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in agricultural, food, and
biological systems*

This is not a peer-reviewed paper.

*Paper Number: 01-6006
An ASAE Meeting Presentation*

Assessment of Heat-Damaged Wheat Kernels Using Near-Infrared Spectroscopy

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**Written for presentation at the
2001 ASAE Annual International Meeting
Sponsored by ASAE
Sacramento Convention Center
Sacramento, California, USA
July 30-August 1, 2001**

Abstract. *Heat damage is a serious problem frequently associated with wet harvests because of improper storage of damp grain or artificial drying of moist grain at high temperatures. Heat damage causes protein denaturation and reduces processing quality. The current visual method for assessing heat damage is subjective and based on color change. Denatured protein related to heat damage does not always cause a color change in kernels. The objective of this research was to evaluate the use of near-infrared (NIR) reflectance spectroscopy to identify heat-damaged wheat kernels. A diode-array NIR spectrometer was used to collect single kernels spectra and Partial least squares and two-wavelength regression models were used for classification of heat-damaged and undamaged wheat.*

Keywords. *Wheat, single kernel measurement, drying, heat damage, protein denaturation, near-infrared spectroscopy.*

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Introduction

Heat damage is an important grading factor for wheat in the U.S. Heat damage can be caused by excessive heating from artificial drying, or spontaneous heating during storage and can injure the gluten quality of wheat (Halverson and Zeleny 1988). Heat-damaged kernels account for about 10-25% of the total damaged kernels from U.S. No.1 to No.5 grades (USDA 1988). They are defined as kernels and pieces of kernels that are materially discolored and damaged by heat. Heat damage results in protein denaturation and reduces gluten quality. In some cases, even if the temperature is not high enough to cause discoloration, gluten quality may be impaired (Halverson and Zeleny 1988). Use of heat-damaged wheat can affect processing, resulting in changes in physical properties of dough and loss of baking quality (McDermott 1971, Hook 1980, Preston et al 1989, Becker and Sallans 1956).

The current inspection method for determining heat-damaged kernels relies on visual examination of wheat kernels and, in some cases, a cross-sectional analysis to determine if the color is reddish-brown or mahogany. Results from this form of analysis vary greatly and depend on inspector's experience. Heat damage due to improper storage usually occurs with a fungal attack, resulting in both heat damage and visual fungal damage. Kernel discoloration caused by fungus infestation on the seed coat is easy to recognize and is the probable reason that heat damage from bin heating can be accurately determined visually. However, denatured protein related to heat damage does not always cause a change in kernel color. Therefore, some heat-damaged kernels cannot be found with visual examination.

Several other methods to assess heat damage in wheat have been studied. Mixograph has been routinely used for heat-damage determination (Kilborn and Tipples 1981, Preston et al 1989, Preston and Symons 1993). Measurements of changes in physical dough properties and baking quality provide the most meaningful tests of heat damage because they relate directly to processing quality. Unfortunately, these tests are time-consuming, require expensive equipment, and a high degree of technical expertise. Researchers have determined heat damage by enzyme inactivation, dye binding capacity, turbidity, and protein-extractability (McDermott 1971, Hook 1980, Every 1987). All four of those methods are based on the effects of heat on grain protein denaturation and are fairly complicated procedures. Seckinger and Wolf (1970) and Preston and Symons (1993) studied the measurement of heat damage in wheat by assessment of protein fibril formation. A small amount of endosperm material obtained from a milling or grinding sample was placed on a microscope slide, wet with a drop of water, and observed. They found that heat damage reduced the protein fibril formation. This procedure requires technical training when reference photographs are used. Thus, the available methods for assessing heat damage are either time-consuming or complicated. An objective method that is rapid, precise, nondestructive, and based on

single kernels would benefit both the food industry and the U.S. Department of Agriculture's Grain Inspection, Packers, and Stockyards Administration (GIPSA).

