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# Visualizing Tissue Molecular Structure of a Black Type of Canola (*Brassica*) Seed with a Thick Seed Coat after Heat-Related Processing in a Chemical Way

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**ABSTRACT:** Heat-related processing of cereal grains, legume seeds, and oil seeds could be used to improve nutrient availability in ruminants. However, different types of processing may have a different impact on intrinsic structure of tissues. To date, there is little research on structure changes after processing within intact tissues. The synchrotron-based molecular imaging technique enables us to detect inherent structure change on a molecular level. The objective of this study was to visualize tissue of black-type canola (*Brassica*) seed with a thick seed coat after heat-related processing in a chemical way using the synchrotron imaging technique. The results showed that the chemical images of protein amides were obtained through the imaging technique for the raw, wet, and dry heated black type of canola seed tissues. It seems that different types of processing have a different impact on the protein spectral profile in the black type of canola tissues. Wet heating had a greater impact on the protein $\alpha$-helix to $\beta$-sheet ratio than dry heating. Both dry and wet heating resulted in different patterns in amide I, the second derivative, and FSD spectra. However, the exact differences in the tissue images are relatively difficult to be obtained through visual comparison. Future studies should focus on (1) comparing the response and sensitivity of canola seeds to various processing methods between the yellow-type and black-type of canola seeds; (2) developing a sensitive method to compare the image difference between tissues and between treatments; (3) developing a method to link images to nutrient digestion, and (4) revealing how structure changes affect nutrient absorption in humans and animals.

**KEYWORDS:** *black-type of canola tissue, synchrotron, imaging, protein amides*

## INTRODUCTION

In recent years, more scientists use synchrotron infrared microspesctroscopy (SR-IMS) plus various newly developed techniques (e.g., array technique) to characterize and diagnose various diseases to detect structure changes on different organisms on a molecular level.[1,2] However, to date, there is little application of the synchrotron-radiation-based technique, SR-IMS, in food and feed quality study as a noninvasive method. SR-IMS as a technique could be used to explore the feed of interest without disturbing it and damaging it. [1] The work can be done in a rapid, sensitive, and automated manner. [1] The technique provides high resolution and is able to detect the system of interest within cellular dimensions.

Heat-related processing is commonly used to improve nutrient availability and metabolizable protein in the small intestine. However, different heat-related processing (toasting, extrusion, dry heating, wet heating, pelleting, microwaving, etc.) may have a different impact on molecular structure changes associated with the interaction of treatments (e.g., enzymatic treatments, chemical treatments, physical−chemical treatments) in the same tissues. For example, processing may affect covalent modification of amino acid side chains due to cross-linking or Maillard reactions and may result in inaccessibility of proteins due to complexation with other feed components (cellulose, etc), which are well known in scientific communities. Heat-related processing often produces contradictory results in terms of nutrient utilization and availability. Part of the reason is that we do not have a direct method or noninvasive method to monitor internal changes induced by processing without damaging internal tissue structure, and we do not have a direct method to find optimal processing conditions.[2] This study aimed at using SR-IMS with the ATR technique to visualize the tissue structure of a black type of canola (*Brassica*) seed (with a thick seed coat) after heat-related processing in a chemical way. The hypothesis of this study was that penetration of the heating effect to the seed tissues differed between different heat methods, and the canola tissue with a thick seed coat (black type) was difficult to be penetrated by heating in comparison with the yellow type of canola with a thin seed coat.

## MATERIALS AND METHODS

**Processing of Seed Tissue and Preparation for Synchrotron Study.** Black canola seed tissues (*Brassica*) with a thick seed coat were dry heated and wet heated at the APS Research Lab, University of Saskatchewan (Saskatoon, Canada). The detailed description of processing methods was reported.[3] The raw (control), wet-heated and dry-heated seeds were cut into thin cross sections (6 $\mu$m thickness) after being frozen using a microtome at the University of Saskatchewan (Saskatoon, Canada), and then unstained cross sections were transferred to $BaF_2$ windows (size 13 × 1 mm disk; Spectral Systems, New York) for transmission mode synchrotron SR-IMS work. Photomicrographs of the cross-section of the tissues were taken with a microscope linked to a digital camera from the $BaF_2$ window at the U2b station in NSLS. Heating was used to test whether the internal protein structure in tissues could be changed, such as protein fine structures, $\alpha$ helix, $\beta$-sheet, etc.

