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Analysis of Hard-to-Cook Red and Black Common Beans Using Fourier Transform Infrared Spectroscopy

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Extracted fractions from black and red common beans (*Phaseolus vulgaris*) were studied using Fourier transform infrared spectroscopy (FT-IR). Beans were stored under three conditions: control at 4 °C; hard-to-cook (HTC) at 29 °C, 65% RH for 3.5 months; and refrigerated at 2 °C, 79% RH for 3.5 months after a HTC period (called HTC-refrigerated). Two fractions isolated from the beans, the soluble pectin fraction (SPF) and the water insoluble residue of the cell wall (WIRCW), were analyzed using diffuse reflectance (DRIFTS) FT-IR. The soaking water and cooking water from the beans were also studied using attenuated total reflectance (ATR) FT-IR. The DRIFTS FT-IR results from the SPF and WIRCW fractions were consistent with previously published data for Carioca beans showing that in general, more phenolic compounds were associated with the SPF of HTC beans than in the control beans. Results also showed that HTC-refrigerated beans had higher concentrations of phenolic compounds than control beans in the SPF. The ATR FT-IR results for soaking and cooking waters from the HTC-refrigerated and HTC beans had higher concentrations of absorbing compounds than the control beans, indicating that they lost more constituents to the water. Additionally, results indicate that the mechanism(s) for reversibility of the HTC defect could be different than the one(s) involved in the development of the defect.

KEYWORDS: Hard-to-cook; common beans; FT-IR; phenolics; bean extraction; hard-to-cook defect reversibility

INTRODUCTION

Common beans (*Phaseolus vulgaris*) stored under the adverse conditions of high temperature and high humidity may develop a hardening defect characterized by extended cooking times for cotyledon softening (*1*−*3*). Beans that have undergone this HTC phenomenon require increased energy (fuel) cost for preparation; are less acceptable to the consumer due to changes in flavor, color, and texture; and have decreased nutritive quality (*4*, *5*). Several hypotheses have been proposed to explain the cause of bean hardening: lipid oxidation and/or polymerization, formation of insoluble pectates, lignification of middle lamella, and multiple mechanisms. However, most researchers do agree that the defect develops mainly in the cotyledons (*6*).

While some researchers believe that the HTC phenomenon is irreversible (*7*), a study by Hentges et al. (*8*) showed that the cooking time of dry beans and cowpeas that had developed the HTC defect decreased continuously with time when stored under refrigerated conditions (6.5 °C, 71% RH) after the HTC defect developed. An understanding of mechanisms leading to reversibility of the HTC phenomenon would provide insight into the development of the defect and would aid in the search for appropriate methods to prevent it (*6*).

Recent advancements in technology have allowed for closer examination of the possible causes of the HTC defect. Garcia et al. (*9*) were the first to use FT-IR to study the role of phenolic compounds and pectates in the development of the HTC phenomenon in Carioca beans. Their study found that the HTC beans had a greater amount of pectates and three times more phenolics in the soluble pectic fraction. They concluded that the presence of more ferulic acid bound to soluble pectin in the HTC beans may cause changes in cell adherence, thereby inhibiting cell separation when the beans are cooked. This study supported previous work by Srisuma et al. (*10*), which showed that hydroxycinnamic acids (especially ferulic acid), associated with hardening, increased in HTC beans as compared to the control beans.

In the Garcia et al. (*9*) study, FT-IR was performed only on the solid (freeze-dried) fractions of the bean extractions, including the SPF and WIRCW. However, advancements in FT-IR instrumentation allow investigations of both liquid and solid fractions of the extractions and therefore might provide more information to explain the HTC phenomenon. Also of interest is whether different varieties of common beans (i.e., black and red beans) show the same structural trends as those reported by

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Table 1. Comparisons of 50% Cook Times of Control, HTC, and HTC-Refrigerated Beans[a]

	bean type	50% cook time (min)
red beans	control	23
	HTC	56
	HTC-refrigerated	38
black beans	control	29
	HTC	70
	HTC-refrigerated	42

[a] A 50% cook time is the average time it takes the 12th and 13th probes of the Mattson type bean cooker to fall through their respective beans.

Garcia et al. (*9*) for Carioca beans. The mechanism of reversibility of the HTC defect has also not been previously studied using FT-IR. By subjecting HTC beans to refrigeration temperatures and analyzing them using FT-IR, structural changes that occur during HTC defect reversal could be evaluated. Finally, analysis of the soaking and cooking waters from the beans has not previously been done but would be appropriate to observe the differences in the types of solids lost to the water during these processes.

The objectives of this research project were to use FT-IR in combination with multivariate analysis techniques to (i) study the solid fractions of extracted phenolic compounds of regular, HTC, and HTC-refrigerated black and red beans and (ii) analyze the soaking and cooking waters of regular, HTC, and HTC-refrigerated black and red beans.

MATERIALS AND METHODS

Beans. Dr. George Hosfield, in the Department of Crop and Soil Science at Michigan State University, East Lansing, MI, provided the common black beans (variety T-39). Red beans (variety Rojo Chiquito) were obtained from Central Bean Company, Quincy, WA. Both varieties were used for the FT-IR studies.

Storage Conditions of the Beans. Control black and red beans were stored in a chamber at 4 °C and 73% RH for the duration of the project.

