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Food Chemistry 87 (2004) 195–203
Food Chemistry
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# Estimation of HRW wheat heat damage by DSC, capillary zone electrophoresis, photoacoustic spectroscopy and rheometry ☆

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Received 9 May 2003; received in revised form 17 November 2003; accepted 17 November 2003

**Abstract**

The effect of wheat heat damage was estimated using hard red winter (HRW) wheat varieties grown in Oklahoma, and analyses were performed on wheat kernels, flour, and isolated starch. Wheat kernels were analyzed by photoacoustic spectroscopy (PAS), flour was analyzed by DSC, capillary zone electrophoresis (CZE) and rheometry, while starch was analyzed by DSC. DSC data showed little difference in the temperatures of starch gelatinization. The $\Delta H$ values increased at 50 °C storage temperature. PAS data revealed differences in sensitivities to heat between the three cultivars. The pericap of cultivar Jagger was about five times more heat resistant than the pericarp of the other two cultivars. CZE maps showed that gluten proteins from cultivar 2137 was more affected by the 60 days and 50 °C treatment than the other two cultivars. Non-linear steady shearing showed that all heat-treated flours had lower viscosity, which suggests a deleterious effect on the potential performance of yeasted baked products. Viscoelastic properties of the Jagger untreated suspension sample showed strong gluten. The linear and non-linear viscoelastic behaviours of Jagger cultivar were less altered by heat treatment than were the other cultivars.
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*Keywords:* Photoacoustic; Hard red winter wheat; DSC; Capillary zone electrophoresis; Glutenin

## 1. Introduction

Heat damage might occur during the artificial drying of newly harvested wheat or when storing conditions are allowed to reach high temperatures, either by faulty management or in areas with extreme environmental temperatures. Wheat proteins can be denatured by kernel heat damage during storage or during the wheat milling process (Hook, 1980; Jeanjean, Damidaux, & Feillet, 1980; McDermott, 1971). Protein denaturation limits the functional properties of the resulting flour. Gluten heat denaturation reduces the viscoelastic properties of flour dough and results in low bread quality (Becker & Sallans, 1956). Gluten aggregates and forms new intermolecular disulphide bonds, which changes dough rheology and gluten extractability as a result of heating (Booth et al., 1980). Wheat heat damage is a grading factor that affects the commercial value of the crop. A near infrared reflectance (NIR) spectroscopy procedure was developed to detect wheat heat damage (Wang, Dowell, & Chung, 2001). Heat-damaged and sound kernels were scanned ($\log(I/R)$) from 400 to 1700 nm to observe the difference between the spectra. The spectra of the heat-damaged kernels were characterized by light scattering. A number of methods were developed to test the effect of heat damage on wheat protein functionality or solubility (Lupino & Anon, 1988). Every (1987) reported a fast and simple procedure based on protein solubility (Protein Solubility Test). The procedure used 1 g of whole meal suspended in 2% NaCl solution and, within 4 min, the absorbance of the supernatant from the suspension was read at 595 nm.

The Grain Inspection, Packers, and Stockyards Administration (GIPSA), a USDA agency, is looking for

☆ Names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by the USDA implies no approval of the product to the exclusion of others that may also be suitable.

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0308-8146/$ - see front matter. Published by Elsevier Ltd.
doi:10.1016/j.foodchem.2003.11.014

objective, simple, and fast methods to inspect grains. Visually checking and selecting discoloured kernels is currently used to test wheat samples for heat damage. The objective of this work is to introduce one more method to evaluate wheat heat damage due to post-harvest drying or storage.

## 2. Materials and methods

### 2.1. Wheat samples preparation

Foundation seed of hard red winter wheat (*Triticum aestivum* L.) cultivars 2174, Jagger, and 2137, were obtained from Oklahoma Foundation Seed Stocks Inc., Stillwater, OK, harvested in 2001. The protein content was determined by near infrared spectroscopy using a Foss NIR System 6500 (NIR System, Silver Spring MD). Protein contents of 9.7%, 9.1%, and 10.2% (12% moisture basis) were obtained for 2174, Jagger, and 2137, respectively. One-kg samples were stored in covered glass jars at 30 and 50 °C for 30 and 60 days. The samples were gently shaken, once every week during storage, to ensure even temperature inside the grain mass. Wheat samples were milled into flour using a Quadrumat Senior Mill (W. Brabender Instruments, Inc., Hackensack, NJ). Starch was isolated according to Eliasson (1983). Briefly, the starch was removed from dough using three washings with distilled water and centrifuged (1000*g*, 20 min, 10 °C ). The protein layer on top of the starch was scraped off with a spatula. The starch was air-dried and ground to pass through a 70 mesh sieve. Protein content ($N \times 5.7$) in the isolated starch was determined by nitrogen combustion analysis, using a LECO CHN-2000 instrument (Leco Corp., St. Joseph MI). Isolated starch contained 2.3% protein, dry basis.

### 2.2. Differential scanning calorimetry

Moisture content of flour and isolated starch samples was adjusted to 65%. DSC pans were hermetically double-sealed in coated aluminium pans and allowed to equilibrate for 30 min. The DSC analysis was carried out on a TA Instrument 2920 – dual cell and single cell runs. Conditions were set at 10 °C/min from ambient to 110 °C with sensitivity set at 2 μW/s. The DSC was calibrated against an indium standard. During each run, nitrogen flow rate was 24 $(cm)^3$/min. The onset or peak temperatures and $\Delta H$ were calculated for each run.