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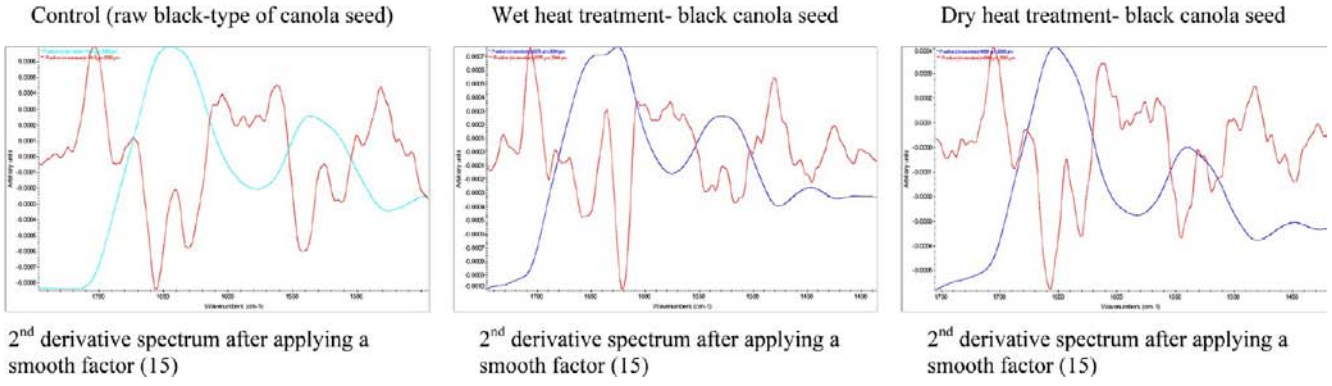


**Figure 1.** Effect of heat-related processing on spectral profile change (typical synchrotron-based spectroscopy spectrum and their second-derivative spectra in a black type of canola seed tissue (*Brassica*) affected by heat-related processing methods at ca. 1718−1480 $cm^{-1}$ to show potentially heat-induced changes of the protein second structure spectral profile (such as changes in the $\alpha$-helix and $\beta$-sheet ratio and their pattern): control vs wet heating vs dry heating).