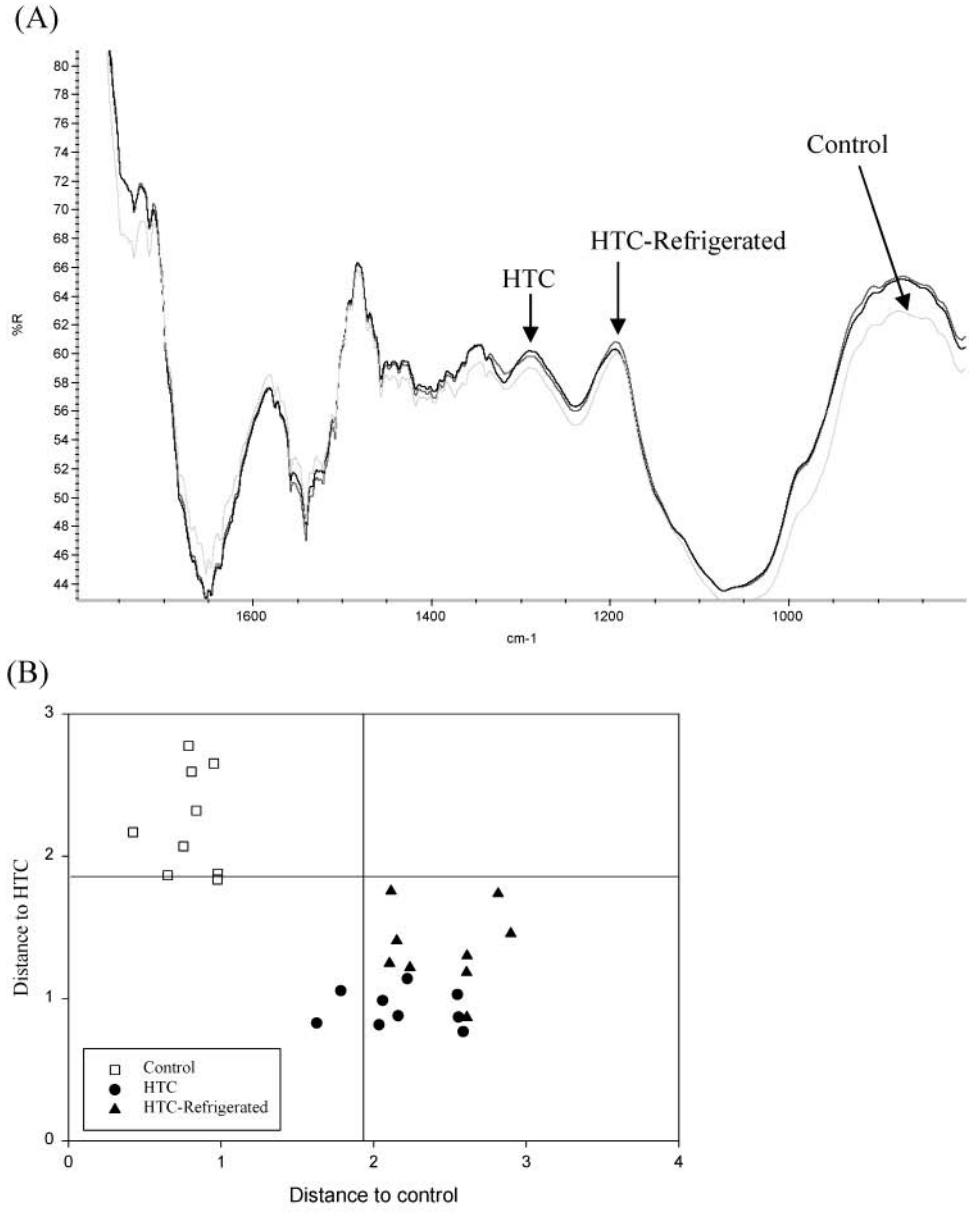

Figure 1. FT-IR DRIFTS results for the SPFs for extracted red bean flours. (**A**) Spectrograph of region used for DA. (**B**) Cooman plot obtained from DA results (1768–714 cm^{-1}). Legend: light gray = control; dark gray = HTC-refrigerated; and black = HTC. HTC-refrigerated = HTC beans stored at refrigeration temperatures for 3.5 months. Horizontal and vertical lines across Cooman plots indicate 95% confidence intervals.

Table 2. FT-IR Peak Areas (Uncorrected[a]) of SPFs and WIRCW Fractions for Extracted Red and Black Bean Flours[b−e]

sample type		region		
		1708–1581 cm^{-1}	1579–1483 cm^{-1}	1192–902 cm^{-1}
red SPF	control	6087.08[a] (282.30)	4281.03[a] (189.01)	14 601.70[a] (781.29)
	HTC	7391.66[b] (20.08)	5148.50[b] (197.33)	16 824.48[b] (511.33)
	HTC-refrigerated	7235.40[b] (297.03)	5065.84[b] (208.40)	16 395.36[ab] (1198.97)
black SPF	control	9149.44[a] (495.40)	6706.20[a] (239.40)	21 106.92[a] (475.71)
	HTC	9456.10[a] (134.60)	6934.26[ab] (55.42)	21 097.06[a] (118.71)
	HTC-refrigerated	9782.02[a] (191.67)	7203.12[b] (114.12)	21 622.70[a] (258.52)
red WIRCW	control	5426.96[a] (339.22)	3670.76[a] (326.66)	14 539.26[a] (693.39)
	HTC	5345.02[a] (522.39)	3745.85[a] (377.21)	13 781.64[a] (904.28)
	HTC-refrigerated	5875.60[a] (316.64)	4090.50[a] (229.82)	14 938.66[a] (703.32)
black WIRCW	control	8557.70[a] (227.08)	6144.53[a] (124.53)	20 014.32[a] (837.72)
	HTC	8286.00[a] (284.68)	5739.89[b] (69.71)	19 575.49[a] (1150.21)
	HTC-refrigerated	8296.26[a] (365.98)	5900.60[ab] (100.60)	18 901.93[a] (834.68)

[a] Uncorrected peak areas were calculated by choosing the area of interest in the spectra but no baseline correction. [b] The region 1708–1581 cm^{-1} corresponds to the phenolic compounds region, 1579–1483 cm^{-1} to the amide region, and 1192–902 cm^{-1} to the pectate region. [c] For every region in each of the fractions, areas with different letters are significantly different from each other. [d] HTC-refrigerated = HTC beans stored at refrigeration temperatures for 3.5 months. [e] Standard deviations shown in parentheses. [a−c]Data in the same column with different superscripts are significantly different ($p < 0.05$). Data were not compared across columns.