### 2.3. Photoacoustic spectroscopy

Photoacoustic spectra were obtained using a Bio-Rad, Digilab Division (Cambridge, MA) FTS 6000 spectrometer equipped with a KBr crystal beamsplitter and an MTEC (Ames, IA) model 200 photoacoustic detector. The light source was a water-cooled ceramic mid-infrared globar, delivering 150 mW energy to the sample compartment. Digital signal processing (DSP) was embedded in Win-IR-Pro software provided by Bio-Rad. The spectrometer was operated under step-scan phase modulation with a step rate of 2.5 Hz at three phase modulation frequencies, 400, 200 and 100 Hz. For the wheat kernels in this study, these frequencies probed three sampling depths of approximately 9, 13 and 18 μm, respectively. The highest available phase modulation amplitude in the range (0.5–2.0 $\lambda_{He-Ne}$) at each frequency was selected.

Wheat kernels were dried under vacuum at room temperature for three days prior to PAS analysis to eliminate water bands interfering with the amide I band. The PAS sample cell was filled with 10–12 intact wheat kernels, depending on their size, and the space inside the cell containing the kernels was purged with helium to maximize the signal-to-noise ratio. PAS spectra were acquired from symmetric interferograms by correcting the phase rotation angle and ratioing the signals against a carbon black reference. Fourier transformation of the interferograms was by triangular apodization for maximum linear response, in a software-based DSP method, developed by Bio-Rad[1], generated in-phase (0°) and quadrature (90°) components. Depth specific phase angle spectra of the wheat kernels were computed by 3D interpolation as the root mean square values of the 0° and 90° components. Spectra of the polyester and protein carbonyl regions (1730 and 1640 $cm^{-1}$) for each wheat sample were computed at 8 $cm^{-1}$ resolution and signal-averaged over two scans. Spectra were baseline-corrected and normalized to the amide I peak to correct for small differences in sample weights. Typical depth-resolved PAS spectra of the untreated and treated wheat kernels are shown in Figs. 3 and 4.

### 2.4. Capillary zone electrophoresis

The high-molecular weight glutenin subunits (HMW-GS) were extracted using a modification of the previously described procedures (Lookhart & Bean, 1995; Marchylo, Kruger, & Hatcher, 1989; Osborn, 1907). Albumins and globulins were extracted from the flour by three consecutive treatments with deionized water (1 g flour/4 ml solvent), and vortexed for 5 min using a Genie 2 at a speed setting #4. After centrifugation (385*g*, 5 min, 4 °C), the pellet was treated two times with 50% *n*-propanol to extract the gliadins. Glutenins were extracted by constantly stirring the gliadin-free pellet with 50% *n*-propanol containing 1% (w/v) DTT (45 min, vortex Genie 2, speed #4) to reduce the glutenin disulphide bonds and by centrifugation (385*g*, 5 min, 4 °C). HMW glutenin subunits in the supernatant were precipitated by the addition of *n*-propanol containing 1%

DTT, to bring the final propanol concentration to 60% v/v. The mixture was vortexed for 60 min and stored at 4 °C for 72 h. The proteins were then collected by centrifugation (1951*g*, 5 min, 4 °C). After vacuum-drying and pulverizing, the HMW-GS were resolubilized in 50% *n*-propanol containing 1% DTT, and sonicated for 45 min before being analyzed by CZE.

The CE analyses were performed on a Beckman MDQ instrument (San Ramon, CA.). In CZE, the separation was carried out at an operating voltage of 15 kV on a 27 cm (20 cm to the detector × 50 μm I.D.) fused-silica capillary from Polymicro Technologies Inc. (Phoenix, AZ, USA), thermostatted at 45 °C. The running buffer was freshly prepared before use and consisted of 100 mM phosphate (pH 2.4) at 20% (v/v) acetonitrile, 0.4% (w/v) glycine and 0.05% (w/v) hydroxypropylmethylcellulose (HPMC), and the detection was performed in the UV at 200 nm.

### 2.5. Rheological test

Three hard red winter wheat flours, 2174, Jagger, and 2137, were used as control one (normal) and heat-damaged one (stored at 50 °C for 60 days). Flour samples, for the rheological experiments, were prepared by dispersing flours in deionized water or sodium phosphate buffer (pH 7.0 at 25 °C) at a concentration of 20% by weight.

Rheological properties of flour suspensions were measured using a Rheometric ARES strain-controlled fluid rheometer with a 50-mm cone-and-plate geometry. The angle of the cone was 0.04 radians. The sample chamber was enclosed in a humidity chamber to avoid evaporation of the solution. The temperature was controlled at 25 ± 0.1 °C in the experiment chamber, using a water circulation system. The linear dynamic rheological measurements were conducted according to the method described by Xu, Bietz, Felker, Carriere, and Wirtz (2001). The non-linear rheological steady shear experiments were conducted over a shear range of 0.006–1000 $s^{-1}$. Measurements of the shear viscosity were obtained throughout the course of the experiment.

