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Using vibrational molecular spectroscopy to reveal association of steam-flaking induced carbohydrates molecular structural changes with grain fractionation, biodigestion and biodegradation

Ningning Xu [a,b], Jianxin Liu [a,*], Peiqiang Yu [b,c,**]

[a] College of Animal Sciences, Zhejiang University, Hangzhou, China
[b] Department of Animal and Poultry Science, University of Saskatchewan, Saskatoon S7N 5A8, Canada
[c] College of Life Science and Engineering, Foshan University, Guangdong, China

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ABSTRACT

Advanced vibrational molecular spectroscopy has been developed as a rapid and non-destructive tool to reveal intrinsic molecular structure conformation of biological tissues. However, this technique has not been used to systematically study flaking induced structure changes at a molecular level. The objective of this study was to use vibrational molecular spectroscopy to reveal association between steam flaking induced CHO molecular structural changes in relation to grain CHO fractionation, predicted CHO biodegradation and biodigestion in ruminant system. The Attenuate Total Reflectance Fourier-transform Vibrational Molecular Spectroscopy (ATR-Ft/VMS) at SRP Key Lab of Molecular Structure and Molecular Nutrition, Ministry of Agriculture Strategic Research Chair Program (SRP, University of Saskatchewan) was applied in this study. The fractionation, predicted biodegradation and biodigestion were evaluated using the Cornell Net Carbohydrate Protein System. The results show that: (1) The steam flaking induced significant changes in CHO subfractions, CHO biodegradation and biodigestion in ruminant system. There were significant differences between non-processed (raw) and steam flaked grain corn ($P < .01$); (2) The ATR-Ft/VMS molecular technique was able to detect the processing induced CHO molecular structure changes; (3) Induced CHO molecular structure spectral features are significantly correlated ($P < .05$) to CHO subfractions, CHO biodegradation and biodigestion and could be applied to potentially predict CHO biodegradation ($R^2 = 0.87$, RSD = 0.74, $P < .01$) and intestinal digestible undegraded CHO ($R^2 = 0.87$, RSD = 0.24, $P < .01$). In summary, the processing induced molecular CHO structure changes in grain corn could be revealed by the ATR-Ft/VMS vibrational molecular spectroscopy. These molecular structure changes in grain were potentially associated with CHO biodegradation and biodigestion.

1. Introduction

Feed processing technology, such as pressure toasting, steam flaking, extrusion, has been used to improve nutritive value and nutrient supply to animal due to several reasons such as limited feed resource, improving feed nutritive value, favourablely changing biodegradation and biodigestion features. However, the research on how processing change internal molecular structure and how molecular structure changes affect nutrient availability are still rare, except the research team at the SRP Key Lab of Molecular Structure and Molecular Nutrition, Ministry of Agriculture Strategic Research Chair Program (SRP) at the University of Saskatchewan, Canada.

The feed resource, especially high quality forage, is seriously insufficient, and is becoming bottleneck of the whole industry. Many studies have tried to supply dairy cattle low quality forage with supplementation of non-fiber carbohydrate (NSC) to maintain the high milk yield [1–4]. Corn grain is the most important NSC source for dairy cattle, and several types of corn products have been used in dairy farm, many researches have studied the effect of corn grain on milk production and gastrointestinal digestion characteristics to make better use of corn [5,6]. However, little research has concentrated on the inherent molecular basis of corn degradation in dairy cow, little research explained the different degradation characteristics at a molecular level.

Advanced Attenuate Total Reflectance Fourier-transform Vibrational Molecular Spectroscopy (ATR-Ft/VMS) has been developed recently as a new non-destructive and non-invasive technology for identifying the inherent molecular structure without destroying the chemical covalent

* Corresponding author at: Institute of Dairy Science, College of Animal Sciences, Zhejiang University, Hangzhou 310058, China.
** Corresponding author at: Ministry of Agriculture Strategic Research Chair, University of Saskatchewan, Room 6D34, 51 Campus Drive, Saskatoon S7N 5A8, SK, Canada.
E-mail addresses: liujx@zju.edu.cn (J. Liu), peiqiang.yu@usask.ca (P. Yu).

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bond. Combining with chemometrics, a linkage between nutrients chemical information and spectral intensity information could be developed [7–10]. Thus, intrinsic structure, basing on spectral intensity features, could be used to quantize nutrients information [11].

The energy availability and CHO supply to dairy cows could be elucidated by Cornell Net Carbohydrate Protein System (CNCPS) carbohydrate subfractions [12]. In the previous study [13], we reported the alteration of biomacromolecule in corn by steam flaking. The objective this study was to use an advanced vibrational molecular spectroscopic approach to reveal association between steam flaking processing induced molecular structural changes of intrinsic carbohydrates (CHO) conformation in cereal grain of corn and CHO fractionation, biodegradation and biodigestion in ruminants. The hypothesis was that CNCPS CHO subfractions and CNCPS-based CHO degradation in dairy cows were highly associated with CHO internal structures of corn.

2. Materials and Methods

2.1. Feed Processing and Samples for Molecular Structure Study

Four raw corn seed samples (Code = C1, C2, C3 and C4) and four steam flaked corn samples (code = SF1, SF2, SF3 and SF4) were used to detect molecular structure features, carbohydrate subfractions and digestion characteristics. The detailed sampling and feed processing condition were reported previously [13]. The raw corn grains were warm-seasoned corn grown in 2015, and the steam flaked corns were produced in the steam flaking plant. All the samples were ground through 1 mm mesh (Retsch ZM-1) for subsequent chemical test and spectral collection at University of Saskatchewan (Saskatoon, Canada).