HTC black and red beans were produced by storing beans at 29 °C and 65% RH for 3.5 months. In addition to control and HTC samples, black and red beans that had become HTC were then placed in refrigerated chambers at 2 °C and 79% RH for 3.5 months to determine if the HTC defect could be reversed under these conditions. These beans were called HTC-refrigerated.

Cooking Trials. Beans were soaked for 15 h prior to cooking in a Mattson type bean cooker following the method described by Hentges et al. (*8*). This cooker had 25 probes, each weighing 105 ± 1 g, which were placed on 25 beans. The 50% cook time was determined as the average of the times that it took for the 12th and 13th probes to go through their respective beans (shown in **Table 1**).

Chemicals. Heat stable α-amylase (A-3306), protease (P-3910), and amyloglucosidase (A-9913) were purchased from Sigma Chemicals Co. (St. Louis, MO). KBr powder packets were obtained from Thermo SpectraTech, Inc. (Madison, WI).

Extraction of Phenolics. All of the bean treatments except the control T-39 black beans were soaked in 4 °C distilled water (60 min) and then dehulled manually. Samples were then finely ground using a 0.5 mm screen (Tecator Cyclotec, 1093 Sample Mill, Tecator, Hoganics, Sweden). The following extraction procedure adapted from Garcia et al. (*9*) and Srisuma et al. (*10*) was used to obtain the SPF and WIRCW fractions used in the FT-IR analyses. (i) Dehulled bean samples were obtained and then were ground. (ii) Bean cotyledon (3 g) was extracted three times with 25 mL of hexane. (iii) The hexanes-extracted cotyledon residues (R_1) then were extracted with 25 mL of absolute methanol (3×) followed by 25 mL of 50% methanol (3×). After each of the extractions, the mixture was centrifuged at 8000*g* for 10 min. (iv) The residue (R_2) left after the last extraction with 50% methanol was allowed to dry overnight. (v) After it was dried, the residue (six beakers containing 500 mg of each sample) was mixed with 25 mL of sodium phosphate buffer, 0.08 M, pH 6.0. (vi) Then, the residue was treated with a thermoresistant α-amylase (0.1 mL of enzyme to 500 mg of dry sample) at pH 6.0 for 30 min in a boiling water bath. The pH was adjusted using 0.325 M HCl and 0.275 M NaOH. (vii) After it was cooled, the pH was adjusted to 7.5, followed by treatment with a protease (at a ratio of 1:100, w/w, or 0.1 mL of a solution containing 50 mg of protease/mL of 0.08 M, pH 6.0, sodium phosphate buffer) for 1 h at 60 °C (while stirring). (viii) The pH was readjusted to 3.0, and then, the enzyme amyloglycosidase (0.3 mL of enzyme to 500 mg of dry sample) was added, and the mixture was incubated for 1 h at 60 °C while stirring. (ix) The suspension was allowed to cool and centrifuged (8000*g* for 10 min), and the supernatant was added to 4 volumes of 95% ethanol. After 12 h at 0 °C, a pectin fraction (SPF) precipitated. (x) The SPF was rinsed three times with 10 mL of 80% ethanol, suspended in water, and freeze-dried in a VirTis Freezemobile 5SL freeze-drier (Gardiner, NY) for storage. (xi) The residue of enzymatic treatments was rinsed three times with 10 mL of H_2O, then twice with each of the following: 10 mL of 0.5 M sodium phosphate buffer, pH 7.2; 10 mL of deionized water; 10 mL of a 1:1 mixture of chloroform and methanol (45 °C for 30 min), 10 mL of methanol, and 10 mL of water (the mixture was centrifuged at 8000*g* for 10 min after each of the extractions and rinses). (xii) The remaining residue was added to 10 mL of 90% DMSO and sonicated for 20 min and then centrifuged (8000*g*, 10 min), added again to 10 mL of 90% DMSO and sonicated for another 20 min, centrifuged (8000*g*, 10 min), rinsed twice with 10 mL of DMSO and then 5× with 10 mL of H_2O, centrifuged, and resuspended in deionized water and freeze-dried. This fraction was called the WIRCW.

FT-IR Procedures. The two fractions isolated (SPF and WIRCW) as well as soaking and cooking waters were analyzed using FT-IR methods. A ThermoNicolet Nexus 670 FT-IR spectrometer (ThermoNicolet Analytical Instruments, Madison, WI) equipped with a liquid nitrogen-cooled mercury cadmium telluride A detector and KBr optics was used to obtain the spectra. To obtain the measurements, a total of 128 scans at 4 cm^{-1} resolution were averaged for both DRIFTS and ATR methods.

Solid Fraction (SPF and WIRCW) Analysis Using DRIFTS FT-IR. The DRIFTS accessory of the FT-IR instrument was used to analyze the freeze-dried samples of the SPF and WIRCW fractions for all six bean types (control, HTC, and HTC-refrigerated black and red beans). Each dried fraction (25 mg) was mixed with 475 mg of KBr powder, the mixture was placed in the DRIFTS sampling cup, and spectra were collected. KBr was also used to collect the background spectra.

Soaking and Cooking Water Analysis Using ATR FT-IR. The soaking and cooking waters from the control, HTC, and HTC-refrigerated black and red beans were analyzed using a multibounce ZnSe−ATR method. Soaking water was obtained by soaking the beans in deionized distilled water in a 3:1 ratio at room temperature for 15 h prior to sample analysis. Cooking water was prepared by cooking beans to their 50% cook time in fresh deionized distilled water (1:1 ratio) in a 250 mL Erlenmeyer flask covered with aluminum foil. The cooled, filtered cooking water was analyzed immediately using the multibounce ZnSe-ATR accessory of the FT-IR.