## 3. Results and discussion

The DSC data showed slight change of the thermal properties of the flour after heat treatment, while isolated starch showed no change. Starch gelatinization is a phenomenon dependent on the amount of water available. Since the moisture content of the stored wheat kernels was relatively low (12–13%), the starch was not affected. The reason for selecting this moisture for our study was because it is the moisture commonly used for wheat storage in most parts of the world. In addition, this moisture content is proven to lower enzymatic activity, such as starch degrading enzymes and proteases. Treated Jagger samples showed similar onset and peak temperatures to the control, while the Δ*H* showed 30% increase, except for the 60 days 30 °C sample (Fig. 1). The 30 days 30 °C-treated 2174 showed no effect on the onset and peak temperatures, while the other treatments lowered these temperatures. The Δ*H* showed an increase at 30 days and decrease at 60 days. The effect of heat treatment on the proteins, as shown by the SDS–PAGE (Fig. 2) and CE data, may be the cause of water migration between the starch and the proteins and thus influenced the DSC Δ*H* values. Fig. 2 includes all three controls and some treated samples to avoid reporting a crowded figure. The CE and SDS–PAGE showed that the 2137 sample was the most affected by the heat treatment, where major changes were noticed in the protein structure. These changes may be responsible for reducing protein water absorption; as a result, more water was available for the starch and thus produced higher Δ*H* values (Fig. 1).

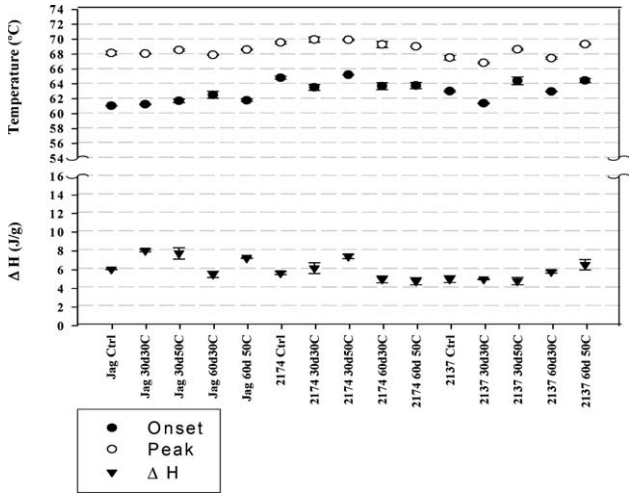


Fig. 1. Onset and peak temperatures or Δ*H* of 2137, 2174, and Jagger cultivars treated at 30 and 60 days or 30 and 50 °C.

The major difference between wheat kernels before and after storage was in the level of moisture, which decreased during storage due to water evaporation in the heat. Water vapour absorbance bands were significantly reduced, especially in spectra of the kernels that were heated to 50 °C for 60 days. This is as would be expected. Therefore, in order to examine effects of storage that were much less dominant than the water evaporation, kernels were dried under vacuum at room temperature prior to PAS analysis. After vacuum-drying, the water band was not noticed.

Intact wheat kernels were examined by PAS at three probing depths before and after storage. In order to characterize the changes in wheat kernels, following heat treatment in storage by PAS, we focussed on the distribution pattern of chemical components at the three depths (9, 13 and 18 μm) in the kernels near the surface

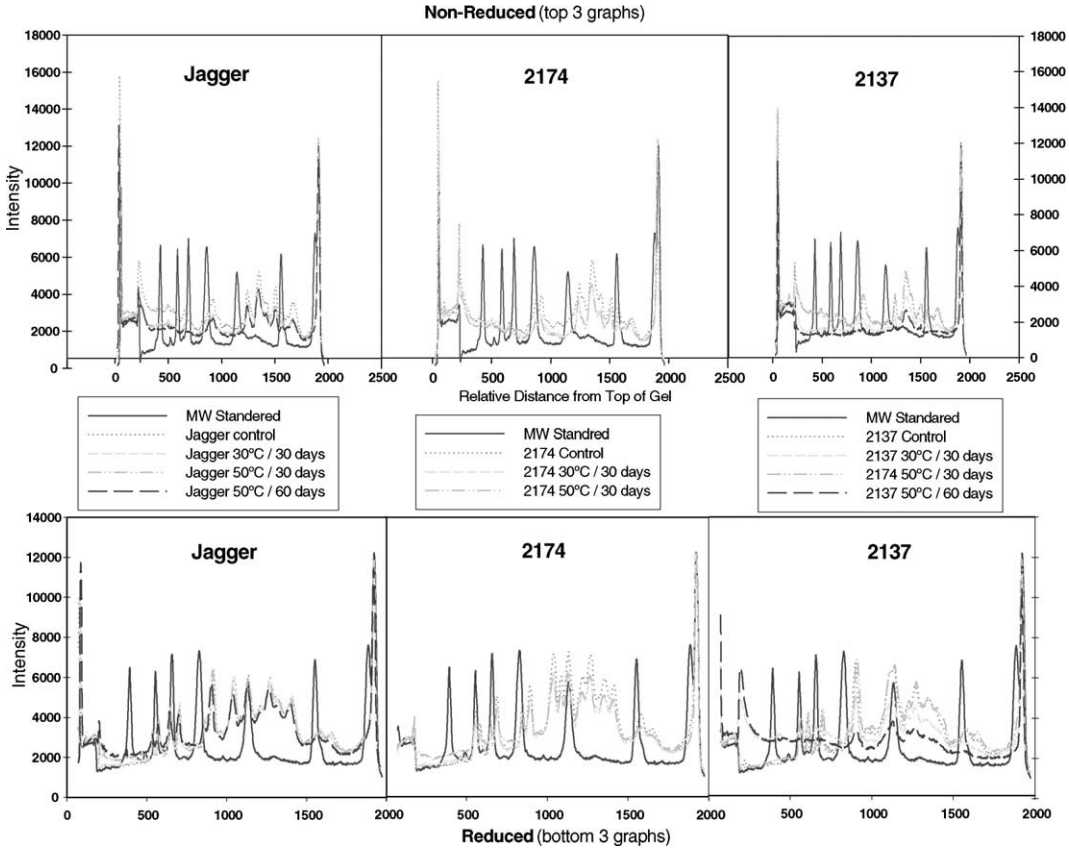


Fig. 2. SDS–PAGE of Jagger, 2174, and 2137 control and some treated samples.

of the pericarp (Drapcho, Curbelo, Jiang, Crocombe, & McCarthy, 1997). Depth profiling by PAS reveals how the distribution pattern changes as the probing depth of the PAS measurement increases from the surface to the interior of the kernel. Change in the chemical distribution pattern with depth may reflect change in the secondary structure of the proteins and polysaccharides and/or the morphology of the wheat kernel itself.

