Effects of conditioning temperature and time during the pelleting process on feed molecular structure, pellet durability index, and metabolic features of co-products from bio-oil processing in dairy cows

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ABSTRACT

The objectives of this study were to systematically determine effects of conditioning temperature (70, 80, and 90°C), time (50 and 75 s), and interaction (temperature × time) during the pelleting process on co-products from bio-oil processing (canola meal) in terms of processing-induced changes on (1) protein molecular structure, (2) pellet durability index, (3) detailed chemical profile, (4) metabolic features and fractions of protein and carbohydrate, (5) total digestible nutrients and energy values, and (6) rumen degradable and undegradable content. Pellet durability was increased with increasing conditioning time. Chemical and carbohydrate profiles of co-products were not altered by pelleting process under different conditioning temperatures and times. With regard to protein fraction profiles, pellets conditioned for 50 s had higher soluble crude protein (SCP) and lower neutral detergent insoluble crude protein (NDICP) contents than those conditioned for 75 s (21.7 vs. 20.1% SCP, 16.0 vs. 16.5% NDICP, respectively). Total digestible nutrients and energy values were not altered by processing. Samples conditioned for 50 s had a higher content of rapidly degradable protein fraction (PA2) than those conditioned for 75 s (21.7 vs. 21.1% crude protein). In addition, the slowly degradable true protein fraction (PB2) was affected by the interaction of conditioning temperature and time. However, carbohydrate fractions did not differ with different conditioning temperatures and time. Different temperatures and time of conditioning during pelleting process greatly affect protein profiles without altering carbohydrate profiles. Molecular structure analyses also showed that pelleting altered inherent protein molecular structures of the co-products from bio-oil processing. Future study is needed to detect how molecular structure changes affect nutrient availability in dairy cattle.

Key words: pelleting, conditioning temperature and time, nutrient utilization and availability, molecular structure

INTRODUCTION

Pelleting is a process that uses moisture, heat, and pressure to agglomerate smaller particles into larger particles (Skoch et al., 1981; Falk, 1985). Its proven benefits include improved palatability and hygienic condition of feed, increased feed bulk density and flowability, elimination of segregation problems, and improved transportation efficiency. Pelleting also affects the metabolic and digestion characteristics of feeds (Thomas and van der Poel, 1996; Abdollahi et al., 2013). However, the interrelations between conditioning, pelleting, and cooling should be considered when evaluating the effects of the pelleting process on feed materials (Thomas et al., 1997). It is also important to understand how the inherent structure of feed is affected by pelleting under different conditioning temperatures and times, and how pelleting-induced structural changes affect nutrient profiles and availability in dairy cattle (e.g., energy value, protein and carbohydrate fractions, true nutrient supply in rumen and small intestine; Yu, 2010).

To date, limited information is available about the effects of conditioning temperature, time, and their interaction of pelleting process on internal protein structure changes at the molecular level in relation to nutrient utilization and availability in dairy cattle. The objectives of this study were to systematically determine effects of conditioning temperature (70, 80, and 90°C), time (50 and 75 s), and their interaction (temperature × time) during the pelleting process on co-products from bio-oil processing (canola meal as model feed) in terms of processing-induced changes on (1) protein molecular structure, (2) pellet durability index, (3) detailed chemical profiles, (4) metabolic features and fractions of protein and carbohydrate, (5) total digestible nutrients and energy values, and (6) rumen degradable and undegradable content in dairy cattle.

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MATERIALS AND METHODS

Sample Preparation, Pelleting Process, and Pellet Durability Test

The co-products from bio-oil processing of canola meal as model feeds were used in this study to investigate the effect of conditioning temperature, time, and their interaction of pelleting process on internal protein structure changes on a molecular level in relation to nutrient utilization and availability in dairy cattle. Two representative sources of co-products were obtained from a commercial feed company (Federated Cooperatives Ltd., Saskatoon, SK, Canada) with different manufacturing dates. A California laboratory pellet mill (NH-397202, California Pellet Mill Co., Crawfordsville, IN) with a capacity of 20 kg/h was used to produce canola meal pellets. The conditioning temperature (70, 80, and 90°C) was set with heating pads wrapped around the conditioning chamber and maintained by using an Omega CN350 controller (Omega Engineering Inc., Stamford, CT). The temperature of the conditioned mash was measured at the outlet of the conditioner continuously by an Omega HH-25TC thermometer. Conditioning time was determined by changing the flowrate at which the canola meal entered the conditioner. The conditioning time was determined by placing colored materials into the conditioner, then measuring time until the materials exited the conditioner. At dial settings of “24” and “30” on the pelleting machine, the production rates were 250 and 814 g/min, respectively; and related transition time of materials in the conditioner were 75 and 50 s, respectively. Steam was injected at 1.0 kg/h at 248.2 kPa during the conditioning stage. A pellet die with a hole diameter of 6.4 mm and a thickness of 44 mm was used. Pellets were cooled before the pellet durability test by exposing pellets to the air for 24 h. This resulted in a total of 12 treatment samples (3 temperatures $\times$ 2 times $\times$ 2 different batches).

