JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

pubs.acs.org/JAFC

Studies on *Brassica carinata* Seed. 2. Carbohydrate Molecular Structure in Relation to Carbohydrate Chemical Profile, Energy Values, and Biodegradation Characteristics

Hangshu Xin,[†,⊥] Kevin C. Falk,[§] and Peiqiang Yu*[,†,#]

[†]Department of Animal and Poultry Science, College of Agricultural and Bioresources, University of Saskatchewan, 51 Campus Drive, Saskatoon, Saskatchewan S7N 5A8, Canada

[§]Agriculture and Agri-Food Canada, 107 Science Place, Saskatoon, Saskatchewan S7N 0X2, Canada

[#]Department of Animal Science, Tianjin Agricultural University, 22 Jinjin Road, Xiqing District, Tianjin 300384, China

ABSTRACT: The objectives of this study were to investigate (1) the carbohydrate chemical profile, (2) the energy values, (3) the rumen neutral detergent fiber (NDF) degradation kinetics, (4) the carbohydrate-related functional group structural features using a Fourier transform infrared (FTIR) spectroscopic technique with attenuated total reflectance (ATR), and (5) the correlations between carbohydrate intrinsic structural features and nutritional profiles in three strains of *Brassica carinata* in yellow and brown seed coats, with comparison to canola seed as a reference. The results showed that yellow *B. carinata* strains 111000EM and AAC A100 were lower for contents of neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL), and carbohydrate (CHO) and higher for contents of total digestible nutrients (TDN), energy values, and effective degradable NDF (EDNDF) than brown-seeded 110915EM. In comparison, brown canola seed (*Brassica napus* L.) had more fiber content and less EDNDF. Also, carinata strains showed significantly different IR intensities in structural carbohydrate (SCHO), cellulosic compounds (CELC), and total CHO profiles. These structural variations might be one of the possible reasons for various fiber profile and biodegradation characteristics for ruminants in oilseeds. However, multivariate analyses within carbohydrate regions indicated there were still some structural relationships among the four oilseed samples. Moreover, the correlation study showed that the changes of CELC and CHO peak intensities were highly related with some changes in CHO chemical profile, energy values, and in situ NDF degradation kinetics in *B. carinata* and canola seeds. Further study with a large sample size is still necessary to figure out whether CHO molecular spectral information could be used to predict nutrient values and biological behavior in oilseeds.

KEYWORDS: *carinata seed, carbohydrate, nutritive, molecular structure, correlation*

INTRODUCTION

Brassica carinata possesses many positive agronomic traits,[1−3] and it can grow well in hot, dry, and semiarid climates typical of the southern prairies of western Canada. Moreover, with the development of high erucic acid types for industrial uses, as well as zero erucic acid lines[4] and zero erucic acid/high oleic acid lines,[5] seed oil from these species and its byproduct (carinata meal) may have potential applications in the food, biofuel, and feed industries. As a result, the expansion of *B. carinata* has been strongly supported by the Saskatchewan Ministry of Agriculture (such as ADF Project 20070130).

Although many years of research have been conducted on the breeding and agronomics of *B. carniata*,[2,6,7] we still know little about its nutritive value and biodegradation behavior, which may allow for its use in animal diets. As documented in our accompanying part 1 of this series of study, characterization of protein structure features in relation to protein supply and availability obtained from yellow and brown carinata seeds has been carried out.

Subsequently, to enhance the seed traits and add new values to the seeds, the current study was designed to characterize (1) the carbohydrate chemical profile. (2) the energy values, (3) the rumen neutral detergent fiber (NDF) degradation kinetics, (4) the carbohydrate-related functional group structural features using a Fourier transform infrared (FTIR) spectroscopic technique with attenuated total reflectance (ATR), and (5) correlations between carbohydrate intrinsic structural features and nutritional profiles in three strains of *B. carinata* with yellow and brown seed coats, in comparison to brown-seeded canola (*Brassica napus* L.).

MATERIALS AND METHODS

Seed Samples. Three strains of carinata seed were used in this study: 111000EM, 110915EM, and AAC A100. *B. napus* canola seed was also included as a reference for nutritive values and protein structure. 111000EM and AAC A100 were yellow-seeded, whereas 110915EM and canola were brown-seeded. The three strains of *B. carinata* seed are newly developed lines (not currently available in the commercial market), and all of the seeds were obtained from the Saskatoon Research Centre, Agriculture and Agri-Food Canada (AAFC) breeding program. Seeds of 111000EM and AAC A100 and the canola sample were obtained from field-grown plots at the AAFC Research Farm in Saskatoon in 2011. Seeds of 110915EM were grown in contra-season Chile during the winter of 2010/2011. All of the

Received: May 11, 2013
Revised: September 20, 2013
Accepted: September 23, 2013
Published: September 23, 2013

dx.doi.org/10.1021/jf402077g | J. Agric. Food Chem. 2013, 61, 10127−10134

Table 1. Carbohydrate Chemical Profile in Three Strains of *B. carinata* Seed in Yellow and Brown Colors[a]

	111000EM yellow	AAC A100 yellow	110915EM brown	canola brown	SEM[b]	*P* value	contrast, *P* value: carinata-yellow vs carinata-brown	contrast, *P* value: carinata vs canola
NDF, %DM	12.0b	9.67c	13.4a	13.2a	0.20	0.001	0.001	0.003
ADF, %DM	6.43c	4.95d	7.49b	9.83a	0.12	<0.0001	0.0003	<0.0001
ADL, %DM	0.96b	0.74b	2.13ab	3.76a	0.34	0.01	0.04	0.003
CHO, %DM	31.2ab	29.8ab	33.1a	27.4b	0.70	0.02	0.04	0.01
NFC, %DM	21.2a	21.6a	21.9a	16.2b	0.64	0.01	0.53	0.002
hemicellulose, %DM	5.62a	4.80a	5.92a	3.43b	0.23	0.01	0.06	0.002
cellulose, %DM	5.47	4.20	5.37	6.06	0.37	0.10	0.31	0.07
NFC, %CHO	68.1ab	72.5a	66.4b	59.0c	0.94	0.002	0.03	0.001
hemicellulose, %CHO	18.0a	16.1ab	17.9a	12.5b	0.71	0.02	0.38	0.004
cellulose, %CHO	17.6ab	14.1b	16.2ab	22.1a	1.13	0.03	0.79	0.01

[a]Means with the different letters in the same row are significantly different ($P < 0.05$). NDF, neutral detergent fiber; ADF, acid detergent fiber; ADL, acid detergent lignin; CHO, carbohydrate, CHO = 100 − EE − CP − ash; NFC, non-fiber CHO; NFC = 100 − (NDF − NDICP) − EE − CP − ash; hemicellulose = NDF − ADF, and cellulose = ADF − ADL. [b]SEM, standard error of the mean.

seeds were cleaned, dried, and then stored at −20 °C for several weeks before analysis. Each kind of seed had two sources (obtained from two replicated yield trials).