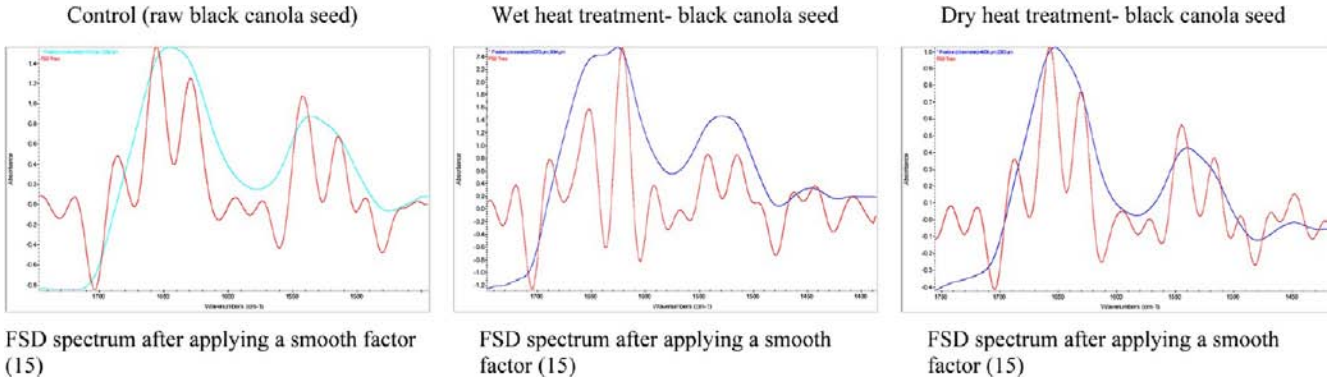


**Figure 2.** Effect of heat-related processing on spectral profile change (typical synchrotron-based Fourier self-deconvolution (FSD) spectra of a black type of canola seed tissue (*Brassica*) affected by heat-related processing methods at ca. 1718−1480 $cm^{-1}$ to show potential heat-induced changes of the protein second structure spectral profile (such as changes in the $\alpha$-helix and $\beta$-sheet ratio and pattern): control vs wet heating vs dry heating).

**Synchrotron-Radiation-Based Microspectroscopy.** The preliminary experiment for testing methodology was carried out at the 01B1-1 station, Canadian Light Sources (CLS, University of Saskatchewan, Canada). The experiment was conducted on beamline U2B at the National Synchrotron Light Source (NSLS, New York). The detailed molecular imaging methodology was described previously. [4] Briefly, tissue spectra were collected in the mid-infrared range, 4000−650 $cm^{-1}$, at a resolution of 4 $cm^{-1}$ with 64−128 scans coadded and an aperture setting of ca. 10 × 10 $\mu$m. Mapping steps were equal to aperture size. OMNIC 7.3 software was used to collect and process the data. The charge-coupled device camera linked to the infrared images (objective ×32) was used to obtain scanned visible images.

**Monitor Tissue Structure Changes through Imaging.** To monitor tissue structure changes in the wet- and dry-heated black type of canola seed tissue in comparison with the control, tissue spectra in protein amides I and II at ca. 1710−1480 $cm^{-1}$ were analyzed. The second-derivative functions and FSD method were used to detect the component peaks of amide I which were used to indicate structure changes. The protein second structure $\alpha$-helix and $\beta$-sheet could be determined. Chemical images were carried out under the peaks centered at ca. 1650 (amide I) and 1550 (amide II) $cm^{-1}$. The above protein functional groups band assignments and their relative biological meaning were obtained from publications.[5−8] False color maps derived from the area under the protein spectral features were used to represent the distribution and intensity of functional groups across the area of interest in the canola seed tissues. [9]

## RESULTS AND DISCUSSION

**Univarite Molecular Spectral Analysis To Monitor Molecular Changes Associated with Heat-Related Processing.** Protein value and functionality depend on its structure (1°, 2°, 3°, and/or 4°) mainly because the structure may affect digestive enzyme attachment in animal digestive tract or affect rumen bacteria attachment in rumen for degradation. Therefore, the structure change may affect the protein value and functionality. For a long time, when check processing the effect, we usually use indirect methods to check processing effects by measuring digestion and degradation without directly measuring the structure change affected by processing because of the technology limitation at that time. Now with the synchrotron-based technique, the structure changes could be checked by monitoring molecular spectral changes within inherent protein structure. SR-IMS makes microlocalization of the tissue structure possible.[5,8−11] Two unique primary features of protein (amides I and II) can be used to check the protein infrared spectral feature.[7,12−14] Because the research shows that the amide I band is sensitive to the protein secondary structure ($\alpha$-helix, $\beta$-sheet, random coil, and/or $\beta$-turn), so it is usually used for protein secondary structure characterization[8,11−14] Figures 1 and 2 show the second-derivative and Fourier self-deconvolution (FSD) spectra for the raw and heated tissue which were used to identify component peaks. The results showed that heat-related processing had some impact on the protein inherent structure


