NOTE

A Method for Separating Light and Dark Kernels of Winter Wheat (*Triticum aestivum* L.) Based on Density

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Wheat (*Triticum aestivum* L.) is subject to a physiological condition known as yellow-berry, which causes the production of an undesirable, off-color grain. Yellow-berry-affected wheat can be distinguished from normal wheat by a softer, lighter-colored starchy endosperm, which lacks the corneous or vitreous texture characteristic of the normal grain (Sharp 1927).

Wheat kernels affected by yellow-berry have been reported to contain more moisture and starch and less protein than do normal wheat kernels (Roberts 1919). A high percentage of yellow-berries in a wheat sample has also been shown to affect the wheat's milling and baking qualities (Phillips and Niernberger 1976, Pomeranz et al 1976).

For experimental purposes, yellow-berry kernels have traditionally been separated from normal kernels on the basis of a visual appraisal and hand separation (Dikeman and Pomeranz 1977, Hubbard et al 1977, Waines et al 1978). A less tedious method of separating these kernels would be desirable.

The density of yellow-berry kernels has been reported to be lower than that of normal wheat (Bailey 1916, Roberts 1919, Sharp 1927). This paper describes a simple method for separating the light and dark kernels of wheat, based on this density difference. A growth trial was also done to evaluate the effects of the separation technique on the nutritive value of the wheat samples for rats.

MATERIALS AND METHODS

Wheat Cultivars

Samples of five cultivars of winter wheat were obtained from various growing locations throughout Alberta. The cultivars obtained were Yogo, Sundance, Nugaines, Winalta, and Kharkov, which represented the most commonly grown varieties of Canadian winter wheat (Grant et al 1974), although Norstar has recently become the most common cultivar.

Methods of Separation of Wheat Kernels

Twenty samples, each containing 100 wheat kernels, were obtained at random for each cultivar and were separated by hand into light and dark kernels of wheat, and the percentage of each color was recorded. The separated samples were weighed and the thousand-kernel weight (weight of 1,000 kernels in grams) determined. The samples were then recombined and separated by the following density gradient method: When a substance floats in a liquid, it displaces a volume of liquid equal to its own weight. If the body sinks in the liquid, it weighs more than the liquid it displaces. By altering the ratio of chloroform to ethanol in a solution, a density gradient can be established whereby the light-colored wheat kernels float to the surface and the heavier, dark-colored kernels sink to the bottom of the solution. Occasionally, a wheat kernel will remain suspended in solution, but making slight alterations in the ratio of chloroform to ethanol ensures that only light-colored kernels rise to the surface and only dark-colored kernels sink.

The percentage of light and dark kernels in each of these samples was also recorded, and the thousand-kernel weight was determined. Twenty more samples of each cultivar were separated by hand, and these samples as well as those separated by density gradient were analyzed for dry matter and protein.

All separations were done at room temperature (21°C); 20 ml of chloroform was used for each separation but the amount of ethanol used varied with the cultivar to be separated. The ratios used for each separation and their densities are shown in Table I.

To determine whether the separation technique removed anything from the wheat, samples of Sinton wheat (5 g) were soaked in a chloroform:ethanol solution or demineralized water. The kernels were then removed, the solvent evaporated, and the residue weighed. Residue weight for the samples was 0.001 g for the chloroform:ethanol-treated sample and 0.003 g for the

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demineralized water-treated sample. This suggests that the treatment extracts little from the wheat sample.

Growth Trial

To evaluate the effects of the separation technique on the nutritive value of the wheat, the performance of rats fed treated and untreated samples of Sinton wheat was compared. Sixteen Sprague-Dawley rats (University of Alberta strain), weighing an average of 75.4 g when the trial began, were randomly assigned to one of two treatments, with an equal number of males and females per group. The experimental diet for this growth trial contained Sinton wheat treated with chloroform:ethanol solution and dried at 75°C for 4 hr. The control diet contained untreated Sinton wheat. Both diets were heated for 20 hr at 60°C before feeding, to ensure a similar dry matter.