Carbohydrate Chemical Profile. All of the seed samples were first ground in a coffee grinder (PC770, Loblaws Inc., Toronto, Canada) for 20 s, as described in the accompanying part 1 of this series (final particle size, 98% < 1.0 mm). The contents of neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined according to AOAC procedures.[8] Acid detergent lignin (ADL) concentration[9] was also measured. Other chemical parameters including total carbohydrate (CHO), nonfiber CHO (NFC), hemicellulose, and cellulose were calculated according to the formulas as described in NRC-2001.[10]

Energy Values. Total digestible nutrient (TDN), as well as digestible energy, metabolizable energy, and net energy, is commonly used for estimation of available energy in feedstuffs. Total digestible nonfiber carbohydrate (tdNFC), crude protein (tdCP), neutral detergent fiber (tdNDF), fatty acid (tdFA), and total digestible nutrient at 1× maintenance ($TDN_{1\times}$), digestible energy at production level of intake ($DE_{3\times}$), metabolizable energy at production level of intake ($ME_{3\times}$), net energy at production level of intake ($NE_{3\times}$), net energy for maintenance (NE_m), and net energy for growth (NE_g) were determined using a summative approach from the NRC-2001 for dairy cattle[10] and the NRC-1996 for beef cattle,[11] respectively.

Rumen Degradation Kinetics of NDF. In situ nylon bag techniques were employed to estimate rumen degradation kinetics of NDF. This procedure was conducted according to the method of Yu et al.[12] The model[13] used for measuring rumen degradation kinetics of NDF was $R(t) = U + (100 - S - U) \times e^{-K_d \times (t - T_0)}$, where $R(t)$ is the residue at t h of incubation (%), S is the soluble fraction (%), U is the undegradable fraction (%), T_0 is lag time (h), and K_d is degradation rate (%/h).

Then rumen undegradable NDF (RUNDF) and rumen degradable NDF (RNDF) were calculated according to the NRC-2001.[10]

FTIR Spectroscopy on Carbohydrate Structure. The carbohydrate molecular spectrum data of three strains of *B. carinata* seed and canola seed were collected using a JASCO FT/IR 4200 with ATR (JASCO Corp., Tokyo, Japan) at the feed molecular structure analysis laboratory, University of Saskatchewan (Canada). The FTIR spectrum of each seed sample was obtained in the mid-IR range from ca. 4000 to 800 cm^{-1} with 128 co-added scans at a resolution of 4 cm^{-1}.

The spectral data were analyzed by using OMNIC 7.2 (Spectra Tech., Madison, WI, USA). By referring to a previous paper,[14] several parameters associated with carbohydrate functional group band assignments were detected as follows: (1) structural carbohydrates (SCHO, peaks height and area baseline ca. 1487−1190 cm^{-1}; there were three peaks in this region with the first, second, and third peaks centered at ca. 1415, 1374, and 1234 cm^{-1}, respectively), which were associated with hemi- and cellulosic compounds; (2) cellulosic compounds (CELC, peak height and area baseline ca. 1306−1191 cm^{-1}); and (3) total carbohydrates (CHO, peaks height and area baseline ca. 1198−896 cm^{-1}, and there were three peaks in this region with the first, second, and third peaks centered at ca. 1132, 1096, and 1050 cm^{-1}, respectively).

Agglomerative hierarchical cluster analysis (AHCA) and principal component analysis (PCA) were performed using Statistica 8.0 software (StatSoft Inc., Tulsa, OK, USA) to clarify whether there were any structural relationships among these four kinds of oilseed within the regions of SCHO (ca. 1487−1190 cm^{-1}) and CHO (ca. 1198−896 cm^{-1}).

Statistical Analysis. All of the data were statistically analyzed using the Mixed Model procedure of SAS 9.2. For carbohydrate chemical profile, energy values, and degradation kinetics, the model was $Y_{ij} = \mu + F_i + e_{ij}$, where Y_{ij} is the observation of the dependent variable ij; μ is the fixed effect of population mean of the variable; F_i is a fixed effect of seed type (i = 4; 111000EM, 110915EM, AAC A100, and canola seed), each seed sources being used as replications; and e_{ij} is the random error associated with the observation ij. For carbohydrate spectral data, the model was $Y_{ijk} = \mu + F+_i + S(F)_j + e_{ijk}$, where Y_{ijk} is the observation of the dependent variable ijk; μ is the fixed effect of population mean of the variable; F_i is a fixed effect of seed type (i = 4; 111000EM, 110915EM, AAC A100, and canola seed); $S(F)_j$ is a random effect of seed source nested within seed; and e_{ijk} is the random error associated with the observation ijk.

Relationships between the carbohydrate-related molecular structure parameters and nutritive values in carinata seed (n = 6) and canola seed (n = 2) were analyzed using PROC CORR of the SAS using the Pearson correlation method.

Multiple treatment comparisons were performed using the Tukey−Kramar test. Statistical significance was declared and detected at $P < 0.05$, whereas trends were declared at $P \leq 0.10$.