Near-infrared (NIR) reflectance spectroscopy is a method that may meet those requirements. NIR can be used to measure both physical and chemical properties of agricultural products, by-products, and food. Grain drying, especially high-temperature drying (over 60 °C), can cause stress cracks, irreversible protein denaturation, and starch gelatinization (Brooker et al 1992, Myers 1990). The stress cracks in the kernel could result in light scattering that in turn affects the energy absorption pattern. Irreversible protein denaturation changes the native folding of protein to an aggregate and forms an unfolding rigid entity. The transition from native protein to denatured protein has two stages, aggregation and cross-linking. Protein aggregation involves the formation of a higher molecular weight complex, and cross-linking forms specific bonding at specific sites on the protein strands (Wall et al 1975, Wight 1981, Myers 1990). Those chemical structure changes may also result in NIR absorption differences between native protein and denatured protein. Thus, classifying heat-damaged wheat and undamaged wheat by NIR reflectance spectroscopy might be possible.

Recent research on single wheat kernel quality measurement using NIR spectroscopy has successfully measured protein content (Delwiche 1995, 1998); wheat class (Song et al 1995, Delwiche and Massie 1996); color class (Dowell 1997, Dowell 1998, Wang et al 1999a, 1999b, 1999c); and insect damage (Dowell et al 1998, Baker et al 1999, Ridgway et al 1999). However, the use of NIR spectroscopy to measure kernel heat damage, especially in combination with other quality factor measurements, has not been studied. Therefore, the objective of this research was to evaluate the use of NIR reflectance spectroscopy to identify heat-damaged wheat kernels.

Materials and Methods

Sample Preparation

Hard red spring wheat (HRS) and hard white wheat (HWW), obtained from GIPSA (Kansas City, MO), were used. Heat-damaged wheat samples were created by drying undamaged wheat (Preston and Symons 1993). Before drying, wheat samples were tempered to 17% moisture content in sealed containers for at least 2 days at 4 °C. Then about 300 g of each wheat class was sealed in a 500-ml Pyrexplus brand media bottle (Fisher Scientific Co., Pittsburgh, PA) and placed in a forced-air oven for 16 h at 70 °C. The bottle with a one-piece teal polypropylene plug-seal cap is autoclavable up to 121 °C. Therefore, there was no moisture loss from the bottle during heating. After drying, wheat samples were removed from the bottle and conditioned for at least 10 days at room temperature before spectra were viewed. Wheat samples without heat

treatment were used as the control. Chemical compositions of the samples used are shown in Table I.

Kernel Color Measurement

Reflectance spectra from 480 to 750 nm were transferred into $L^*a^*b^*$ color space. Grams/32 (Galactic Industries, Salem, NH) software was used for kernel color determination. In the $L^*a^*b^*$ color space, L^* varies from 0 (black) to 100 (perfect white); a^* ranges from -100 to 100 and measures green when negative and red when positive; b^* varies from -100 to 100 and, is a measure of blue when negative and yellow when positive.

Mixograph Assessment of Heat Damage

A mixogram was used for determination of heat damage to wheat. Mixograph curves were obtained using standard laboratory procedures (Approved Method 54-40A, AACC 1999). Flour (10g) was mixed with the optimum amount of water for 8 min, or until mix time could be determined, at 26 °C in a mixograph bowl. A mixograph pattern was analyzed to provide mixing requirement, mixing tolerance, and optimum water absorption. Wheat flour used for obtaining mixograph curves was prepared using a laboratory mill (60-65% extraction).

Rapid Viscosity Analyzer Assessment of Heat Damage

A model 3-CR rapid viscosity analyzer (RVA) (Newport Scientific Pty. Ltd., Warriewood, NSW, Australia) was used. Sample preparation and testing was based on Approved Method 76-21 (AACC 1999). Gelatinization, pasting, and setback profiles were measured with 12.2 °C/min ramping rate in 13 min. The temperature profile was divided into three sections, low temperature, high temperature, and cooling/setback. The temperature profile started at 50 °C and was held for about 1 min, then the temperature started to increase. In about 4 min, it reached 95 °C and was held at this temperature for 3 min. After about 4 min cooling, the temperature decreased to 50 °C and was held at this temperature for about 1 min.