Both treated and untreated wheat were fed at a level of 93.0%; vitamins and minerals were included to meet or exceed the rats' nutrient requirements (National Academy of Sciences-National Research Council 1978). To facilitate mixing, the wheats were ground in a Wiley mill through a 1.0-mm screen.

Rats were individually housed in 18 × 24 × 18-cm stainless steel metabolism cages, maintained in an environmentally controlled room (22°C, 45% RH) with automatic lighting, which provided 12 hr of darkness and 12 hr of light. Feed and water were supplied *ad libitum*. Weight gain and feed consumption were recorded weekly for each rat and summarized at the end of the 21-day feeding period to obtain total weight gain and total feed consumption.

Chemical and Statistical Analysis

Dry matter was analyzed according to methods of the Association of Official Analytical Chemists (1975). Protein content was determined by near-infrared reflectance, using a Technicon 2.5 Infralyzer. The Kjeldahl method was used to standardize the Infralyzer. Kjeldahl nitrogen was converted to protein using the conversion factor, 5.7. The correlation coefficient between the protein content of a sample determined by the Kjeldahl method and that determined by near-infrared reflectance is highly significant (Williams 1975, Watson et al 1976, Winch and Major 1981). Analyses of variance, involving an equal number of observations, were conducted according to the procedures of Steel and Torrie (1960).

RESULTS AND DISCUSSION

The percentage of light and dark kernels obtained by hand separation was not significantly different from that obtained by density gradient (Table II). The percentage of yellow-berries in the winter wheat cultivars averaged 25.2% for Yogo, 34.6% for Kharkov, 58.3% for Winalta, 78.5% for Nugaines, and 84.6% for Sundance. The occurrence of yellow-berries has been shown to be cultivar-related (Wiese 1977, Dikeman and Pomeranz 1976).

The thousand-kernel weight of the light-colored kernels (average, 30.6 g) was generally lower than that of the dark-colored kernels (average, 31.7 g). Earlier studies have also shown that the density of yellow-berry kernels is lower than that of normal wheat (Bailey 1916, Roberts 1919, Sharp 1927). The change in density is due to differences in cell structure, related to the size of the interstitial air spaces. Sharp (1927) suggested that if the spaces between starch grains were filled with protein, the kernel would be corneous, but if these spaces contained air, the kernel would be starchy and opaque (i.e., yellow-berry).

No significant differences were seen in the thousand-kernel weight of samples separated either by hand or density gradient (Table II). The thousand-kernel weight of the light-colored kernels separated by hand averaged 30.9 g, and that of the light-colored kernels separated with chloroform:ethanol averaged 30.8 g. The thousand-kernel weight of the dark-colored kernels separated by hand averaged 31.6 g, and that of the dark-colored kernels separated by density gradient averaged 31.9 g.

The thousand-kernel weight of the light-colored kernels was the greatest for the cultivar, Sundance (34.3 g), followed by Kharkov

TABLE I
Ratio of Chloroform to Ethanol Used for Color Separation of Winter Wheat Cultivars

Cultivar	Chloroform (ml)	Ethanol (ml)	Density
Sundance	20	3.2	1.416
Kharkov	20	2.6	1.435
Nugaines	20	3.4	1.414
Yogo	20	2.4	1.442
Winalta	20	2.7	1.430

TABLE II
Effects of Method of Separation on the Percentage of Yellow-Berries and Thousand-Kernel Weight of Winter Wheats

