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Studies on *Brassica carinata* Seed. 1. Protein Molecular Structure in Relation to Protein Nutritive Values and Metabolic Characteristics

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ABSTRACT: The objectives of this study were to investigate (1) the protein chemical profile, (2) the protein subfractions partitioned by the Cornell Net Carbohydrate and Protein System (CNCPS), (3) the rumen crude protein (CP) degradation kinetics, (4) the protein supply predicted by the DVE/OEB system, (5) the protein structural features using a Fourier transform infrared (FTIR) spectroscopic technique with attenuated total reflectance (ATR), and (6) the correlations between protein 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 carinata seed strains were different in both nutritional values and IR absorbance within the protein spectral region (ca. 1720−1482 cm^{-1}). The comparison between yellow and brown *B. carinata* seeds indicated that the former was lower in acid detergent insoluble crude protein (ADICP; $P = 0.002$) and undegradable protein fraction (PC; $P = 0.002$) and greater in the degradable (D) fraction ($P = 0.004$) and true absorbed protein in the small intestine (DVE; $P = 0.02$) as well as feed milk value (FMV; $P = 0.02$) than the latter. The brown canola seed (*Brassica napus* L.) was also not in full accordance with *B. carinata* seed on these parameters. The FTIR studies showed significant differences in protein amide II peak height, amide I peak area, and β-sheet height among different *B. carinata* strains. However, multivariate spectral analyses indicated a similarity in protein structural makeup in these four kinds of oilseed. The not very strong correlations shown in this study implied that the limited sample size and narrow range in biological and spectral variation might be responses for the weak relationships between chemical profile and mid-IR spectral data. Further studies using sufficient samples with wide and diverse range in nutritional properties are needed to illustrate the actual relationship between spectroscopic data and nutritional profiles in oilseeds.

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

INTRODUCTION

Brassica carinata, also known as Ethiopia mustard, originated in Ethiopia and is regarded as one of the oldest crops in east Africa; its cultivation can be traced back to 4000 BC.[1,2] This plant is ideally suited to grow in a Mediterranean climate and has strong resistance to biotic or abiotic stressors.[3−5] Compared with conventional canola seed, *B. carinata* has a larger seed size,[6] resulting in higher protein content and lower crude fiber concentration. Therfore, research on breeding development and nutrition evaluation of this vigorous crop has been conducted in countries with semiarid climates such as western Canada.[7−9]

Canola seed, which is an excellent source of essential fatty acids and protein, has gained widespread acceptance in feed rations for dairy cattle,[10] goats,[11] and steers.[12] Similar to canola, *B. carinata* may also have a potential application in the animal industry. However, as far as we know, great efforts and progress have been made in breeding works for the development of *B. carinata* in various applications,[4,13,14] but nutrient availability and quality evaluation of carinata seed for animals are extremely lacking.

Additionally, different microchemical structural makeup of biopolymer has been proved to be responsible for the various digestive behaviors and biodegradation characteristics in the feedstuffs using molecular spectroscopy with chemometrics, such as feather meal.[15] Therefore, besides nutritional information, molecular structure features of *B. carinata* seed also need to be explored. Consequently, a series of research studies were designed in our group to collect detailed information on both metabolic and molecular structural bases. The objectives of the current study were to investigate (1) the protein chemical profile, (2) the protein subfractions partitioned by the Cornell Net Carbohydrate and Protein System (CNCPS), (3) the rumen crude protein (CP) degradation kinetics, (4) the protein supply predicted by the DVE/OEB system, (5) the protein-structural features using a Fourier transform infrared (FTIR) spectroscopic technique with attenuated total reflectance (ATR), and (6) the correlation between protein 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.).

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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 are yellow-seeded, whereas 110915EM is brown-seeded and canola was 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 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).

Protein Chemical Profile and Subfractions. All of the seed samples were first ground before chemical analysis. To prevent heavy sticking and clumping in a common feed grinder during the grinding process because of the high content of oil in the seed, all of the samples were ground in a coffee grinder (PC770, Loblaws Inc., Toronto, Canada) for 10 s, then chilled, and reground for another 10 s (final particle size, 98% < 1.0 mm). The cooling process was needed to prevent the samples from getting too warm because the higher temperature would affect the nutritive value in the carinata and canola seeds. The content of CP was measured using the Kjeldahl method according to the AOAC procedure.[16] The neutral detergent insoluble crude protein (NDICP) and acid detergent insoluble crude protein (ADICP) concentrations were determined according to the method of Licitra et al.[17] The methods for determining the contents of soluble crude protein (SCP) and non-nitrogen protein (NPN) were previously described by Xin and Yu.[18] The protein subfractions were partitioned into non-protein nitrogen (PA), rapidly degradable fraction (PB1), intermediately degradable fraction (PB2), slowly degradable fraction (PB3), and undegradable fraction (PC) using the CNCPS.[19]

Rumen in Situ Incubation. This trial was approved by the Animal Care Committee of the University of Saskatchewan, and all of the animals were cared for under the Canadian Council on Animal Care (CCAC).[20] Nylon bag techniques were conducted using six ruminally fistulated Holstein dairy cows in late lactation stage in this experiment. The cows were fed a 50:50 silage to concentrate ration twice daily at 8:00 a.m. and 4:00 p.m. The concentrate included barley, wheat, oats, and dairy supplement pellets. All of the seed samples (particle size, 1.0 mm) were incubated in the rumens for 0, 2, 4, 8, 12, 24, and 48 h according to the "gradual addition − all out" schedule. There were 2, 2, 2, 2, 3, 4, and 5 bags (10 × 20 cm, pore size = 40 μm, Nitex 03-41/31 monofilament open mesh fabric; Screen Tech, Mississagua, ON, Canada) for the corresponding time point, and the total bags put into the rumen was not more than 20 at any time point. After incubation, all of the bags were removed from the rumen and washed with cold tap water without any detergent and then dried, weighed, reground, and stored at 4 °C for further analysis.