RESULTS AND DISCUSSION

Carbohydrate Chemical Profile among Different Strains of *B. carinata* Seed. Similar patterns were observed on fiber contents among different carinata seeds (Table 1). Brown 110915EM seed was in the highest level on NDF (P = 0.001), ADF ($P < 0.0001$), and ADL (P = 0.01) contents, followed by yellow-seeded 111000EM and AAC A100. These results were partially in agreement with the data of earlier studies in which yellow-seeded strains of canola had been shown to have thinner hulls and contain lower NDF and ADL contents than brown-seeded strains.[15,16] Simbaya et al.[17] also reported that the dietary fiber content in yellow-seeded *Brassica* species was lower than that of the brown-seeded ones. Also, brown-seeded canola had more fiber but less CHO content

Table 2. Energy Values in Three Strains of *B. carinata* Seed in Yellow and Brown Colors[a]

							contrast, *P* value	
	111000EM yellow	AAC A100 yellow	110915EM brown	canola brown	SEM[b]	*P* value	carinata-yellow vs carinata-brown	carinata vs canola
tdNFC, %DM	20.9a	21.6a	21.5a	16.5b	0.71	0.02	0.78	0.004
tdCP, %DM	23.0ab	24.7a	23.1ab	22.3b	0.37	0.04	0.19	0.03
tdNDF, %DM	2.02a	1.51a	1.62a	0.59b	0.16	0.01	0.50	0.003
tdFA, %DM	40.4b	40.4b	38.5b	45.3a	0.63	0.01	0.07	0.002
TDN_{1x}, %DM	129.8ab	131.6a	125.8b	134.2a	0.83	0.01	0.01	0.01
TDN_{3x}, %DM	103.7ab	104.8a	101.1b	106.5a	0.53	0.01	0.01	0.01
DE_{1x}, Mcal/kg, NRC-2001 dairy	5.75ab	5.85a	5.59b	5.92a	0.03	0.01	0.01	0.01
DE_{3x}, Mcal/kg, NRC-2001 dairy	4.59ab	4.66a	4.49b	4.70a	0.02	0.01	0.01	0.01
ME_{3x}, Mcal/kg, NRC-2001 dairy	4.19ab	4.26a	4.08b	4.30a	0.02	0.01	0.01	0.01
NE_{L3x}, Mcal/kg, NRC-2001 dairy	2.75ab	2.80a	2.68b	2.83a	0.02	0.01	0.01	0.01
ME, Mcal/kg, NRC-1996 beef	4.72ab	4.80a	4.58b	4.85a	0.03	0.01	0.01	0.01
NE_m, Mcal/kg, NRC-1996 beef	3.37ab	3.43a	3.27b	3.48a	0.02	0.01	0.01	0.01
NE_g, Mcal/kg, NRC-1996 beef	2.46ab	2.50a	2.38b	2.54a	0.02	0.01	0.01	0.01

[a]Means with the different letters in the same row are significantly different ($P < 0.05$). tdNFC, truly digestible nonfiber carbohydrate; tdCP, truly digestible crude protein; tdNDF, truly digestible neutral detergent fiber; tdFA, truly digestible fatty acid; TDN_{1x}, total digestible nutrient at 1 times maintenance estimated from NRC dairy model 2001; ME, metabolizable energy estimated from NRC beef model 1996; ME_{3x}, metabolizable energy at production level of intake (3×) estimated from NRC dairy model 2001; NE_{L3x}, net energy for lactation at production level of intake (3×) estimated from NRC dairy model 2001; NE_m, net energy for maintenance estimated from NRC beef model 1996; NE_g, net energy for growth estimated from NRC beef model 1996. [b]SEM, standard error of the mean.

Table 3. Characteristics of NDF in Situ Rumen Degradability of Three Strains of *B. carinata* Seed in Yellow and Brown Colors[a]

							contrast, *P* value	
	111000EM yellow	AAC A100 yellow	110915EM brown	canola brown	SEM[b]	*P* value	carinata-yellow vs carinata-brown	carinata vs canola
K_d, %/h	6.80b	8.48b	8.12b	12.9a	0.65	0.01	0.58	0.002
T_0, h	0.14	0.50	1.01	0.44	0.53	0.72	0.34	0.86
S, %	6.10	13.7	12.4	6.30	1.79	0.08	0.31	0.10
D, %	64.1a	55.6a	40.3b	36.5b	2.12	0.002	0.002	0.002
U, %	29.8b	30.7b	47.3a	57.3a	2.38	0.003	0.004	0.002
RUNDF, %NDF	60.0ab	53.9b	64.4ab	68.8a	2.18	0.03	0.048	0.02
RUNDF, g/kg DM	71.9b	51.9c	86.0a	90.8a	1.98	0.001	0.001	0.001
EDNDF, %NDF	40.0ab	46.2a	35.6ab	31.2b	2.18	0.03	0.048	0.02
EDNDF, g/kg DM	48.0	44.7	47.5	41.1	3.10	0.47	0.78	0.19

[a]Means with the different letters in the same row are significantly different ($P < 0.05$). K_d, rate of degradation of D fraction; T_0, lag time; S, soluble fraction in the in situ incubation; D, insoluble but potentially degradable fraction in the in situ incubation; U, potential undegradable fraction in the in situ incubation; RUNDF, rumen undegradable neutral detergent fiber; EDNDF, effective degradable fraction of neutral detergent fiber in the rumen. [b]SEM, standard error of the mean.

than *B. carinata* seed. The contents of CHO and NFC were calculated by the difference method and negatively correlated with crude fat,[10] so the far more oil (46.3 vs 40.8%; unpublished data) should be directly responsible for the lower CHO and NFC contents in canola seed compared with *B. carinata* seed. No differences were found in hemicellulose ($P = 0.06$) and cellulose concentrations ($P = 0.31$) between yellow (111000EM and AAC A100) and brown (110915EM) *B. carinata* strains.

