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# Chemical Profile, Energy Values, and Protein Molecular Structure Characteristics of Biofuel/Bio-oil Co-products (Carinata Meal) in Comparison with Canola Meal

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**ABSTRACT:** To our knowledge, little information exists on nutritive values and molecular structural characteristics associated with protein biopolymers of carinata meal from biofuel and bio-oil processing. The objectives of this study were to investigate (1) chemical compositions; (2) protein and carbohydrate subfractions partitioned by the Cornell Net Carbohydrate and Protein System (CNCPS); (3) truly digestible nutrients and energy values; (4) protein conformation spectral characteristics using the ATR-FT/IR technique; and (5) the correlation between protein intrinsic structural features and nutrient profiles of carinata meal in comparison with conventional canola meal as references. The results showed that carinata meal was higher ($p < 0.05$) in soluble crude protein (SCP, 55.6% CP) and nonprotein nitrogen (NPN, 38.5% CP) and lower in acid detergent insoluble crude protein (ADICP, 1.3% CP) compared to canola meal. Although no differences were found in CP and carbohydrate (CHO) contents, CNCPS protein and carbohydrate subfractions were different ($p < 0.05$) between carinata meal and canola meal. Carinata meal has similar contents of total digestible nutrient (TDN) and predicted energy values to canoal meal ($p > 0.05$). As for protein spectral features, much greater IR absorbance in amide I height and area as well as $\alpha$-helix and $\beta$-sheet height for carinata meal by 20−31% ($p < 0.05$) was found compared with canola meal; however, results from agglomerative hierarchical cluster analysis (CLA) and principal component analysis (PCA) indicated these two meals could not be distinguished completely within the protein spectrum (ca. 1728−1478 $cm^{-1}$). Additionally, close correlations were observed between protein structural parameters and protein nutrient profiles and subfractions. All the comparisons between carinata meal and canola meal in our study indicated that carinata meal could be used as a potential high-protein supplement source for ruminants. Further study is needed on more information associated with nutrient degradability, utilization, and availability of carinata meal to ruminants for its better and effective application in animal industry.

**KEYWORDS:** *co-products from biofuel and bio-oil processing, carinata and canola meal, nutrient, protein spectral profile, correlation*

## INTRODUCTION

With the expansion of the biofuel and bio-oil industry in North America, a variety of co-products is left after oil extraction. Although these oilseed meals cannot be directly used for human consumption because of their antinutritional components and undigestible fiber content, they are good protein sources for animal feed or organic fertilizer[1,2] since protein is concentrated during the manufacturing process, for example, canola meal.[3,4] *Brassica carinata*, commonly known as Ethiopian mustard, has an oil profile optimized for use in the biofuel or bio-oil industry. Compared with canola crop, carinata is ideally suited to grow in semiarid regions and has excellent harvestability with good lodging and shatter resistance.[5] Therefore, some regions with semiarid climates, such as the southern prairies of Canada and the Northern Plains of the United States, are showing more and more interest in this vigorous crop for biofuel or bio-oil production, resulting in substantial carinata meal left as co-product. However, information on protein nutrient profiles of carinata meal is extremely rare, and this situation is a real obstacle for its effective utilization in animals.

In regard to feed protein quality, published results have demonstrated that the true biological value of feed protein depends not only on its protein content but also on internal molecular structures.[6−8] Consequently, protein's inherent structures including its secondary structures, such as $\alpha$-helix and $\beta$-sheet, are highly associated with protein quality, utilization, and availability to animals.[6,9] Moreover, Fourier transform infrared spectroscopy (FT/IR) technique with ATR, a promising tool, has been successfully applied recently to detect structural changes of molecular makeup and conformation of biopolymers among different kinds of feedstuff.[10−12] Again, studies are lacking to date on protein structural features of carinata meal from biofuel/bio-oil processing plants.

Therefore, as far as we know, this is the first paper to show detailed nutritional characteristics of carinata meal, one of the co-products from biofuel or bio-oil processing. As another major member plant in the *Brassica* family, canola processing co-product (canola meal) is widely and successfully used as a supplementary protein resource in the animal industry.[3,4] Thus, canola meal was used in our study as a reference. The objectives of this study were to reveal (1) chemical compositions; (2) protein and carbohydrate subfractions partitioned by the Cornell Net Carbohydrate and Protein System (CNCPS); (3) truly digestible nutrients and energy values; (4) protein

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conformation spectral characteristics using the ATR-FT/IR technique; and (5) the correlation between protein intrinsic structural features and nutrient profiles in comparison with conventional canola meal.

## MATERIALS AND METHOD

**Carinata Meal Co-products from Biofuel and Bio-oil Processing.** Carinata meal ($n$ = 2 sources) was produced by Agrisoma Sciences, Canada, and canola meal ($n$ = 2 sources) was obtained from Cargill (Manitoba, Canada).

**Chemical Analysis.** The samples were ground through a 1 mm screen (Retsch ZM-1; Brinkmann Instruments, Mississauga, ON, Canada) and analyzed for dry matter (DM, AOAC official method 930.15), ash (AOAC official method 942.05), crude protein (CP, AOAC official method 984.13), ether extract (EE, AOAC official method 920.39), neutral detergent fiber (NDF, AOAC official method 2002.04), and acid detergent fiber (ADF, AOAC official method 973.18) according to AOAC methods.[13] The concentration of NDF was analyzed with the addition of sodium sulfite and heat-stable amylase according to previous literature.[14] The contents of neutral detergent-insoluble crude protein (NDICP) and acid detergent-insoluble crude protein (ADICP) were determined according to Licitra et al. (1996).[15] Acid detergent lignin (ADL)[14] and soluble crude protein (SCP)[16] concentrations were determined in our study. Nonprotein nitrogen (NPN) concentrations were obtained by precipitating true protein in the filtrate with trichloroacetic acid (TCA, 10%) and determined as the difference between total N and the N content of the residue after filtration. Nitrogen-adjusted NDF (NDFn) and nitrogen-adjusted ADF (ADFn) were calculated as NDF − NDICP and ADF − ADICP. As given by the NRC (2001),[17] total carbohydrate (CHO), nonfiber CHO (NFC), hemicellulose, and cellulose were calculated as follows: CHO = 100 − EE − CP − ash, NFC = 100 − (NDF − NDICP) − EE − CP − ash, hemicellulose = NDF − ADF, and cellulose = ADF − ADL. All samples were analyzed in duplicate and repeated if error exceeded 5%.