Rumen degradation kinetics of CP was measured according to the model described by Ørskov and McDonald:[21]

$$R(t) = \mathrm{U} + (100 - \mathrm{S} - \mathrm{U}) \times \mathrm{e}^{-K_\mathrm{d} \times (t - T_0)}$$

$R(t)$ was residue at t h of incubation (%), S was soluble fraction (%), U was undegradable fraction (%), T_0 was lag time (h), and K_d was degradation rate (%/h).

Then rumen undegradable protein (RUP), bypassed protein (BCP), and rumen degradable protein (RDP) were calculated according to the NRC-2001 model[22] and the DVE/OEB system.[23]

Protein Supply and Availability Predicted by the DVE/OEB System. *True Protein Digested in the Intestine.* In the DVE/OEB system, the protein supply is expressed as the protein truly digested in and absorbed from the small intestine.[23] It includes three parts: (1) digestible feed true protein escaping rumen degradation (ABCP); (2) digestible true microbial protein synthesized in the rumen (AMCP); and (3) endogenous protein losses in the digestive tract (ENDP). The true protein digested in the intestine (DVE) value = ABCP + AMCP − ENDP.

The content of ABCP can be estimated on the basis of the content and digestibility of BCP,[23] which was calculated as

$$\mathrm{BCP\ (g/kg\ DM)} = 1.11 \times [\mathrm{CP\ (g/kg\ DM)} \times \mathrm{RUP\ (\%CP)}/100]$$

$$\mathrm{ABCP\ (g/kg\ DM)} = [\mathrm{dRUP\ (\%)} \times \mathrm{BCP\ (g/kg\ DM)}]/100$$

where the factor 1.11 is the regression coefficient between in situ RUP and in vivo RUP[24] and dRUP can be obtained from the three-step in vitro procedure described by Calsamiglia and Stern.[25]

The content of AMCP depends on rumen microbial protein synthesis ($\mathrm{MCP_{FOM}}$), which is based on fermented organic matter (FOM).

$$\mathrm{MCP_{FOM}\ (g/kg\ DM)} = 0.15 \times \mathrm{FOM\ (g/kg\ DM)}$$

$$\mathrm{AMCP\ (g/kg\ DM)} = 0.75 \times 0.85 \times \mathrm{MCP_{FOM}\ (g/kg\ DM)}$$

The factor 0.15 means that 150 g of microbial protein is assumed to be produced per kilogram of FOM;[23] the factor 0.75 means that 750 g/kg of microbial nitrogen is present in amino acid and the remaining part of nitrogen in nucleic acids; and the factor 0.85 is the assumed digestibility of the true protein in $\mathrm{MCP_{FOM}}$.[23]

The content of ENDP is based on the amount of undigested dry matter (UDM).

$$\mathrm{UDM\ (g/kg\ DM)} = \mathrm{UAsh\ (g/kg\ DM)} + \mathrm{UOM\ (g/kg\ DM)}$$

$$\mathrm{UAsh\ (g/kg\ DM)} = 0.35 \times \mathrm{Ash\ (g/kg\ DM)}$$

$$\mathrm{UOM\ (g/kg\ DM)} = \mathrm{OM\ (g/kg\ DM)} - (\mathrm{OM\ (g/kg\ DM)} \times \mathrm{dOM\ (\%)}/100)$$

$$\mathrm{ENDP\ (g/kg\ DM)} = 0.075 \times \mathrm{UDM\ (g/kg\ DM)}$$

The factor 0.35 means 35% of ash can be digested; dOM is digestibility of organic matter (OM) after 120 h of rumen incubation;[23] and the factor 0.075 means that 75 g CP/kg UDM is absorbed in the small intestine.

Degraded Protein Balance (OEB).

$$\mathrm{MCP_{RDP}\ (g/kg\ DM)} = \mathrm{CP\ (g/kg\ DM)} - \mathrm{BCP\ (g/kg\ DM)}$$

$$\mathrm{OEB\ (g/kg\ DM)} = \mathrm{MCP_{RDP}\ (g/kg\ DM)} - \mathrm{MCP_{FOM}\ (g/kg\ DM)}$$

Feed Milk Value (FMV) Predicted by Metabolic Characteristics of Protein. The FMV was determined by metabolic characteristics of protein from the DVE/OEB system. The efficiency of use of metabolizable protein for lactation is assumed to be 0.67,[22] and protein composition in milk is assumed to be 33 g protein/1000 g milk.