Energy Values among Different Strains of *B. carinata* Seed. Contents of truly digestible nutrients (TDN), digestible energy (DE), metabolizable energy (ME), and net energy (NE) predicted according to the NRC-2001 model[10] and the NRC-1996 model[11] of different strains of oilseed are presented in Table 2. No variations were observed in the determinations of total digestible nonfiber carbohydrate (tdNFC), crude protein (tdCP), and neutral detergent fiber (tdNDF) among the three *B. carinata* seed strains. As for the total digestible fatty acids (tdFA), the yellow *B. carinata* strains tended to be greater ($P = 0.07$) than the brown seed. The content of total digestible nutrients (TDN) was eliminated from tdNFC, tdCP, tdNDF, and tdFA, and yellow-seeded AAC A100 had the highest level of TDN_{1x} and the brown-seeded strain 110915EM had the lowest level, whereas 111000EM was in the middle. Other energy parameters had similar trends as TDN_{1x} among these three strains. Comparing *B. carinata* with canola, predicted values of DE_{1x}, ME, NE_m, NE_g, and NE_L in carinata seed were 5.73, 4.70, 3.36, 2.45, and 2.74 Mcal/kg, which were significantly lower than those in canola seed. As reviewed by Bell,[18] greater fiber content would dilute the digestible nutrients in canola meal, which was also supported by our data in oilseed samples (see Tables 1 and 2). As is well-known, oil is the main source of energy in the feedstuff. Consequently, the energy values of intact carinata seed were 23−40% higher than those in defatted carinata meal reported in our earlier study.[19] In addition, selection of yellow-seeded strains with reduced fiber content and more energy would be regarded as one of the breeding goals according to the data in the present study.

Table 4. Carbohydrate Structure Profiles of Three Strains of *B. carinata* Seed in Yellow and Brown Colors, Revealed Using Infrared Molecular Spectroscopy[a]

	111000EM	AAC A100	110915EM	canola			contrast, *P* value	
	yellow	yellow	brown	brown	SEM[b]	*P* value	carinata-yellow vs carinata-brown	carinata vs canola
structural carbohydrate (SCHO) profiles[c]								
SCHO peak 1 height	0.019	0.019	0.018	0.019	0.0008	0.74	0.39	0.71
SCHO peak 2 height	0.015a	0.013b	0.013b	0.015a	0.0004	<0.0001	0.01	0.001
SCHO peak 3 height	0.019a	0.019ab	0.017bc	0.016c	0.0005	0.0002	0.004	0.0003
SCHO area	3.507	3.357	3.179	3.361	0.0926	0.12	0.03	0.90
cellulosic compounds (CELC) profiles[d]								
CELC height	0.018a	0.017a	0.016	0.014c	0.0004	<0.0001	0.0002	<0.0001
CELC area	0.873ab	0.899a	0.797b	0.664c	0.0259	<0.0001	0.01	<0.0001
total carbohydrate (CHO) profiles[e]								
CHO peak 1 height	0.045a	0.037b	0.036b	0.038b	0.0006	0.002	0.003	0.12
CHO peak 2 height	0.068b	0.066b	0.062b	0.080a	0.0018	<0.0001	0.03	<0.0001
CHO peak 3 height	0.099ab	0.101ab	0.093b	0.104a	0.0028	0.06	0.04	0.07
CHO peak 1 area	1.937a	1.459b	1.472b	1.402b	0.0177	<0.0001	0.001	0.0004
CHO peak 2 area	2.376b	2.282b	2.121b	3.931a	0.1344	<0.0001	0.21	<0.0001
CHO peak 3 area	9.680	9.992	9.163	9.059	0.3223	0.15	0.10	0.15
total CHO area	13.994ab	13.733ab	12.757b	14.392a	0.3668	0.02	0.02	0.04
spectral ratio profiles[f]								
height ratio of SCHO peak 1:2	1.291bc	1.456a	1.423ab	1.260c	0.0278	0.02	0.22	0.02
height ratio of SCHO peak 1:3	0.986	0.971	1.055	1.172	0.0751	0.35	0.45	0.13
height ratio of SCHO peak 2:3	0.765ab	0.669b	0.732ab	0.929a	0.0384	0.03	0.77	0.01
height ratio of CHO peak 1:2	0.657a	0.564b	0.586b	0.478c	0.0127	<0.0001	0.12	<0.0001
height ratio of CHO peak 1:3	0.453a	0.369b	0.391b	0.367b	0.0103	<0.0001	0.12	0.004
height ratio of CHO peak 2:3	0.688b	0.654b	0.667b	0.772a	0.0099	<0.0001	0.77	<0.0001
area ratio of CHO peak 1:2	0.819a	0.642b	0.703b	0.379c	0.0265	<0.0001	0.40	<0.0001
area ratio of CHO peak 1:3	0.203a	0.147b	0.162b	0.158b	0.0066	<0.0001	0.12	0.09
area ratio of CHO peak 2:3	0.247b	0.229b	0.232b	0.443a	0.0180	<0.0001	0.77	<0.0001
area ratio of SCHO:CELC	4.026	3.751b	4.025b	5.066a	0.1287	0.01	0.43	0.002
area ratio of SCHO:total CHO	0.251a	0.245ab	0.249a	0.234b	0.0026	0.03	0.71	0.01
area ratio of CELC:total CHO	0.062a	0.066a	0.063a	0.046b	0.0016	0.003	0.47	0.001

[a]Means with the different letters in the same row are significantly different ($P < 0.05$). [b]SEM, standard error of the mean. [c]Carbohydrate data unit, IR absorbance unit; structural carbohydrate (SCHO) peak baseline, ca. 1487−1190 cm^{-1}, and there were three peaks in this region with the first, second, and third peaks at 1415, 1374, and 1234 cm^{-1}, respectively. The first, second, and third peak regions were ca. 1418−1415, 1377−1374, and 1238−1233 cm^{-1}, respectively. [d]The cellulosic compounds (CELC) peak baseline, ca. 1306-1191 cm^{-1}, the peak region was 1238−1233 cm^{-1}. [e]The carbohydrate (CHO) peak baseline, ca. 1198−896 cm−1, and there were three peaks in this region with the first, second, and third peaks at 1132, 1096, and 1050 cm^{-1}, respectively. The first, second, and third peak regions were ca. 1158−1137, 1099−1078, and 1053−1034 cm^{-1}, respectively. [f]Different ratios were calculated on the basis of relevant data.