**Fractionation of Protein Fractions and Carbohydrate Fractions.** The protein and carbohydrate subfractions were partitioned using the Cornell Net Carbohydrate and Protein System.[18] Individual protein fractions in this system are described as fractions PA, PB, and PC. Fraction PA is nonprotein nitrogen (its degradation rate is infinity); fraction PB is partitioned according to the differences in ruminal degradation rates. PB1 is rapidly degraded protein (its degradation rate is 1.20−4.00/h); fraction PB2 is intermediately degraded protein (its degradation rate is 0.03−0.16/h); fraction PB3 is slowly degraded protein (its degradation rate is 0.0006−0.0055/h); fraction PC is considered to be undegradable protein (like ADICP), which is highly resistant to digestion by microorganisms and host enzyme systems.[18]

Carbohydrate is partitioned into the following fractions: (1) CA fractions are composed of soluble sugars with a rapid degradation rate of 3.00/h; (2) CB1 is an intermediately degradable fraction such as starch or pectin with a degradation rate of 0.20−0.50/h; (3) CB2 is a slowly degradable fraction such as available cell wall with a degradation rate of 0.02−0.10/h; and (4) CC is a fraction such as unavailable cell wall.[18]

**Energy Values.** Total digestible nutrient (TDN), as well as digestible energy, metabolizable energy, and net energy, are commonly used for estimation of available energy in feedstuffs. Total digestible nonfiber carbohydrate (tdNFC), crude protein (tdCP), neutral detergent fiber (tdNDF), and fatty acid (tdFA), total digestible nutrient at 1× maintenance ($TDN_{1x}$), digestible energy at production level of intake ($DE_{3x}$), metabolizable energy at production level of intake ($ME_{3x}$), and net energy at production level of intake ($NE_{3x}$) were determined using a summative approach from the NRC-2001 dairy.[17] Net energy for maintenance ($NE_m$) and net energy for growth ($NE_g$) were predicted using NRC-1996 beef.[19]

**Molecular Spectroscopy on Protein Structure.** The protein molecular spectrum data of different sources of carinata meal and canola meal samples were collected using a JASCO FT/IR 4200 with ATR (JASCO Corporation, Tokyo, Japan), at the Feed Molecular Structure Analysis Lab at the Department of Animal and Poultry Science, University of Saskatchewan (SK, Canada). The samples were ground through a 1 mm screen before spectral analysis. The IR spectrum of each sample was obtained within the mid-IR range (ca. 4000−800 $cm^{-1}$) with 32 scans at a resolution of 4 $cm^{-1}$. Five replicates were randomly carried out for each meal sample.

Subsequently, the spectral data were analyzed by OMNIC 7.2 software (Spectra Tech., Madison, WI, USA). The spectral parameters, which were associated with protein molecular structure, detected in our study included amide I and II height and area, $\alpha$-helix and $\beta$-sheet peak height, and their ratios. Chemical functional groups were identified according to previous publications.[20,21] The protein baseline was ca. 1728−1478 $cm^{-1}$ for both amide I and II, and their peaks fell within the range ca. 1728−1576 and 1576−1478 $cm^{-1}$, respectively. For detection of $\alpha$-helix and $\beta$-sheet in the protein secondary structures, two steps were applied as described by Yu (2005).[22] In our study, the peaks of $\alpha$-helix and $\beta$-sheet fell within the range ca. 1654−1650 and ca. 1627−1620 $cm^{-1}$, respectively. Spectral peak intensity height and area ratios were calculated based on respective spectral data.

**Multivariate Analyses.** Two molecular spectral multivariate analyses, agglomerative hierarchical cluster analysis (CLA) and principal component analysis (PCA), were performed using Statistica 8.0 software (StatSoft Inc., Tulsa, OK, USA), to clarify whether there were spectral differences between carinata meal and canola meal within the protein fingerprint spectra (ca. 1728−1478 $cm^{-1}$).

**Statistical Analysis.** Chemical and nutrient profile data of carinata meal and canola meal were statistically analyzed using the mixed model procedure of SAS 9.2, and the model was

$$Y_{ij} = \mu + F_i + e_{ij}$$

where $Y_{ij}$ was the observation of the dependent variable $ij$; $\mu$ was the fixed effect of population mean of the variable; $F_i$ was a fixed effect of meal type ($i$ = 2; carinata meal and canola meal), each meal source as replications; and $e_{ij}$ was the random error associated with observation $ij$.

The spectral data of carinata meal and canola meal were statistically analyzed using the mixed model procedure of SAS 9.2, and the model was

$$Y_{ij} = \mu + F_i + S(F)_j + e_{ij}$$

where $Y_{ij}$ was the observation of the dependent variable $i$; $\mu$ was the fixed effect of population mean of the variable; $F_i$ was a fixed effect of meal type ($i$ = 2; carinata meal and canola meal); $S(F)_j$ was a random effect of meal source nested within the meal; and $e_{ij}$ was the random error associated with observation $i$.