It is known that photoacoustic peak ratios of different peaks of a polymeric material may vary at different probing depths (Jones & McClelland, 1996). This photoacoustic effect was observed in the wheat kernels: the ester to amide I peak ratio (peak height at 1730 $cm^{-1}$ to peak height at 1640 $cm^{-1}$) in wheat kernels varied at different probing depths. This variance with depth was exploited to detect effects of heat in storage on the three cultivars of wheat studied in our work. Results of PAS analyses of the dried wheat kernels (cultivars Jagger, 2137; 2174), before and after storage at 30 °C for 60 days, are shown in Figs. 3–5, respectively. The spectra show small differences in the polyester and protein carbonyl (amide I) regions, which suggest that the kernels probably underwent morphological changes during heating and perhaps secondary structural changes but experienced few, if any, chemical changes such as ester or amide hydrolysis.

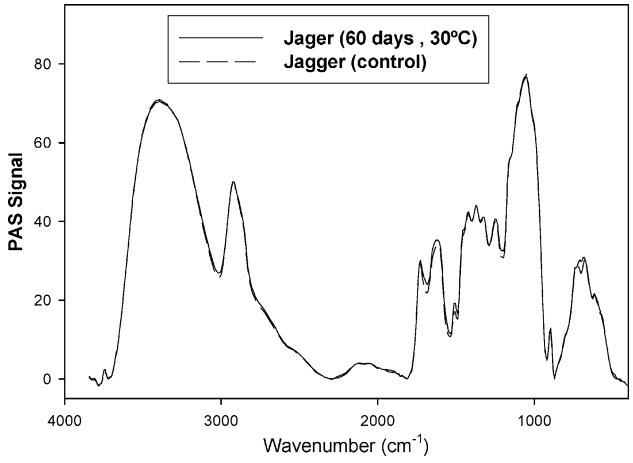


Fig. 3. Photoacoustic spectra of wheat 2000 over the entire infrared range. Kernels stored for 60 days at 30 °C (heavy trace). Unheated control kernels (– – –).

PAS depth-profiles indicate that a fairly consistent pattern of change occurred during heat-storage. Fig. 6 is a typical example of the difference spectra between kernels heated for 60 days and 30 °C or unheated wheat kernels. The difference across the spectral range 1750–1550 $cm^{-1}$ varies with the wavenumbers, as predicted by photoacoustic theory[2], but the variance in the heated

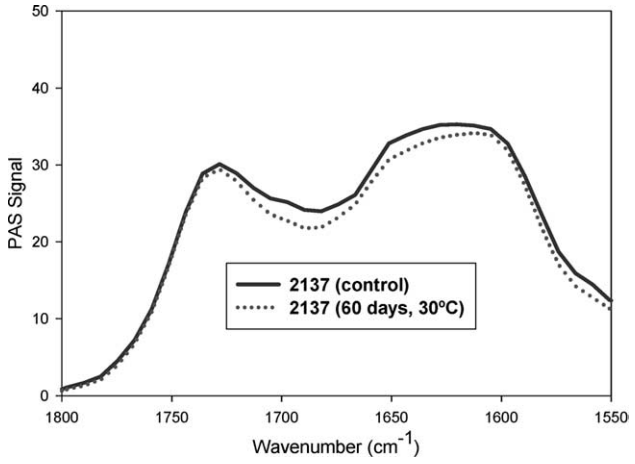


Fig. 4. Photoacoustic spectra of wheat 2137 over the 1750–1550 $cm^{-1}$ range. Kernels stored for 60 days at 30 °C (heavy trace). Unheated control kernels (light trace).

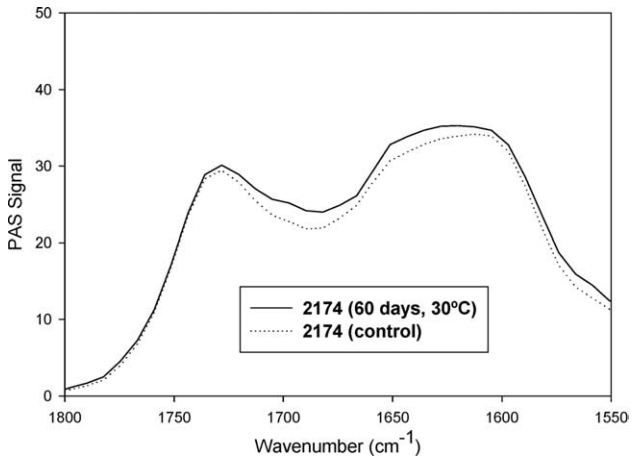


Fig. 5. Photoacoustic spectra of wheat 2174 over the 1750–1550 $cm^{-1}$ range. Kernels stored for 60 days at 30 °C (heavy trace). Unheated control kernels (···).