Characteristics of NDF in Situ Rumen Degradability in Different Strains of *B. carinata* Seed. Table 3 shows the characteristics of NDF in situ rumen degradability among different strains of *B. carinata* seed in brown and yellow colors. The three *B. carinata* strains had similar degradation rates but lower ($P = 0.002$) than that of canola seed, which meant *B. carinata* seed would be degraded more slowly than canola seed. As for rumen fractions, yellow-seeded *B. carinata* was higher for the D faction ($P = 0.002$) and lower for the U fraction ($P = 0.004$). In relation to the lower U fraction, yellow 111000EM and AAC A100 had a higher ($P = 0.048$) content of effective degradability of NDF (EDNDF, %NDF) than brown 110915EM, and all *B. carinata* seeds had more EDNDF than brown canola seed ($P = 0.02$). The degradability of NDF is highly associated with the fiber profile in the feedstuffs such as cellulose, hemicelluloses, and lignin. Therefore, variations in these fiber components (Table 1), especially in lignin, might result in the differences of EDNDF among the four kinds of oilseed. In addition, some form of processing might be helpful for improving ruminal digestibility of NDF in oilseeds, as noted by Khorasani et al.[20]

Absorbed IR Intensity of Carbohydrates and Their Spectral Ratios in Different Strains of *B. carinata* Seed. When carbohydrate molecular structure features in feedstuff are explored, structural carbohydrates (SCHO) such as cellulose and hemicellulose and nonstructural carbohydrates (NSCHO) such as starch are usually included.[21] Because of negligible amounts of starch in oilseed,[22] the carbohydrate-related

Table 5. Correlation between Carbohydrate Structural Characteristics and Chemical and Nutrient Profiles of *B. carinata* and Canola Seeds

	SCHO peak area		CELC peak area		CHO peak area	
	r	*P*	*r*	*P*	*r*	*P*
carbohydrate chemical profile						
NDF, %DM	−0.287	0.49	−0.729	0.04	−0.089	0.83
ADF, %DM	−0.175	0.68	−0.973	<.0001	0.263	0.53
ADL, %DM	−0.355	0.39	−0.964	0.0001	0.137	0.75
CHO, %DM	−0.294	0.48	0.474	0.24	−0.751	0.03
NFC, %DM	−0.115	0.79	0.819	0.01	−0.649	0.08
hemicellulose, %DM	−0.113	0.79	0.658	0.08	−0.603	0.11
cellulose, %DM	0.485	0.22	−0.564	0.15	0.550	0.16
energy values						
tdNFC, %DM	−0.096	0.82	0.821	0.01	−0.621	0.10
tdCP, %DM	−0.136	0.75	0.742	0.04	−0.320	0.44
tdNDF, %DM	0.278	0.51	0.813	0.01	−0.292	0.48
tdFA, %DM	0.282	0.50	−0.705	0.05	0.769	0.03
$TDN_{1\times}$, %DM	0.459	0.25	−0.351	0.39	0.831	0.01
$TDN_{3\times}$, %DM	0.459	0.25	−0.352	0.39	0.831	0.01
$DE_{1\times}$, Mcal/kg	0.440	0.28	−0.266	0.52	0.794	0.02
$DE_{3\times}$, Mcal/kg	0.448	0.27	−0.239	0.57	0.790	0.02
$ME_{3\times}$, Mcal/kg	0.438	0.28	−0.258	0.54	0.784	0.02
$NE_{L3\times}$, Mcal/kg	0.431	0.29	−0.278	0.51	0.793	0.02
ME, Mcal/kg	0.458	0.25	−0.245	0.56	0.797	0.02
NE_m, Mcal/kg	0.454	0.26	−0.273	0.51	0.803	0.02
NE_g, Mcal/kg	0.444	0.27	−0.300	0.47	0.802	0.02
characteristics of NDF in situ rumen degradability						
K_d, %/h	−0.101	0.81	−0.836	0.01	0.496	0.21
T_0, h	−0.143	0.74	−0.143	0.74	−0.078	0.86
S, %	−0.409	0.32	0.406	0.32	−0.393	0.34
D, %	0.672	0.07	0.814	0.01	0.123	0.77
U, %	−0.516	0.19	−0.923	0.001	0.011	0.98
RUNDF, %	−0.366	0.37	−0.842	0.01	−0.021	0.96
RUNDF, g/kg DM	−0.349	0.40	−0.827	0.01	−0.053	0.90
EDNDF, %	0.366	0.37	0.842	0.01	0.021	0.96
EDNDF, g/kg DM	0.255	0.54	0.427	0.29	−0.123	0.77

functional group band assignments in our study involved only SCHO, cellulosic compounds (CELC), and total carbohydrates (CHO) as well as their spectral ratios calculated on the basis of relevant spectral data (Table 4). First, the presence of bands of moderate intensity at approximately ca. 1415, 1374, and 1234 cm^{-1} represented three characteristics of SCHO, and these regions were mainly associated with hemicelluose and cellulosic compounds. The SCHO second and third peak heights as well as SCHO area absorbed intensities were 0.013, 0.017, and 3.179 IR units for brown-seeded *B. carinata* and 0.014, 0.019, and 3.432 IR units for yellow-seeded *B. carinata*. These tiny but significant differences between these two *B. carinata* strains implied that concentrations of hemicellulose and cellulosic compounds were greater for the yellow-coated seed compared to the brown. Within the SCHO region (ca. 1487−1190 cm^{-1}), an absorption band appeared at ca. 1234 cm^{-1}, which was associated with cellulosic compounds (baseline ca. 1306−1191 cm^{-1}). Consistent with SCHO peak parameters, 111000EM was similar to AAC A100, and both were 9−11% greater (P = 0.01) than the brown-seeded 110915EM in CELC peak and area absorbed intensities. These results were not in accordance with fiber data shown in Table 1. Although CELC IR absorbance statistically differed between two strains of *B. carinata* seed, the variations were in narrow ranges. This phenomenon probably indicated that compared to conventional "wet" chemical analyses, a spectroscopic technique like FTIRM had relatively greater ability to identify subtle differences in chemical compounds among different feedstuffs. Cellulosic compounds include phenolic−carbohydrate complexes, hemicellulose encrustation, and cellulose crystallinity, having structures that are sensitive to digestive enzymes in ruminants.[23−25] Along with cellulose in cell walls are different types of hemicelluloses, which have quite different digestibilities from cellulose.[26] As a result, these variable structural characteristics of SCHO and CELC between yellow (111000EM and AAC A100) and brown (110915EM) seeds could partially explain their different biodegradation behaviors seen in Table 3. Analysis of the three peaks falling in the spectral region of carbohydrate (CHO), ca. 1198−896 cm^{-1}, revealed similar trends on CHO first, second, and third peak heights, CHO first peak area, and total CHO peak area. Yu et al.[27] also reported the greater IR intensities of CHO peak and area in the yellow-seeded canola (*Brassica rapa*) compared to the brown-seeded canola (*Brassica napus*) by using synchrotron-based FTIR spectroscopy. Spectral ratios associated with carbohydrate molecular structure were relatively constant in 111000EM, AAC A100, and 110915EM seeds despite dramatic differences found in individual CHO parameters mentioned above. However, when it came to the reference seed, canola exhibited notably different performance on almost all of the structural

