

# **Detect Protein Molecular Structure of Canola Meal and Presscake due to Processing Conditions, in Relation with their Protein Digestive Behavior and Nutritive Value**

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## **Abstract**

Canola is a major oil-seed crop in western Canada and was developed from rapeseed by Canadian plant breeders in 1970's. The intermediate product in the manufacturing process of canola oil and canola meal called canola presscake. Heat treatment of animal feed has been employed to improve protein utilization. Research studies had been showed that heat processing affected protein molecular structures, which may influence protein quality and availability. However, studies on protein structures due to processing conditions, in relation to nutritive value and digestive behavior of protein are extremely rare. Results indicated that the yellow-seeded canola meal had higher crude protein and low fiber content compared to the brown-seeded one. Furthermore, since presscake has not passed through the solvent extraction process had a lower protein but higher oil content than canola meal and is a potential great energy supplement source for ruminants. The results of this study indicated the possibility of using Fourier-transformed infrared-vibration spectroscopy (FT/IR), in order to characterize rapidly feed structures at molecular level and also relate nutrient utilization to the specific chemical make-up of intrinsic structures of the feed.

## **Introduction**

Canola is a major oil-seed crop in western Canada and was developed from rapeseed by Canadian plant breeders in 1970's. Unlike with traditional rapeseed, canola contains low levels of "erucic acid" in the oil portion (<2% of total fatty acids in the oil) and low levels of anti-nutritional compounds called "glucosinolates" in the meal portion (<30  $\mu\text{mol}$  of any one or any combination of the four aliphatic glucosinolates in its defatted meal).<sup>1</sup> The intermediate product in the manufacturing process of canola oil and canola meal called canola presscake. Heat treatment of animal feed has been employed to improve the utilization and the availability of its protein<sup>2</sup> and inactivate any anti-nutrition factors<sup>3</sup> by reducing fermentation and metabolism in the rumen. Research showed that heat processing affected protein molecular structures, which may influence protein quality, nutrient utilization, availability or digestive behavior<sup>4</sup>. However, the study on protein structures and alteration of their inherent structures due to processing conditions, in relation to nutritive value and digestive behavior of protein are extremely rare. A new approach is the use of Fourier-transformed infrared-vibration spectroscopy (FT/IR), a technique for studying the secondary structural composition, stability and conformational changes. The hypothesis of

this study is that processing conditions changes the protein molecular structure in canola meal and canola presscake and that these changes are associated with protein nutrient utilization and availability. The aim of this study was to 1) characterize the effect of processing conditions on the nutritive value, for ruminants, of the yellow-seeded (*B. juncea*) and brown-seeded (*B. napus*) canola meal, in comparison with brown-seeded (*B. napus*) canola presscake, 2) to reveal protein molecular structures of canola meal and presscake by using FT/IR-ATR molecular spectroscopy and 3) to investigate the relationship between protein molecular structures and nutrient utilization.

## Results

**Table 1.** Chemical profiles: Comparison among yellow canola meal (CM: *B. Juncea*), brown canola meal (CM: *B. Napus*), and canola presscake brown (CPC: *B. Napus*).

| Item                        | Canola treatment              |                             |                              | Contrast |         | CM vs CPC<br>P value |
|-----------------------------|-------------------------------|-----------------------------|------------------------------|----------|---------|----------------------|
|                             | CM-Yellow<br><i>B. Juncea</i> | CM-Brown<br><i>B. Napus</i> | CPC-Brown<br><i>B. Napus</i> | SEM      | P value |                      |
| Feed milk value             | 5.83a                         | 3.91b                       | 3.43b                        | 0.10     | 0.001   | 0.001                |
| EE (g kg <sup>-1</sup> DM)  | 22.8 <sup>b</sup>             | 40.4 <sup>b</sup>           | 147.2 <sup>a</sup>           | 14.42    | 0.016   | 0.007                |
| NDF (g kg <sup>-1</sup> DM) | 237.3 <sup>b</sup>            | 342.0 <sup>a</sup>          | 337.9 <sup>a</sup>           | 5.77     | 0.002   | 0.007                |
| ADF (g kg <sup>-1</sup> DM) | 103.5 <sup>b</sup>            | 182.9 <sup>a</sup>          | 193.4 <sup>a</sup>           | 3.08     | < 0.001 | 0.001                |
| CP (g kg <sup>-1</sup> DM)  | 469.5 <sup>a</sup>            | 399.8 <sup>b</sup>          | 345.6 <sup>b</sup>           | 11.31    | 0.010   | 0.008                |

SEM= Standard error of mean; a, b, c Means with different letters within the same row differ ( $P < 0.05$ ).

