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Synchrotron-Based Microspectroscopic Study on the Effects of Heat Treatments on Cotyledon Tissues in Yellow-Type Canola (*Brassica*) Seeds

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ABSTRACT: Synchrotron-based infrared (IR) microspectroscopy is able to reveal structural features of biomaterials within intact tissue at both cellular and molecular levels. Heat-related treatments have been used to improve nutrient availability of canola seeds and meal. However, hitherto, there has been no study on the sensitivity and response of each layer in canola seeds to heat-related treatments. It is not known which layer (epiderm/mucllage, spermoderm, endosperm, or cotyledon) is the most sensitive to heat when heat treatment is applied to the seeds. Traditional wet chemical analysis is unable to answer such questions. The objective of this study is to use synchrotron IR microspectroscopy with multivariate molecular spectral analyses as a research tool to study heat treatment effects in a fast way on the structural changes in cotyledon tissues of yellow-type canola (*Brassica*) seeds among raw (treatment code "A"), wet heating (autoclaving at 121 °C for 60 min, treatment code "B"), and dry heating (dry roasting at 120 °C for 60 min, treatment code "C"). The hypothesis of this study was that different heat treatments have different heat penetration abilities on cotyledon tissues in yellow-type canola seeds. The multivariate analytical tools principal component analysis (PCA) and agglomerative hierarchal cluster analysis (AHCA) were applied to investigate variance and groupings within the spectral data set [whole spectral range of ca. 4000−650 cm^{-1}, spectral range of ca. 1300−900 cm^{-1} (cellulose or saccarides), spectral range of ca. 1800−1500 cm^{-1} (secondary structures of protein) and spectral range of ca. 1500−1300 cm^{-1} (bending motion of methylene and methyl group; this change is consistent with the change in the range of ca. 3000−2800 cm^{-1})]. The results showed that there were no clear cluster and groups formed in the cotyledon tissues among the three treatments (A, B, and C). There were no clear distinguished responses of the cotyledon tissues to different types of heat treatments using multivariate molecular spectral analyses. The results indicate that the cotyledon tissues might not be sufficiently penetrated by both heat treatments (autoclaving and dry roasting) under the specified conditions. A future study is needed to analyze individual functional group band intensity among the treatments using univariate molecular spectral analysis to confirm multivariate PCA and cluster analyses.

KEYWORDS: *Synchrotron IR microspectroscopy, cotyledon tissue, heat treatment, canola seeds*

INTRODUCTION

Canola seeds usually contain high protein, balanced amino acids, and unique nutrient and chemical profiles.[1,2] However, protein degradation is still high, which can result in an unbalance between protein breakdown and nutrient uptake/absorption in the rumen. Heat treatment could possibly be used as a method to decrease protein degradation, shift protein digestion from rumen to intestine, and thus increase metabolizable protein and high feed milk value.

Canola seeds have a unique inherent structure. From outside to inside, the layers of a canola seed include the epiderm/mucllage, spermoderm, endosperm, and cotyledon. It is not known which layer is the most sensitive to heat treatments because of the limited analytical techniques available.

Synchrotron-based infrared (IR) microspectroscopy is able to reveal structural features of biomaterials from different layers within intact tissue at both the cellular and molecular levels.[3−6] The objective of this study was to use synchrotron IR microspectroscopy as a research tool to study the effects of heat treatment on the structural changes of cotyledon tissues in yellow-type canola (*Brassica*) seeds among the raw, wet-heated (autoclaving at 121 °C for 60 min), and dry-heated (dry roasting at 120 °C for 60 min) yellow-type canola seeds. The hypothesis of this study was that different heat treatments had different penetration ability to cotyledon tissues in yellow-type canola seeds, which could be detected by synchrotron IR microspectroscopy.

MATERIALS AND METHODS

Wet- and Dry-Heat Processing. Yellow-type canola seeds (*Brassica*) were wet-heated (autoclaving) at 121 °C for 60 min and dry-roasted at 120 °C for 60 min at APS Research Laboratory, University of Saskatchewan (Saskatoon, Saskatchewan, Canada).

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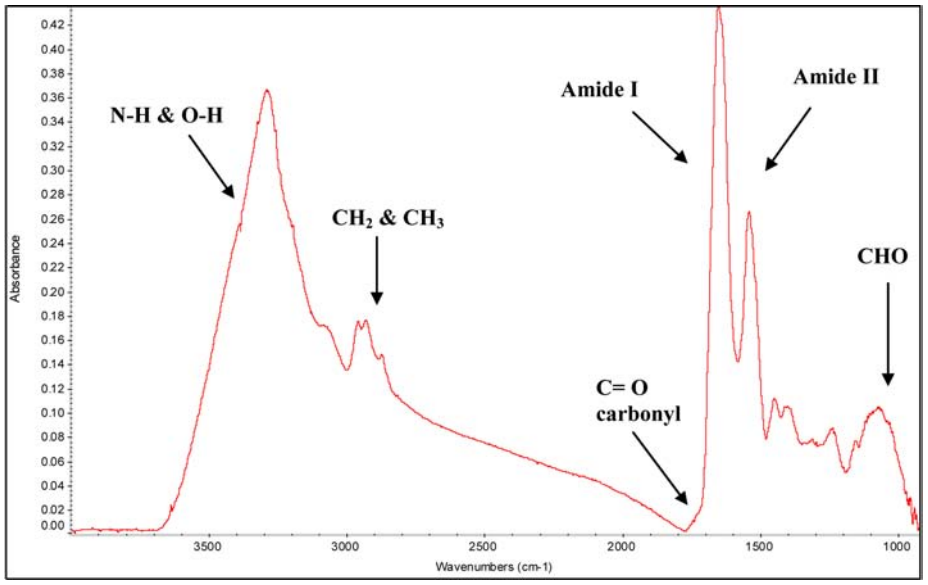