Statistical Analysis. All measurements were performed in triplicate. The infrared spectra were analyzed and classified by the classical multivariate procedure of discriminant analysis (DA) using TQ Analyst software (ThermoNicolet). Cooman plots were created using DA results following general statistical procedures described by Rencher (*11*). Regions where significant differences were observed in the spectra were chosen for the statistical analysis. Models for the DRIFTS measurements of the SPF and WIRCW fractions were constructed using the 1768−714 cm^{-1} region with no baseline correction. Models for the bean soaking waters were constructed using the regions 1450−1365 and 1180−1010 cm^{-1}, while the regions of 1450−1365 and 1180−975 cm^{-1} were used to construct the models for the bean cooking waters.

Analysis of variance was performed on the peak areas using the general linear models procedure of the Statistical Analysis System

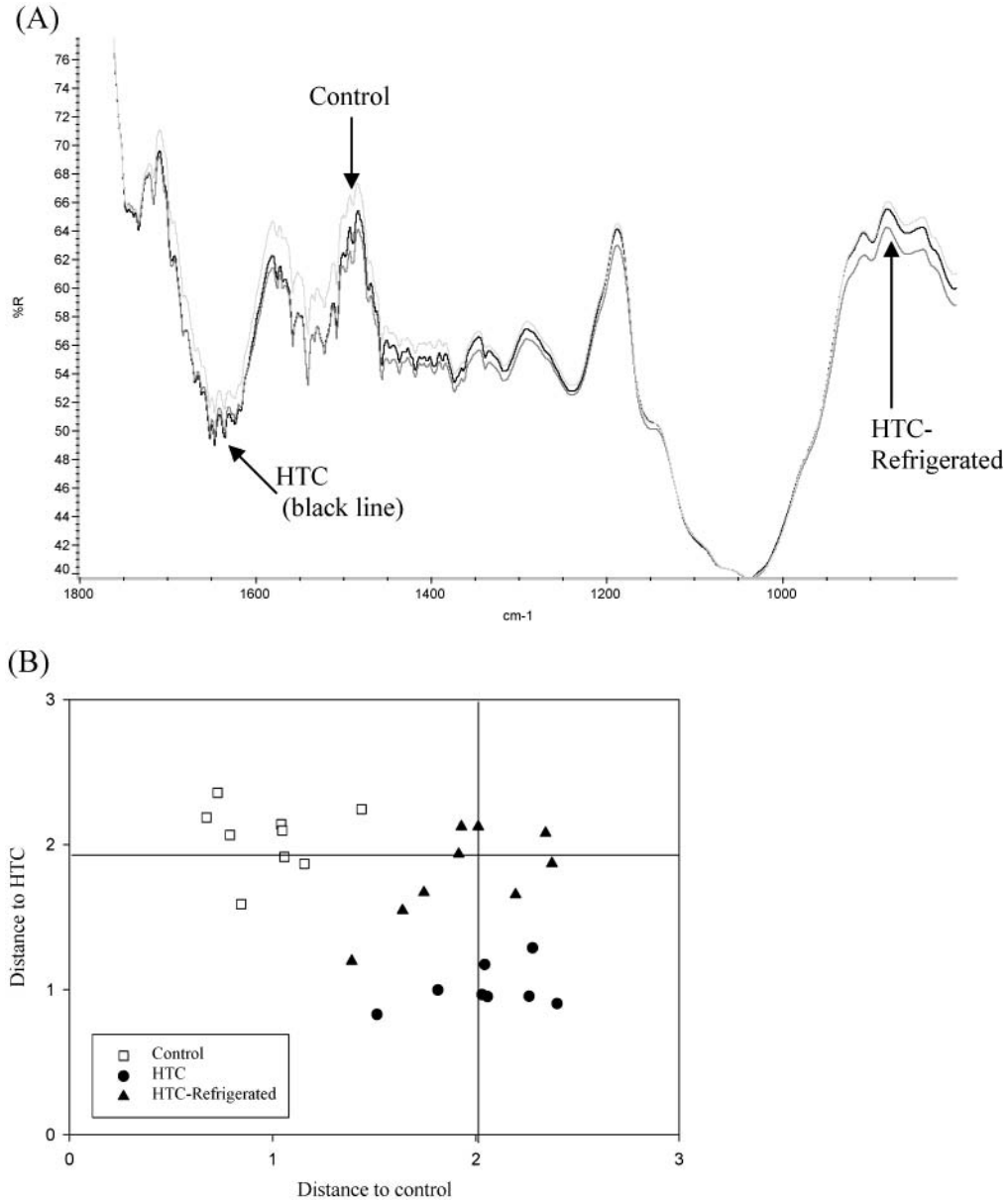

Figure 2. FT-IR DRIFTS results for the WIRCW fraction of extracted red bean flours. (**A**) Spectrograph of region used for DA. (**B**) Cooman plot obtained from DA results (1768–714 cm^{-1}). Legend: light gray = control; dark gray = HTC-refrigerated; and black = HTC. HTC-refrigerated = HTC beans stored at refrigeration temperatures for 3.5 months. Horizontal and vertical lines across Cooman plot indicate 95% confidence intervals.

(SAS). Tukey groupings in SAS were used to compare the peak areas of the control, HTC, and HTC-refrigerated beans for the various regions of interest.

RESULTS AND DISCUSSION

Cooking Times for Control, HTC, and HTC-Refrigerated Beans. The 50% cook times of the HTC red and black beans were 2.45 and 2.41 times greater than for the control red and black beans, respectively, as shown in **Table 1**. The results from the cooking tests of the HTC-refrigerated beans (**Table 1**) showed that the red HTC-refrigerated beans took 1.64 times longer to cook as compared to the red bean control, and the black HTC-refrigerated beans took 1.45 times longer to cook as compared to the black bean control. Therefore, the 50% cook time of the HTC-refrigerated beans was between that of the HTC beans and the control, indicating the reversibility of the HTC defect as shown previously by Hentges et al. (*8*).