Protein Molecular Structure by ATR-FTIR Spectroscopy. The protein molecular spectral data were collected from *B. carinata* and canola seed samples using JASCO FT/IR 4200 with ATR (JASCO Corp., Tokyo, Japan) at the University of Saskatchewan. This spectrometer was equipped with a MIRacle ATR accessory module and a ZnSe crystal and pressure clamp (Pike Technologies, Madison, WI, USA). The IR spectra were obtained in the mid-IR range (ca. 4000−800 $\mathrm{cm^{-1}}$) with 128 co-added scans. The protein spectral parameters involved the protein amide profile such as amide I and II, as well as the protein secondary structure profile such as α-helix and β-sheet. The detailed procedure has been reported in Xin and Yu.[18]

Univariate and Multivariate Spectral Analyses. Univariate spectral analysis was performed using OMNIC 7.2 software (Spectra Tech., Madison, WI, USA) on protein amide I (ca. 1652 $\mathrm{cm^{-1}}$), protein amide II (ca. 1541 $\mathrm{cm^{-1}}$), α-helix (ca. 1652 $\mathrm{cm^{-1}}$), and β-sheet (ca. 1626 $\mathrm{cm^{-1}}$). The baseline was ca. 1720−1482 $\mathrm{cm^{-1}}$.

Multivariate spectral analyses, agglomerative hierarchical cluster analysis (AHCA) and principal component analysis (PCA), were performed within the protein fingerprint spectra region, ca. 1720−

Table 1. Protein Chemical Profile 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
CP, %DM	23.1	24.8	23.4	22.6	0.38	0.06	0.30	0.06
NDICP, %DM	1.38b	1.09b	1.42ab	1.95a	0.10	0.01	0.18	0.004
ADICP, %DM	0.22c	0.11c	0.56b	0.84a	0.04	0.001	0.002	0.0003
SCP, %DM	11.3b	12.5a	11.7ab	11.0b	0.14	0.01	0.41	0.01
NPN, %DM	6.47	6.53	4.47	6.04	0.77	0.33	0.10	0.82
NDICP, %CP	5.98b	4.38b	6.09ab	8.63a	0.45	0.01	0.17	0.004
ADICP, %CP	0.95c	0.42c	2.38b	3.70a	0.14	0.0003	0.001	0.0001
SCP, %CP	49.1b	50.4a	50.2a	48.6b	0.31	0.04	0.26	0.02
NPN, %CP	28.0	26.5	19.1	26.6	3.31	0.35	0.12	0.61

[a]Means with different letters in the same row are significantly different ($P < 0.05$). CP, crude protein; NDICP, neutral detergent insoluble crude protein; ADICP, acid detergent insoluble crude protein; SCP, soluble crude protein; NPN, non-protein nitrogen. [b]SEM, standard error of the mean.

Table 2. Protein Subfractions in Three Strains of *B. carinata* Seed in Yellow and Brown Colors Using the CNCPS[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
PA, %DM	6.47	6.53	4.47	6.04	0.77	0.33	0.10	0.82
PB1, %DM	4.86	5.94	7.26	4.94	0.76	0.23	0.12	0.29
PB2, %DM	10.4	11.2	10.2	9.68	0.28	0.07	0.16	0.05
PB3, %DM	1.16	0.98	0.87	1.11	0.12	0.39	0.23	0.47
PC, %DM	0.22c	0.11c	0.56b	0.84a	0.04	0.001	0.002	0.0003
PA, %CP	28.0	26.5	19.2	26.6	3.31	0.35	0.12	0.61
PB1, %CP	21.0	23.9	31.1	22.0	3.14	0.24	0.09	0.40
PB2, %CP	45.0	45.3	43.7	42.8	0.61	0.13	0.13	0.06
PB3, %CP	5.02	3.95	3.71	4.94	0.55	0.34	0.31	0.33
PC, %CP	0.95c	0.42c	2.38b	3.70a	0.14	0.0003	0.001	0.0001
true protein (TP), %CP	71.0	73.1	78.5	69.7	3.36	0.38	0.20	0.31
PB1, %TP	30.4	34.6	44.9	31.8	4.55	0.24	0.09	0.40
PB2, %TP	65.0	65.5	63.2	61.9	0.89	0.13	0.13	0.06
PB3, %TP	7.27	5.72	5.37	7.14	0.80	0.34	0.31	0.33

[a]Means with the different letters in the same row are significantly different ($P < 0.05$). CNCPS, Cornell net carbohydrate and protein system; PA, non-protein nitrogen (K_d = assumed to be infinity); PB1, rapidly degradable protein subfraction as per CNCPS (K_d = 120−400% h^{-1}); PB2, intermediately degradable protein subfraction as per CNCPS (K_d = 3−16% h^{-1}); PB3, slowly degradable protein subfraction as per CNCPS (K_d = 0.06−0.55% h^{-1}); PC, undegradable protein subfraction as per CNCPS. [b]SEM, standard error of the mean.

1482 cm^{-1}. Both AHCA and PCA applications in spectral analysis have been reviewed by Yu.[26]

Statistical Analysis. Data of the protein chemical profile, subfractions, degradation kinetics, and protein supply predicted by the DVE/OEB system were statistically analyzed using the Mixed Model procedure of SAS 9.2 and 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 source being as replications; and e_{ij} is the random error associated with the observation ij.