Figure 1. Typical synchrotron-based IR spectrum in the cotyledon area of canola seeds (raw, pixel size = 10 × 10 μm) showing various function groups: N−H and O−H, C−H [CH_3 asymmetric stretch at ca. 2960 cm^{-1}, CH_2 asymmetric stretch at ca. 2930 cm^{-1}, CH_3 symmetric stretch at ca. 2872 cm^{-1}, and CH_2 symmetric stretch at ca. 2850 cm^{-1})], amide I and II, C═O carbonyl, CHO, and cellulosic compounds.

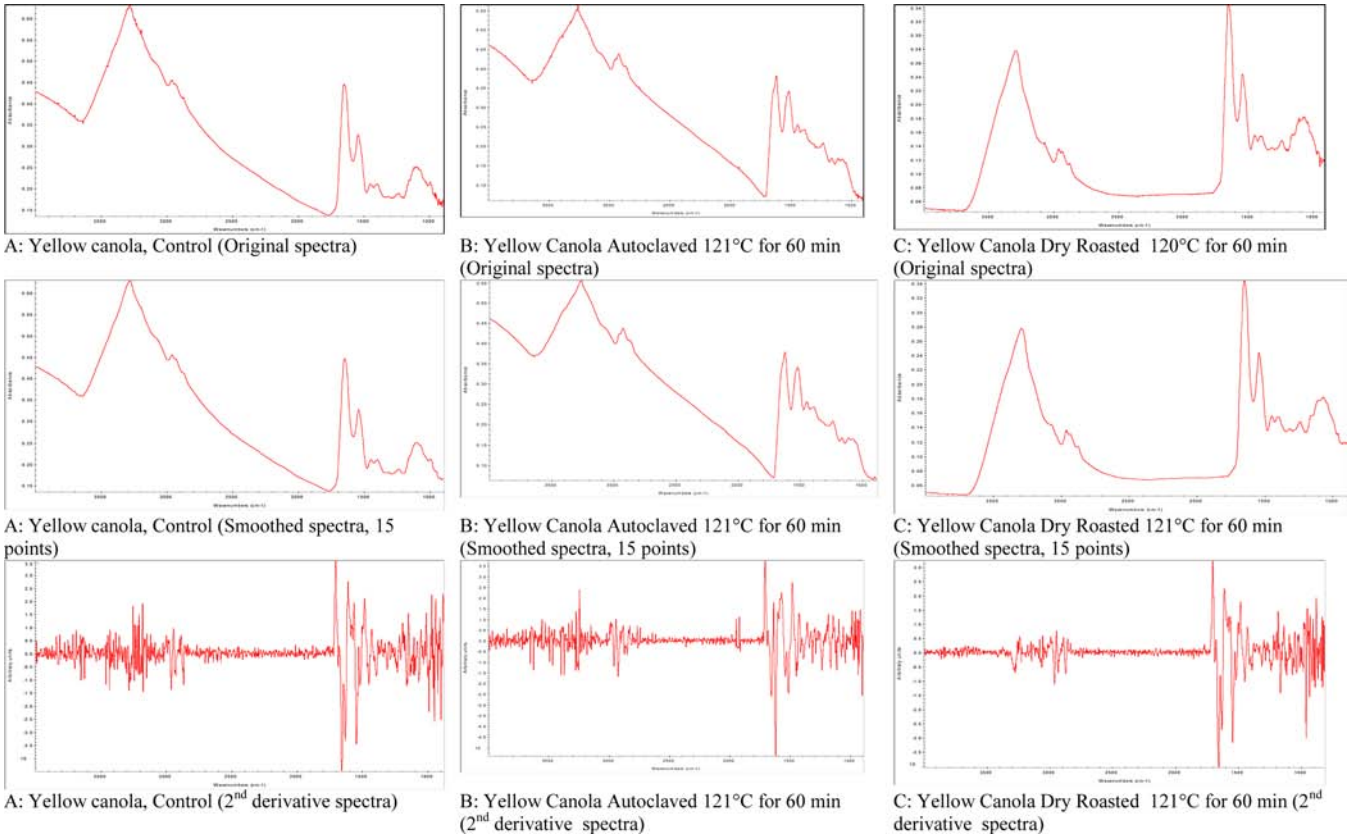

Figure 2. Typical synchrotron-based raw spectra along the corresponding smoothed spectra (15 points) and also second-derivative spectra in the cotyledon layer of yellow-type canola tissues (control, autoclaved at 121 °C for 60 min, and dry roasted at 121 °C for 60 min), revealed by synchrotron-based FTIR microspectroscopy.