Analysis of SPF and WIRCW Fractions for Control and HTC Beans. For comparison purposes, it is important to note that in the Garcia et al. (*9*) study, the HTC beans were stored for longer times than in the current study; therefore, it is reasonable to expect that the results obtained in this study were not as pronounced as the ones obtained in the Garcia et al. (*9*) work. HPLC analysis of phenolic compounds bound to soluble pectin (SPF) and to the cell wall (WIRCW) for Carioca beans showed that both *p*-coumaric and ferulic acids were present in the SPF, but only ferulic acid was found in the WIRCW (*9*). Such phenolic compounds appear in a peak centered around 1650 cm^{-1} in FT-IR spectra.

The spectra of red bean SPF fractions showed the same trend as that observed for the Carioca beans in the Garcia et al. (*9*) study (**Figure 1A**). The HTC beans had lower reflectance in the peak centered around 1650 cm^{-1}, indicating the presence of more phenolics in the HTC beans as compared to the control beans. DA results in this study using the 1768−714 cm^{-1} region (**Figure 1B**) showed clear separation between the control and the HTC red beans. According to peak areas calculated, the concentration of phenolic compounds (∼1708−1581 cm^{-1}),

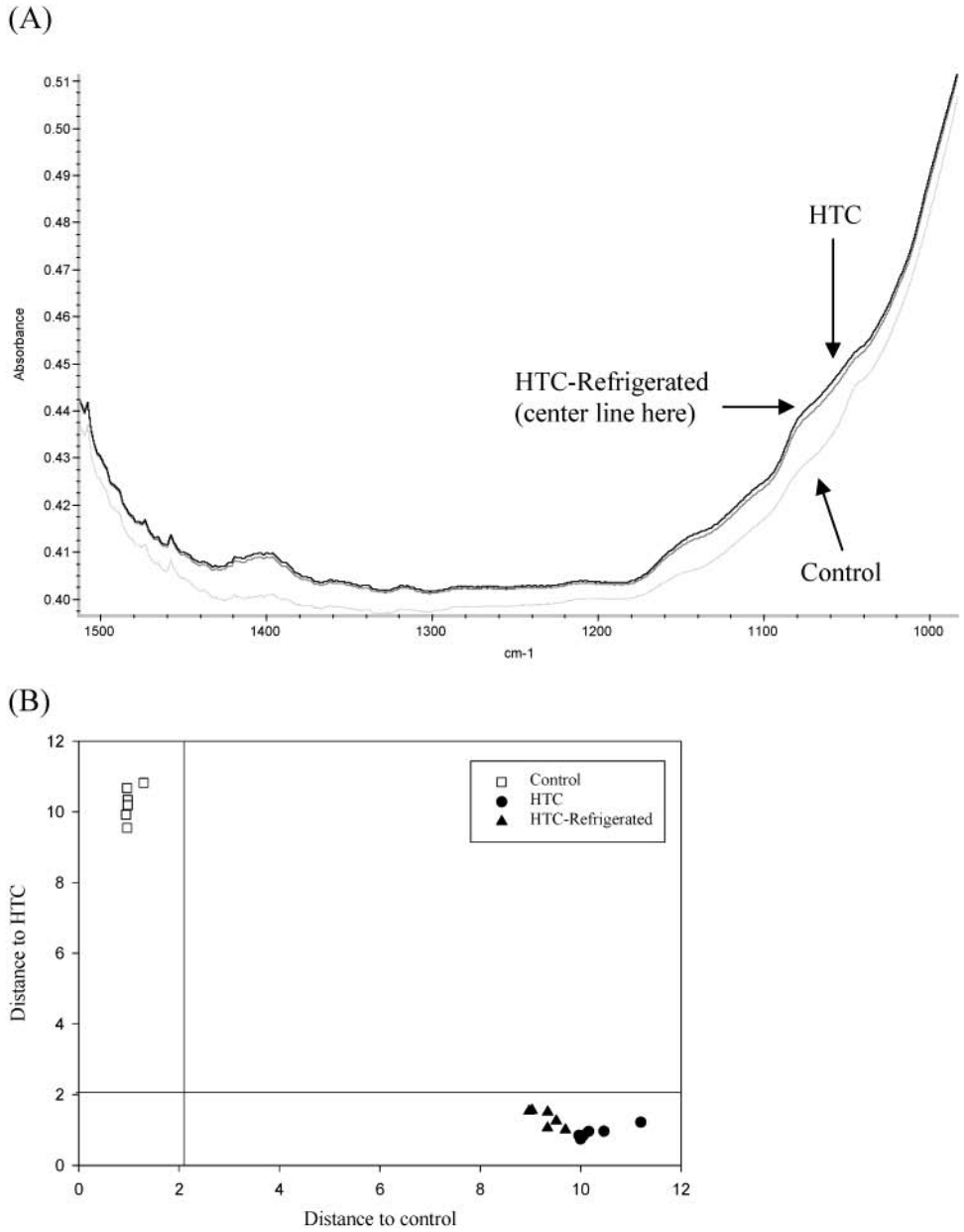

Figure 3. FT-IR ATR results for the soaking water of black beans. (**A**) Spectrograph of region used for DA. (**B**) Cooman plot obtained from DA results (1450–1365 and 1180–1010 cm^{-1}). Legend: light gray = control; dark gray = HTC-refrigerated; and black = HTC. HTC-refrigerated = HTC beans stored at refrigeration temperatures for 3.5 months. Horizontal and vertical lines across Cooman plot indicate 95% confidence intervals.

amide region (~1579−1483 cm^{-1}), and the pectates (~1192−903 cm^{-1}) for red HTC beans was larger than for the control beans (**Table 2**). Peak areas in spectra collected using FT-IR methods are directly related to concentration of absorbing compounds; therefore, greater peak areas correlate to greater compound concentrations.