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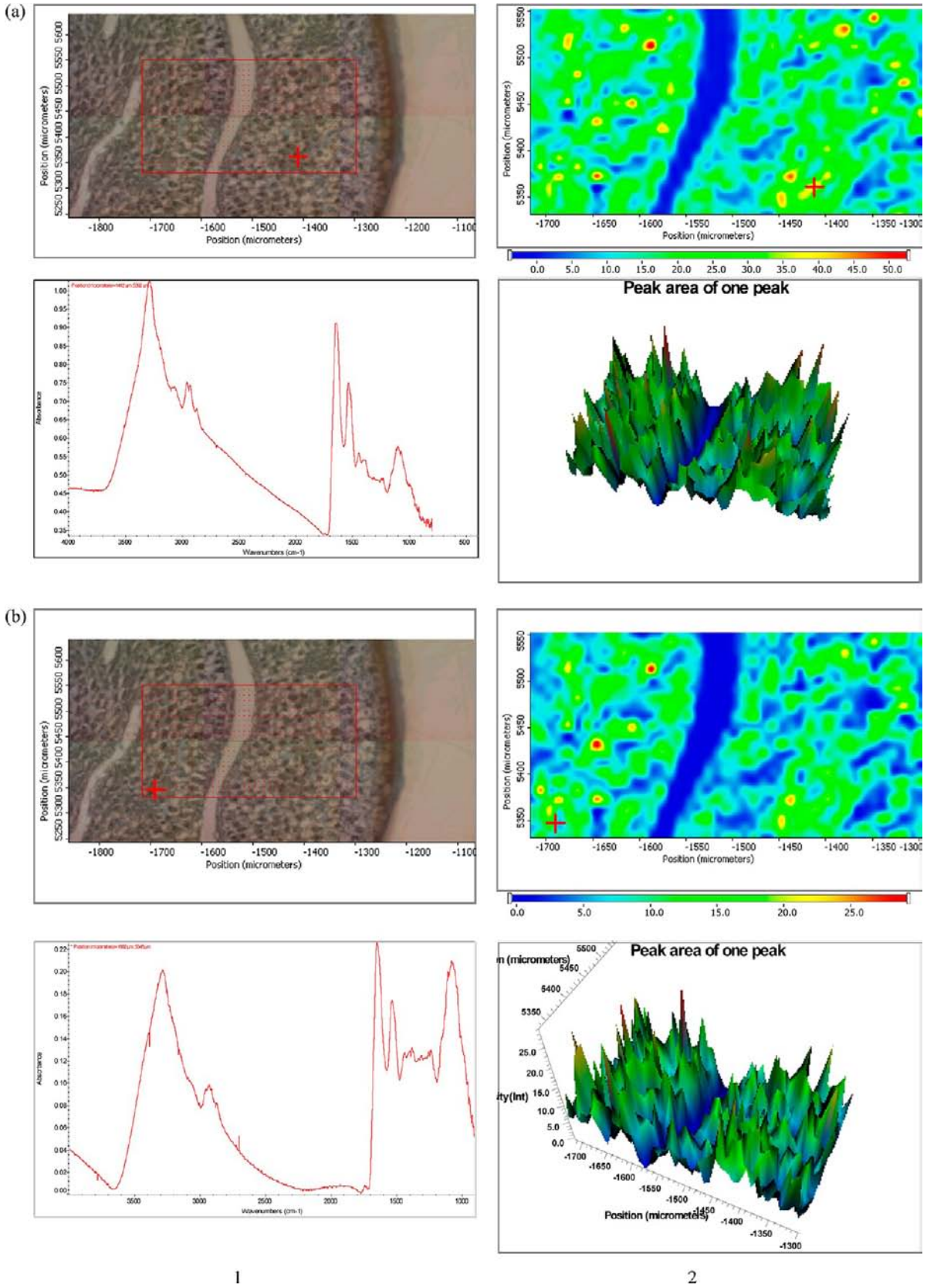