Fig. 6. Photoacoustic signal difference spectrum at 9 and 18 μm probing depths for Jagger cultivar over the 1750–1550 $cm^{-1}$ range. Difference between kernels stored for 60 days at 30 °C and unheated control kernels.

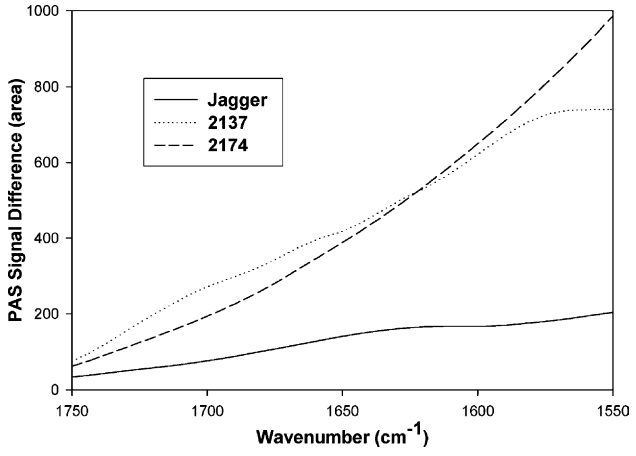


Fig. 7. Photoacoustic signal difference spectrum at 9 m probing depth into cultivars 2137 (···), 2174 (– – –), and Jagger (—) over the 1750–1550 $cm^{-1}$ wavenumber range. Differences in relative peak areas between kernels stored for 60 days at 50 °C.

kernels is significantly larger than in the unheated kernels. At probing depths of 9 and 18 μm into the kernels, the PAS signal differences in the ester and amide I peaks (1730 and 1640 $cm^{-1}$) are particularly large, as seen in Fig. 6. Similar PAS signal differences were measured at depths between 9 and 13 μm and between 13 and 18 μm. In theory, if the treated samples are unchanged from the control samples, these difference spectra in the photoacoustic depth profiles should be flat zero lines. Therefore, the difference spectra seen in this work are strong evidence of minor secondary structural changes and/or subtle morphological changes in the surfaces of the heated wheat kernels.

PAS depth-profiling of kernels of the three cultivars also revealed that the surface changes were generally smaller in Jagger than either 2137 or 2174, possibly indicating that the surface of the Jagger cultivar is less sensitive to heat-storage than the other two cultivars. Fig. 7 shows plots of the change in the PAS difference spectra between heat-treated (50 °C for 60 days) and untreated control kernels across the spectral range 1750–1550 $cm^{-1}$ for each of the three cultivars. The areas under the difference spectra estimate measures of the effects of heat storage. The total area under the difference spectra for cultivar Jagger is 174 (mean = −0.60, SD = 0.41), while the total areas for cultivar 2137 and cultivar 2174 are 693 (mean = −2.04, SD = 1.60) and 1002 (mean = 3.71, SD = 2.27), respectively, which indicates that the pericarp of the Jagger cultivar is possibly 4–5 times more resistant to heat than the pericarps of the other two cultivars. This result awaits statistical verification by planned tests of larger data sets using partial least squares and stepwise discriminant analysis. However, this preliminary result is in accord with

expectation that the different cultivars will show different sensitivities to heat due to structural variation expressed in genetic phenotypes. Pericarps in the different cultivars likely have different resistances to heat-induced changes, such as stress cracks, protein denaturation and hemicellulose structural change. Light scattering effects of these surface changes are readily detected by photoacoustic spectroscopy (Gordon, Schudy, Wheeler, Wicklow, & Green, 1997).

The CE data up to 20 min and the 50 °C/60 days are included in the discussion because the major changes took place around that time and temperature/days. The profiles of glutenin and gliadin extracts from the control (25 °C) and the stored wheat at 50 °C showed overall differences in intensity and resolution of peaks. The extracts were loaded in the cathode and those proteins with more negatively charged surface to mass ratios migrated first. CE is a powerful tool that has been used to detect small changes in charges. As the prolamin proteins were modified as a result of storage temperature and time, small changes in the charge to mass ratio of the polypeptide species in the extract cause change in migration time. This suggests that gliadin and glutenin fractions of the three cultivars have different susceptibilities to heat storage.

Hydrophilic gliadin peaks of the cv. 2137, treated at 50 °C, eluting between 3 and 5 min, were unchanged, while a relatively hydrophilic peak, eluting at 12.2–12.4 min, increased by 235% in area (Fig. 8(a)). The 2174 cv. early hydrophilic and less hydrophobic peaks, eluting up to 10 and 10–20 min, respectively, displayed no overall changes in pattern separation or peak area at 50 °C (Fig. 9(a)). The relatively more hydrophobic gliadin peaks, eluting between 10 and 20 min, showed peaks with reduced area of up to 100 (Fig. 9(a)). Jagger showed no change in gliadin peaks (Fig. 10(a)).