NIR Spectra Collection

A diode-array NIR spectrometer (DA7000, Perten Instruments, Springfield, IL) was used to quantify single kernels of heat-damaged and undamaged wheats. The diode-array spectrometer measures visible (400-750 nm) and NIR (750-1,700 nm) reflectance at a rate of 30 spectra per second. Single wheat kernels were placed in a black V-shaped trough (12 mm long, 10 mm wide, and 5 mm deep) and illuminated with white light via a fiber bundle (8 mm diameter) positioned 13 mm from the top of the trough and oriented 45° from vertical. The reflectance probe (2-mm diameter) was oriented vertically 9.5 mm from the top of the trough. The reflectance probe carried the reflected energy to a spectrometer. The procedures included collecting a baseline, collecting 8 spectra from each of the kernels, and averaging the 8 spectra for each

kernel. A total of 260 heat-damaged and 260 undamaged kernels from HRS and HWW were measured, for a grand total of 1040 kernels. A spectrum of the empty trough was measured as a reference before kernel measurement and again after every 100 kernels.

Statistical Analysis and Model Development

Partial least squares (PLS) (Galactic Industries, Salem, NH) was used for data analysis and modeling. PLS is a multivariate data analysis technique designed to handle intercorrelated regressors. Two-class models were developed to classify heat-damaged and undamaged kernels. Wheat kernels first were separated equally into calibration and testing sets, based on even and odd numbers. Heat-damaged kernels and undamaged kernels were assigned constant values of 1.0 and 2.0, respectively. A kernel was considered to be correctly categorized if the predicted value lay on the same side of the midpoint of assigned values. The model performance is reported as the cross-validation of each calibration sample set and prediction of testing sample sets. The number of PLS factors used was the minimum required to give the best classification results. Stepwise discriminant (STEPDISC) analysis was used to develop two-wavelength models for classification of heat-damaged and undamaged wheat kernels. STEPDISC procedure uses forward selection and backward elimination to select a subset of the wavelengths for classifying each spectrum into one of the classes. The wavelengths are chosen to enter or leave the model according to the significant level of an *F*-test from an analysis of covariance and the model with highest classification accuracy.

Results and Discussion

Mixograph and RVA Assessments of Heat-Damaged Wheat

The mixograph curve of undamaged wheat showed a strong and high peak at 180 seconds after mixing (Fig. 1). The mixograph curve of heat-damaged wheat showed a flat line through the mixing period. This indicates that protein in wheat was denatured, and dough lost baking quality (McDermott 1971, Hook 1980, Preston et al 1989, Becker and Sallans 1956). Disulfide bonds of the gluten play an important role in determining gluten and dough properties (Lásztity 1996). Dough structure is based on an extensive three-dimensional bonding of the protein subunits joined together by disulfide cross-links, and heat-damage breaks these bonds.

RVA curves showed that heat-damaged wheat had a higher peak viscosity and lower final viscosity than undamaged wheat. Also, the heat-damaged wheat reached peak viscosity earlier than undamaged wheat. This probably was due to starch damage, resulting in easier water absorbing and faster swelling than undamaged

starch. However, no significant difference was found between damaged and undamaged wheat. Therefore, the detailed results from the RVA test are not reported.

Assessment of Heat-Damaged Wheat by PLS Models

Classification results of cross-validation of calibration sample sets and prediction of testing sample sets using PLS models with 7 PLS factors are summarized in Table II. The NIR wavelength region of 750-1,700 nm gave the highest percentage of correct classification for both cross-validation and prediction (100%). The visible wavelength region gave the lowest percentage of classification. Figure 2 shows the average spectra of heat-damaged and undamaged wheat kernels. In general, the $\log(1/R)$ values of heat-damaged kernels were lower. Differences in $\log(1/R)$ value between heat-damaged and undamaged kernels increased as wavelength increased. In addition, differences in $\log(1/R)$ value between heat-damaged and undamaged kernels were smaller in the visible region than in the NIR region.