The detailed chemical and nutrient profiles and rumen and intestinal digestion of the same samples were reported previously.[7]

Tissue Preparation. Five seeds were randomly selected from each treatment (A, raw; B, wet heating; and C, dry roasting). Each seed was cut into thin cross-sections (6 μm thickness) using a microtome at the University of Saskatchewan (Saskatoon, Saskatchewan, Canada), and then unstained cross-sections were transferred to BaF_2 windows (size = 13 × 1 mm disk; Spectral Systems, Hopewell Junction, NY) for transmission mode synchrotron IR microspectroscopic study. Photomicrographs of the cross-section of the tissues on BaF_2 windows were taken with a microscope linked to a digital camera from the U2b station in National Synchrotron Light Source at Brookhaven National

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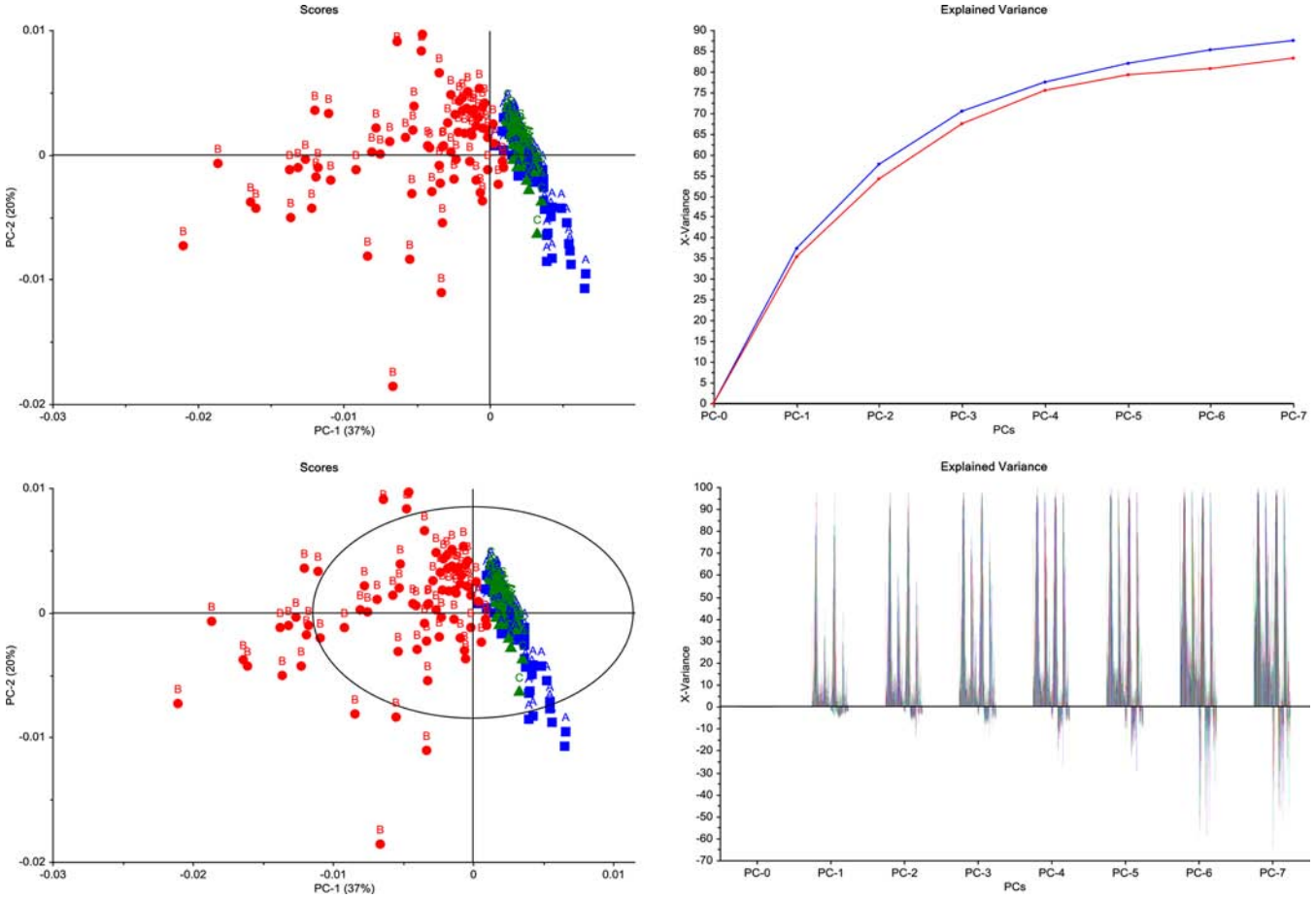

Figure 3. PCA of the second-derivative spectra in the cotyledon layer of yellow-type canola seeds without deleting outliers (A, control; B, autoclaved at 121 °C for 60 min; and C, dry roasted at 121 °C for 60 min), collected by synchrotron FTIR microspectroscopy.