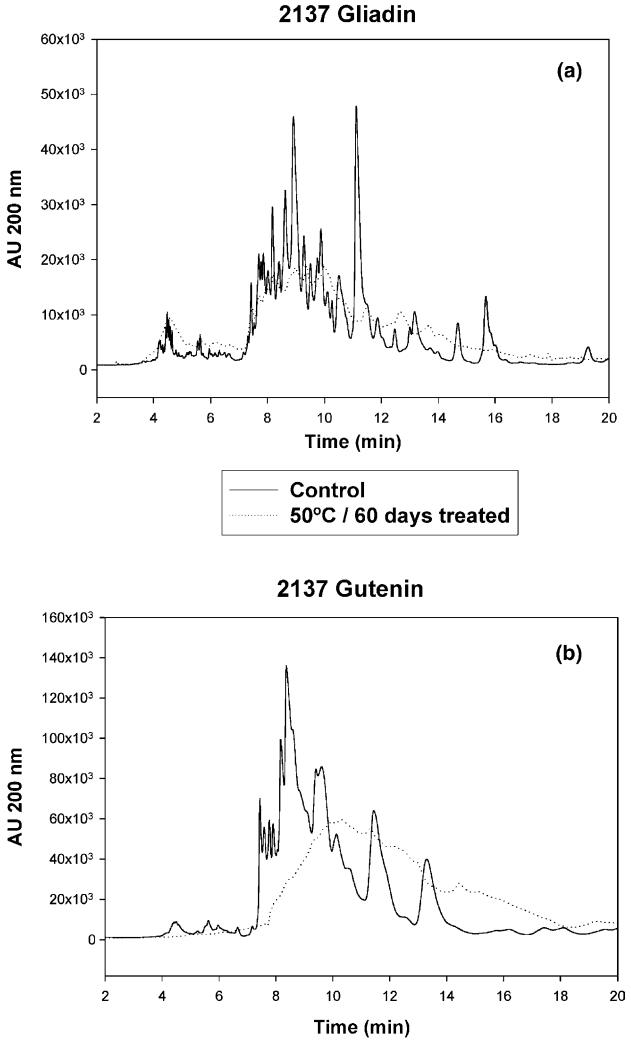


Fig. 8. CE profile of 2137 cultivars, control and treated at 50 °C for 60 days: (a) gliadins, (b) glutenins.

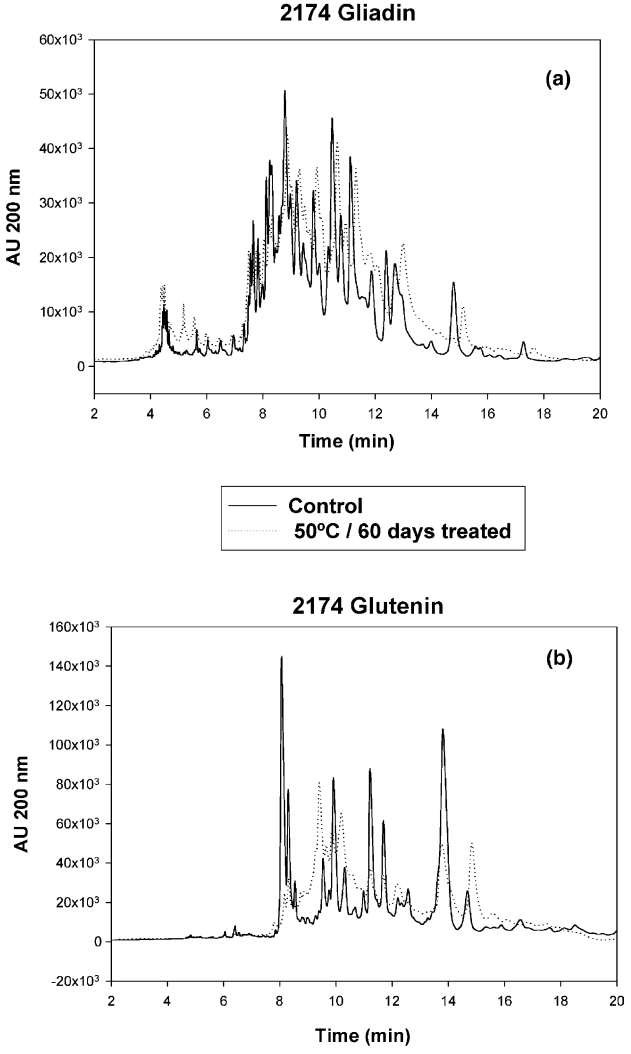


Fig. 9. CE profile of 2174 cultivars, control and treated at 50 °C for 60 days: (a) gliadins, (b) glutenins.