Criterion	Color	Method	Cultivar				
			Sundance	Kharkov	Nugaines	Yogo	Winalta
Percentage[a]	Light	Hand	85.4 ± 2.0	35.3 ± 8.8	80.1 ± 4.9	24.1 ± 5.5	59.9 ± 6.0
	Light	Density	83.8 ± 2.8	34.0 ± 7.4	77.0 ± 4.7	26.2 ± 4.6	56.7 ± 4.2
	Dark	Hand	14.5 ± 2.0	64.7 ± 8.8	19.8 ± 4.9	75.9 ± 5.5	40.1 ± 6.0
	Dark	Density	16.1 ± 2.8	65.9 ± 7.4	23.0 ± 4.7	73.8 ± 4.6	43.3 ± 4.2
Thousand-kernel weight (g)[a]	Light	Hand	34.0 ± 1.3	34.4 ± 1.2	28.4 ± 0.4	27.7 ± 2.0	30.2 ± 1.1
	Light	Density	34.6 ± 0.8	33.5 ± 0.8	27.5 ± 1.0	28.7 ± 0.9	29.6 ± 2.2
	Dark	Hand	34.0 ± 0.9	33.5 ± 0.8	30.8 ± 1.7	29.4 ± 0.6	30.5 ± 1.1
	Dark	Density	34.7 ± 1.2	33.8 ± 0.9	29.7 ± 0.6	30.2 ± 1.3	30.9 ± 2.0

[a] Means ± standard deviations.

TABLE III
Effects of Method of Separation and Color on the Chemical Analyses of Winter Wheats

Criterion	Color	Method	Cultivar				
			Sundance	Kharkov	Nugaines	Yogo	Winalta
Dry matter (%)[a]	Light	Hand	92.9 ± 0.1	92.7 ± 0.2	92.6 ± 0.1	93.0 ± 0.1	92.9 ± 0.1
	Light	Density	92.8 ± 0.1	92.2 ± 0.1	92.3 ± 0.1	92.7 ± 0.1	92.7 ± 0.1
	Dark	Hand	93.1 ± 0.3	92.6 ± 0.1	92.9 ± 0.1	92.8 ± 0.1	93.1 ± 0.1
	Dark	Density	92.7 ± 0.1	92.4 ± 0.1	92.6 ± 0.1	92.6 ± 0.1	92.6 ± 0.1
Protein (%)[a]	Light	Hand	12.9 ± 0.2	14.2 ± 0.2	12.4 ± 0.2	12.0 ± 0.4	13.2 ± 0.3
	Light	Density	12.4 ± 0.2	14.1 ± 0.2	12.5 ± 0.2	11.7 ± 0.3	13.1 ± 0.2
	Dark	Hand	12.1 ± 0.4	13.2 ± 0.1	12.1 ± 0.2	12.1 ± 0.2	12.6 ± 0.3
	Dark	Density	12.4 ± 0.5	13.0 ± 0.2	11.7 ± 0.2	12.2 ± 0.1	12.8 ± 0.1

[a] Means ± standard deviations.

TABLE IV
Performance of Rats Fed Treated and Untreated Sinton Wheat

	Control	Treated	SE[a]
Intake (g)	270.1	259.5	1.45
Gain (g)	65.7	64.1	0.84
Feed efficiency	4.1	4.0	0.18

[a]Standard error of the mean.

(33.9 g), Winalta (29.9 g), Yogo (28.2 g), and Nugaines (28.0 g). For the dark-colored kernels, the thousand-kernel weight was greatest for Sundance (34.3 g), followed by Kharkov (33.6 g), Winalta (30.7 g), Nugaines (30.3 g), and Yogo (29.8 g).

Method of separation did not affect the dry matter content of separated wheat kernels (Table III). The dry matter content of light kernels separated by hand averaged 92.8%, and that of light kernels separated by density gradient averaged 92.5%. The dry matter content of dark kernels separated by hand averaged 92.9%, and that of dark kernels separated by density gradient averaged 92.6%. Also, no significant differences were noted in the dry matter content of the various cultivars tested. The relatively high dry matter content of all samples reflects air drying that occurred during storage.

The protein content was also significantly similar for wheat kernels separated by hand or chemical means. The protein content of the light kernels separated by hand averaged 12.9%, and that of the light kernels separated by density gradient averaged 12.7%. The protein content of the dark kernels was lower than that of the light kernels, averaging 12.4% for both the hand-separated and density gradient-separated kernels. This observation does not support previous work, which reported that wheat kernels affected by yellow-berry contained a lower level of protein than did normal wheat (Waines et al 1978, Dikeman and Pomeranz 1977, Hubbard et al 1977).

