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Explore protein molecular structure in endosperm tissues in newly developed black and yellow type canola seeds by using synchrotron-based Fourier transform infrared microspectroscopy

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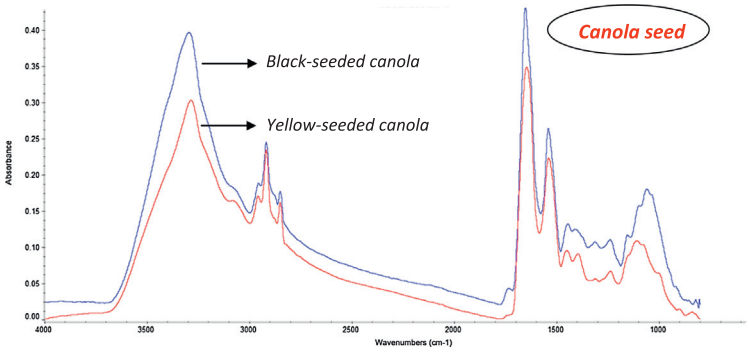
HIGHLIGHTS

- Yellow canola seeds contained relatively higher percentage of a-helix and β-sheet than black canola seeds.
- Yellow canola seeds contained higher percentage of amide I and amide II area compared to the black canola seed.
- Not significant molecular structural differences in protein amide I and II region between yellow and black canola seed.

GRAPHICAL ABSTRACT



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ABSTRACT

This study was conducted to characterize the protein molecular structure in endosperm tissues in newly developed black and yellow-type canola seeds by using synchrotron-based Fourier transform infrared microspectroscopy. The results showed that the yellow canola seeds contained relatively lower ($P < 0.05$) percentage of β-sheet and amide I and amide II area compared to the black-type canola seed. This might be an indication that the protein value of the yellow canola seeds as food or feed is different from that of the black canola seeds. The multivariate molecular spectral analyses (AHCA, PCA) showed that there were not significant molecular structural differences in the protein amide I and amide II fingerprint region (*ca.* 1720–1480 cm^{-1}) between the yellow and the black-type of canola seed. It can be concluded that both the yellow and the black-seeded canola contain the same proteins but in different ratios.

Introduction

Canola is a major oil-seed crop in western Canada and was developed from rapeseed by Canadian plant breeders in 1970s. Unlike with the traditional rapeseed, canola contains low levels of "erucic acid" in the oil portion (<2% of total fatty acids in the oil) and low levels of anti-nutritional compounds called "glucosinolates" in the meal portion (<30 μmol in its defatted meal) [1]. The most common canola varieties used in Western Canada are the yellow-seeded and dark-brown-seeded varieties. It has been reported that yellow-seeded varieties are lower in fibre than brown-seeded types [2–4].

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Research studies are mainly focused on total chemical composition of canola seeds including total protein, carbohydrates and dietary fibre components using the traditional "wet" chemical analysis [3,4]. The traditional analytical chemistry is currently employed to study a specific tissue component which is often separated it from the whole complex matrix. Consequently, the object prepared for analysis is usually destroyed and the information about the spatial origin and the component distribution is lost [5]. According to our knowledge, little research has been carried out to study the chemical bonding and functional groups of a feed seed tissue within cellular dimensions. It is supposed that these features are closely related to feed's quality and nutritive value for humans as well as for animals' diets.

One of the most important nutrients in animal and human diets is protein. Traditionally, when we determine protein quality in a feed or a food, we usually determine total protein or amino acid content. Accordingly, the resulting values are compared to a standard to determine the specific protein quality. Due to the fact that, quality of protein relies not only on its content but also on its inherent structures (i.e. nutrient matrix and protein secondary structures), the method mentioned before appears to have a weakness [6]. Protein quality, nutrient utilization, availability or digestive behavior is associated with the access of gastrointestinal digestive enzymes to the protein, which is affected by the inherent protein structure [7–10]. The mostly occurring protein secondary structures include the α-helix and the β-sheet [11–13]. For the reasons mentioned, examine the protein secondary structure may be vital for understanding the digestive behavior, utilization and protein availability in both humans and animals [9].