The results for the SPF fraction of the black beans were, in general, similar to the ones obtained for the red beans (**Figure 1**). The spectra indicated the same trend as for the red beans, meaning that the HTC beans had a higher absorbance in the phenolic region and therefore had higher concentrations of phenolic compounds. The DA results using the 1768−714 cm^{-1} region also showed significant separation between the control and the HTC beans. However, the results obtained from calculating the peak areas from the phenolic, amide, and pectate regions did not show significant differences for any of the regions (**Table 2**). For most of the fractions, the results for the black beans were in general not as clear as results for the red beans. This might be due to the differences in bean handling prior to dehulling; the control black beans were not soaked prior to dehulling. Also, it is not known if environmental conditions under which the beans were grown affected bean cookability, a phenomenon observed by Proctor and Watts (*12*).

The spectra for the WIRCW fraction of the red control and HTC beans were very similar (**Figure 2**), which is consistent with the results obtained by Garcia et al. (*9*). The DA using the 1768−714 cm^{-1} region showed less separation between the control and the HTC samples as compared to that of the SPF fractions (as expected since the spectra were very similar). The results from the peak areas showed that in general, there was no significant difference between the control and the HTC beans (**Table 2**). The results for the WIRCW fractions of the black beans also showed comparable spectra for the control and HTC beans. However, the DA results using the 1768−714 cm^{-1} region showed a significant separation of the control and HTC

Table 3. FT-IR Peak Areas (Uncorrected[a]) of the Soaking and Cooking Water of the Black and Red Beans[b−e]

sample type		region		
		1708–1502 cm^{-1}	1475–1325 cm^{-1}	1192–902 cm^{-1}
red soaking water	control	152.76[a] (0.09)	59.86[a] (0.04)	138.99[a] (0.09)
	HTC	153.09[ab] (0.21)	60.04[ab] (0.07)	139.53[ab] (0.35)
	HTC-refrigerated	153.27[b] (0.26)	60.17[b] (0.13)	139.64[b] (0.26)
black soaking water	control	153.13[a] (0.04)	60.24[a] (0.04)	140.37[a] (0.05)
	HTC	154.39[b] (0.04)	61.20[b] (0.02)	142.39[b] (0.10)
	HTC-refrigerated	154.30[b] (0.11)	61.11[b] (0.08)	142.09[b] (0.17)
red cooking water	control	157.64[a] (0.29)	63.53[a] (0.17)	148.06[a] (0.38)
	HTC	161.99[b] (0.30)	66.32[b] (0.19)	154.95[b] (0.48)
	HTC-refrigerated	160.25[c] (0.53)	65.08[c] (0.18)	151.76[c] (0.37)
black cooking water	control	158.78[a] (0.29)	64.16[a] (0.22)	149.61[a] (0.61)
	HTC	160.10[b] (0.08)	65.50[b] (0.13)	154.47[b] (0.53)
	HTC-refrigerated	159.43[c] (0.10)	64.72[c] (0.13)	152.18[c] (0.42)

[a] Uncorrected peak areas were calculated by choosing the area of interest (the wavenumbers where the peak began and ended) in the spectrograph but no baseline correction. [b] The region 1708–1581 cm^{-1} corresponds to the phenolic compounds region, 1579–1483 cm^{-1} to the amide region, and 1192–902 cm^{-1} to the pectate region. [c] For every region in each of the fractions, areas with different letters are significantly different from each other. [d] HTC-refrigerated = HTC beans stored at refrigeration temperatures for 3.5 months. [e] Standard deviations shown in parentheses. [a−c]Data in the same column with different superscripts are significantly different ($p < 0.05$). Data were not compared across columns.

samples. Calculation of the peak areas showed no significant differences (**Table 2**).

The HPLC data obtained by Garcia et al. (*9*) showed that the WIRCW fraction of the control beans had four times more ferulic acid as compared to the HTC beans. However, they found no clear differences between the control and the HTC samples in the WIRCW fraction analyzed using FT-IR. Overall, the FT-IR results for the WIRCW fractions of red and black beans in the current study are consistent with FT-IR results for Carioca beans from Garcia et al. (*9*).

Analysis of SPF and WIRCW Fractions for HTC-Refrigerated Beans. The FT-IR results for the HTC-refrigerated red and black beans were similar to those from the HTC beans. These results were unexpected because the 50% cook time of the HTC-refrigerated beans indicated that the beans were reversing in cooking time closer to that of the control beans. However, the mechanism by which the HTC defect becomes reversed is not yet known, and it might be that this mechanism is different than the one through which the beans become HTC.

For both the red and the black bean SPF fractions, the spectra of the HTC-refrigerated beans were almost identical to those of the HTC beans (**Figure 1A**). The DA plots of the 1768−714 cm^{-1} region likewise showed close proximity of the HTC-refrigerated and HTC beans; however, the analysis was able to separate the HTC-refrigerated beans from the control beans (**Figure 1B**). Looking at the peak areas for the phenolic region (∼1650 cm^{-1}) of the red bean SPF, the results showed again that the HTC-refrigerated beans had a higher concentration of phenolics than the control beans (**Table 2**). The results for the other two peak regions (∼1520 and ∼1000 cm^{-1}) for the red bean SPF showed that the HTC-refrigerated beans had a higher concentration of components related to these peaks than the control. For the black bean SPF, the results of the peak areas showed that, in general, no significant difference was found between the different types of beans. The only exception was for the amide region, where the HTC-refrigerated black bean was shown to have a higher concentration of amide compounds than the control (**Table 2**).