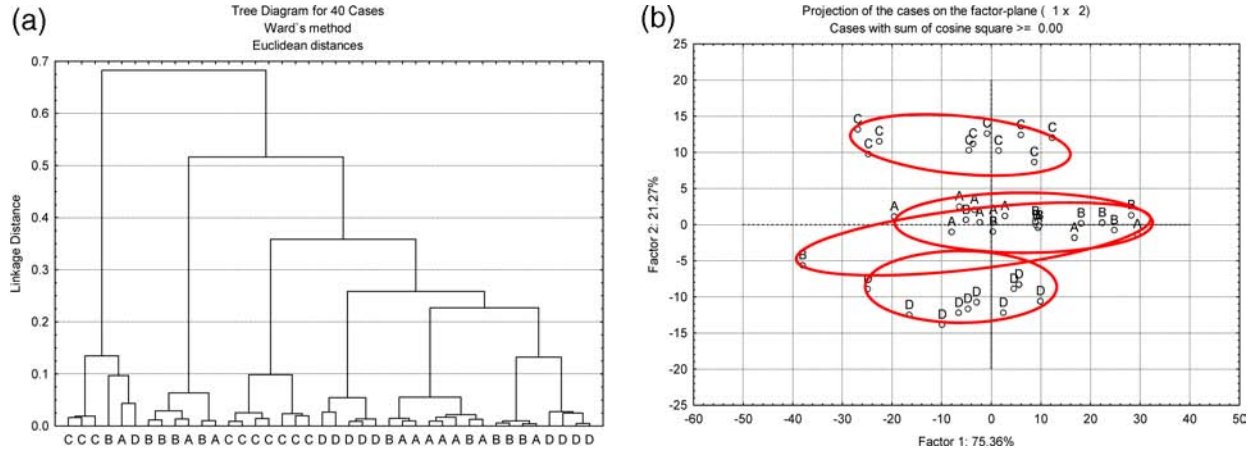

Figure 1. Multivariate molecular spectral analyses of the SCHO (1487−1190 cm^{-1}) on a molecular basis among different oilseeds: A, AAC A100; B, 110915EM; C, 111000EM; D, canola seed. (a) CLA spectral analysis of the structural CHO region (1487−1190 cm^{-1}) obtained from four oilseed samples. [CLA, (1) region of structural CHO ca. 1487−1190 cm^{-1}; (2) distance method, Euclidean; (3) cluster method, Ward's algorithm.] (b) Scatter plot of the first principal component versus the second principal component of PCA of spectrum obtained from four oilseed samples: the first and second principal components explain 75.36 and 21.27% of the total variance, respectively.

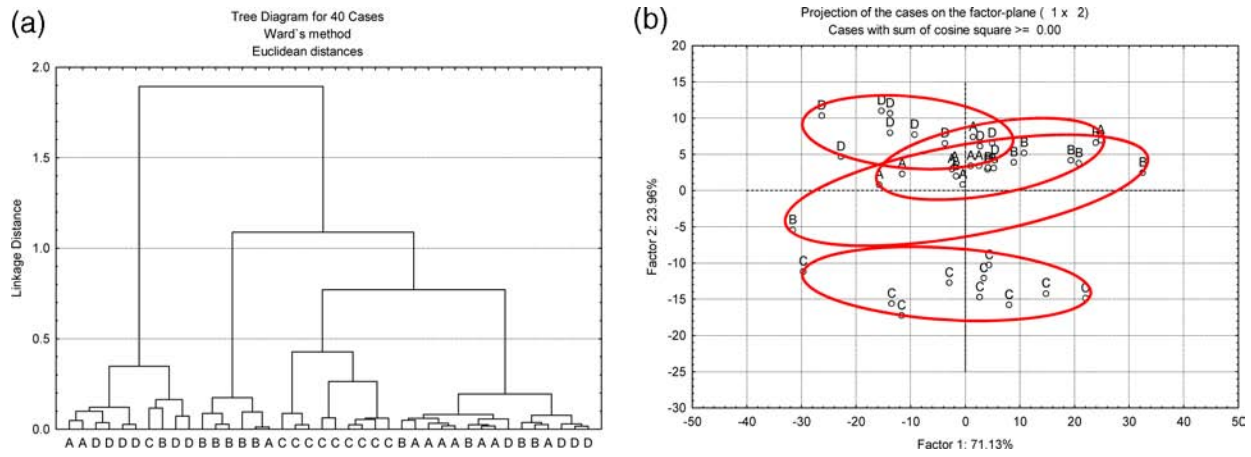

Figure 2. Multivariate molecular spectral analyses of the total CHO (1198−896 cm^{-1}) on a molecular basis among different oilseeds: A, AAC A100; B, 110915EM; C, 111000EM; D, canola seed. (a) CLA spectral analysis of the total CHO region (1198−896 cm^{-1}) obtained from four oilseed samples [CLA, (1) region of total CHO ca. 1198−896 cm^{-1}; (2) distance method, Euclidean; (3) cluster method, Ward's algorithm.] (b) Scatter plot of the first principal component versus the second principal component of PCA of spectrum obtained from four oilseed samples: the first and second principal components explain 71.13 and 23.96% of the total variance, respectively.

ratios. Many researchers have documented nutritive values in relation to carbohydrate molecular structure characteristics in various kinds of feed/food in recent studies.[14,28,29] This was also partly supported by our study on oilseed samples (Tables 1−5) in which *B. carinata* seed exhibited different nutritive and biological profiles from canola seed resulting from various microchemical structural features.