**Figure 3.** Imaging protein amides I and II of the raw black type of canola (*Brassica*) seed tissue (control): (1) visible image and spectra corresponding to the pixel at the cross-hair in the visible image; (2) chemical image. (a) Area under ca. 1655 $cm^{-1}$ peak (amide I). (b) Area under ca. 1550 $cm^{-1}$ peak (amide II).

in the black type of canola tissues because the spectral pattern/feature at the amide I and II region were apparently different in the original spectra and their second-derivative spectra (Figure 1) and FSD spectra (Figure 2). The results showed that with


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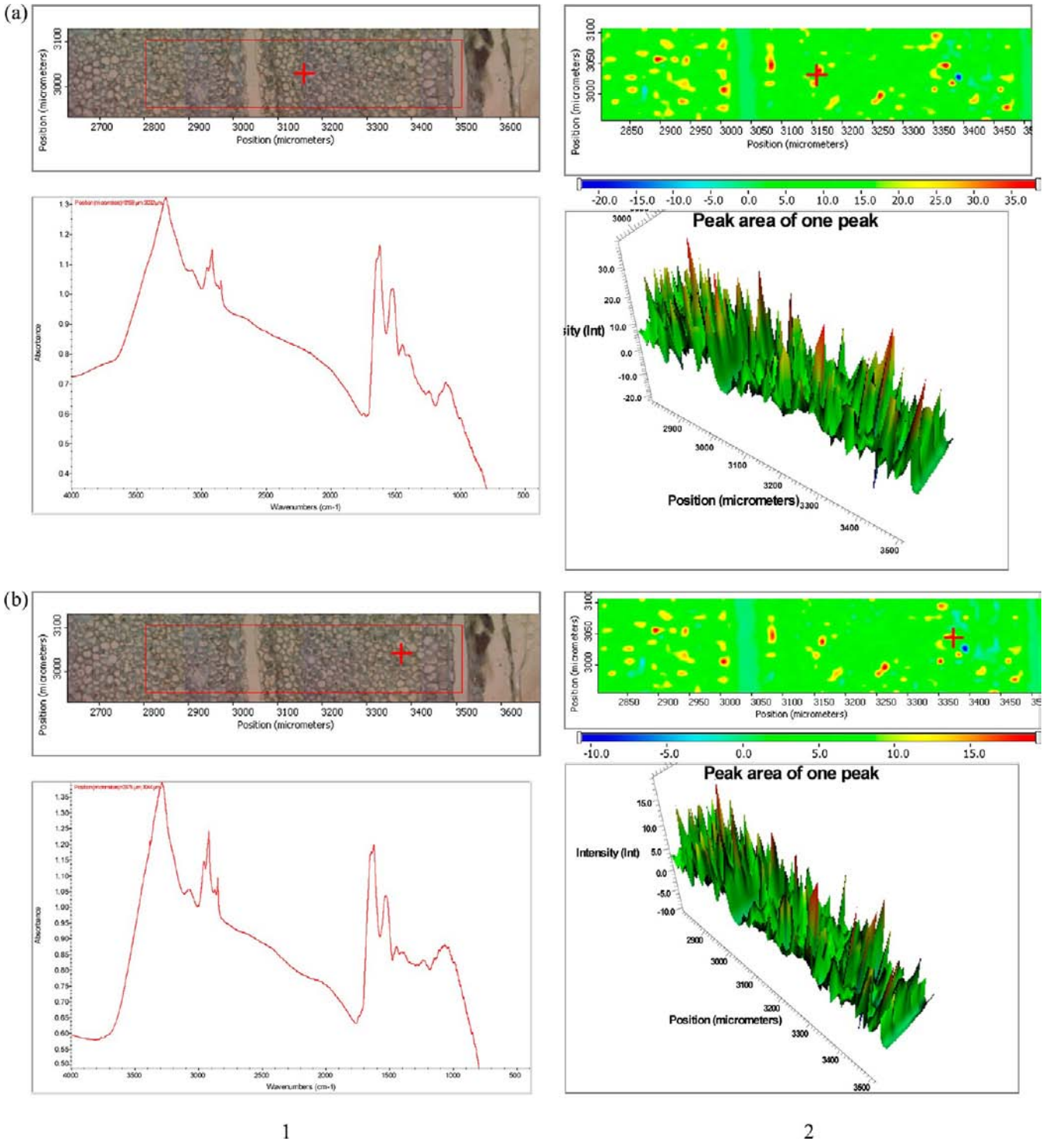


