at 530 mμ. The readings reflect the amount of bran present and are correlated with the flour extraction rate. The method has found wide use in England and in Canada, but its use in other countries has been, so far, limited.

Numerous colorimetric methods have been proposed to determine the bran content of flours. Meyer (11) recommended use of dyes (especially naphthylene blue) as an aid in microscopy of wheat and flour. Recently, Larkin *et al.* (10) have developed a test based on color intensity of bran and germ particles stained with crystal violet. Their method was satisfactory with some flours but not others. Deatherage and MacMasters (4) described a method for direct determination of approximately one-third of the bran in flour.

This study was undertaken to investigate the utility of a dye-adsorption technique to determine the amount of bran in flour. The dye used was methyl green, a basic triaminotriphenyl dye (C.I. 684; synonyms: double green, light green), a coal-tar dye, which has been recommended for staining cutinized and suberized cell walls (3). Chamberlain (2) and Johansen (8) recommend a solution of methyl green in water to stain lignified structures.

The relations between the extent of dye adsorption, the ash content, and color grade (as determined with the Kent-Jones and Martin Flour Color Grader) were studied on commercially and experimentally milled flours.

Materials and Methods

Ninety-two samples of flour were obtained from five commercial mills and one experimental Buhler mill. Moisture and ash determinations were made according to the official methods of analyses of the AOAC (1). Ash content was determined by direct weighing. The results are expressed on 14% moisture basis. Grade color determinations, with the Kent-Jones and Martin Color Grader (9), were made according to the procedure outlined by the manufacturer, involving measurement of light reflected from the surface of a paste prepared from 30 g. of flour and 50 ml. of water. The dye used, methyl green,[2] sometimes contains small amounts of methyl violet but it can be purified. It has an adsorption maximum at 6338A. Methyl green is generally considered to stain more intensely in the presence of acetic acid. Preliminary tests confirmed this view, but an acid suspension was not used because of difficulties in obtaining clear centrifugates. Subsequent tests were continued on a water suspension.

Technical difficulties in measuring adsorbed dye made it easier to measure the concentration of dye in the supernatant and to determine the quantity of bound dye by difference than to measure dye adsorption directly.

The effect of a number of variables in analytical procedure was established. These included: concentration of dye, shaking period, particle size of flour, ratios of dye to bran, and dye adsorption of flour containing different amounts of bran.

After a series of experiments, the following general procedure was followed unless otherwise stated: 2 g. of flour were shaken for 1 hour at room temperature in a 125-ml. stoppered Erlenmeyer flask with 20 ml. of 0.01% methyl green solution. The solution was prepared by diluting a 0.1% stock solution; the stock solution remained stable for more than 1 month at room temperature. After 30 minutes' standing, the supernatant was transferred into a 15-ml. tube and centrifuged for about 15 minutes at 2,000 r.p.m. (1,350 × g). Five milliliters of the clear solution were diluted with 10 ml. of water and the optical density measured with a Beckman (model DU) spectrophotometer at a wave length of 6340A. Amount of adsorbed dye was calculated from the results.

Results and Discussion

Variables Affecting Dye Adsorption. Adsorption of methyl green was found to depend on the concentration of the dye, on the shaking

[2] Coleman and Bell Co., Norwood, Ohio.

period, and on the particle size of flour. The effect of dye concentration is summarized in Table I.

These results show that bran adsorbs far more dye than flour does.

Essentially the same results were obtained when various increments of bran were added to flour and the amount of dye was held constant (20 ml. of a 0.01% solution). Results in Table II are based on tests in which two samples of coarse commercial bran, ground to pass a 40-mesh sieve, were added at various levels to 2-g. samples of flour. Amount of dye adsorbed in each case was calculated after deducting the amount adsorbed by the flour alone, assuming that the addition of bran did not affect the dye adsorption of the flour. The

TABLE I

INFLUENCE OF THE RATIO OF DYE TO FLOUR OR BRAN ON DYE ADSORPTION, EXPRESSED AS MG. METHYL GREEN PER GRAM OF FLOUR OR BRAN

Weight of Flour or Bran	Methyl Green Dye									
	Added	Adsorbed	Added	Adsorbed	Added	Adsorbed	Added	Adsorbed	Added	Adsorbed
	mg	*mg*	*mg*	*mg*	*mg*	*mg*	*mg*	*mg*	*mg*	*mg*
Flour										
100	5.0	2.5	10.0	4.2	20.0	5.0				
200	2.5	1.6	5.0	2.7	10.0	3.4				
500	1.0	0.5	2.0	1.2	4.0	2.2				
1,000			1.0	0.6	2.0	1.2				
1,500			0.7	0.4	1.4	0.8				
2,000	0.25	0.2	0.5	0.4	1.0	0.7				
Bran										
10	50	35	100	44	200	47				
20	25	17	50	36	100	45				
50	10	8	20	18	40	32				
100			10	9.5	20	18	40	33	100	42
150			8	8	16	13	32	24	55	42
200			5	5	10	10	20	19	40	40