The model for spectral data analysis in carinata and canola seeds 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 protein structure amide I and II height, structure amide I and II area, α-helix and β-sheet and their ratios, and protein chemical composition, subfractions, degradation kinetics, and supply predicted by the DVE/OEB system in *B. carinata* seed (n = 6) and canola seed (n = 2) were analyzed using the PROC CORR of 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

Protein Chemical Profile and Subfractions among Different Strains of Carinata Seed. The results of protein chemical profiles in three strains of carinata seed with different seed coat colors in comparison with canola seed are presented in Table 1. The CP content was not changed among different strains of carianta seed with average of 23.8% of DM, which tended to be higher (P = 0.06) than that in canola seed (22.6% of DM). Warwick et al.[8] summarized seed quality traits in 66 accessions of *B. carinata* grown in a field trial at Saskatoon in 1998, and they found the mean value for seed protein content was 34.1% of DM. However, in a very recent study,[9] the seed

Table 3. Characteristics of CP 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	13.9	15.4	13.5	12.6	0.69	0.16	0.25	0.10
T_0, h	0.0	0.52	0.07	0.42	0.21	0.35	0.49	0.41
S, %	29.4	31.3	34.9	30.0	1.04	0.06	0.02	0.19
D, %	66.1a	65.7a	60.5b	65.3a	0.75	0.02	0.004	0.24
U, %	4.58	3.05	4.57	4.76	0.38	0.09	0.18	0.19
RUP, %CP	24.5	21.5	23.2	25.9	0.89	0.09	0.87	0.054
RUP, g/kg DM	56.6	53.4	54.3	58.5	2.84	0.61	0.84	0.32
EDCP, %CP	75.5	78.5	76.8	74.1	0.89	0.09	0.87	0.054
EDCP, g/kg DM	174.2bc	194.3a	179.3b	167.6c	1.94	0.003	0.11	0.003

[a]Means with the different letters in the same row are significantly different ($P < 0.05$). K_d, the 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; RUP, rumen undegraded feed crude protein; EDCP, the effective degradable fraction of crude protein in the rumen. [b]SEM, standard error of the mean.

Table 4. Prediction of the Potential Nutrient Supply (Using the DVE/OEB System) and Feed Milk Value (Using the NRC-2001) to Dairy Cattle from 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
rumen fermentable organic matter, g/kg DM								
FOM	419.6a	432.6a	414.1a	337.4b	8.81	0.01	0.33	0.001
truly absorbed rumen synthesized microbial protein in the small intestine, g/kg DM								
MCP_{FOM}	62.9a	64.9a	62.1a	50.6b	1.32	0.01	0.33	0.001
MCP_{RDP}	167.9bc	188.4a	173.4b	161.1c	1.89	0.002	0.11	0.002
AMCP	40.1a	41.4a	39.6a	32.3b	0.84	0.01	0.33	0.001
truly absorbed rumen undegraded feed protein in the small intestine, g/kg DM								
BCP	62.8	59.3	60.2	64.9	3.15	0.61	0.84	0.32
ABCP	46.5	45.9	40.2	39.9	2.53	0.25	0.12	0.21
endogenous protein losses in the digestive tract, g/kg DM								
UDM	75.2b	67.8b	104.3a	110.7a	4.65	0.01	0.005	0.01
ENDP	5.56b	5.09b	7.82a	8.30a	0.35	0.01	0.005	0.01
total truly absorbed protein in the small intestine, g/kg DM								
DVE	81.0a	82.2a	71.9ab	63.8b	2.12	0.01	0.02	0.004
degraded protein balance, g/kg DM								
OEB	105.0	123.5	111.3	110.5	3.12	0.054	0.48	0.49
feed milk value, g/kg DM								
FMV	1.79a	1.82a	1.59ab	1.41b	0.05	0.01	0.02	0.004

[a]Means with the different letters in the same row are significantly different ($P < 0.05$). AMCP, truly absorbed rumen synthesized microbial protein in the small intestine; ABCP, truly absorbed undegraded feed protein in the small intestine; OEB, degraded protein balance; DVE, truly absorbed protein in the small intestine; ENDP, endogenous protein losses; FOM, OM fermented in the rumen; MCPFOM, microbial protein synthesized in the rumen based on available energy; MCPRDP, microbial protein synthesized in the rumen based on rumen degraded feed CP; BCP, ruminally undegraded feed CP fraction; UDM, undigested DM. The efficiency of use of metabolizable protein for lactation is assumed to be 0.67 (NRC, 2001), and protein composition in milk is assumed 33 g protein/1000 g milk. [b]SEM, standard error of the mean.

protein ranged from 25.9 to 30.5% of DM among 10 selected carinata strains in Saskatoon in 2009. In contrast with these data, the possible reason for the lower protein value found in our study might be associated with (1) the aim of oilseed breeding work, which emphasizes increasing the seed oil content at the expense of the seed protein content[27] [correspondingly, the *B. carinata* seeds used in our study had much higher oil content with an average of 40.8% (unpublished data)] and (2) different selected lines or harvest time (1998, 2008−2009 vs 2011−2012) among these studies, because seed protein content could be influenced by these two factors.[9] Variance in seed coat color could not bring in significant differences in CP content among *B. carinata* seeds, which agreed with previous study targeting brown- and yellow-seeded carinata meals.[28] As for the protein fractions bonded with NDF and ADF, they showed similar trends in all cases. Brown-seeded canola had the highest contents of NDICP and ADICP, followed by brown-seeded 110915EM and yellow-seeded 111000EM and AAC A100. Little information could be found on protein subfraction profile in brown or yellow carinata seed in previous literature. However, Theodoridou and Yu[29] reported that the content of ADICP was significantly lower for the yellow-seeded canola meal compared to the brown-seeded one, which was partially in agreement with our data. The carinata seed had more SCP than canola seed ($P = 0.01$), and for different carinata strains, the SCP content was at