The results for the WIRCW fraction of the HTC-refrigerated red and black beans were again very similar to those of the HTC beans, as shown in the spectra and DA Cooman plots using the 1768−714 cm^{-1} region (**Figure 2**). The peak areas of the amide and pectate regions of the red bean WIRCW showed that the HTC-refrigerated beans did not have significantly higher concentrations of compounds than the control beans (**Table 2**). For the black WIRCW fractions, there was no significant difference found between the HTC-refrigerated, the HTC, and the control samples for any of the regions (**Table 2**).

Overall, the DRIFTS FT-IR results for the black and red HTC-refrigerated beans were very similar to those of the HTC beans, indicating that the same types of components were present in both bean types. If the mechanism to develop the HTC defect involves the accumulation of phenolic compounds, it appears likely that the mechanism of reversibility occurs through a different pathway than the pathway to initially develop the HTC defect.

Analysis of Soaking and Cooking Waters. The spectra of red and black bean soaking waters collected using ATR-FTIR showed that the HTC beans released more solids as compared to the controls (the differences were significantly different for the black beans as shown in **Figure 3A**; **Table 3**). The DA of the regions of interest (1450−1365 and 1180−1010 cm^{-1}) indicated a significant separation between the soaking water of the control and the HTC red and black beans (**Figure 3B**). These results are consistent with the work of Hincks et al. (*13*) and Shomer et al. (*14*) who found that solid loss and electrolyte leakage during soaking were greater for HTC beans than for control beans. These researchers also observed that HTC beans had higher water absorption. Several researchers believe that the reason for the increased release of solutes from the HTC beans is due to damage caused to the cell during the adverse conditions of high temperature and humidity under which the HTC beans are stored (*14*, *15*). Shomer et al. (*14*) analyzed the soaking water of control and HTC beans and found that the soaking water of HTC beans released a greater amount of total sugars, pectin (galacturonic acid), protein, and phosphate. One can relate the components found in the soaking water in the Shormer et al. (*14*) study to the peaks obtained in the FT-IR spectra from the current study, since the most well-defined peaks present are centered around the carbohydrate and protein regions (1600, 1400, and 1100 cm^{-1}). Other types of compounds that are likely present in the soaking water are polyphenols, specifically tannins. A study by Martin-Cabrejas et al. (*16*) showed that HTC beans had higher levels of tannins as compared to control beans.

The ATR FT-IR results obtained for the soaking water of the HTC-refrigerated red and black beans were almost identical to the ones obtained from the HTC beans (**Figure 3**; **Table 3**).