EE: Ether Extract (crude fat); NDF: Neutral Detergent Fiber; ADF: Acid Detergent Fiber; CP: Crude Protein; SCP: Soluble Crude Protein.

Results from this study showed that CM-Yellow (*B. Juncea*) had higher ( $P < 0.001$ ) feed milk value than CM-Brown (*B. Napus*) and CPC-Brown (*B. Napus*) (5.8 kg milk vs. 3.9 kg milk and 3.4 kg milk, respectively). The chemical profiles of the canola meal yellow-seeded (CM\_Y), the brown-seeded (CM\_B) and the canola presscake brown-seeded (CPC\_B) are presented in Table 1. The CM\_Y had significantly lower ether extract content than CM\_B. The values of EE for CPC\_B were almost 7 times higher ( $P < 0.05$ ) compared to the values obtained for CM\_Y and CM\_B. Also, CM\_Y was lower in NDF (237 vs. 340 g kg<sup>-1</sup> DM  $P < 0.005$ ) and ADF (104 vs. 183 g kg<sup>-1</sup> DM,  $P < 0.05$ ) compared to CM\_B. Regarding the protein profile of the feedstuffs used in this study, CM\_Y had higher ( $P < 0.05$ ) values for CP than CM\_B Canola brown-seeded presscake had significant lower CP values (346 vs. 470 g kg<sup>-1</sup> CP) compared to CM\_Y.

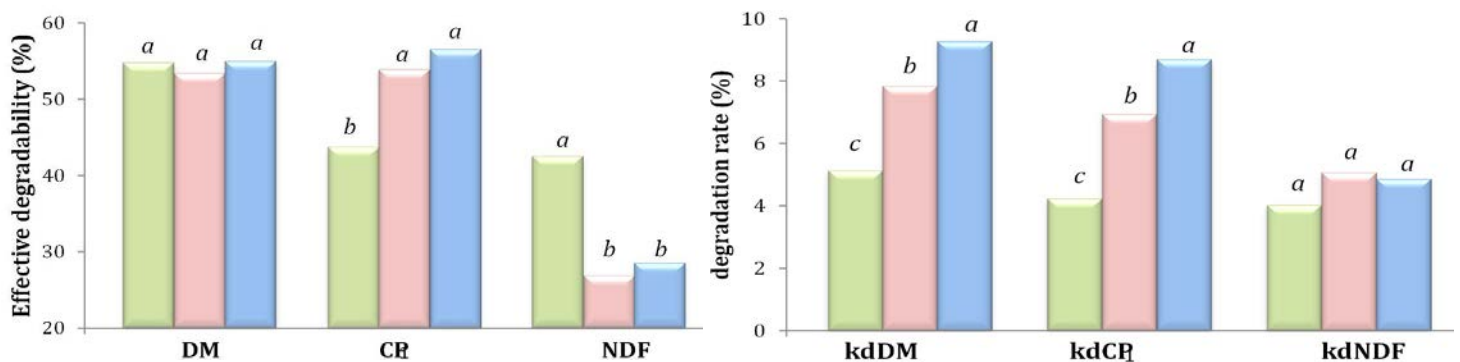
**Table 2.** The structural characteristics of protein by using ATR-FT/IR molecular spectroscopy: comparison among yellow canola meal (CM: *B. Juncea*), brown canola meal (CM: *B. Napus*), and canola presscake brown (CPC: *B. Napus*).