The relationships between the changes in protein structure amide I and II height, structure amide I and II area, $\alpha$-helix and $\beta$-sheet, and their ratios and the changes in chemical protein profile, CNCPS protein fractions, and estimated energy values in carinata meal ($n$ = 2 sources) and canola meal ($n$ = 2 sources) were analyzed using the PROC CORR of SAS using the Pearson correlation method.

For all statistical analysis, model assumption checking was carried out by residual analysis using Proc Univariate with normal and plot options. Multiple treatment comparisons were performed using the Tukey−Kramar test with letter groupings using the pdmix800 macro.[23] Statistical significance was declared and detected at $p < 0.05$, while trends were declared at $p \leq 0.10$.

## RESULTS AND DISCUSSION

**Chemical Profiles, Protein and Carbohydrate Fractions, and Energy Values of Carinata Meal in Comparison with Canola Meal.** The chemical profiles of carinata meal in comparison with canola meal are presented in Table 1. The contents of DM, OM, NDF, ADF, CP, and EE ($p > 0.05$) were not different between the two meals. The CP content of carinata meal in our study was consistent with a previous


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**Table 1. Chemical Profile of Carinata Meal in Comparison with Canola Meal**

| | carinata | canola | SEM[a] | $p$ |
|---|---|---|---|---|
| DM, %[b] | 93.31 | 91.55 | 1.217 | 0.41 |
| ash, % DM | 7.11 | 7.47 | 0.472 | 0.64 |
| OM, % DM | 92.89 | 92.53 | 0.472 | 0.64 |
| EE, % DM | 2.17 | 2.17 | 0.610 | 0.10 |
| CP, % DM | 48.17 | 40.41 | 3.523 | 0.26 |
| SCP, % DM | 25.01 | 12.86 | 1.962 | 0.048 |
| SCP, % CP | 55.61 | 34.77 | 0.415 | 0.001 |
| NPN, % DM | 17.33 | 10.07 | 1.483 | 0.07 |
| NPN, % CP | 38.48 | 27.23 | 0.475 | 0.004 |
| NDICP, % DM | 4.57 | 6.91 | 1.140 | 0.28 |
| NDICP, % CP | 9.52 | 17.15 | 2.891 | 0.20 |
| ADICP, % DM | 0.59 | 1.34 | 0.113 | 0.04 |
| ADICP, % CP | 1.26 | 3.32 | 0.335 | 0.049 |
| NDF, % DM | 18.79 | 27.54 | 2.404 | 0.12 |
| ADF, % DM | 11.36 | 18.58 | 1.784 | 0.10 |
| ADF, % NDF | 59.84 | 67.67 | 3.766 | 0.28 |
| ADL, % DM | 2.97 | 8.51 | 0.909 | 0.05 |
| ADL, % NDF | 15.37 | 30.90 | 2.509 | 0.048 |
| ADL, % ADF | 25.37 | 45.73 | 3.075 | 0.04 |
| NDFn, % DM | 14.22 | 20.62 | 2.042 | 0.16 |
| ADFn, % DM | 10.77 | 17.24 | 1.695 | 0.11 |
| CHO, % DM | 42.55 | 49.95 | 3.646 | 0.29 |
| NFC, % DM | 28.33 | 29.33 | 1.705 | 0.72 |
| NFC, % CHO | 66.90 | 58.72 | 2.020 | 0.10 |
| hemicellulose, % DM | 7.43 | 8.96 | 1.025 | 0.40 |
| hemicellulose, % CHO | 17.69 | 17.92 | 2.385 | 0.95 |
| cellulose, % DM | 8.38 | 10.07 | 0.937 | 0.33 |
| cellulose, % CHO | 19.61 | 20.15 | 0.524 | 0.54 |

[a]SEM, standard error of the mean. [b]DM, dry matter; CP, crude protein; EE, ether extract; NDF, neutral detergent fiber; ADF, acid detergent fiber; ADL, acid detergent lignin; NDICP, neutral detergent-insoluble crude protein; ADICP, acid detergent-insoluble crude protein; SCP, soluble crude protein; NPN, nonprotein nitrogen; NDFn, nitrogen-adjusted NDF; ADFn, nitrogen-adjusted ADF; CHO, total carbohydrate; NFC, nonfiber carbohydrate.

report,[24] in which protein contents of brown- and yellow-seeded *Brassica carinata* were 48.8% and 52.6% DM, respectively. However, another study[25] showed that the CP content of carinata meal was much lower (38.9% DM) than that observed in our study. This large difference might be partially due to the different processing of carinata meal, since the meal used in their research was obtained from extraction with hexane in a Soxhlet extractor in the lab.[25] As for the other protein profiles, carinata meal was remarkably higher in SCP (55.6% vs 34.8% CP; $p = 0.001$) and NPN (38.5% vs 27.2% CP; $p = 0.004$) compared to canola meal. However, the protein fraction associated with the ADF in carinata meal was lower than that of canola meal by 62% ($p = 0.049$). Since ADICP is considered nondegradable during ruminal fermentation, the lower content of ADICP in the feedstuff may indicate its better protein quality. The DM, CP, and ADICP contents in canola meal found in our study were similar to those obtained in the report of Brito and Broderick.[4] The contents of NDF and ADF of carinata meal were 18.8% DM and 11.4% DM, respectively, which were similar to those of canola meal. However, a lower value was observed in the content of ADL (% DM, % NDF, or % ADF) for carinata meal, and ADL content was almost tripled in canola meal (2.97% vs 8.51% DM; $p = 0.05$). According to Mustafa et al. (1996),[26] a close relationship was found between canola seed hull and lignin concentration. The lower ADL content of carianta meal in our result might be explained in large part by the bigger seed size and fewer hulls in the carinata seed.[27] Similar results were observed in carinata meal for contents of NDFn, ADFn, CHO, NFC, hemicellulose, and cellulose compared to canola meal ($p > 0.05$). Also the NDF, ADF, and hemicellulose contents of canola meal were in general agreement with a previous study.[4]