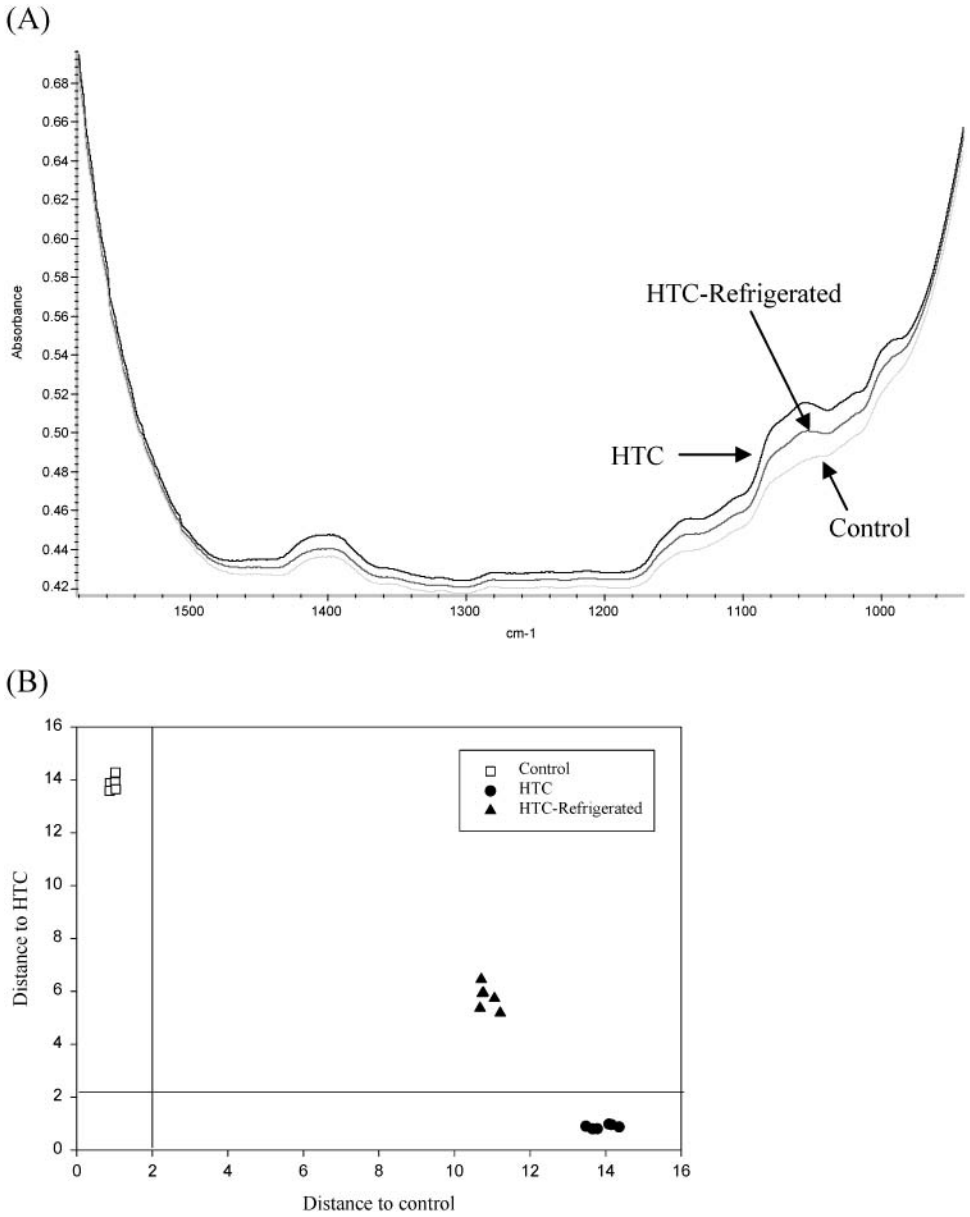

Figure 4. FT-IR ATR results for the cooking water of black beans. (**A**) Spectrograph of region used for DA. (**B**) Cooman plot obtained from DA results (1450–1365 and 1180–975 cm^{-1}). Legend: light gray = control; dark gray = HTC-refrigerated; and black = HTC. HTC-refrigerated = HTC beans stored at refrigeration temperatures for 3.5 months. Horizontal and vertical lines across Cooman plot indicate 95% confidence intervals.

This similarity indicates that the cell wall structure of the HTC-refrigerated beans must be disrupted or damaged, as is the case for HTC beans, since the HTC and HTC-refrigerated beans lost the same amount of solutes to the soaking water. Because the loss of membrane integrity has been related to the proposed mechanism of lipid oxidation and/or polymerization, it is expected that the mechanism of reversibility for HTC-refrigerated beans acts through a different pathway that allows the beans to cook in less time than the HTC beans, even when the cell wall is still fractured and damaged. This is also consistent with the results explained in the previous section.

FT-IR analysis of the cooking waters of the control and HTC red and black beans, which were cooked to the same tenderness point, indicated that the HTC beans lost more constituents to the water as compared to the control beans (**Figure 4A**; **Table 3**). DA plots using the 1450−1365 and 1180−975 cm^{-1} regions showed a clear separation of the control and HTC beans (**Figure 4B**). Peak areas for the control and HTC beans were significantly different. In all regions, the HTC beans lost significantly more constituents to the water as compared to the control beans. This might be due to the damaged structure of the HTC beans, which allows for more components to leak into the water from the inside of the bean.

Other researchers who measured the amount of solids lost during cooking for control and HTC beans concluded that control beans lost more constituents to the water than the HTC beans during the cooking process. This observation is contradictory to what was observed in the current study. However, the cooking methods used in the previous studies differed from the current study. In the work by Hincks et al. (*13*) and Shomer et al. (*14*), the control and HTC beans were cooked for the same period of time (chosen by the researcher). In the present study, the beans were cooked to their 50% cook time so that they would all have the same tenderness at the end. In the method used by the other researchers, it seems logical that the control beans lost more constituents to the water as compared to the HTC beans. By the end of the chosen time, the control beans were

much softer than the HTC beans (*13*), and thus, it would have been easier for them to lose more components to the water.

Analysis of FT-IR spectra of the cooking water from HTC-refrigerated beans showed that concentrations of absorbing compounds for HTC-refrigerated beans were between those of the control and HTC beans (**Figure 4A**; **Table 3**). DA results showed significant separation of the HTC-refrigerated beans from both the control and the HTC beans (**Figure 4B**). Likewise, in peak areas, the HTC-refrigerated beans lost significantly more constituents to the water than the control beans but significantly less than the HTC beans. These results are consistent with the 50% cook time of the beans, which showed that the 50% cook time of the HTC-refrigerated beans was between that of the control and HTC beans (**Table 1**). This indicates that the same mechanism that shortened the cooking time of the HTC-refrigerated beans changed the structure of the HTC-refrigerated beans in a way that they were able to lose less constituents to the water during cooking as compared to the HTC beans. It is also important to note that since reversal of some of the proposed mechanisms for the HTC defect does not appear to be possible (i.e., lignification of the middle lamella or the loss of structural integrity), this might help to narrow down the possibilities for the mechanism of reversibility.

In conclusion, the results from the FT-IR analysis of the SPF fractions using DRIFTS were consistent with previous work by Garcia et al. (*9*) showing that in general, more phenolic compounds were associated with the SPF fraction of HTC beans as compared to control beans for Carioca, red, and black beans. The presence of increased phenolics in the HTC beans is thought to have an effect on cell wall separation during cooking, which makes HTC beans take longer to cook to the same tenderness (*9*, *10*). The HTC-refrigerated bean results were very similar to those of the HTC beans, indicating that the mechanism of reversibility is likely different, at least in part, than the one responsible for developing the HTC defect (i.e., by accumulation of phenolics in the pectin fraction).

When the soaking water was analyzed using ATR FT-IR, the HTC-refrigerated and HTC beans had significantly higher absorbance values than did the control beans, indicating that the HTC-refrigerated and HTC beans lost more constituents to the water as compared to the control samples. This was consistent with previous studies (*13*, *14*), which also indicated a disrupted cell in the beans as a possible explanation. The bean cooking water results also showed significant differences between the control, the HTC, and the HTC-refrigerated beans. The HTC beans lost the most constituents to the water, followed by the HTC-refrigerated beans, and finally the control beans with the least amount of solids loss. The disrupted or damaged cell of the HTC beans is likely the cause for the greater loss. However, the HTC-refrigerated bean results were between those of the HTC and control beans, as was the case for the 50% cook times. This indicates that the mechanism of reversibility might have allowed a change in the structure of the HTC-refrigerated beans in a way that allowed greater cell separation during cooking, thus shorter cooking times and less solids loss than the HTC beans.

ABBREVIATIONS USED

HTC, hard-to-cook; FT-IR, Fourier transform infrared spectroscopy; DRIFTS, diffuse reflectance; ATR, attenuated total reflectance; SPF, soluble pectin fraction; WIRCW, water insoluble residue of the cell wall.

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