In Figures 1 and 2, AHCA and PCA were performed to discriminate carbohydrate-related functional group internal structure among different strains of *B. carinata* seed and canola seed in the regions of SCHO (ca. 1487−1190 cm^{-1}) and CHO (ca. 1198−896 cm^{-1}). None of the oilseed samples could form a separate group from any of the other three kinds below a linkage distance of less than 0.5 and 1.0 according to the AHCA results in SCHO and CHO regions. Consistently, heavy overlaps of each group shown in PCA also indicated that 111000EM, AAC A100, 110915EM, and canola seed could not be distinguished from each other within SCHO and CHO spectra. Therefore, both AHCA and CLA results implied that the four strains might have some structural relationship in molecular makeup within SCHO and CHO spectra.

Compared with univariate results (Table 4), multivariate analyses (AHCA and PCA) seemed to present contradictory results. It was not true. Univariate analysis was carried out on single-band intensities to find characteristics of individual components, whereas multivariate analyses were performed on the basis of the entire spectral information within specific regions (such as SCHO and CHO regions) to distinguish molecular structural features among different feedstuffs.[30,31] In our study, although four kinds of oilseeds were significantly different in carbohydrate-related spectral profiles, they could not be fully discriminated from each other within carbohydrate spectral regions.

Correlations between Carbohydrate Nutritive Properties and Carbohydrate Spectral Characteristics in *B. carinata* and Canola Seeds. Correlations of carbohydrate chemical profile, truly digestible nutrients, energy values, and in situ degradation kinetics in relation to carbohydrate spectral

characteristics obtained from the oilseed samples (n = 8) are presented in Table 5.

The results showed that the molecular spectral intensity of SCHO peak area had no relationship with all of the nutritive parameters in *B. carinata* and canola seeds, but the spectral intensity of the CELC peak area was sensitive to different types of oilseed and had strong negative correlations with NDF (r = −0.73), ADF (r = −0.97), ADL (r = −0.96), degradation rate of NDF (r = −0.84), the U fraction (r = −0.92), and RUNDF (r = −0.84) but strong positive correlations with NFC (r = 0.82), tdNFC (r = 0.82), tdCP (r = 0.74), tdNDF (r = 0.81), the D fraction (r = 0.81), and EDNDF (r = 0.84). However, the spectral intensity of the CHO peak area only negatively related (P = 0.03) with CHO content (r = −0.75) in the carbohydrate chemical profile and positively related with most energy values (r = 0.77−0.83) with the exception of tdNFC, tdCP, and tdNDF. No published paper could be found on the relationships between carbohydrate structural features and nutritive profiles in intact oilseed samples. However, our data were to some extent consistent with the findings of Zhang and Yu,[29] who described the relationship of CHO spectroscopic characteristics to CHO nutritive properties in combined barley and wheat dried distillers grains with solubles (DDGS). In the current study of correlation analysis, only four strains were tested, and the sample size may not be sufficient for finding the actual relationship between molecular structural makeup and nutritional values in oilseeds.

In conclusion, within the three strains of *B. carinata* seeds, yellow strains 111000EM and AAC A100 were lower for NDF, ADF, ADL, and CHO and higher for total digestible nutrients, energy values, and EDNDF than the brown-seeded 110915EM. In comparison, brown canola seed had more fiber content and less EDNDF. Subsequently, the remarkable spectral differences in spectroscopic analysis among the four seeds might be one of the possible reasons for various fiber profile and biodegradation characteristics for ruminants. However, multivariate analyses using the entire spectral information within carbohydrate regions indicated there were still some structural relationships among the four oilseed samples. Moreover, the changes of cellulosic compounds and total carbohydrate peak areas were highly related with some changes in the CHO chemical profile, energy values, and in situ NDF degradation kinetics in *B. carinata* and canola seeds. Further study with a larger sample size is still necessary to determine whether CHO molecular spectral information could be used to predict nutrient values and biological behavior in oilseeds.

AUTHOR INFORMATION

Corresponding Author

*(P.Y.) Mailing address: Professor and Ministry of Agriculture Strategic Research Chair, College of Agricultural and Bioresources, University of Saskatchewan, 51 Campus Drive, Saskatoon, SK, Canada S7N 5A8. Phone: +1 (306) 966-4132. E-mail: peiqiang.yu@usask.ca.

Present Address

⊥College of Animal Science and Technology, Northeast Agricultural University, Harbin, China 150030.

Funding

The Chair (Professor Dr. Peiqiang Yu) research programs have been financially supported by the Ministry of Agriculture Strategic Research Chair Program, Saskatchewan Canola Development Commission (SaskCanola), Natural Sciences and Engineering Research Council of Canada (NSERC-Individual Discovery Grant and CRD grant), and ADF-Saskatchewan.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

We are grateful to Zhiyuan Niu (University of Saskatchewan) for helpful assistance in chemical analysis and spectral data collection.

REFERENCES

(1) Gugel, R. K.; Seguin-Swartz, G.; Petrie, G. A. Pathogenicity of three isolates of *Leptosphaeria maculans* on *Brassica* species and other crucifers. *Can. J. Plant Pathol.* **1990**, *12*, 75−82.

(2) Singh, H.; Singh, D.; Yadava, T. P. Comparative performance of the genotypes of Indian and Ethiopian mustard under semi-arid region of India. *Cruciferae Newsl.* **1988**, *13*, 36−37.

(3) Malik, R. S. Prospects for Brassica carinata as an oilseed crop in India. *Exp. Agric.* **1990**, *26*, 125−129.

(4) Getinet, A.; Rakow, G.; Raney, J. P.; Downey, R. K. Development of zero erucic acid Ethiopian mustard through an interspecific cross with zero erucic acid Oriental mustard. *Can. J. Plant Sci.* **1994**, *74*, 793−795.

(5) Velasco, L.; Nabloussi, A.; De Haro, A.; Fernández-Martínez, J. M. Development of high-oleic, low-linolenic acid Ethiopian-mustard (*Brassica carinata*) germplasm. *Theor. Appl. Genet.* **2003**, *107*, 823−830.