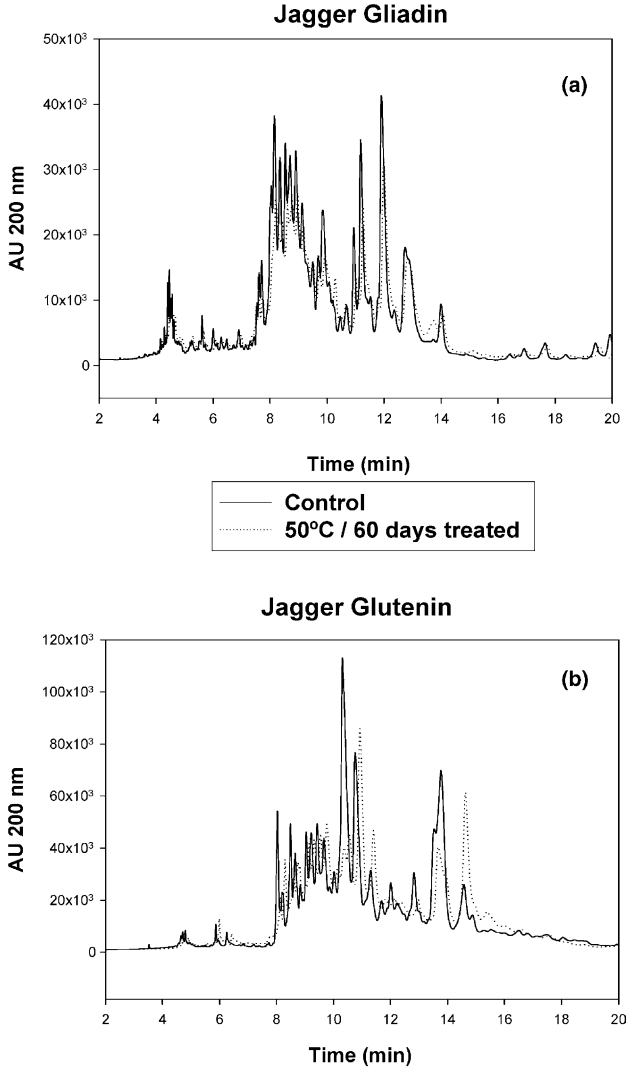


Fig. 10. CE profile of Jagger cultivars, control and treated at 50 °C for 60 days: (a) gliadins, (b) glutenins.

The CE data showed that all three cultivars went through major changes in the glutenin peaks, especially in the medium and more hydrophobic peaks. Major changes were observed with the 2137 cultivar stored at 50 °C, where peaks were reduced or disappeared around the medium and more hydrophobic protein between 8 and 20 min (Fig. 8(b)). Cultivar 2174 hydrophobic peak areas (10–20 min elution) were decreased. Four glutenin peaks, 10.9, 12.4, 14.9, and 16 min, were not present in the 2174 cultivar treated at 50 °C (Fig. 9(b)). Jagger cv. showed major changes, where peaks intensities were reduced, the less hydrophilic peak eluting at 8–20 min (Fig. 10(b)). Heat treatment generated reductions of both glutenin and gliadin patterns, as recorded by CE. The 2137 cv. showed more reduction than 2174 or Jagger. The SDS–PAGE profiles of all three cultivars supported the CE results; 2137 was the most effected by heat and Jagger the least affected.

Based on the data from PAS and CE, only 50 °C and 60 days treatment was selected for rheological analysis. The three hard red winter wheat flours, 2174, Jagger, and 2137, were tested using 20% suspension samples. Rheological measurements made showed no differences at all when suspensions were prepared in de-ionized water or sodium phosphate buffer. This indicated that

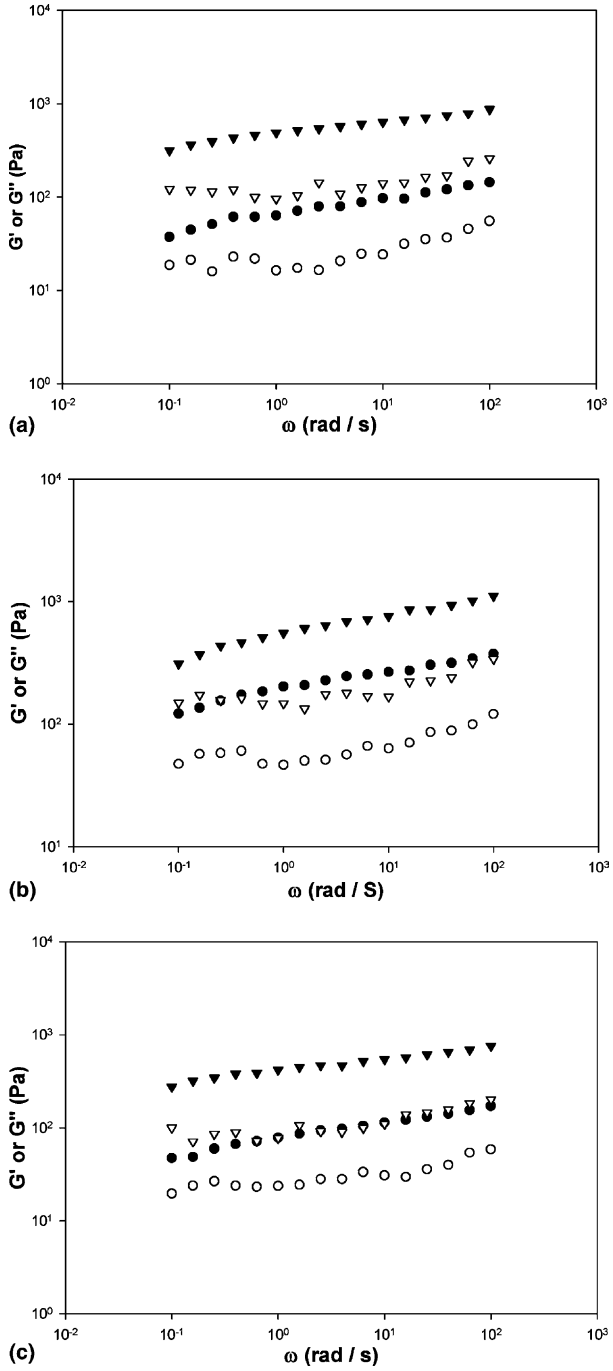


Fig. 11. Linear dynamic frequency-dependence moduli of non-damaged (circled symbol) and heat-damaged (triangle symbol) flour suspensions; (a) for flour sample 2174, (b) for flour sample Jagger, and (c) for flour sample 2137. Suspension concentration was 20%. Filled symbol $G'$, opened symbol $G''$.