Table 5. Protein Amide I and II Profiles and Protein Secondary Structure Profiles of Three Strains of Carinata Seed in Yellow and Brown Colors, Revealed Using Infrared Molecular Spectroscopy[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
protein amides profiles[c]								
amide I height	0.063	0.061	0.053	0.072	0.006	0.27	0.26	0.12
amide II height	0.033ab	0.035a	0.030b	0.035a	0.001	0.02	0.01	0.12
height ratio of amide I:II	1.879	1.776	1.809	2.097	0.174	0.61	0.93	0.24
amide I area	4.231ab	4.147ab	3.745b	4.455a	0.137	0.01	0.01	0.01
amide II area	1.736	1.827	1.583	1.915	0.089	0.19	0.14	0.12
area ratio of amide I:II	2.457	2.275	2.405	2.328	0.081	0.49	0.71	0.62
protein second structure[d]								
α-helix height	0.063	0.061	0.053	0.072	0.006	0.27	0.26	0.12
β-sheet height	0.046b	0.044b	0.041b	0.053a	0.002	<0.0001	0.03	<0.0001
height ratio of α-helix:β-sheet	1.361	1.380	1.312	1.370	0.097	0.96	0.63	0.87

[a]Means with the different letters in the same row are significantly different ($P < 0.05$). [b]SEM, standard error of the mean. [c]Protein amide data unit, IR absorbance unit; the protein peak baseline, ca. 1720−1482 cm^{-1}; protein amide I region, ca. 1720−1576 cm^{-1}; protein amide II region, ca. 1576−1482 cm^{-1}. [d]The peaks of α-helix and β-sheet fell within the ranges of ca. 1653−1650 and 1630−1624 cm^{-1}, respectively.

the highest level for AAC A100 and at the lowest level for 111000EM. All of the oilseeds had similar contents of NPN ($P = 0.35$).

The protein subfractions partitioned by the CNCPS[19] were not changed among three strains of carinata seed with the expectation of PC fraction (expressed as % of DM or % of CP; Table 2). The 110915EM in brown seed coat had more PC fraction ($P = 0.002$) than the other two yellow strains. However, when compared to canola seed, the PC fraction of carinata strains was significantly lower ($P = 0.0003$). Fraction PC in the feed represents the unavailable part of protein,[19] which cannot be used effectively by the ruminants. Therefore, our data implied that the yellow carinata seeds (111000EM and AAC A100) were expected to show comparatively better protein utilization than the brown seed (110915EM), and all of the carinata seeds were better than canola seed.

Characteristics of CP in Situ Rumen Degradability in Different Strains of *B. carinata* Seed. The degradation rate (K_d), rumen fractions, and effective degradable fraction of CP (EDCP) in three strains of *B. carinata* seed in comparison with canola seed are presented in Table 3. Compared with yellow-seeded 111000EM and AAC A100, brown-seeded 110915EM was notably lower ($P = 0.03$) in the soluble (S) fraction and higher ($P = 0.004$) in the degradable (D) fraction. Similarly, Theodoridou and Yu[29] found lower content of D fraction in brown-seeded canola meal. Previously, rumen CP degradation kinetics were studied for carinata meal (our unpublished data), but when incubated as ground seed, large differences were found in K_d (seed vs meal = 14 vs 33%/h), S fraction (seed vs meal = 32 vs 18%), and U fraction (seed vs meal = 4 vs 15%). These inconsistencies might be due to the interaction of oil with protein in the seed, heating effect during meal processing,[30] or different rumen conditions[31] between the two studies. The *B. carinata* seed had 182.6 g/kg of DM EDCP on average, which was remarkably higher than that of canola seed. Although little knowledge has been reported on EDCP (% of CP) in *B. carinata* and canola seeds, the data in the current study were consistent with results from our previous research on *B. carinata* and canola meals (unpublished data). In terms of other measured kinetics parameters such as lag time (T_0; $P = 0.35$) and rumen undegradable protein (RUP; $P = 0.09$), all of the carinata seeds in different colors preformed similarly.

Metabolic Characteristics of the Protein and Feed Milk Value to Dairy Cows in Different Strains of *B. carinata* Seed. Predicted by the DVE/OEB system and the NRC-2001 model, metabolic characteristics of the protein and feed milk value to dairy cattle among different strains of *B. carinata* seed are shown in Table 4. As described in the DVE/OEB system,[23] microbial protein synthesis is estimated on the basis of FOM. Therefore, similarity in FOM among three carinata strains resulted in similarities in MCP_{FOM} and AMCP, which were significantly greater ($P = 0.001$) than those in canola seed. The yellow AAC A100 had the highest MCP_{RDP}, and the other two strains were less far from each other in MCP_{RDP}. With regard to truly absorbed RUP in the small intestine (ABCP), no changes were found among the three strains of *B. carinata* seed ($P = 0.12$). Two yellow-coated *B. carinata* seeds were similar to each other, but were lower than brown-seeded *B. carinata* ($P = 0.01$) or canola in UDM and ENDP. According to the principles of the DVE/OEB system, the DVE value is influenced by AMCP (positively), ABCP (positively), and ENDP (negatively). As a result, yellow-coated strains were greater than the brown 110915EM ($P = 0.02$) as well as the brown canola seed ($P = 0.004$) in DVE value. Although obvious variance existed among these samples, all of the oilseeds were not shown to be rich in metabolizable protein (64−82 g/kg of DM). As illustrated clearly by Tamminga et al.,[23] a positive value of degraded protein balance (OEB) indicates a potential nitrogen loss from the rumen. Our data with an average of 113 g/kg of DM in OEB value showed that the availability of protein far exceeded the availability of energy for MCP synthesis in all strains of carinata seed. Very limited studies have been conducted on protein supply and availability in oilseed samples; however, the present results showed carinata seed had an approximately 13% higher DVE value and a similar OEB value when compared to Vimy flaxseed (DVE = 70 g/kg of DM; OEB = 100 g/kg of DM).[30]

Feed milk value (FMV) was estimated from metabolizable protein efficiency for lactation according to the NRC-2001.