(6) Barro, F.; Fernández-Escobar, J.; De La Vega, M.; Martín, A. Doubled haploid lines of *Brassica carinata* with modified erucic acid content through mutagenesis by EMS treatment of isolated microspores. *Plant Breed.* **2011**, *120*, 262−264.

(7) Gil-Humanes, J.; Martín, A.; Barro, F. Characterization of a collection of *Brassica carinata* genotypes for *in vitro* culture response and mode of shoot regeneration. *Am. J. Plant Sci.* **2011**, *2*, 27−34.

(8) AOAC. *Official Methods of Analysis*; Association of Official Analytical Chemists: Washington, DC, 1990.

(9) Van Soest, P. J.; Robertson, J. B.; Lewis, B. A. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. *J. Dairy Sci.* **1991**, *74*, 3583−3597.

(10) NRC. *Nutrient Requirements of Dairy Cattle*, 7th rev. ed.; National Academy of Sciences: Washington, DC, 2001.

(11) NRC. *Nutrient Requirements of Beef Cattle*, 7th ed.; National Academy of Sciences: Washington, DC, 1996.

(12) Yu, P.; Meier, J.; Christensen, D. A.; Rossnagel, B. G.; McKinnon, J. J. Using the NRC-2001 model and the DVE/OEB system to evaluate nutritive values of Harrington (malting-type) and Valier (feed-type) barley for ruminants. *Anim. Feed Sci. Technol.* **2003**, *107*, 45−60.

(13) Ørskov, E. R.; McDonald, I. The estimation of protein degradability in the rumen from incubation measurements weighted according to rate of passage. *J. Agric. Sci.* **1979**, *92*, 499−503.

(14) Yu, P. Short communication: relationship of carbohydrate molecular spectroscopic features to carbohydrate nutrient profiles in co-products from bioethanol production. *J. Dairy Sci.* **2012**, *95*, 2091−2096.

(15) Theander, O.; Aman, P.; Miksche, G. E.; Yasunda, S. Carbohydrates, polyphenols and lignin in seed hulls of different colors from turnip rapeseed. *J. Agric. Food Chem.* **1977**, *25*, 270−273.

(16) Bell, J. M.; Shires, A. Composition and digestibility by pigs of hull fractions from rapeseed cultivars with yellow or brown seed coats. *Can. J. Anim. Sci.* **1982**, *62*, 557−565.

(17) Simbaya, J.; Slominski, B. A.; Rakow, G.; Campbell, L. D.; Downey, R. K.; Bell, J. M. Quality characteristics of yellow-seeded *Brassica* seed meals: protein, carbohydrates, and dietary fiber components. *J. Agric. Food Chem.* **1995**, *43*, 2062−2066.

(18) Bell, J. M. Factor affecting the nutritional value of canola meal: a review. *Can. J. Anim. Sci.* **1993**, *73*, 679−697.
(19) Xin, H.; Yu, P. Chemical profile, energy values, protein molecular structure characteristics of bio-fuel/bio-oil co-products (carinata meal) in comparison with canola meal. *J. Agric. Food Chem.* **2013**, *61* (16), 3926−3933.
(20) Khorasani, G. R.; de Boer, G.; Robinson, P. H.; Kennelly, J. J. Effect of canola fat on ruminal and total tract digestion, plasma hormones, and metabolites in lactating dairy cows. *J. Dairy Sci.* **1992**, *75*, 492−501.
(21) Wetzel, D. L.; Eilert, A. J.; Pietrzak, L. N.; Miller, S. S.; Sweat, J. A. Ultraspatially resolved synchrotron infrared microspectroscopy of plant tissue *in situ*. *Cell. Mol. Biol.* **1998**, *44*, 145−167.
(22) Blair, R.; Reichert, R. D. Carbohydrate and phenolic constituents in a comprehensive range of rapeseed and canola fractions: nutritional significance for animals. *J. Sci. Food Agric.* **1984**, *35* (1), 29−35.
(23) Van Soest, P. J. Physico-chemical aspects of fibre digestion. In *Digestion and Metabolism in the Ruminant*; McDonald, I. W., Warner, A. C. I., Eds.; University of New England Publishing Unit: Armidale, NSW, Australia, 1975; pp 351−365.
(24) Jung, H. G.; Fahey, G. C., Jr.; Garst, J. E. Simple phenolic monomers of forages and effects of in vitro fermentation on cell wall phenolics. *J. Anim. Sci.* **1983**, *57*, 1294−1305.
(25) Iiyama, K.; Lam, T. B. T. Structural characteristics of cell walls of forage grasses-their nutritional evaluation for ruminants- review. *Asian−Aust. J. Anim. Sci.* **2001**, *14*, 862−879.
(26) Morrison, I. M. Carbohydrate chemistry and rumen digestion. *Proc. Nutr. Soc.* **1979**, *38*, 269−274.
(27) Yu, P.; Christensen, C. R.; Christensen, D. A.; McKinnon, J. J. Ultrastructual-chemical makeup of yellow-seeded (*Brassica rapa*) and brown-seeded (*Brassica napus*) canola within cellular dimensions, explored with synchrotron reflection FTIR microspectroscopy. *Can. J. Plant Sci.* **2005**, *85*, 533−541.
(28) Liu, N.; Yu, P. Characterize microchemical structure of seed endosperm within a cellular dimension among six barley varieties with distinct degradation kinetics, using ultraspatially resolved synchrotron-based infrared microspectroscopy. *J. Agric. Food Chem.* **2010**, *58*, 7801−7810.
(29) Zhang, X.; Yu, P. Relationship of carbohydrate molecular spectroscopic features in combined feeds to carbohydrate utilization and availability in ruminants. *Spectrochim. Acta Part A: Mol. Biomol. Spectrosc.* **2012**, *92*, 225−233.
(30) Yu, P. Applications of hierarchical cluster analysis (CLA) and principal component analysis (PCA) in feed structure and feed molecular chemistry research, using synchrotron-based Fourier transform infrared (FTIR) microspectroscopy. *J. Agric. Food Chem.* **2005**, *53*, 7115−7127.
(31) Miller, L. M. Infrared microsperctroscopy and imaging, http://www.nsls.bnl.gov/newsroom/publications/otherpubs/imaging/workshopmiller.pdf, 2010 (accessed March 2013).