**Figure 4.** Imaging protein amides I and II of the wet-heated black type of canola (*Brassica*) seed tissue: (1) visible image and spectra corresponding to the pixel at the cross-hair in the visible image; (2) chemical image. (a) Area under ca. 1655 $cm^{-1}$ peak (amide I). (b) Area under ca. 1550 $cm^{-1}$ peak (amide II).

the synchrotron technique the heat-induced molecular changes of protein second structure (Figure 1) could be monitored and characterized within intact tissues (without damaging the tissues). Wet heating had a greater impact than dry heating. It seems that it had a higher $\beta$-sheet to $\alpha$-helix spectral intensity ratio. Dry heating had less impact on the black type of canola tissue with a similar $\beta$-sheet to $\alpha$-helix intensity ratio to the raw canola tissue by visual checking. The results indicated that this noninvasive technique could be used as a potential tool to directly measure various treatment effects on inherent structure not only on the protein amide spectral region but also on other biopolymer spectral regions of interest.

**Visualizing Tissue Molecular Structure of a Black Type of Canola (*Brassica*) Seed after Processing through Imaging.** The results showed that using the synchrotron imaging technique we could obtain chemical imaging without


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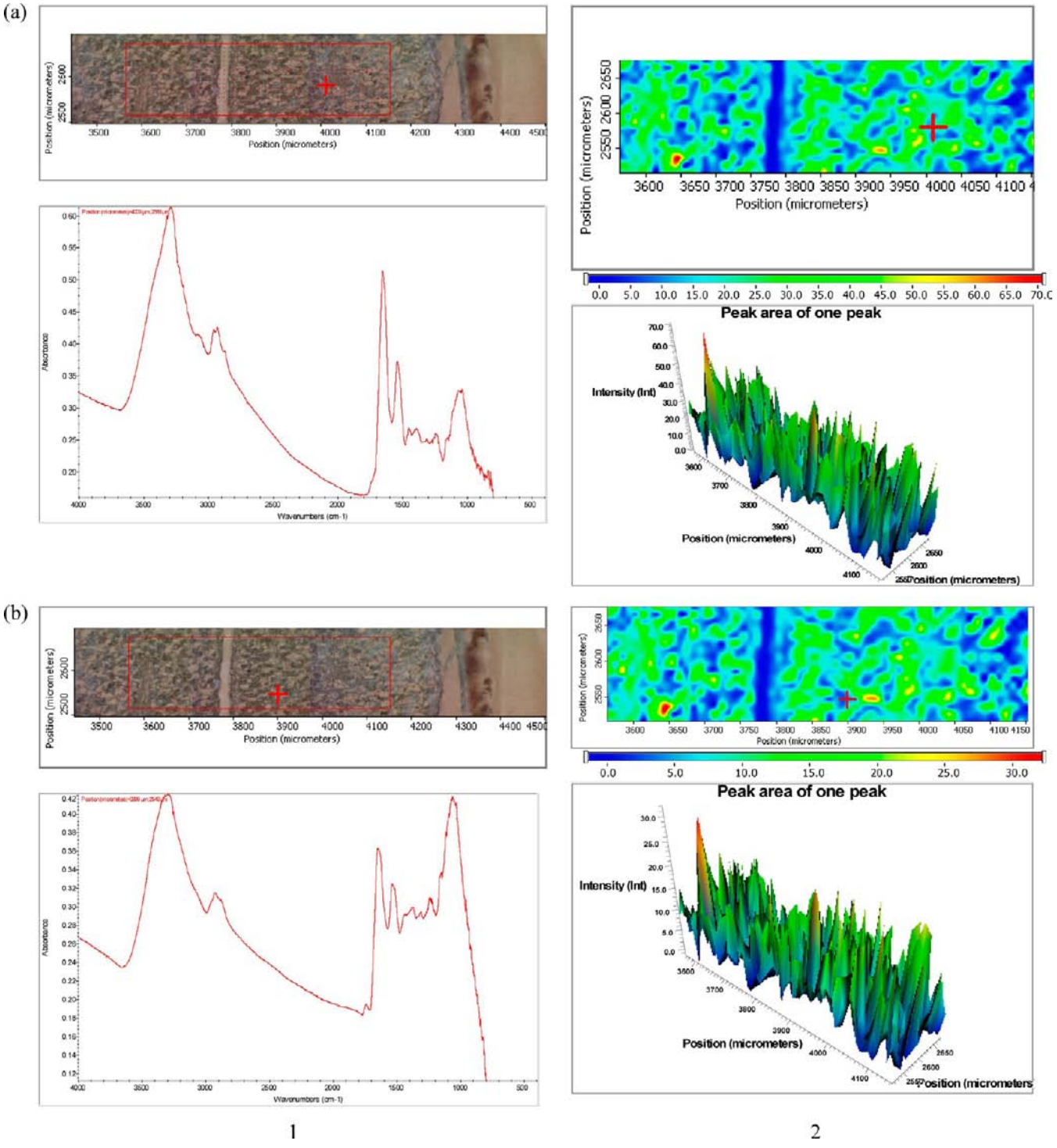