| Item  | Canola treatment   |                    |                   | SEM   | P value | Contrast  |
|---|--------------------|--------------------|-------------------|-------|---------|-----------|
|   | CM-Yellow          | CM-Brown           | CPC-Brown         |       |         | CM vs CPC |
|   | <i>B. Juncea</i>   | <i>B. Napus</i>    | <i>B. Napus</i>   |       |         | P value   |
| Protein molecular structure spectra profiles (Unit: Absorbance) |                    |                    |                   |       |         |           |
| Amide I area  | 3.21 <sup>a</sup>  | 2.89 <sup>a</sup>  | 3.21 <sup>a</sup> | 0.157 | 0.262   | 0.414     |
| Amide II area   | 1.13 <sup>ab</sup> | 0.93 <sup>b</sup>  | 0.07 <sup>a</sup> | 0.074 | 0.046   | 0.090     |
| Ratio amide I to amide II area                                  | 2.90 <sup>ab</sup> | 3.19 <sup>a</sup>  | 2.70 <sup>b</sup> | 0.110 | 0.013   | 0.014     |
| Protein secondary structure profile                             |                    |                    |                   |       |         |           |
| a-helix (height)  | 0.03 <sup>ab</sup> | 0.03 <sup>b</sup>  | 0.04 <sup>a</sup> | 0.002 | 0.002   | 0.002     |
| β-sheet (height)  | 0.04 <sup>a</sup>  | 0.03 <sup>b</sup>  | 0.04 <sup>a</sup> | 0.004 | 0.134   | 0.825     |
| Ratio a-helix to β-sheet  | 0.86 <sup>b</sup>  | 0.96 <sup>ab</sup> | 1.04 <sup>a</sup> | 0.033 | 0.002   | 0.003     |

SEM= Standard error of mean; a, b, c Means with different letters within the same row differ ( $P < 0.05$ ).

The amide II area as well as the amide I to amide II ratio was different ( $P < 0.05$ ) between the brown-seed canola meal (CM\_B) and the brown-seeded canola presscake (CPC\_B). In terms of a-helix, β-sheet and their ratio was found that processing conditions resulted in a significant higher value for a-helix and β-sheet for CPC\_B compared to CM\_B and a higher value ( $P < 0.05$ ) for the R\_a\_β compared to CM\_Y (Table 2).

Comparing CM\_Y with CM\_B, no significant differences were observed in terms of the effective degradability of dry matter. CM\_B had higher ( $P < 0.05$ ) effective degradability of NDF compared to CM\_Y or CPC\_B (Figure 1). Also, the crude protein degradation of CM\_Y was significant lower compared to that of CM\_B.



**Figure 1.** *In situ* rumen degradation kinetics of dry matter (DM), crude protein (CP) and neutral detergent fiber (NDF) of canola meal and canola presscake.

■ CM\_B ■ CM\_Y ■ CPC\_B

**Table 3.** Correlations between protein structures and chemical protein and nutrient profiles

|              | Amide I | Amide II | Amide I to Amide II | a-helix | β-sheet | a-helix to β-sheet |
|--------------|---------|----------|---------------------|---------|---------|--------------------|
| <b>DM</b>    | -0.44   | -0.45    | -0.94               | -0.21   | -0.99   | 0.84               |
| <b>NDF</b>   | -0.1    | 0.26     | 0.24                | 0.01    | -0.60   | 0.80               |
| <b>CP</b>    | 0.54    | 0.54     | -0.74               | 0.73    | -0.42   | -0.03              |
| <b>NDICP</b> | 0.95    | 0.95     | -0.18               | -0.65   | -0.51   | 0.95               |
| <b>PB1</b>   | -0.76   | -0.76    | 0.70                | -0.58   | -0.90   | 0.94               |
| <b>tdCP</b>  | -0.13   | -0.09    | 0.33                | -0.32   | 0.38    | -0.88              |
| <b>EDCP</b>  | -0.93   | -0.93    | -0.46               | -0.81   | -0.77   | 0.96               |
| <b>RUCP</b>  | -0.76   | -0.76    | -0.68               | -0.59   | -0.89   | 0.94               |
| <b>IVCPD</b> | 0.89    | 0.89     | -0.32               | 0.98    | 0.08    | -0.53              |

For chemical profiles, dry matter was tended to be negatively correlated to amide I to amide II ratio ( $R = -0.94$ ,  $P = 0.05$ ). A positive correlation between protein structure a-helix to β-sheet ratio and *in situ* protein degradation in the rumen and no correlation was found between the intestinal digestibility of rumen undegraded protein and a-helix to β-sheet ratio.

## Conclusion

The comparison between yellow-seeded and brown-seeded canola meals showed that the first one had higher crude protein and low fiber content. Furthermore, since presscake has not passed through the solvent extraction process had a lower protein but higher oil content than canola meal and is a potential great energy supplement source for ruminants. The results of this study indicated the possibility of using Fourier-transformed infrared-vibration spectroscopy (FT/IR), in order to characterize rapidly feed structures at molecular level and also relate nutrient utilization to the specific chemical make-up of intrinsic structures of the feed.

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