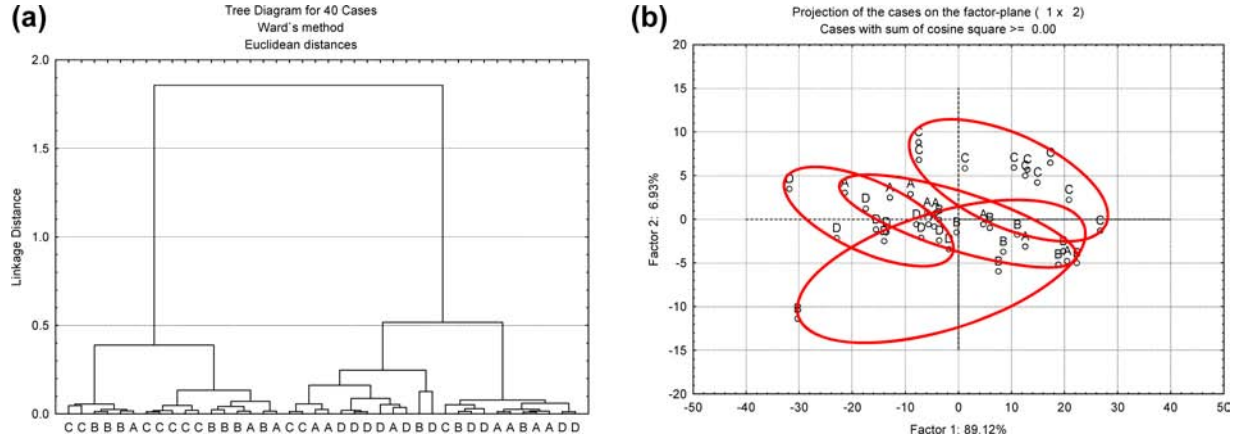

Figure 1. Multivariate molecular spectral analyses of the protein amide (1720−1482 cm^{-1}) on a molecular basis among different oilseeds: A, AAC A100; B, 110915EM; C, 111000EM; D, canola seed. (a) CLA spectral analysis of the protein amide region (1720−1482 cm^{-1}) obtained from four oilseed samples [CLA: (1) region of protein amide ca. 1720−1482 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 89.12 and 6.93% of the total variance, respectively.

Table 6. Correlation between Protein Structural Characteristics and Chemical and Nutrient Profiles of *B. carinata* and Canola Seeds