The protein content of the light kernels was highest for the cultivar, Kharkov (14.1%), followed by Winalta (13.1%), Sundance (12.6%), Nugaines (12.5%), and Yogo (11.9%). The protein content of the dark kernels was greatest for Kharkov (13.1%), followed by Winalta (12.7%), Sundance (12.2%), Yogo (12.1%), and Nugaines (11.9%).

The results of the growth trial with rats are shown in Table IV. The feed intake of rats fed the treated wheat was slightly lower than that of rats fed the control. This difference was not statistically significant, however. No significant differences were noted in growth rate or feed efficiency between rats fed treated or untreated wheat.

The overall results of this study indicate that density gradient separation is similar to hand separation as far as the percentage of light and dark kernels obtained in a wheat sample is concerned. The method does not appear to alter the chemical composition or nutritive value of the wheat, is considerably faster and less tedious than visual appraisal and hand separation, and may be useful where large samples of colored wheat kernels are required for experimental purposes.

LITERATURE CITED

ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS. 1975. Official methods of analysis. 12th ed. Washington, DC.

BAILEY, C. H. 1916. The relation of certain physical characteristics of the wheat kernel to milling quality. J. Agric. Sci. 7:432–442.

DIKEMAN, E., and POMERANZ, Y. 1977. Note on mineral contents of dark hard and yellow hard kernels separated from red winter wheat. Cereal Chem. 54:183–186.

GRANT, M. N., PITTMAN, U. J., HORRICKS, J. S., HOLMES, N. D., ANDERSON, D. T., and SMITH, A. D. 1974. Winter wheat production in western Canada. Publ. 1056, Agriculture Canada.

HUBBARD, J. D., POMERANZ, Y., and LAI, F. S. 1977. Note on protein contents and amino acid composition of dark hard and yellow hard kernels separated from red winter wheat. Cereal Chem. 54:778–783.

NATIONAL ACADEMY OF SCIENCES-NATIONAL RESEARCH COUNCIL. 1978. Nutrient requirements of domestic animals. No. 10. Nutrient requirements of laboratory animals. 3rd. ed. NAS-NRC, Washington, DC.

PHILLIPS, D. P., and NIERNBERGER, F. F. 1976. Milling and baking qualities of yellow berry and dark, hard, and vitreous wheats. Bakers Dig. 50:42–48.

POMERANZ, Y., SHOGREN, M. D., BOLTE, L. C., and FINNEY, K. F. 1976. Functional properties of dark hard and yellow hard red winter wheat. Bakers Dig. 50:35–40.

ROBERTS, H. F. 1919. Yellow-berry in hard winter wheat. J. Agr. Res. 18:155–169.

SHARP, P. F. 1927. Wheat and Flour Studies. IX. Density of wheat as influenced by freezing, stage of development, and moisture content. Cereal Chem. 4:14–46.

STEEL, R. G., and TORRIE, J. H. 1960. Principles and Procedures of Statistics. McGraw-Hill Book Co. Inc., New York, NY.

WAINES, J. G., LABANAUSKAS, C. K., HANDY, M. F., GILL, B. S., and LEHMAN, W. F. 1978. Protein and amino acid profiles of normal and yellow-berry bread. Crop Sci. 18:590–592.

WATSON, C. A., CARVILLE, D., DIKEMAN, E., DAIGGER, G., and BOOTH, G. D. 1976. Evaluation of two infrared instruments for determining protein content of hard red winter wheat. Cereal Chem. 53:214–222.

WIESE, M. V. 1977. Compendium of Wheat Diseases. American Phytopathological Society, St. Paul, MN.

WILLIAMS, P. C. 1975. Application of near infrared reflectance spectroscopy to analysis of cereal grains and oilseeds. Cereal Chem. 52:561–576.

WINCH, J. E., and MAJOR, H. 1981. Predicting nitrogen and digestibility of forages using near infrared reflectance photometry. Can. J. Plant Sci. 61:45–51.

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