**Figure 5.** Imaging protein amides I and II of the dry heated black type of canola (*Brassica*) seed tissue: (1) visible image and spectra corresponding to the pixel at the cross-hair in the visible image; (2) chemical image. (a) Area under ca. 1655 $cm^{-1}$ peak (amide I). (b) Area under ca. 1550 $cm^{-1}$ peak (amide II).

damaging the tissues themselves. Unlike the "wet" chemical method (such as "lab digestion" before GC, HPLC analyses), it always damages the tissue intrinsic structure due to the use of chemicals and digestion during analyses. The SR-IMS imaging technique is able to show seed tissue architecture, conformation, and biopolymer distribution (e.g., cellulosic compound, lignin compound) based on the functional group spectral feature. [4] Figures 3−5 show that the images (intensity and distribution) of the amides I and II functional groups were visualized among the raw (control), wet-heated, and dry-heated modeled black type of seed tissues. The results indicated that the heat-related methods may affect structure in different ways. These different imaging types might indicate potentially different biological values (e.g., protein functionality, biological utilization) between the heated treatments in humans and animals. However, we are still not sure of the accurate differences between the images. Hopefully in the future we can develop a rapid, reliable, and accurate method to link tissue


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image features to nutrient utilization and the digestion feature, which will be very useful. In the previous study, the results showed the yellow type of canola seeds were also affected by processing. However, the yellow type of canola seeds is completed different from the black type of canola seeds, because the yellow-type of canola seeds has a much thinner seed coat and lower fiber and lignin contents. It is expected that the yellow type of canola seed might be more sensitive to heat-related processing because of the thinner seed coat. Heating might easily penetrate tissues to produce a more profound effect in the yellow type of canola seed than the black type of canola seed. Therefore, inherent structures of the yellow type of canola tissues are more sensitive to be changed by the processing. Future study is needed to compare the response and sensitivity of canola seed to various processing between the yellow type and the black type of canola seeds at the same time, to develop a rapid and sensitive method to compare the exact chemical image difference between tissues and between treatments, to develop a method to link images to nutrient digestion, and to reveal how the structure changes affect nutrient absorption in human and animals. There is a long way to go.

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### Notes

The authors declare no competing financial interest.

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