Light scattering probably caused the $\log(1/R)$ value differences between heat-damaged and undamaged wheat. Grains are frequently physically damaged internally during high-temperature drying (Brooker et al 1992). During high-temperature drying, stress-cracks may originate in the endosperm at the center of the kernels and propagate outward to the aleurone layer along the boundary of the starch granules. These cracks increase the air space inside the kernels. Therefore, heat-damaged kernels have more air spaces that diffract and diffuse light. This results in more incident energy reflected to the NIR sensor; hence, lower absorbance occurs in heat-damaged kernels. Undamaged kernels have no internal cracks or have less air space to diffract light. Thus, light entering undamaged kernels is more likely to pass through the kernel, which results in a higher absorbance measured by the NIR sensor.

Assessment of Heat-Damaged Wheat by Two-Wavelength Model

Classification results of calibration sample sets and prediction of testing sample sets using two-wavelength models are summarized in Table III. The wavelengths used in each model were those that produced the highest r^2 and highest percentage of correct classification for the calibration sample sets. Three pairs of wavelengths in the visible and near-infrared regions were compared. Wavelengths selected from the NIR region yielded better testing results (96.8%) than wavelengths selected from the visible region (94.3%). An instrument with single or two wavelengths is less expensive than an instrument with a longer wavelength region because a simple filter could be applied in the instrument. The lowest classification accuracy of 94.3% from the two-wavelength model may meet the requirements for industry and grain inspection applications. Therefore, the two-wavelength model is more practical.

Difference in color between heat-damaged and undamaged wheat kernels was the major contributor to the classification accuracy of the two-wavelength model using wavelengths of 505 nm and 560 nm. Kernel color measurement results showed that heat-damaged kernels had larger a^* and b^* values than undamaged kernels (Table IV). This indicates that heat-damaged kernels are darker and more yellow than undamaged

kernels. Heat damage causes comprehensive physical and chemical changes in wheat. The wavelengths used for the other two models may represent scattering effects and interactions of moisture, starch, protein, oil, and cellulose caused by heat-damage in the wheat.

Conclusions

The log (1/R) values of undamaged wheat kernels were higher than those of heat-damaged wheat kernels over the entire wavelength region. The main contributors to the spectral characteristics of heat-damaged kernels are light scattering and color that changes the log (1/R) values of the absorption spectrum. For PLS models, classification accuracy of 100% was obtained for both cross-validation and prediction from the NIR wavelength region of 750-1,700 nm. The visible wavelength region (400-750 nm) gave the lowest classification accuracy. For two-wavelength models, the average of correct classification for the calibration sample set was more than 97%. The average of correct classification for the testing sample set was about 96%. Although the classification accuracies of two-wavelength models were lower than those of the PLS models, the classification accuracy of about 97% may meet requirements for industry and grain inspection applications. Therefore, the simple two-wavelength model is recommended.

Results from this limited sample set and heat treatment suggest that NIR technology could be used to detect heat-damage in wheat. More research is needed with a larger number of samples and a wider range of heat treatments to confirm these results.

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Table 1. Composition of Wheat Samples (% db).

Wheat Class	Protein	Starch	Ash	Fiber
HWW	15.88	64.9	1.66	2.05
HRS	19.89	58.5	1.75	1.32

Table 3. Classification of Heat-Damaged and Undamaged Wheat kernels Using Two-Wavelength Models.