Laboratory (NSLS-BNL, U.S. Department of Energy, Upton, NY). The pre-methodology study was performed at 01B1-1 station, Canadian Light Sources (CLS).

Synchrotron-Radiation-Based Microspectroscopy. The preliminary experiments were carried out at 01B1-1 station, CLS (Saskatoon, Saskatchewan, Canada). The experiments were conducted on beamline U2B at the NSLS-BNL (Upton, NY) in 2012. The detailed method has been previously described.[8] Briefly, the tissue spectra were collected in the mid-IR range, 4000−650 cm^{-1}, at a resolution of 4 cm^{-1} with 128 scans co-added and an aperture setting of ca. 10 × 10 μm. For each seed, a total of 15 spectra were collected from the cotyledon area. Each treatment had a total of 75 spectra (=5 seeds × 15 spectra/seed). The OMNIC 7.3 software was used to collect and process the data. The charge-coupled device camera coupled to the IR microscope (32× objective) was used to obtain scanned visible images.

Multivariate Molecular Spectral Analysis. The spectral differences in whole mid-IR region (ca. 4000−650 cm^{-1}), spectral ranges of ca. 1300−900 cm^{-1} (cellulose or saccarides), spectral ranges of ca. 1800−1500 cm^{-1} (secondary structures of protein), and spectral ranges of ca. 1500−1300 cm^{-1} (bending motion of methylene and methyl group; this change is consistent with the change in the range of ca. 3000−2800 cm^{-1}) in cotyledon tissue affected dry and heat treatments were analyzed by multivariate analyses following pre-processing using the second-derivative spectra (full-spectra region) or original spectra in individual spectral regions and the removal of outlier spectra. The multivariate analyses included agglomerative hierarchical cluster analysis (AHCA) and principal component analysis (PCA), which were performed using Statistica 8.0 and the Unscramble X 10.2 (CAMO Software AS, Oslo, Norway).

RESULTS AND DISCUSSION

Processing Effect on Whole Seeds and Synchrotron IR Microspectroscopy. A previous study by Samadi et al.[7] showed that heat processing did have a significant effect on the same and ground whole canola seeds. It was found that the heat processing changed protein solubility, non-protein nitrogen, neutral detergent fiber, and neutral detergent insoluble protein. It also changed *in situ* rumen degradation, *in vitro* intestinal digestibility, and rapidly and slowly degradable protein fractions of whole canola seeds. However, in that study, it was not known which layer in the canola seeds was the most sensitive to heating because ground whole canola seeds were used in the study. Usually canola seeds have four layers, which include the first layer of epiderm/mucllage, second layer of spermoderm, third layer of endosperm, and fourth layer of cotyledon. A number of critical points were not addressed in the study by Samadi et al.,[7] including (1) was the heating equally applied to all layers, (2) was the heating only effective to certain layer within the seeds, and (3) did wet heating and dry heating have an equal effect on the internal structure?

With synchrotron-based IR microspectroscopy, it is possible to investigate each layer without damaging inherent tissue layers to determine whether the processing or treatment affects the internal structure change. In this study, synchrotron-based IR microspectroscopy was applied to obtain spectral features in the fourth layer of canola seeds in cotyledon tissue after both wet and dry heating was applied to whole canola seeds. It is our goal to develop a rapid method to study heat treatment effects and detect