Results for protein and carbohydrate subfractions partitioned by the CNCPS[18,28] are presented in Table 2. In our study, PA

**Table 2. Protein and Carbohydrate Subfractions of Carinata Meal in Comparison with Canola Meal Using CNCPS[a]**

| | carinata | canola | SEM[b] | $p$ |
|---|---|---|---|---|
| Protein Subfractions | | | | |
| PA, % DM | 18.53 | 11.01 | 1.354 | 0.06 |
| PB1, % DM | 8.23 | 3.05 | 0.466 | 0.02 |
| PB2, % DM | 19.45 | 16.85 | 2.018 | 0.49 |
| PB3, % DM | 3.98 | 5.57 | 1.061 | 0.40 |
| PC, % DM | 0.59 | 1.34 | 0.114 | 0.04 |
| PA, % CP | 38.48 | 27.23 | 0.475 | 0.004 |
| PB1, % CP | 17.13 | 7.54 | 0.619 | 0.01 |
| PB2, % CP | 34.87 | 48.09 | 2.986 | 0.09 |
| PB3, % CP | 8.27 | 13.83 | 2.622 | 0.27 |
| PC, % CP | 1.26 | 3.32 | 0.335 | 0.049 |
| true protein (TP), % CP | 60.26 | 69.45 | 0.343 | 0.003 |
| PB1, % TP | 28.42 | 10.84 | 1.002 | 0.006 |
| PB2, % TP | 57.86 | 69.27 | 4.481 | 0.21 |
| PB3, % TP | 13.72 | 19.89 | 3.714 | 0.36 |
| Carbohydrate Subfractions | | | | |
| CA, % DM | 26.06 | 26.36 | 2.022 | 0.93 |
| CB1, % DM | ND | ND | | |
| CB2, % DM | 8.55 | 1.00 | 0.712 | 0.02 |
| CC, % DM | 7.94 | 22.59 | 2.220 | 0.04 |
| CA, % CHO | 61.36 | 52.78 | 1.200 | 0.04 |
| CB1, % CHO | ND | ND | | |
| CB2, % CHO | 20.45 | 2.01 | 2.503 | 0.04 |
| CC, % CHO | 18.20 | 45.22 | 3.621 | 0.03 |

[a]CNCPS, Cornell Net Carbohydrate and Protein System. PA, rapidly degradable protein subfraction as per CNCPS ($K_d$ = 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; CA, rapidly fermented carbohydrate subfraction as per CNCPS ($K_d$ = 200-350% $h^{-1}$); CB1, intermediately degraded carbohydrate subfraction as per CNCPS ($K_d$ = 20−50% $h^{-1}$); CB2: slowly degraded carbohydrate subfraction as per CNCPS ($K_d$ = 2−10% $h^{-1}$); CC, unavailable cell wall as per CNCPS; ND, not detected. [b]SEM, standard error of the mean.

and PB1 fractions were higher in carinata meal than those in canola meal (38.5% vs 23.2% CP and 17.1% vs 7.5% CP, respectively; $p < 0.05$), and these results were undoubtedly consistent with NPN and SCP data shown in Table 1. Fraction PC consists of protein bound to lignin or tannins and Maillard reaction protein; this fraction is considered undegradable in the rumen and consequently poorly used by the animals.[18,29] Carinata meal had a lower content of PC fraction ($p < 0.05$), which might be an indication of an improvement of protein nutritional value for ruminants when compared to canola meal. No differences were found between these two meals for the

intermediately (PB2) and slowly (PB3) degradable protein fractions ($p > 0.05$), whether expressed as percentage of DM, CP, or true protein (TP). Little information on protein subfractions of carinata meal could be found from previous publications, but as for canola meal, we obtained higher values for PA, PB1, and PB3, a lower value for PB2, and a similar value for PC than those obtained in another study (in press) in our group,[30] which might be due to different canola varieties or different meal processing between these two studies. The TP is considered as the PB fraction (the sum of PB1, PB2, and PB3) in CNCPS, and this part can be degraded in the rumen.[18] The content of TP in carinata meal accounted for 60.3% of the total CP, which was nine percentage units below ($p = 0.003$) that of canola meal.

Although carinata and canola meals had similar contents of CHO (Table 1), they differed in carbohydrate subfractions including CA, CB2, and CC ($p < 0.05$; Table 2). Fraction CA, representing rapidly fermented soluble sugars, was markedly higher in the present study for carinata meal (61.4% CHO) compared to canola meal. No CB1 fraction was observed in our study, as no starch could be detected in both meals. As for other subfractions, carinata meal contained approximately 10-fold more slowly degraded carbohydrate fraction (CB2) and 2.5 times less unavailable fiber (CC) compared with canola meal. These results indicated that carinata meal had a higher level of degradable carbohydrate during rumen fermentation. As we know, protein and carbohydrate subfractions partitioned by the CNCPS are highly associated with ruminal degradation behavior and nutrient availability to ruminants; therefore carinata meal may not have exactly the same characteristics of ruminal degradation as canola meal even though its contents of CP and CHO were similar to those of canola meal.