Model No.	Wavelengths (nm)	Calibration Results (%)			Testing Results (%)		
		Undamaged	Damaged	Average	Undamaged	Damaged	Average
1	505, 560	98.1	96.5	97.3	96.8	91.8	94.3
2	985, 1050	97.0	98.1	97.5	96.1	97.4	96.8
3	1550, 1575	96.2	98.3	97.3	94.1	99.3	96.7

Table 4. The Color Variations Between Heat-Damaged and Undamaged Wheat Kernels Measured as *L*, *a*, and *b* Values in the L*a*b Color Space.

Class	<i>L</i>	<i>a</i>	<i>b</i>
Hard red spring wheat			
Heat-damaged	61.14a	16.02a	25.09a
Undamaged	60.61a	12.96b	16.15b
Hard white wheat			
Heat-damaged	71.33a	14.96a	23.34a
Undamaged	68.53b	12.14b	19.07b
Heat-damaged	66.24a	15.91a	24.21a
Undamaged	64.56a	12.54b	17.61b

^a Values within the same column followed by different letters are significantly different at $P < 0.05$.

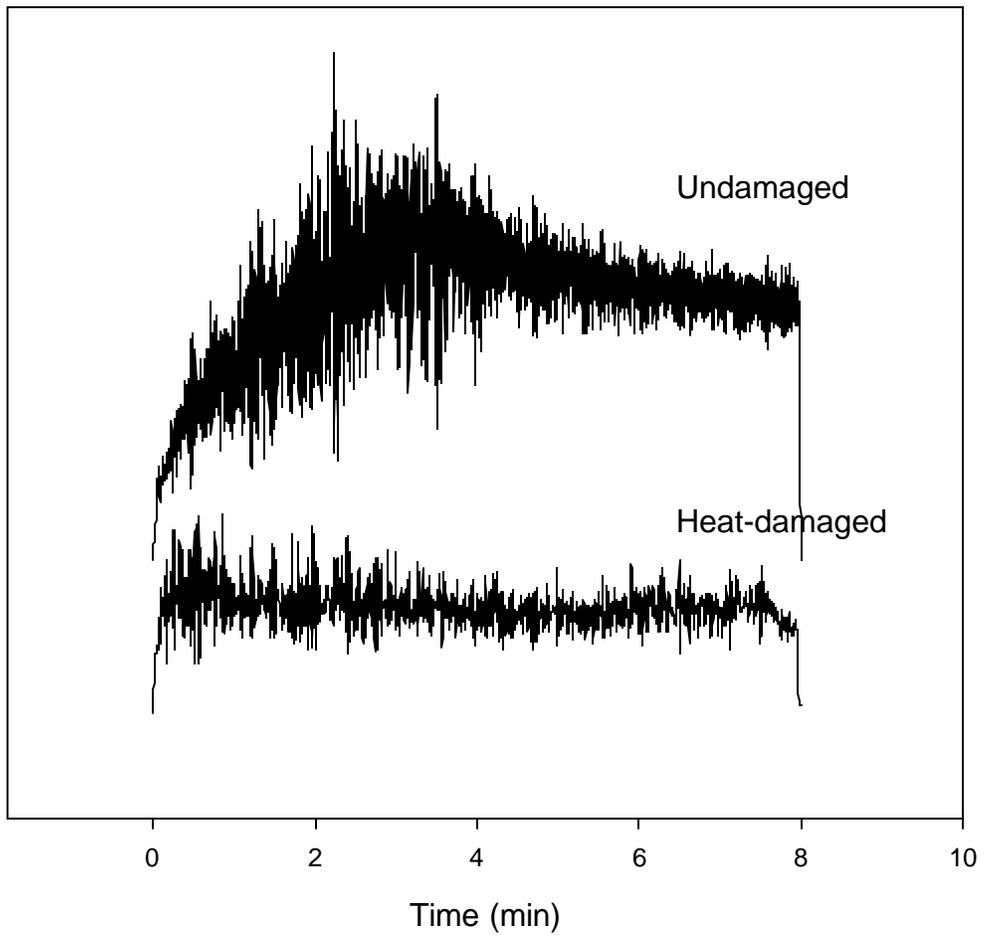


Figure 1. Mixograph curves for heat-damaged wheat (bottom) and undamaged wheat (top).