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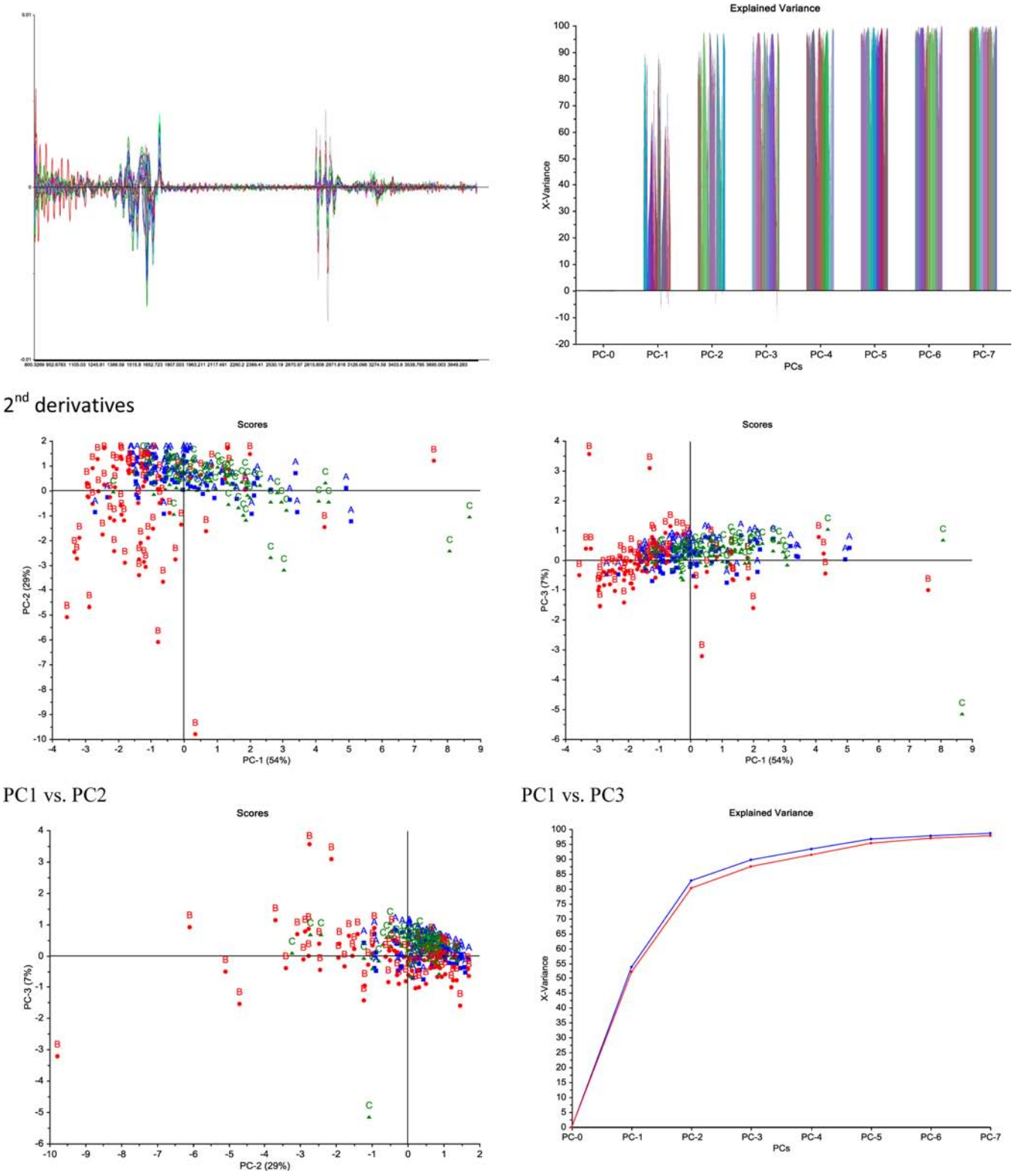

Figure 4. PCA of the second-derivative spectra (after removal of the outliers) in the cotyledon tissue of yellow-type canola tissues (A, control; B, autoclaved at 121 °C for 60 min; and C, dry roasted at 121 °C for 60 min), collected by synchrotron FTIR microspectroscopy.

internal structural changes without damaging tissue inherent structures/layers.

Multivariate Spectral Analysis of Heat-Induced Changes in Cotyledon Tissues. To rapidly detect heating effects on the inside of canola tissue in the cotyledon layer, multivariate data analysis in the whole spectral region was applied. Unlike univariate analysis,[3] the multivariate spectral analyses makes use of the entire spectral data set with little parametrization, and detailed knowledge on the band assignments are not required to determine correlations and clustering in the data. In the first instance, we applied PCA to investigate the variance in the data set. PCA transforms the original set of