these flours were stable under mild conditions, regardless of heat treatment. The linear dynamic frequency sweep results are shown in Fig. 11(a)–(c). The elasticity ($G'$) and loss moduli ($G''$) of the three suspensions from untreated wheat flours exhibited strong viscoelastic solid behaviour. The plateaux of $G'$ were 80, 200, and 100 Pa for untreated 2174, Jagger, and 2137, respectively. However, the $G'$ and $G''$ of all three cultivar flour suspensions, treated at 50 °C and 60 days, increased 2.5–8.5 times. But linear ranges were extremely small for all three damaged flour suspensions, namely less than 0.1% (data not shown). The bread making process is usually done under much larger shearing than this linear range. So, we also measured the non-linear rheological properties over a shear rate range of 0.006–1000 $s^{-1}$ (Fig. 12). With the shear rate increase, both untreated and heat-treated flours exhibited shear thinning behaviour. However, all three treated flours had lower viscosities than untreated flours at 1–100 $s^{-1}$, which is the actual mixing shear rate range for breadmaking (Bloksma, 1988). Apparently, the quality of flours stored at 50 °C for 60 days had indeed been negatively altered. The CE data provided evidence that heat treatment damaged the gluten. It is also possible that heat treatment affected the starch in a way beyond the limit of detection by DSC. Heating at the temperature levels used in this work can cause conformational changes of proteins, as shown by the data presented here. These conformational changes will result in the "gel" behaviour shown in Fig. 11. However, this "gel" behaviour was only exhibited at a shear strain of less than 0.1%, which is very small. Non-linear steady shearing measurements showed that all three heat-damaged flours had lower viscosities than did untreated flours at 1–100 $s^{-1}$, which indicated that the breadmaking quality of the heat-damaged flours would be worse than the untreated flours. Among the three untreated flours, the linear elasticity ($G'$) of Jagger was about twice higher than those of flours 2174 and 2137, meaning that Jagger had better protein quality since their protein contents were similar. The heat-treated Jagger flour was less altered at both linear and non-linear viscoelastic behaviours than the two cultivars (Figs. 11 and 12). This result suggested that Jagger has better protein quality, or a heat resistant pericarp, as shown by PAS analysis. Pericarp heat damage could cause lower quality flour with more fibre content. Higher fibre content in wheat flour will increase water absorption, thus changing the physicochemical properties of the dough. Heat damage may also cause wheat proteins to become insoluble by way of aggregation. That was shown clearly to be the case from the CZE data. The loss of gluten protein extensibility, as a result of heat damage, limits gluten water absorption, and the plasticizing effect of water is limited, thus changing the rheological properties.

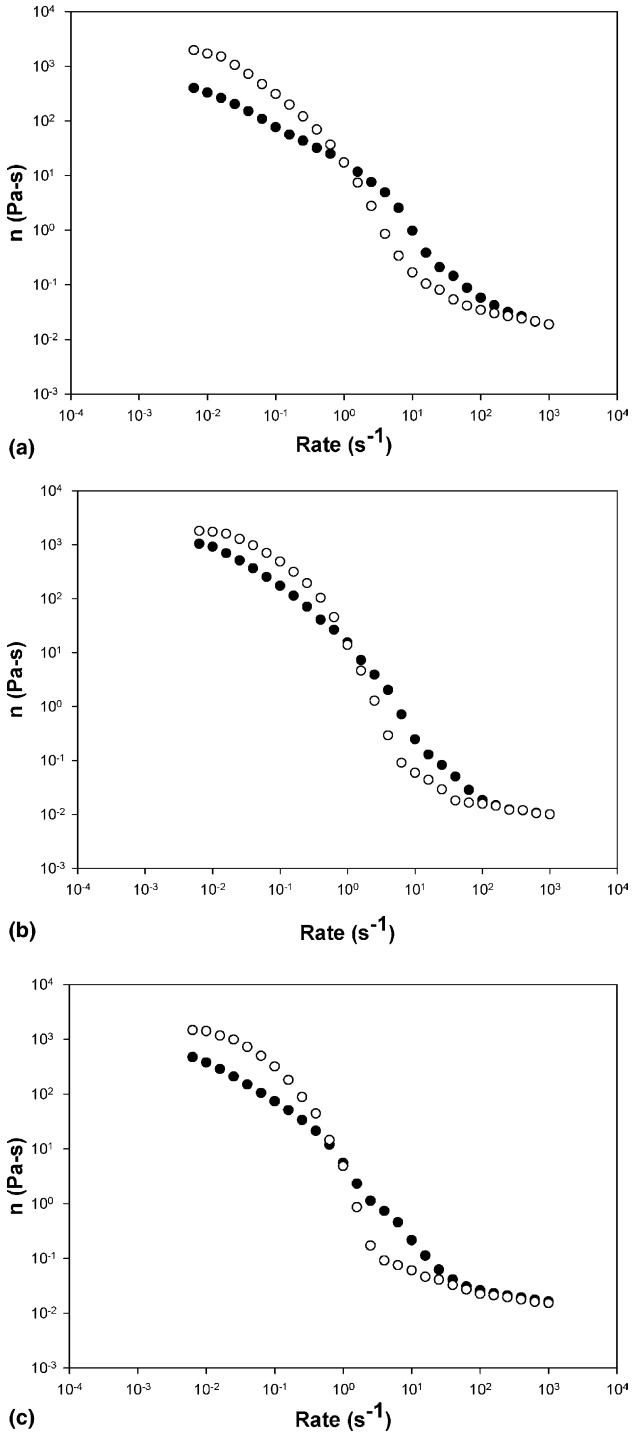


Fig. 12. Non-linear steady shear viscosity of non-damaged (filled circle) and heat-damaged (opened circle) flour suspensions; (a) for flour sample 2174, (b) for flour sample Jagger, and (c) for flour sample 2137. Suspension concentration was 20%.

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