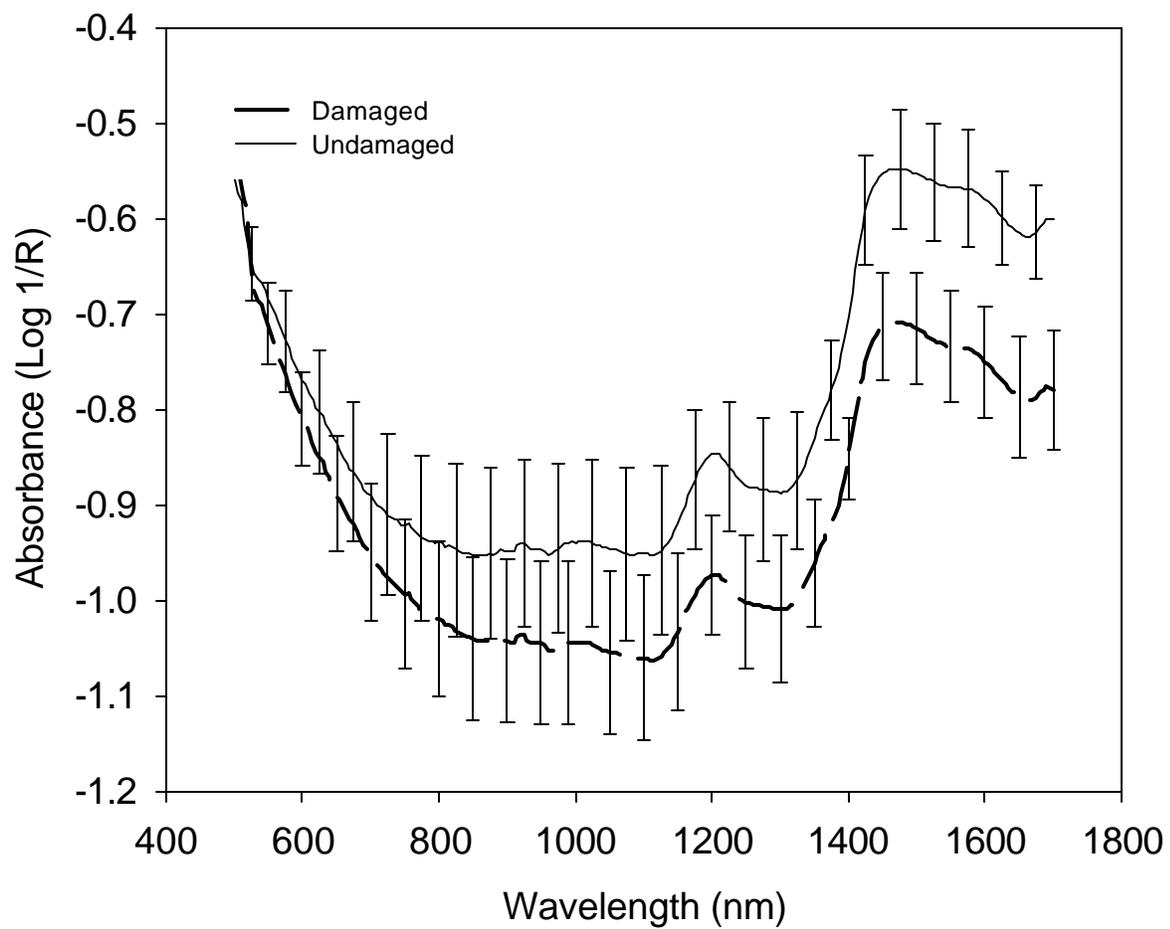


Figure 2. NIR absorption curves for heat-damaged and undamaged wheat. Vertical bar represents one standard deviation.