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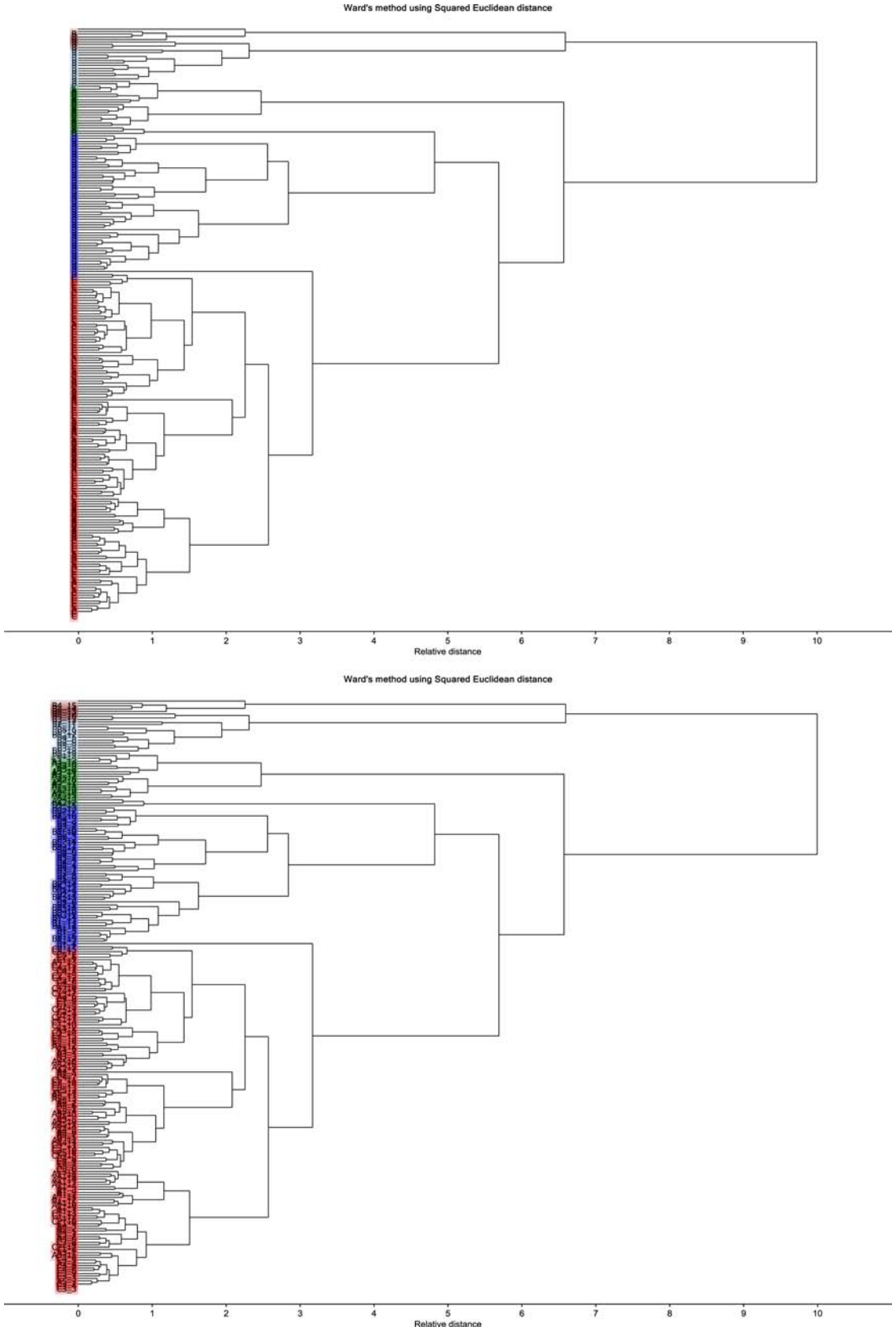

Figure 5. AHCA of the second-derivative spectra (after removal of the outliers) in the cytoledon tissue of yellow-type canola seeds (A, control; B, autoclaved at 121 °C for 60 min; and C, dry roasted at 121 °C for 60 min), collected by synchrotron FTIR microspectroscopy.

variables to a new set of uncorrelated variables called principal components. The first few principal components typically account for the majority of the variance in the data set. The purpose of PCA is to derive a small number of independent