Synchrotron-based vibrational FTIR microspectroscopy has been employed in order to reveal molecular structural features within the tissue in different kind of materials [14]. This technique is able to explore the molecular chemistry of biological samples with high signal-to-noise ratio at ultraspatial resolutions as fine as 3–10 μm due to the synchrotron light brightness [14–19]. This technique can be applied to detect information on ultrastructural chemistry by imaging or mapping without destruction of the intrinsic microstructures of plant tissue [20,21]. Research studies carried out previously, mentioned that using synchrotron-based FTIR microspectroscopy is feasible to (i) compare plant tissue (i.e. species or varieties) according to spectroscopic characteristics, functional groups, spatial distribution and chemical intensity within cellular dimensions and (ii) relate plant molecular structures to availability and utilization of plant tissue components [20–24].

The objective of this study was to use synchrotron light sourced FTIR microspectroscopy as an approach to define the molecular structural differences between the proteins of two newly developed canola seed varieties in the whole tissues at cellular and subcellular levels. Therefore is hypothesized that different types of canola seed (yellow, black) have different protein molecular structure profiles which may be detected by synchrotron infrared microspectroscopy.

Materials and methods

Feed samples

Yellow (*B. napus*) and black (*B. napus*) canola seeds were supplied by the Agriculture and Agri-Food Canada, Saskatoon Research Center, Canada and used as feed sources. Yellow and black canola seeds were collected from two different harvest years 2010 (total 4 kg) and 2011 (total 4 kg).

Synchrotron-based BaF2 window preparation

Seven seeds were randomly selected from each treatment (1 = CS_Y10, 2 = CS_Y11, 3 = CS_B10 and 4 = CS_B11) and then each seed was cut into thin cross sections (6 μm thickness per section; five sections per treatment) using a Microm 330 microtome (Microm Laborgerate GmbH, Sandhausen, Germany) at the Western College of Veterinary Medicine, University of Saskatchewan. A more detailed methodology concerning the slide preparation was reported previously by Yu et al. [21], however here it will be briefly described. Unstained cross-sections were transferred to BaF2 windows (size: 13 × 1 mm disc; Spectral Systems, Hopewell Junction, NY, USA) for synchrotron-based microspectroscopic analysis in transmission mode. Photomicrographs of tissues cross-section were taken with a microscope linked to a digital camera from the BaF_2 window at U2b station in National Synchrotron Light Source at Brookhaven National Lab (NSLS-BNL, US Department of Energy, New York).

Synchrotron-based Fourier transform infrared microspectroscopy data collection and analysis

Synchrotron experiment was carried out at the National Synchrotron Light Source in the Brookhaven National Laboratory (NSLS-BNL, US Department of Energy, NY). The detailed methodology has been reported previously [25] and the following is a brief explanation. The beamline U2B was equipped with a FTIR spectrometer (Magna-IR 860 Spectrometer, Nicolet Instruments, Madison, WI, USA) outfitted with a KBr beamsplitter and a liquid nitrogen-cooled mercury cadmium telluride (MCT) detector coupled to a Nic-Plan IR microscope, a Schwartzshild 32×objective, and a 10×condenser. Synchrotron radiation from the Vacuum Ultraviolet storage ring (with an energy level of 800 MeV) entered the interferometer via a port in the instrument designed for infrared emission. A range of 30–50 spot samples for each seed was randomly scanned in the relatively pure protein area in the endosperm region approximately 100–600 μm from the epidermis. The regions of high protein content were used in order to collect randomly the spectra within the mid-infrared range from 4000 to 800 cm^{-1} at a resolution of 4 cm^{-1} (128 co-added scans) and an aperture of 10_m × 10_m. Scanned visible images were obtained by using a charge-coupled device camera connected to the infrared images.

Univariate and multivariate spectral data analyses

For the analysis of the spectral data obtained at the synchrotron-based Fourier transform infrared microspectroscopy, the univariate and multivariate approaches were adapted. The univariate method consists of various spot sampling and mapping displays of spectral data [26,27]. On the other hand, the multivariate analysis method utilizes the entire spectral information and creates spectral corrections. The agglomerative hierarchical cluster analysis (AHCA) which uses the Ward's algorithm method without prior parameterization, and the principal component analysis (PCA) are encompassed in the multivariate method.

Univariate molecular spectral analysis of protein structure profiles

The spectrum collection, the corrections with the background spectrum, the classification of protein's structure functional spectral bands and the data analysis was carried out using the software program OMNIC 7.3 (Spectra Tech, Madison, WI, USA).

Protein amide I and II peak area intensities as well as absorption peak heights for secondary protein structure α-helices (*ca.* 1660 cm^{-1}) and β-sheets (*ca.* 1630 cm^{-1}) are detectable in the