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# Detect molecular spectral features of newly developed *Vicia faba* varieties and protein metabolic characteristics in ruminant system using advanced synchrotron radiation based infrared microspectroscopy: A preliminary study

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ABSTRACT

Recently, advanced synchrotron radiation-based Fourier transform infrared microspectroscopy (SR-IMS) has been developed as a rapid, direct, non-destructive and bioanalytical technique. To date, there has been very little application of this technique to study the molecular structure make-up in pulse seeds. Thus, the objectives of this study were to detect the interactive association between protein molecular structure and nutrient availability of newly developed *Vicia faba* varieties. Two different varieties of faba beans (CDC Snowdrop = low-tannin variety; vs. FB9-4 = high-tannin variety) were selected for this study. The molecular spectra data were collected by using SR-IMS. The ratio of both amide I to II area and height were higher ($P < 0.01$), while the ratio of α-helix to β-sheet was lower ($P < 0.05$) in CDC Snowdrop compared to FB9-4. The crude protein (CP) content and the predicted truly digestible nutrients as well as the bioenergy values did not vary between two varieties. The CDC Snowdrop had exhibited a higher ($P < 0.01$) rapidly degradable CP fraction (75.99 vs. 71.45% on CP) and a lower ($P < 0.01$) moderately degradable CP fraction (19.43 vs. 22.85% on CP), resulting in a higher ($P < 0.01$) rumen degradable protein and a lower ($P < 0.01$) rumen undegradable protein content than that of FB9-4 variety. However, the total supply of digestible rumen undegraded feed protein was higher ($P < 0.05$) in FB9-4 than CDC Snowdrop. Strong positive correlations were found between the ratio of α-helix to β-sheet and CP contents ($R = 0.86$, $P < 0.01$) as well as the truly digestible CP contents ($R = 0.83$, $P < 0.01$); respectively. In conclusion, the results of this study reveal that the protein are metabolized differently between different type of faba bean varieties and the advanced SR-IMS molecular spectroscopy can be used to rapidly delineate protein molecular structure motifs along with their nutritive value in ruminant livestock system.



## 1. Introduction

Synchrotron radiation-based infrared microspectroscopy (SR-IMS) has recently been developed as a rapid, direct and non-destructive technique to reveal internal chemical structure changes at a cellular or subcellular dimension among various food/feed [1–3]. The advantage of using SR-IMS is being its brighter lights (100–1000 times) compared to conventional globar source light, which can provide a higher signal-to-noise ratio at high-spatial resolutions within plant cellular dimensions [4–6]. Moreover, recent studies revealed that nutrient utilization, bioavailability and overall feed quality are highly linked to the intrinsic molecular structures of feed [7,8]. For instance, protein amide I and II spectral profiles (e.g., peak intensity, ratio of functional groups) were found to be highly related to the protein value [9].

Faba beans (*Vicia faba* L.), also widely known as fava or horse beans, could potentially be used as protein supplements in ruminant diets due to its high protein (25–30%) and starch (30–40%) contents [10–12]. The faba bean protein is extensively and rapidly degradable in rumen and the portion that is not degraded in the rumen are usually accessible later in the intestinal tract [13]. The highly soluble nature of the protein in faba beans that makes them easily degraded in the rumen though provide a surge of nitrogen (N) substrate for ruminal microbes, the excessive degradation may cause protein loss through urinary N excretion. However, the presence of some tannins in faba beans (mostly in hulls) can reduce the protein degradation in rumen but allow it be

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subsequently digested post-ruminally [14]. In Canada, a 350% increase in faba bean production between 2013 and 2016 has been reported, with further increases expected. The most commonly grown faba bean varieties in the industry to-date are classified as "low" or "high-tannin" varieties. Recently, there many new varieties of faba been have been introduced in Canada. However, to the best of our knowledge, no research has been conducted yet on protein structural motifs and protein metabolic characteristics of these new varieties (low vs. high-tannin varieties) of faba beans. Moreover, as feed intrinsic structures often being destroyed in wet chemical analyses, the association between feed nutrient molecular structure and its utilization and availability cannot be detected by these traditional processes.

In contrast to traditional "wet" chemical methods, synchrotron radiation-based Fourier transform infrared microspectroscopy (SR-IMS), taking advantage of the synchrotron light brightness (million times brighter than sunlight) and small effective source size, is capable of exploring the molecular chemistry within microstructures of a biological tissue without destruction the inherent structures of feed at high diffraction-limited spatial resolution. To date there has been very little application of this advanced technique to study the structural motifs related to protein region in pulse seeds.

Therefore, the objectives of this study were to compare: (a) molecular structure features using the advanced synchrotron technique-SR-IMS; (b) protein related chemical profiles; (c) metabolic characteristics of protein and the bio-energy values between two varieties of faba beans; and (d) to specify the association between molecular structure and protein chemical profiles as well as the predicted metabolic profiles of protein and energy.

## 2. Materials and Methods

### 2.1. Faba Bean Samples

Two distinct varieties of faba beans, namely CDC Snowdrop (low-tannin variety) and FB9-4 (high-tannin to variety) were collected from Crop Development Center, University of Saskatchewan, Canada. For synchrotron-based structure study, randomly five seeds were taken from each sample. The samples were ground through a 1 mm screen using a laboratory mill (Retsch ZM-1, Brinkmann Instruments Ltd., Mississauga, ON, Canada) for wet chemical analysis.