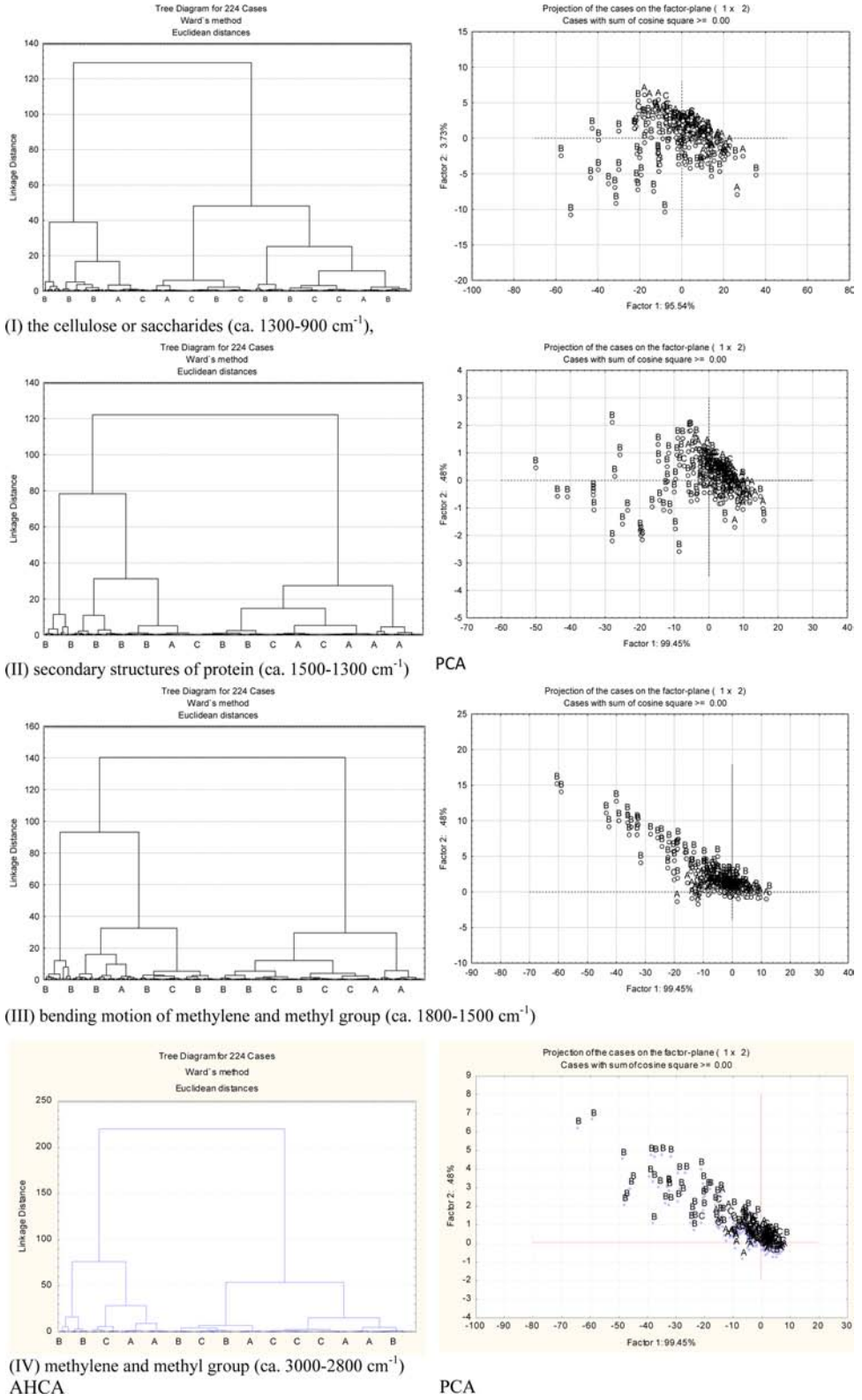

Figure 6. Multivariate molecular spectral analyses of (I) cellulose or saccharides (ca. 1300−900 cm^{-1}), (II) secondary structures of protein (ca. 1500−1300 cm^{-1}), (III) bending motion of methylene and methyl group (ca. 1800−1500 cm^{-1}), and (IV) methylene and methyl group (ca. 3000−2800 cm^{-1}) in the cotyledon tissue of yellow-type canola seeds on a molecular basis (A, control; B, autoclaved at 121 °C for 60 min; and C, dry roasted at 121 °C for 60 min), collected by synchrotron FTIR microspectroscopy.

linear combinations (principal components) from the original set of variables that retains as much of the information in the original spectra. It displays the results as score plots between the various components (Unscramble X 10.2, CAMO Software AS, Oslo, Norway). The second multivariate spectral analysis was AHCA. It performs an AHCA of a spectra data set and displays the results of cluster analysis as dendrograms.[9] In this study, Ward's algorithm was used on second-derivative

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spectral data over the entire mid-IR region (ca. 4000−800 cm^{-1}).[9]

Figure 1 is a typical synchrotron Fourier transform infrared (FTIR) microspectroscopy spectrum in the cotyledon layer of canola seeds, showing various function group bands: N−H and O−H, C−H, amide I and II, C═O carbonyl, CHO, and cellulosic compounds. The C−H group includes a CH_3 asymmetric stretch at ca. 2960 cm^{-1}, CH_2 asymmetric stretch at ca. 2930 cm^{-1}, CH_3 symmetric stretch at ca. 2872 cm^{-1}, and CH_2 symmetric stretch at ca. 2850 cm^{-1}. From this spectrum, it was found that the cotyledon layer contain relatively high protein. The ratio of protein/CHO is also relatively high.

Figure 2 shows typical raw spectra along the corresponding smoothed spectra (15 points) and also second-derivative spectra in the cotyledon layer of yellow-type canola seeds (control, autoclaved at 121 °C for 60 min, and dry roasted at 121 °C for 60 min), revealed by synchrotron FTIR microspectroscopy. It is hard to visually detect spectral differences between the raw, autoclaved, and dry-roasted cotyledon tissues; hence, a multivariate approach is more applicable. Figure 3 shows the PCA of the second-derivative spectral data obtained from the raw ("A"), autoclaved ("B"), and dry-roasted ("C") cotyledon tissues of yellow-type canola seeds. The results show that the autoclave heating (B) versus raw (A) and dry heating (C) can be almost grouped in separate ellipses. However, the raw (A) versus dry heating (C) cannot be grouped in separate ellipses, and there is significant overlap (Figure 3). These results indicate that autoclaving (wet heating) might have a profound effect on canola seeds. However, there are some potential outliers in the spectral data sets [Figure 3 (2)]. Total variance that can be explained by first and second principal components is still relatively low (57%) [Figure 3 (1)]. After removal of the outliers, PCA was again performed, and the results are displayed in Figure 4. The results showed that the raw (A), autoclave heating (B), and dry heating (C) cannot be fully grouped in separate ellipses and there is some overlap. Total variance that can be explained by first and second principal components increased from 57% (Figure 2) to 83% (Figure 4). These results indicate that the heat treatment effects on cotyledon tissue are minimal. There is no study found in the literature that can be used in comparison to our study.