No differences could be observed in truly digestible nutrients including tdNFC, tdCP, tdNDF, and tdFA as well as $TDN_{1x}$ between carinata meal and canola meal in this study (Table 3). According to the equations in the NRC-2001 model, predicted energy values of $DE_{1x}$ and $DE_{3x}$ were 3.89 and 3.53 Mcal/kg, respectively, for carinata meal. Again no published data on energy values of carinata meal were available for our comparison, but our results on canola meal were consistent with those predicted by other researchers.[3,30] The calculated values for $ME_{3x}$ and $NE_{L3x}$ for dairy and ME, $NE_m$, and $NE_g$ for beef cattle of carinata meal were 3.12, 2.01, 3.19, 2.19, and 1.51 Mcal/kg, respectively. As for the canola meal, these energy values were closely consistent with those obtained from brown-seeded canola meal (*Brassica napus*) in recent research.[30]

**Molecular Structure Characteristics of Protein in Carinata Meal in Comparison with Canola Meal.** It has been proved that ATR-FT/IR molecular spectroscopy can be used as a rapid tool to detect changes in intrinsic spectral features in relation to protein structures.[10,11] Infrared molecular spectroscopic characteristics of protein structures of carinata meal in comparison with canola meal are shown in Table 4, revealing the IR absorbance of peak height or area for amide I, amide II, $\alpha$-helix, $\beta$-sheet, and their spectral ratios. We found greater IR absorbance ($p < 0.05$) in amide I height (0.041 vs 0.033 IR unit) and area (3.245 vs 2.693 IR unit) as well as $\alpha$-helix height (0.042 vs 0.032 IR unit) and $\beta$-sheet height (0.037 vs 0.030 IR unit) for carinata meal by 20−31% when compared with canola meal. The amide I and II bands are the two primary features within the protein spectrum. The amide I band is particularly sensitive to changes in protein secondary structure,[31−34] and $\alpha$-helix and $\beta$-sheet are the two typical structures in protein secondary structure,[35,36] which closely relates to feed quality, digestive behavior, and nutrient availability to animals.[6,37,38] In protein secondary structure, a higher percentage of $\beta$-sheet may cause lower protein degradability and utilization in ruminants.[6] Though protein $\alpha$-helix and $\beta$-sheet are peak absorption intensity values rather than exact determinations, they can still be used for treatment comparison.[7] As a result, it might be inferred from our data that carinata meal would have a different degradability or utilization of protein during ruminal fermentation from canola meal, resulting from its greater amide I height and area as well as $\alpha$-helix and $\beta$-sheet heights in the protein structural makeup.

**Table 3. Energy Values[a] of Carinata Meal in Comparison with Canola Meal**

| | carinata | canola | SEM[b] | $p$ |
|---|---|---|---|---|
| tdNFC, % DM | 27.77 | 28.74 | 1.673 | 0.72 |
| tdCP, % DM | 47.94 | 39.87 | 3.548 | 0.25 |
| tdNDF, % DM | 5.48 | 4.06 | 0.395 | 0.13 |
| tdFA, % DM | 1.17 | 1.17 | 0.610 | 0.10 |
| $TDN_{1x}$, % DM | 76.82 | 68.30 | 2.934 | 0.18 |
| $TDN_{3x}$, % DM | 69.77 | 63.97 | 1.846 | 0.16 |
| $DE_{1x}$, Mcal/kg, NRC-2001 dairy | 3.89 | 3.42 | 0.174 | 0.20 |
| $DE_{3x}$, Mcal/kg, NRC-2001 dairy | 3.53 | 3.22 | 0.116 | 0.20 |
| $ME_{3x}$, Mcal/kg, NRC-2001 dairy | 3.12 | 2.80 | 0.120 | 0.20 |
| $NE_{L3x}$, Mcal/kg, NRC-2001 dairy | 2.01 | 1.78 | 0.082 | 0.20 |
| ME, Mcal/kg, NRC-1996 beef | 3.19 | 2.80 | 0.142 | 0.19 |
| $NE_m$, Mcal/kg, NRC-1996 beef | 2.19 | 1.87 | 0.113 | 0.18 |
| $NE_g$, Mcal/kg, NRC-1996 beef | 1.51 | 1.23 | 0.097 | 0.19 |

[a]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 one 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 4. Protein Amide I and II Profiles and Protein Secondary Structure Profiles of Carinata Meal and Canola Meal, Revealed Using Infrared Molecular Spectroscopy**

| | carinata | canola | SEM[c] | $p$ |
|---|---|---|---|---|
| protein amides profiles[a] | | | | |
| amide I height | 0.041 | 0.033 | 0.002 | 0.003 |
| amide II height | 0.023 | 0.021 | 0.001 | 0.43 |
| height ratio of amide I:II | 1.844 | 1.559 | 0.057 | 0.07 |
| amide I area | 3.245 | 2.693 | 0.136 | 0.01 |
| amide II area | 1.358 | 1.217 | 0.063 | 0.13 |
| area ratio of amide I:II | 2.390 | 2.235 | 0.164 | 0.57 |
| protein secondary structure[b] | | | | |
| $\alpha$-helix height | 0.042 | 0.032 | 0.002 | 0.003 |
| $\beta$-sheet height | 0.037 | 0.030 | 0.002 | 0.01 |
| height ratio of $\alpha$-helix:$\beta$-sheet | 1.132 | 1.063 | 0.045 | 0.39 |

[a]Protein amide data unit, IR absorbance unit; the protein peak baseline, 1728−1478 $cm^{-1}$; protein amide I region, 1728−1576 $cm^{-1}$; protein amide II region, 1576−1478 $cm^{-1}$. [b]The peaks of $\alpha$-helix and $\beta$-sheet fell within the range ca. 1654−1650 and 1627−1620 $cm^{-1}$, respectively. [c]SEM, standard error of the mean.