### 2.2. Synchrotron Radiation-based Infrared Microspectroscopy

The randomly selected seeds for the molecular structure study from each sample were first cut into thin cross-sections (6 μm thickness) using a microtome at the Western College of Veterinary Medicine at the University of Saskatchewan, Canada. The unstained cross-sections were then transferred to $BaF_2$ windows (size = 13 × 1 mm disk; Spectral Systems, Hopewell Junction, NY) for transmission mode synchrotron infrared microspectroscopic study. Photomicrographs of cross-section of the tissues on $BaF_2$ windows were taken with a microscope linked to a digital camera from the Beamline 1.4 (Infrared microspectroscopy) station in Advanced Light Source at Lawrence Berkeley National Laboratory (ALS-LBNL, U.S. Department of Energy, 6 Cyclotron Rd, Berkeley, CA 94720, USA). The ALS synchrotron beamtime was arranged by Dr. Lisa Miller at National Synchrotron Light Source at Brookhaven National Laboratory (NSLS-BNL, U.S. Department of Energy, Upton, NY). Mr. Randy Smith and Dr. Lisa Miller (NSLS-BNL, New York) and Dr. Hans Bechtel (ALS-LBNL, Berkeley) provided helpful synchrotron data collection at ALS and NSLS. The pre-methodology study on general feed structure was performed at 01B1-1 beamline station, Canadian Light Sources (CLS). The detailed procedure has been previously described [3]. In brief, the tissue spectra were collected in the mid-IR range, 4000–800 $cm^{-1}$, at a resolution of 4 $cm^{-1}$ with 128 scans co-added and an aperture setting of ca. 10 × 10 μm. A background spectrum was collected from an area free of sample with 256 scans co-added. The baseline correction was done for each spectrum. For each seed, a total of 10 spectra were collected from the cotyledon area. Each sample had a total of 50 spectra (=5 seeds/sample × 10 spectra/seed). The OMNIC 7.3 (Spectra-Tech Inc., Madison, WI) software was used to collect and process the data. The assignment of functional group bands were referred to in the literature published previously [9,15].

### 2.3. Univariate Spectral Analysis

Univariate molecular spectral analysis from SR-IMS was carried out by using OMNIC 7.3 software (Spectra Tech., Madison, WI, USA). Regions of specific interest in our study involved protein functional group band assignments. Protein parameters included amides I and II height and area, α-helix and β-sheet peak height and their corresponding spectral ratios. The amide spectral region in this study was approximately ca. 1724–1478 $cm^{-1}$. Protein amides I and II peaks fell within the range of ca. 1662–1641 $cm^{-1}$ and ca. 1552–1529 $cm^{-1}$, respectively, and the peaks of modeled α-helix and β-sheet ranged ca. 1660–1641 $cm^{-1}$ and ca. 1635–1621 $cm^{-1}$, respectively, which were identified by using 2nd derivative spectral function. Spectral peak intensity height and area ratios were calculated based on spectral data.

### 2.4. Multivariate Molecular Spectral Analysis

The spectral differences in the finger print region (ca. 1724–1478 $cm^{-1}$) in cotyledon tissue were analyzed by multivariate analyses. In the current study, two different multivariate methods were employed to perform multivariate spectral analysis using Statistical 8.0 (StatSoft Inc., Tulsa, OK, USA). Agglomerative hierarchical cluster analysis (CLA), which uses Ward's Algorithm method without parameterization for clustering, presents results as dendrograms [16–18]. Principal Component Analysis (PCA), which is the other multivariate analysis method, transforms all interrelated variances into new uncorrelated variances called principles components (PCs) [16–18]. The result of PCA is presented as a scatter plot using two main PCs, which took >90% of variance of PC1 vs. PC2. The detailed procedures of these two methods were reviewed in series of papers [16–18].

### 2.5. Chemical Analysis

The standard methods of the AOAC [19] were used to analyze the contents of dry matter (DM; method 930.15), CP (method 984.13; adopted for Kjeltec 2400 autoanalyzer; Foss Analytical A/S, Hillerød, Denmark), ash (method 942.05), and ether extract (EE, method 920.39). The content of soluble crude protein (SCP) was analyzed according to the method of Roe et al. [20] with slight modifications as described by Peng et al. [21]. The contents of acid detergent-insoluble protein (ADICP), neutral detergent-insoluble protein (NDICP), and non-protein N (NPN) were analyzed according to the methods described by Licitra et al. [22].

### 2.6. Protein Subfractions

The Cornell Net Carbohydrate Protein System (CNCPS) version 6.5 was used to estimate protein fractions, as well as rumen degradable protein (RDP), rumen undegradable protein (RUP), in the two newly developed varieties of faba bean [23]. The CP was divided into five fractions based on different degradation rates in the rumen. The subfraction PA1, which represents ammonia N in version 6.5 and is considered to degrade in the rumen at the rate of 200%/h, was estimated to be zero for these faba bean samples. The soluble portion of CP (PA2) is the rapidly degradable CP fraction (10–40%/h), was estimated as (SCP-PA1). The subfraction PB1 is considered as moderately degradable CP fraction (3–20%/h) and was calculated as (CP-PA1-PA2-PB2-PC). The PB2 subfraction is the slowly degradable CP fraction (1–18%/h) and was estimated as (NDICP-ADICP). The PC was considered as undegradable