TABLE II

DYE ADSORPTION OF BRAN ADDED AT VARIOUS LEVELS TO 2 GRAMS OF FLOUR

Bran Sample Number	Bran Added to 2 g. Flour	Dye Adsorption of Bran
	mg	*mg dye/g bran*
1	20	4.8
	40	4.5
	60	4.4
	100	4.6
2	40	6.0
	60	5.2
	100	5.3

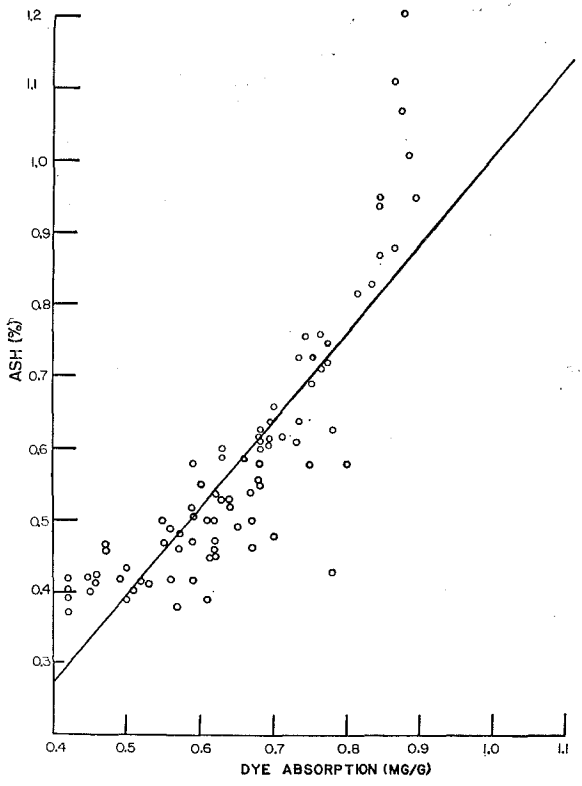

Fig. 1. Scattergram showing relationship between dye adsorption and ash content. (r = +0.86)

dye adsorption of the flour used in this series was 0.47 mg. per g.

Dye adsorption of each numbered bran was fairly constant, despite variations from 1 to 5% in the amount added to flour. The ratio of dye adsorption of bran to flour was 10 to 1 for bran No. 1 and almost 12 to 1 for bran No. 2. This ratio essentially did not change by a tenfold increase in added dye. The only advantage of increasing the quantity of dye was that the solutions clarified more easily on centrifugation and did not become turbid even after standing 24 hours. When weaker concentrations of dye were used, the absorbance of the clear solution had to be measured within 4 hours after centrifugation to avoid opalescence. But a more concentrated solution involved additional dilution and difficulties from dye adhering to glassware and stoppers. Stronger dye concentration did not affect relative placing of flours by ash content or grade color; hence, the tests were made with the weaker solution.

To test the effect of particle size on dye adsorption, pure semolina was ground for various time periods to reduce the original product from a size retained on a 20-mesh sieve to that which would pass a 50-mesh sieve. The dye adsorption of the original product increased gradually with grinding from 0.24 mg. to 0.36 mg. dye per g. of product. Though such wide variations in particle size are unlikely to be encountered under normal commercial conditions, particle size affects dye adsorption. Finer flour adsorbs more dye than does coarser flour.

When flour was shaken with the dye solution, there was no significant effect on dye adsorption if the flour was shaken at least 30 minutes. To secure complete adsorption, the products were shaken 1 hour. Shaking times beyond 1 hour sometimes made solutions difficult to clarify and they required longer centrifugation.

Determinations were made in duplicate. The standard error of a dye adsorption determination was 0.011.

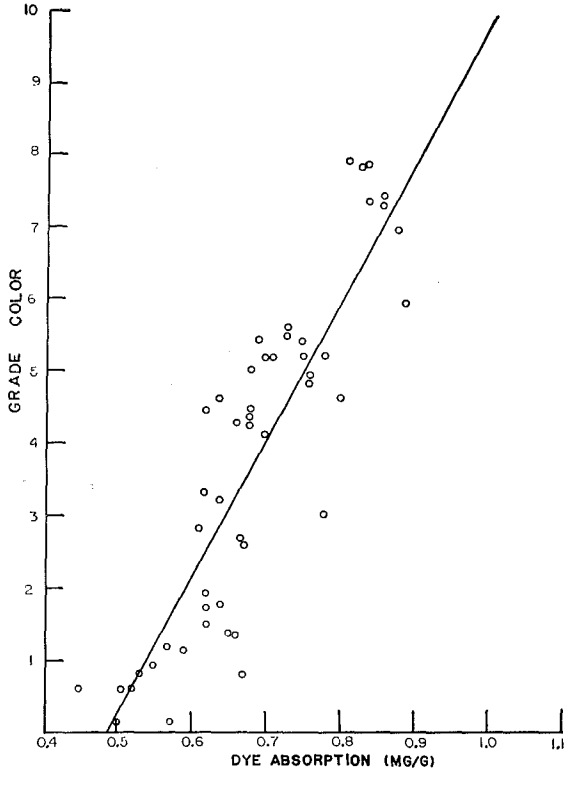

Fig. 2. Scattergram showing relationship between dye adsorption and color grade. (r = +0.88)

Relations between Dye Adsorption, Ash Content, and Color Grade. The results on the main series of samples are shown in Figs. 1, 2, and 3.