2.2. Chemical Profiles

According to standard methods by AOAC [14] (2005), content of dry matter (DM), crude protein (CP), Ash and ethanol extract (EE) were obtained, Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were analyzed by Ankom Fiber Analyzer (ANKOM Technology Corp., Fairport NY) based on filtration method with ash included [15]. Residues of NDF and ADF were applied to detect neutral detergent insoluble CP (NDICP) and acid detergent insoluble CP (ADICP) using Kjeldahl–N Method [16]. Starch content was determined by Megazyme Total Starch Assay Kit (Megazyme International Ltd., Wicklow, Ireland). Water soluble carbohydrates (WSC) were determined using anthrone sulfuric acid colorimetric method [17]. A 288 h of in situ degradation procedure was performed to get undigested NDF content (iNDF) [18].

2.3. Carbohydrate Fractionation

Based on the most recent updated CNCPS version 6.5 [19], carbohydrate was partitioned into eight subfractions (CA1, CA2, CA3, CA4, CB1, CB2, CB3, CC). All organic acids were redefined as CA1, CA2 and CA3, water soluble carbohydrates were characterized as CA4 (with degradation rate as 40–60%/h). Starch, soluble fiber and digestible fiber were identified as CB1 (with degradation rate as 20–40%/h), CB2 (with degradation rate as 20–40%/h) and CB3 (with degradation rate as 4–9%/h), respectively. Indigestible fiber was identified as CC (with degradation rate as 0%/h). Since no organic acids were expected in corn and flaked corn [12]. The CHO subfractions of interest in this study were CA4, CB1, CB2, CB3 and CC. CNCPS was used to predict rumen degradable (RD) and undegradable (RU) carbohydrate. The intestine digestible rumen undegradable CHO (DRUC) was calculated by subtracting the completely undegradable CHO (CC) subfraction from rumen undegradable carbohydrate (RUCHO) [20].

2.4. Carbohydrate Molecular Spectral Study

The molecular spectral intensity of various corn samples was collected by a JASCO (4200, JASCO Corp., Tokyo, Japan) Fourier-transform Vibrational Molecular Spectroscopy with Attenuated Total Reflectance (ATR-Ft/VMS) at the Molecular Spectroscopy Lab of Ministry of Agriculture Strategic Research Chair, Department of Animal and Poultry Science, University of Saskatchewan, Canada. All samples were ground through 0.12 mm mesh, and then molecular spectra data was collected at mid-infrared fingerprint region (ca. 4000–800 cm^{-1}) with 128 co-added scans of the samples and 256 co-added scans of background at a resolution of 4 cm^{-1}. Five replicates of each corn sample. Then OMINC 7.3 (Spectra Tech, Madison, WI, USA) software was applied to analyze the spectra data.

2.5. Chemometrics and Molecular Spectral Analyses

CHO functional group bands associated parameters [21,22] were obtained as follows: total CHO region was ca. 1187–950 cm^{-1} depending on the bond linkage [23] and C═O stretching vibrations. Three major sub-peaks were detected within CHO fingerprint region as CHO Peak1 (P1: ca. 1187–1132 cm^{-1}), CHO Peak2 (P2: ca. 1132–1066 cm^{-1}) and CHO Peak3 (P3: ca. 1066–950 cm^{-1}). Structure CHO (SCHO) fingerprint region was ca. 1265–1214 cm^{-1}, non-structure CHO fingerprint region including two distinguishable peaks: Peak_928 (ca. 950–881 cm^{-1}) and Peak_860 (ca. 881–820 cm^{-1}). β-glucan [24] area was ca. 1448–1390 cm^{-1} and height at ca. 1413 cm^{-1}. Values of spectral peak heights and areas were obtained through OMNIC 7.3.

Statistica 8.0 software (StatSoft Inc., Tulsa, OK, USA) was applied to agglomerative hierarchical cluster analysis (CLA) and principal component analysis (PCA) of spectroscopic data within the total CHO region (ca. 1187–950 cm^{-1}) and non-structure CHO region (ca. 950–820 cm^{-1}). Multivariate analysis was performed to distinguish inherent spectral intensity difference among different types of corn.

2.6. Statistical Analysis

PROC MIXED program of SAS 9.4 was performed for CHO subfractions, rumen degradable and undegradable CHO fractions with the following model: $Y_{ij} = \mu + trt_i + e_{ij}$, where, Y_{ij} is the dependent variable ij, trt_i is the effect of treatments, e_{ij} is the random error related to ij. Residual test was performed to test the model assumption.

2.6.1. Correlation Analysis

PROC CORR of SAS 9.4 was applied to detect (1) association between CHO subfractions and molecular spectral feature, (2) relationships between rumen degradable and undegradable CHO subfractions and molecular spectral features. PROC UNIVARIATE with plot and normal option was applied to test data normality.

2.6.2. Multiple Regression Analysis With Model Variable Selection

PROC REG procedure of SAS 9.4 with reversed stepwise option was performed to select most associated molecular spectral parameters to predict CHO nutrients supply. PROC UNIVARIATE program with normal and plot options was applied to test residual normality of the regression model.

For all statistical analyses, $P < .05$ declare as significant difference and $P \leq .10$ as a trend.

3. Results and Discussion

3.1. Processing Induced Changes in Carbohydrate Molecular Structure Profiles (Raw vs Steam Flaked)

Multivariate analysis of the whole spectral data generated in CHO associated fingerprint region (ca. 1187–950 cm^{-1}) was performed to compare the internal biopolymers differences among different corn