In a previous study, cluster analysis enabled the feed intrinsic structures to be distinguished, and features that differed between treatments were identified.[10−12] Our results, which are displayed in the form of a dendrogram (Figure 5), show that no different spectral clusters between the different heat treatments (control versus autoclave versus dry heating) of the cotyledon layer of yellow-type canola seeds were observed in the spectral range. From this diagram, the heating in the cotyledon layer of canola seeds was not distinguished from each other. This is contrary to the study by Samadi et al.,[7] who did show the differences between the heat treatments when applied to ground whole seeds.

The similar results were also found when using multivariate spectral methods to analyze individual original spectral ranges of ca. 1300−900 cm^{-1} (cellulose or saccarides), ca. 1800−1500 cm^{-1} (secondary structures of protein), and ca. 1500−1300 cm^{-1} (bending motion of methylene and methyl group; this change is consistent with the change in the range of ca. 3000−2800 cm^{-1}) (Figure 6) in the cotyledon tissue. It was found that dry and heat treatments did not form different clusters or groups.

The previous results by Samadi et al.[7] plus our results indicate that heat treatments only have a profound effect on the outside layers/structures of canola seeds. Again, no study has been found in the literature that can be used in comparison to our study.

In conclusion, there were no clear distinguished responses of the cotyledon tissues to different types of heat treatments using multivariate molecular spectral analyses. The results indicated that the cotyledon tissues might not be sufficiently penetrated by both autoclaving and dry roasting at the specified conditions. A future study is needed to analyze individual functional group band intensity among the treatments using univariate molecular spectral analysis to confirm multivariate PCA and cluster analyses.

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Notes

The authors declare no competing financial interest.

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REFERENCES

(1) Theodoridou, K.; Yu, P. Effect of processing conditions on the nutritive value of canola meal and presscake: Comparison of the yellow and brown-seeded canola meal with the brown-seeded canola presscake. *J. Sci. Food Agric.* **2013**, *93*, 1986−1995.

(2) Theodoridou, K.; Yu., P. Metabolic characteristics of the proteins in yellow-seeded and brown-seeded canola meal and presscake in dairy cattle: Comparison of three systems (PDI, DVE, and NRC) in nutrient supply and feed milk value (FMV). *J. Agric. Food Chem.* **2013**, *61*, 2820−2830.

(3) Yu, P. Application of advanced synchrotron-based Fourier transform infrared microspectroscopy (SR-FTIR) to animal nutrition and feed science: A novel approach. *Br. J. Nutr.* **20004**, *92*, 869−885.

(4) Marinkovic, N. S.; Chance, M. R. Synchotron infared microscopy. In *Encyclopedia of Molecular Cell Biology and Molecular Medicine*, 2nd ed.; Meyers, R., Ed.; Wiley, Inc.: Hoboken, NJ, 2006; Vol. *13*, pp 671−708.

(5) Miller, L. M.; Dumas, P. Chemical imaging of biological tissue with synchrotron infrared light. *Biochim. Biophys. Acta* **2006**, *1758*, 846−857.

(6) Dokken, K. M.; Davis, L. C.; Marinkovic, N. S. Use of infrared microspectroscopy in plant growth and development. *Appl. Spectrosc. Rev.* **2007**, *40* (4), 301−326.

(7) Samadi; Theodoridou, K.; Yu, P. Detect the sensitivity and response of protein molecular structure of canola seed (yellow and brown) to different heat processing and relation to protein utlization and availability using ATR-FT/IR molecular spectroscopy with chemometrics. *Spectrochim. Acta, Part A* **2013**, *105*, 304−313.

(8) Yu, P.; McKinnon, J. J.; Christensen, C. R.; Christensen, D. A. Imaging molecular chemistry of pioneer corn. *J. Agric. Food Chem.* **2004**, *52*, 7345−7352.

(9) CytoSpec. *Software for Infrared Spectral Imaging, Version 1.1.01*; CytoSpec: Berlin, Germany, 2004.
(10) Doiron, K. J.; Yu, P.; McKinnon, J. J.; Christensen., D. A. Heat-induced protein structures and protein subfractions in relation to protein degradation kinetics and intestinal availability in dairy cattle. *J. Dairy Sci.* **2009**, *92*, 3319−3330.
(11) Doiron, K. J.; Yu., P.; Christensen, C. R.; Christensen, D. A.; McKinnon, J. J. Detecting molecular changes in vimy flaxseed protein structure using synchrotron FTIRM and DRIFT spectroscopic techniques: Structural and biochemical characterization. *Spectroscopy* **2009**, *23*, 307−322.
(12) Liu, B.; McKinnon, J. J.; Thacker, P.; Yu, P. Molecular structure and metabolic characteristics of the proteins and energy in triticale grains and dried distillers grains with solubles for dairy cows. *J. Agric. Food Chem.* **2012**, *60*, 10064−10074.