	amide I height		amide I area		amide II height		amide II area		area ratio of amide I:II		height ratio of amide I:II	
	r	*P*	*r*	*P*	*r*	*P*	*r*	*P*	*r*	*P*	*r*	*P*
protein chemical profile												
CP, %DM	−0.140	0.74	−0.282	0.50	0.041	0.92	0.021	0.96	−0.438	0.28	−0.213	0.61
NDICP, %DM	0.352	0.39	0.401	0.32	0.090	0.83	0.218	0.60	0.143	0.74	0.426	0.29
ADICP, %DM	0.373	0.36	0.173	0.68	−0.087	0.84	0.136	0.75	−0.012	0.98	0.558	0.15
SCP, %DM	−0.310	0.46	−0.436	0.28	−0.098	0.82	−0.122	0.77	−0.369	0.37	−0.340	0.41
NPN, %DM	0.626	0.10	0.508	0.20	0.515	0.19	0.311	0.45	0.089	0.83	0.483	0.23
protein subfractions												
true protein, %CP	−0.772	0.03	−0.625	0.10	−0.497	0.21	−0.352	0.39	−0.174	0.68	−0.690	0.06
PA, %CP	0.664	0.07	0.566	0.14	0.510	0.20	0.309	0.46	0.174	0.68	0.537	0.17
PB1, %CP	−0.732	0.04	−0.647	0.08	−0.551	0.16	−0.364	0.38	−0.179	0.67	−0.597	0.12
PB2, %CP	−0.018	0.97	−0.087	0.84	0.158	0.71	0.018	0.97	−0.173	0.68	−0.166	0.70
PB3, %CP	0.056	0.90	0.496	0.21	0.290	0.49	0.172	0.68	0.350	0.40	−0.094	0.83
PC, %CP	0.371	0.37	0.193	0.65	−0.071	0.87	0.146	0.73	−0.005	0.99	0.544	0.16
characteristics of CP in situ rumen degradation												
K_d, %/h	−0.225	0.59	−0.190	0.65	0.054	0.90	−0.159	0.71	0.019	0.96	−0.338	0.41
T_0, h	−0.140	0.74	0.335	0.42	0.451	0.26	0.444	0.27	−0.459	0.25	−0.472	0.24
S, %	−0.711	0.048	−0.875	0.004	−0.846	0.01	−0.749	0.03	0.282	0.50	−0.342	0.41
D, %	0.613	0.11	0.842	0.01	0.902	0.002	0.768	0.03	−0.369	0.37	0.186	0.66
U, %	0.313	0.45	0.122	0.77	−0.145	0.73	−0.039	0.93	0.258	0.54	0.480	0.23
RUP, %CP	0.538	0.17	0.525	0.18	0.277	0.51	0.408	0.32	−0.061	0.89	0.508	0.20
RUP, g/kg DM	0.572	0.14	0.460	0.25	0.345	0.40	0.497	0.21	−0.323	0.44	0.504	0.20
EDCP, %	−0.538	0.17	−0.525	0.18	−0.277	0.51	−0.408	0.32	0.061	0.89	−0.508	0.20
EDCP, g/kg DM	−0.321	0.44	−0.409	0.32	−0.083	0.85	−0.153	0.72	−0.276	0.51	−0.362	0.38
protein supply predicted by the DVE/OEB system												
FOM, g/kg DM	−0.679	0.06	−0.543	0.16	−0.288	0.49	−0.441	0.27	0.104	0.81	−0.713	0.047
MCP_{FOM}, g/kg DM	−0.679	0.06	−0.544	0.16	−0.288	0.49	−0.442	0.27	0.104	0.81	−0.713	0.047
MCP_{RDP}, g/kg DM	−0.337	0.42	−0.419	0.30	−0.094	0.82	−0.169	0.69	−0.259	0.54	−0.375	0.36
AMCP, g/kg DM	−0.679	0.06	−0.543	0.16	−0.288	0.49	−0.441	0.27	0.104	0.81	−0.713	0.047
ABCP, g/kg DM	0.149	0.73	0.143	0.74	0.324	0.43	0.219	0.60	−0.240	0.57	−0.051	0.90
UDM, g/kg DM	0.268	0.52	−0.025	0.95	−0.254	0.54	0.001	1.00	−0.010	0.98	0.530	0.18
ENDP, g/kg DM	0.270	0.52	−0.025	0.95	−0.255	0.54	0.0001	1.00	−0.009	0.98	0.532	0.18
DVE, g/kg DM	−0.294	0.48	−0.179	0.67	0.078	0.86	−0.096	0.82	−0.073	0.86	−0.462	0.25
OEB, g/kg DM	0.062	0.89	−0.155	0.71	0.093	0.83	0.109	0.80	−0.437	0.28	0.036	0.93
feed milk value predicted by the NRC-2001												
FMV, g/kg DM	−0.296	0.48	−0.177	0.68	0.083	0.85	−0.089	0.83	−0.083	0.84	−0.468	0.24

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Table 7. Correlation between Protein Secondary Structural Characteristics and Chemical and Nutrient Profiles of *B. carinata* and Canola Seeds

	α-helix height		β-sheet height		ratio of α-helix and β-sheet	
	r	*P*	*r*	*P*	*r*	*P*
protein profile						
CP, %DM	−0.140	0.74	−0.372	0.36	0.340	0.41
NDICP, %DM	0.352	0.39	0.600	0.12	−0.175	0.68
ADICP, %DM	0.373	0.36	0.494	0.21	0.032	0.94
SCP, %DM	−0.310	0.46	−0.538	0.17	0.205	0.63
NPN, %DM	0.626	0.10	0.497	0.21	0.577	0.13
protein subfractions						
true protein, %CP	−0.772	0.03	−0.719	0.045	−0.536	0.17
PA, %CP	0.664	0.07	0.575	0.14	0.528	0.18
PB1, %CP	−0.732	0.04	−0.671	0.07	−0.531	0.18
PB2, %CP	−0.018	0.97	−0.270	0.52	0.415	0.31
PB3, %CP	0.056	0.90	0.359	0.38	−0.444	0.27
PC, %CP	0.371	0.37	0.505	0.20	0.008	0.99
characteristics of CP in situ rumen degradation						
K_d, %/h	−0.225	0.59	−0.372	0.36	0.053	0.90
T_0, h	−0.140	0.74	0.125	0.77	−0.474	0.24
S, %	−0.711	0.048	−0.737	0.04	−0.445	0.27
D, %	0.613	0.11	0.634	0.09	0.406	0.32
U, %	0.313	0.45	0.331	0.42	0.129	0.76
RUP, %CP	0.538	0.17	0.652	0.08	0.185	0.66
RUP, g/kg DM	0.572	0.14	0.569	0.14	0.437	0.28
EDCP, %	−0.538	0.17	−0.652	0.08	−0.185	0.66
EDCP, g/kg DM	−0.321	0.44	−0.527	0.18	0.152	0.72
protein supply predicted by the DVE/OEB system						
FOM, g/kg DM	−0.679	0.06	−0.810	0.02	−0.227	0.59
MCP_{FOM}, g/kg DM	−0.679	0.06	−0.810	0.02	−0.227	0.59
MCP_{RDP}, g/kg DM	−0.337	0.42	−0.538	0.17	0.133	0.75
AMCP, g/kg DM	−0.679	0.06	−0.810	0.02	−0.227	0.59
ABCP, g/kg DM	0.149	0.73	−0.039	0.93	0.452	0.26
UDM, g/kg DM	0.268	0.52	0.322	0.44	0.073	0.86
ENDP, g/kg DM	0.270	0.52	0.323	0.44	0.075	0.86
DVE, g/kg DM	−0.294	0.48	−0.463	0.25	0.113	0.79
OEB, g/kg DM	0.062	0.89	−0.114	0.79	0.358	0.38
feed milk value predicted by the NRC-2001						
FMV, g/kg DM	−0.296	0.48	−0.463	0.25	0.110	0.80

Consequently, the calculated values of FMV from the lowest to the greatest were 1.41, 1.59, 1.79, and 1.82 g/kg of DM for the canola and *B. carinata* 110915EM, 111000EM, and AAC A100, respectively, which was the same trend seen for the DVE value from the Dutch system.