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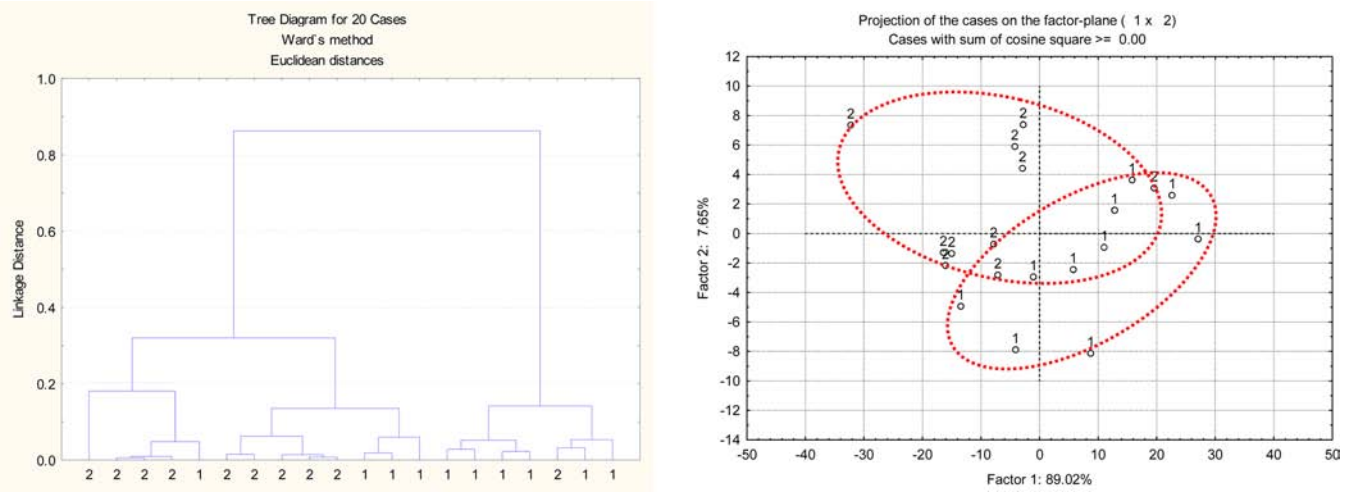


**Figure 1.** Multivariate molecular spectral analyses of amide I and amide II (1728−1478 $cm^{-1}$) on a molecular basis between canola meal and carinata meal: 1 = canola meal; 2 = carinata meal.

**Table 5. Correlation between Protein Structural Characteristics and Chemical and Nutrient Profiles of Carinata Meals and Canola Meals**

| | amide I height | | amide II height | | height ratio of amide I and II | | amide I area | | amide II area | | area ratio of amide I and II | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | $r^a$ | $p$ | $r$ | $p$ | $r$ | $p$ | $r$ | $p$ | $r$ | $p$ | $r$ | $p$ |
| Chemical Profiles | | | | | | | | | | | | |
| DM, % | 0.651 | 0.349 | 0.026 | 0.974 | 0.674 | 0.326 | 0.533 | 0.468 | 0.644 | 0.356 | 0.082 | 0.918 |
| CP, % DM | 0.798 | 0.202 | 0.163 | 0.837 | 0.819 | 0.181 | 0.706 | 0.294 | 0.704 | 0.296 | 0.244 | 0.756 |
| NDICP, % CP | −0.848 | 0.152 | −0.237 | 0.763 | −0.946 | 0.054 | −0.945 | 0.055 | −0.348 | 0.652 | −0.866 | 0.135 |
| ADICP, % CP | −0.982 | 0.018 | −0.455 | 0.545 | −0.996 | 0.005 | −0.974 | 0.026 | −0.695 | 0.305 | −0.583 | 0.417 |
| SCP, % CP | 0.988 | 0.012 | 0.728 | 0.272 | 0.921 | 0.079 | 0.948 | 0.052 | 0.823 | 0.177 | 0.429 | 0.571 |
| NPN, % CP | 0.995 | 0.005 | 0.668 | 0.332 | 0.952 | 0.048 | 0.972 | 0.028 | 0.780 | 0.220 | 0.500 | 0.500 |
| NPN, % SCP | −0.909 | 0.091 | −0.865 | 0.135 | −0.770 | 0.230 | −0.809 | 0.191 | −0.931 | 0.069 | −0.155 | 0.845 |
| Protein Fractions Partitioned by CNCPS | | | | | | | | | | | | |
| PA, % CP | 0.995 | 0.005 | 0.668 | 0.332 | 0.952 | 0.048 | 0.972 | 0.028 | 0.780 | 0.220 | 0.500 | 0.500 |
| PB1, % CP | 0.968 | 0.032 | 0.791 | 0.210 | 0.875 | 0.125 | 0.909 | 0.091 | 0.864 | 0.136 | 0.343 | 0.657 |
| PB2, % CP | −0.861 | 0.139 | −0.890 | 0.110 | −0.700 | 0.300 | −0.739 | 0.261 | −0.953 | 0.047 | −0.047 | 0.953 |
| PB3, % CP | −0.783 | 0.217 | −0.168 | 0.832 | −0.902 | 0.098 | −0.907 | 0.093 | −0.239 | 0.761 | −0.918 | 0.082 |
| PC, % CP | −0.982 | 0.018 | −0.455 | 0.545 | −0.996 | 0.005 | −0.974 | 0.026 | −0.695 | 0.305 | −0.583 | 0.417 |
| true protein (TP), % CP | −0.988 | 0.012 | −0.711 | 0.289 | −0.932 | 0.068 | −0.962 | 0.038 | −0.791 | 0.209 | −0.476 | 0.524 |
| PB1, % TP | 0.972 | 0.028 | 0.783 | 0.218 | 0.884 | 0.116 | 0.920 | 0.081 | 0.850 | 0.150 | 0.368 | 0.632 |
| PB2, % TP | −0.717 | 0.283 | −0.917 | 0.083 | −0.513 | 0.487 | −0.557 | 0.443 | −0.955 | 0.045 | 0.184 | 0.816 |
| PB3, % TP | −0.703 | 0.297 | −0.056 | 0.944 | −0.849 | 0.151 | −0.849 | 0.152 | −0.124 | 0.876 | −0.951 | 0.049 |
| Truly Digestible Nutrients and Energy Values | | | | | | | | | | | | |
| tdCP, % DM | 0.808 | 0.192 | 0.174 | 0.826 | 0.828 | 0.172 | 0.718 | 0.282 | 0.707 | 0.293 | 0.255 | 0.745 |
| $TDN_{1x}$, % DM | 0.875 | 0.125 | 0.249 | 0.751 | 0.896 | 0.104 | 0.805 | 0.195 | 0.722 | 0.278 | 0.350 | 0.650 |
| $TDN_{3x}$, % DM | 0.887 | 0.113 | 0.312 | 0.688 | 0.886 | 0.114 | 0.801 | 0.199 | 0.775 | 0.225 | 0.296 | 0.705 |
| $DE_{1x}$, Mcal/kg | 0.858 | 0.142 | 0.227 | 0.773 | 0.879 | 0.121 | 0.782 | 0.218 | 0.718 | 0.282 | 0.326 | 0.674 |
| $DE_{3x}$, Mcal/kg | 0.850 | 0.150 | 0.218 | 0.782 | 0.871 | 0.129 | 0.773 | 0.227 | 0.716 | 0.284 | 0.315 | 0.685 |
| $ME_{3x}$, Mcal/kg | 0.846 | 0.154 | 0.213 | 0.787 | 0.868 | 0.132 | 0.768 | 0.232 | 0.714 | 0.286 | 0.311 | 0.689 |
| $NE_{L3x}$, Mcal/kg | 0.854 | 0.146 | 0.232 | 0.768 | 0.872 | 0.129 | 0.774 | 0.226 | 0.727 | 0.273 | 0.307 | 0.693 |
| ME, Mcal/kg | 0.856 | 0.144 | 0.221 | 0.779 | 0.879 | 0.121 | 0.782 | 0.218 | 0.712 | 0.288 | 0.331 | 0.669 |
| $NE_m$, Mcal/kg | 0.865 | 0.135 | 0.233 | 0.767 | 0.888 | 0.112 | 0.794 | 0.206 | 0.716 | 0.284 | 0.342 | 0.659 |
| $NE_g$, Mcal/kg | 0.865 | 0.135 | 0.234 | 0.766 | 0.887 | 0.113 | 0.793 | 0.207 | 0.716 | 0.284 | 0.340 | 0.660 |