The correlation coefficients for the above samples were: ash vs. grade color, r = +0.87; ash vs. dye adsorption, r = +0.86; grade color vs. dye adsorption, r = +0.88. All the correlations were significant at the 0.1% point.

The regression equations computed were: A = 1.249C − 0.227 (for calculating ash from dye adsorption); B = 18.985C − 9.193 (for calculating grade color from dye adsorption), and B = 11.630A − 3.160 (for calculating grade color from ash). In the above equations, A = ash content (%), B = grade color, and C = dye adsorption (mg. per g. of material).

Assuming that a flour fairly free from bran contains about 0.35% ash, its dye adsorption would be 0.46 mg. per g. flour, a figure higher than that of ground semolina (0.36 mg. per g.), probably because of the smaller particle size of commercially milled flour.

An increase of 1% ash in flour corresponded to an increase in dye

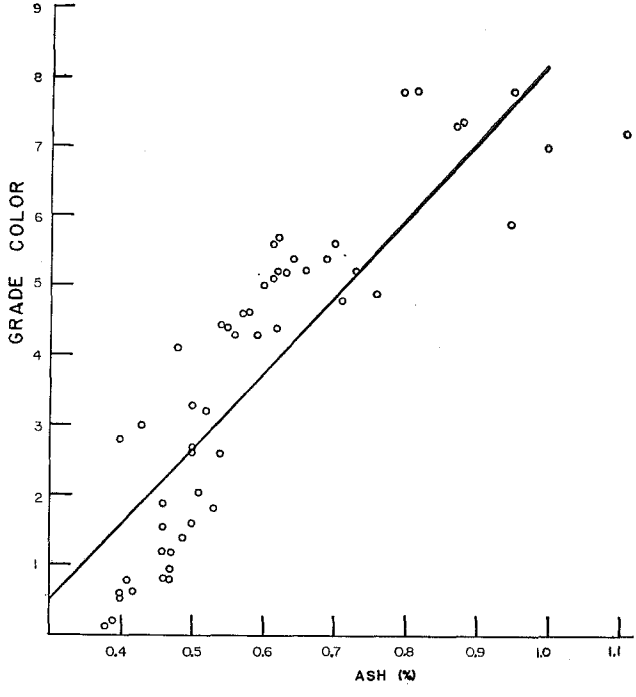

Fig. 3. Scattergram showing relationship between ash content and color grade. (r = +0.87)

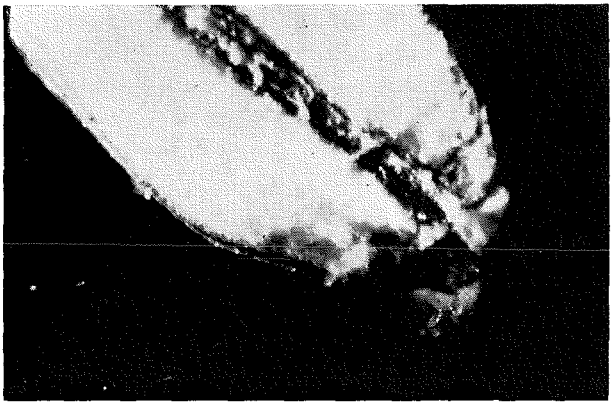

Fig. 4. Longitudinal section of wheat kernel stained with methyl green. (10×)

uptake of 0.8 mg. per g. If the mineral matter of bran is 5%, the dye uptake of bran would be 4 mg. per g., which confirms results given in Table II.

Staining Wheat Sections. Figure 4 shows a longitudinal cross-section of a wheat kernel stained with methyl green. There is no measurable difference in dye uptake of the various pericarp layers, and the staining intensity of the aleurone layer is practically the same as that of the endosperm.

Wheat kernel sections showed a deep green pericarp and germ, contrasted with a faint blue-violet color of the endosperm. Use of 0.01% solution of methyl green to stain microtome wheat sections alone, or in a staining schedule after a 0.1% solution of Xylidine Ponceau, provided additional contrast between layers of wheat. The pericarp and germ are stained green; the endosperm, a deep red.

Staining has generally a broad specificity, governed mainly by solubility or adsorption. Both the flour and bran adsorb the dye, but they vary in their dye-adsorption capacity. This variation is the basis of the proposed method. Methyl green was found to be very well suited for qualitative testing of flour grade. Although the dye is adsorbed by flour, flour has a blue-violet tint from traces of methyl violet in the dye; bran particles are deep green and stand out well in either a slick test or microscopic examination. Staining with methyl green also helps detect insect fragments—a special advantage in long-extraction flour containing quantities of bran particles. In stained preparations, bran particles are green, whereas insect fragments are unstained and retain their natural brown color.

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