Protein Molecular Structural Features in Different Strains of Carinata Seed. Table 5 shows the absorbance peak height and area intensities of the protein amide I and II and protein secondary structure in yellow- and brown-seeded *B. carinata* strains in comparison to canola seed. Brown-coated carinata 110915EM showed the lower values (P = 0.01) in protein amide II height and amide I area when compared to the yellow strains (111000EM and AAC A100). Although statistically significant, these differences (0.034 vs 0.030 IR unit; 4.189 vs 3.745 IR units) were quite small. By analyzing protein secondary structural parameters, the yellow-seeded strains were still greater (P = 0.03) in β-sheet height. *Brassica* oilseed protein involves two fractions, cruciferin and napin, which are regarded as storage proteins highly associated with the nutritional properties and quality of the total seed protein.[32] The cruciferin fraction has a greater proportion of β-sheet conformation than the napin fraction (50 vs 12%),[33,34] so our results indicated that yellow-seeded *B. carinata* (111000EM and AAC A100) might have a greater concentration of cruciferin fraction than the brown seed (110915EM). With respect to the reference sample, canola seed in this study, it exhibited the highest values in protein amide I peak area (P = 0.01) and β-sheet height (P < 0.0001).

Although differences were observed among these oilseed samples in some protein structure parameters, all of the seeds could not be distinguished from each other within the protein spectral region ca. 1720−1482 cm^{-1} from AHCA and PCA analyses (Figure 1). This might imply a similarity and some relationship in protein structural makeup among different oilseeds, which was not unexpected as the seeds used in our study were all from the *Brassica* family. Also, our data to a large extent agreed with those of Xin and Yu,[18] who reported *B. carinata* meal had an internal structural relationship on molecular makeup of protein with canola meal.

Correlations between Protein Nutritive Properties and Protein Spectral Characteristics among Oilseed Strains. The Pearson correlation method was employed to find the relationship between FTIR spectroscopic information on protein molecular structure and protein chemical profile,

subfractions, and degradation kinetics, as well as predicted protein supply and feed milk value (Tables 6 and 7).

For protein amide spectral parameters, amide I height negatively correlated with true protein ($r = -0.77$, $P = 0.03$), PB1 fraction ($r = -0.73$, $P = 0.04$), and the S fraction ($r = -0.71$, $P = 0.048$). Absorbance intensities of amide I area and amide II height and area had strongly negative correlation with the S fraction but positive correlation with the D fraction. Height ratio of amide I to II was in the close relationship ($r = -0.71$, $P = 0.047$) with FOM, also with MCP_{FOM} and AMCP. This implied that protein values of oilseed might be predicted by spectral traits of protein amide I and II from FTIR spectroscopy. For protein secondary structure spectral parameters, similar findings were observed. There were negative correlations between α-helix height and true protein ($r = -0.77$, $P = 0.03$), PB1 fraction ($r = -0.73$, $P = 0.04$), and the S fraction ($r = -0.71$, $P = 0.048$). Also, β-sheet height strongly negatively correlated with true protein ($r = -0.72$, $P = 0.045$) and the S fraction ($r = -0.74$, $P = 0.04$) as well as FOM, MCP_{FOM}, and AMCP ($r = -0.81$, $P = 0.02$). These results indicated that higher absorbance in α-helix and β-sheet might cause lower values in protein features in oilseed samples. Although these correlations mentioned above were significant, most of the nutritive parameters showed weak relationships with protein spectral data. Numerous publications have reported that mid-IR spectroscopic data are highly linked to nutritional values in various kinds of feedstuff[18,30,35] but was not strongly supported by our data. This phenomenon might be attributed to the small sample size (only four oilseed varieties were examined) and narrow range of sample in biological and spectral variation (Tables 1−5). Therefore, sufficient samples with a wide and diverse range in nutritional properties would be necessary to illustrate the actual relationship between spectroscopic data and nutritional profiles in oilseed samples.

In summary, the three *B. carinata* seed strains showed different profiles for both nutritional values and protein internal structure makeup. The comparison between yellow (111000EM and AAC A100) and brown *B. carinata* seed (110915EM) indicated that the former was lower in ADICP and PC fraction and greater in the D fraction and DVE as well as FMV than the latter. Brown-seeded canola was also not in full accordance with *B. carinata* seed on these parameters. With the ATR-FTIR spectroscopic technique, the protein inherent molecular structural features were illuminated among different types of oilseed within a cellular dimension. The differences in protein structural parameters in seed samples might explain the variations on nutritive values and biological behaviors for animals. The four oilseeds exhibited a similarity in protein structural makeup from multivariate spectral analyses (AHCA and PCA). The few correlations shown in our study implied that the limited sample size and narrow range in biological and spectral variation might be a response for the weak relationships between chemical profile and mid-IR spectral data. Further studies using sufficient samples with a wide and diverse range in nutritional properties are still needed to illustrate the actual relationship between spectroscopic data and nutritional profiles in oilseeds.

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Notes

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