[a] $r$, Pearson correlation coefficient.

The advantages and application of both cluster and principal component analysis, which are data reduction methods, in classifying inherent chemical structure differences have been described in detail by Yu (2005).[39] Figure 1 shows the results of CLA and PCA of protein spectral features (region ca. 1728−1478 $cm^{-1}$), comparing carinata meal with canola meal, and Ward's algorithm method was applied in CLA analysis with the exception of any prior parametrization in the protein IR region in our study. No clear separate classes could be distinguished between carinata meal and canola meal in cluster analysis. Also these two meals could not be fully grouped into separate ellipses since overlapping of groups was obviously found in

PCA figures as the first and second principal components, explaining 89.02% and 7.65% of the total variance in our study. These results indicated that an inherent structural relationship in molecular makeup of protein existed between carinata meal and canola meal. To our knowledge, no publications could be found on the internal protein structure of carinata meal, and no comparison therefore can be made.

**Correlation Analysis between Protein Spectral Features and Nutrient Profiles in Carinata Meal and Canola Meal.** Protein structural characteristics, such as amide I and II profiles, and protein secondary structural characteristics, such as $\alpha$-helix and $\beta$-sheet profiles, in relation to chemical composition, protein subfractions, truly digestible nutrients, and energy values in carinata meal and canola meal are shown in Tables 5 and 6.

**Table 6. Correlation between Protein Secondary Structural Characteristics and Chemical and Nutrient Profiles of Carinata Meals and Canola Meals**

| | $\alpha$-helix height | | $\beta$-sheet height | | height ratio of $\alpha$-helix and $\beta$-sheet | |
|---|---|---|---|---|---|---|
| | $r$[a] | $p$ | $r$ | $p$ | $r$ | $p$ |
| Chemical Profiles | | | | | | |
| DM, % | 0.468 | 0.532 | 0.549 | 0.451 | 0.290 | 0.710 |
| CP, % DM | 0.639 | 0.361 | 0.720 | 0.280 | 0.336 | 0.664 |
| NDICP, % CP | −0.782 | 0.218 | −0.944 | 0.056 | −0.006 | 0.994 |
| ADICP, % CP | −0.914 | 0.086 | −0.978 | 0.022 | −0.369 | 0.631 |
| SCP, % CP | 0.995 | 0.005 | 0.949 | 0.051 | 0.615 | 0.385 |
| NPN, % CP | 0.988 | 0.012 | 0.973 | 0.027 | 0.542 | 0.458 |
| NPN, % SCP | −0.953 | 0.047 | −0.811 | 0.189 | −0.823 | 0.177 |
| Protein Fractions Partitioned by CNCPS | | | | | | |
| PA, % CP | 0.988 | 0.012 | 0.973 | 0.027 | 0.542 | 0.458 |
| PB1, % CP | 0.992 | 0.008 | 0.911 | 0.089 | 0.693 | 0.307 |
| PB2, % CP | −0.915 | 0.085 | −0.742 | 0.258 | −0.880 | 0.120 |
| PB3, % CP | −0.720 | 0.281 | −0.905 | 0.096 | 0.097 | 0.903 |
| PC, % CP | −0.914 | 0.086 | −0.978 | 0.022 | −0.369 | 0.631 |
| true protein (TP), % CP | −0.996 | 0.005 | −0.963 | 0.038 | −0.577 | 0.424 |
| PB1, % TP | 0.995 | 0.005 | 0.920 | 0.080 | 0.675 | 0.325 |
| PB2, % TP | −0.795 | 0.205 | −0.560 | 0.440 | −0.968 | 0.032 |
| PB3, % TP | −0.630 | 0.370 | −0.846 | 0.154 | 0.218 | 0.782 |
| Truly Digestible Nutrients and Energy Values | | | | | | |
| tdCP, % DM | 0.651 | 0.349 | 0.731 | 0.269 | 0.339 | 0.661 |
| $TDN_{1x}$, % DM | 0.737 | 0.263 | 0.816 | 0.184 | 0.353 | 0.647 |
| $TDN_{3x}$, % DM | 0.761 | 0.239 | 0.812 | 0.188 | 0.427 | 0.573 |
| $DE_{1x}$, Mcal/kg | 0.714 | 0.286 | 0.794 | 0.206 | 0.348 | 0.652 |
| $DE_{3x}$, Mcal/kg | 0.704 | 0.296 | 0.785 | 0.216 | 0.346 | 0.654 |
| $ME_{3x}$, Mcal/kg | 0.699 | 0.301 | 0.780 | 0.220 | 0.344 | 0.656 |
| $NE_{L3x}$, Mcal/kg | 0.711 | 0.289 | 0.786 | 0.214 | 0.361 | 0.640 |
| ME, Mcal/kg | 0.711 | 0.289 | 0.794 | 0.206 | 0.341 | 0.659 |
| $NE_m$, Mcal/kg | 0.723 | 0.277 | 0.805 | 0.195 | 0.345 | 0.655 |
| $NE_g$, Mcal/kg | 0.723 | 0.277 | 0.805 | 0.195 | 0.346 | 0.654 |

[a] $r$, Pearson correlation coefficient.

For protein chemical profiles, ADICP was negatively strongly correlated with protein amide I height ($r = -0.982$, $p = 0.02$), amide I and II height ratio ($r = -0.996$, $p = 0.005$), and amide I area ($r = -0.974$, $p = 0.026$), whereas these spectral profiles showed positive correlations with NPN ($r = 0.952-0.995$, $p < 0.05$). Also SCP had a positive correlation with amide I height ($r = 0.988$, $p = 0.012$). These results might imply that a high level of amide I height (or area) was closely associated with higher contents of NPN but lower levels of ADICP in carianta and canola meals. There were strongly positive correlations between PA fraction and amide I height ($r = 0.995$, $p = 0.005$), height ratio of amide I and II ($r = 0.952$, $p = 0.048$), and amide I area ($r = 0.972$, $p = 0.028$), whereas the PC fraction had a negative relationship with these spectral parameters ($r = -0.974$ to $-0.996$, $p < 0.05$). True protein (the sum of PB1, PB2, and PB3) content negatively correlated with IR absorbance of both amide I height ($r = -0.988$, $p = 0.012$) and amide I area ($r = -0.962$, $p = 0.038$), which meant higher values in amide I features might cause lower TP content in oilseed meal. Little information could be found for our comparison on correlations between amide I and II profiles and chemical composition in rapeseed meals; however, amide I area intensity, which was obtained from diffuse reflectance infrared Fourier transform spectroscopy (DRIFT) and synchrotron-based Fourier transform infrared microspectroscopy (SR-IMS), had moderate negative relationships with ADICP ($r = -0.57$) and PC fraction ($r = -0.57$) and weakly positively correlated with true protein ($r = 0.45$) in triticale DDGS and cereal grains.[40] These results implied that some nutrient values of oilseed meal might be estimated by spectral data from IR microspectroscopy. No correlations were found between amide I and II profiles and truly digestible nutrients and energy values in our study.

In regard to protein secondary structures, there were positive correlations between $\alpha$-helix height and SCP ($r = 0.995$, $p = 0.005$) and NPN ($r = 0.988$, $p = 0.012$) and between $\beta$-sheet height and NPN ($r = 0.973$, $p = 0.027$) and negative relationships between $\beta$-sheet height and ADICP ($r = -0.978$, $p = 0.022$). A recent study targeting hulless barley and blend DDGS combinations gave similar results on NPN in relation to protein secondary characteristics.[11] For CNCPS protein subfractions, $\alpha$-helix height closely correlated with PA ($r = 0.988$, $p = 0.012$), PB1 ($r = 0.992$, $p = 0.008$), and true protein ($r = -0.996$, $p = 0.005$), and $\beta$-sheet height was associated with PA ($r = 0.973$, $p = 0.027$), PC ($r = -0.978$, $p = 0.022$), and true protein ($r = -0.963$, $p = 0.038$). These findings were not fully in accordance with the results reported by Liu et al. (2012, in press),[40] who found protein secondary structure profiles had close correlations with most protein subfractions except the PA fraction. Again, no remarkable relationship could be observed between protein secondary structural characteristics and truly digestible nutrients and energy values, which was partially consistent with a previous study.[11]

On the basis of the data mentioned above, it was concluded that carianta meal had different protein nutrient profiles, such as SCP, NPN, and ADICP, when compared with canola meal. Although CP and CHO concentrations of carinata meal were similar to those of canola meal, protein and carbohydrate subfractions partitioned by CNCPS differed from each other. This was also the case for protein intrinsic structural features, as carinata meal showed a much higher level of amide I height and area as well as $\alpha$-helix and $\beta$-sheet heights, and these spectral

parameters were correlated with protein nutrient profiles and subfractions. Results from CLA and PCA showed that protein inherent spectral features at ca. 1728−1478 $cm^{-1}$ were not fully distinguished between these two meals. All the comparisons between carinata meal and canola meal in our study indicated that carinata meal could be used as a potential high-protein supplement source for ruminants. Further study is needed on more information associated with nutritive values, utilization, and availability of carinata meal for its better and effective application in the animal industry.

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