Pellet durability was presented as a pellet durability index (PDI), which was measured using the “Pfast” method and expressed on a percentage basis (Thomas and van der Poel, 1996). Around 500 g of sieved pellets was put in a drum with specified dimensions (30.5 $\times$ 30.5 $\times$ 12.7 cm) and tumbled for 10 min at 50 rpm. After tumbling, the pellets were screened with a sieve (US Sieve Size 6 with 3.36 mm diameter) to determine the pellets that could not pass through the sieve. The PDI was calculated as follows (Thomas and van der Poel, 1996):

$$\text{PDI} = \frac{(\text{weight of pellets before tumbling})}{(\text{weight of pellets after tumbling})} \times 100\%.$$

Pelleting-Induced Changes in Chemical Profiles

For chemical analysis, all samples were ground through a 1-mm screen using a Retsch ZM 200 Rotor mill (Rose Scientific Ltd., Edmonton, AB, Canada). Dry matter, ash, ether extract (EE), and CP were analyzed according to AOAC (1990) official methods 930.15, 942.05, 954.02, and 984.13, respectively. The methods described in Van Soest et al. (1991) combined with an Ankom A200 filter bag technique (Ankom Technology Corp., Fairport, NY) were used to determine ADF, NDF, and ADL in the samples. Adjusted NDF was determined when sodium sulfite was added together with heat-stable amylase before neutral detergent extraction. The methods in Licitra et al. (1996) were applied to analyze acid detergent insoluble CP (ADICP) and neutral detergent insoluble CP (NDICP). All samples were incubated with a bicarbonate-phosphate buffer, and then filtered through Whatman #54 filter papers to determine the total soluble CP (SCP) content (Roe et al., 1990). The method used to determine NPN was in accordance with Licitra et al. (1996), in which tungstic acid is used to precipitate the true protein fraction and then the difference between total CP and precipitated CP is considered the NPN.

Pelleting-Induced Changes in Energy Values

Summative approaches from the National Research Council (NRC, 2001) dairy were used to estimate values of total digestible (td) NFC (tdNFC), total digestible CP (tdCP), total digestible NDF (tdNDF), total digestible FA (tdFA), TDN at 1 $\times$ maintenance (TDN_{1x}), TDN at 3 $\times$ maintenance (TDN_{3x}), digestible energy (DE) at 1 $\times$ maintenance (DE_{1x}), DE at a production level of 3 $\times$ maintenance (DE_{p3x}), ME at a production level of 3 $\times$ maintenance (ME_{p3x}), and net energy at a production level of 3 $\times$ maintenance (NE_{Lp3x}). The methods to determine ME, NE_M, and NE_G were in accordance with NRC (1996).

Pelleting-Induced Changes in Protein and Carbohydrate Fractions

In this study, the Cornell Net Carbohydrate and Protein System (CNCPS, version 6.1; Van Amburgh et al., 2010) was applied to partition the CP and carbohydrate (CHO) fractions (Van Amburgh et al., 2010; Higgs et al., 2012). In CNCPS version 6.1, protein fractions are profiled based on their ruminal degradation characteristics: ammonia (PA1), nonammonia soluble true protein (PA2), moderately degradable true protein (PB1), slowly degradable true protein (PB2), and completely undegradable CP (PC; Higgs et al.,

2012; Van Amburgh et al., 2010, 2013). Fraction PA1 degrades at 200%/h; PA2 degrades at 10 to 40%/h; PB1 contains neutral detergent soluble protein and degrades at 3 to 20%/h; PB2 contains proteins that bind in fiber and slowly degrades at 4 to 9%/h in forages. The CHO fraction scheme is expanded into 8 fractions: VFA (CA1), lactate (CA2), organic acids (CA3), sugars (CA4), starch (CB1), soluble fibers (CB2), slowly degradable and available NDF (CB3), and lignin (CC). Fraction CA2 degrades at 7%/h, CA3 degrades at 5%/h, and CA4 degrades at 40 to 60%/h; CB1 is starch and degrades at 20 to 40%/h, CB2 degrades at 20 to 40%/h, and CB3 has a degradation rate of 4 to 9%/h. Fractional degradation and passage rates were obtained from the CNCPS feed library and literature (Tylutki et al., 2008; Van Amburgh et al., 2010; Higgs et al., 2012). In canola meal, CA1, CA2, and CA3 are not present, and only a small amount of CB1 (5%) was found (Newkirk, 2009). The CHO fractions of interest in this study were CA4, CB2, CB3, and CC (Newkirk, 2009). Fractions of rumen degradable and undegradable protein and carbohydrate were calculated as well.

Models in CNCPS version 6.1 become more sensitive to feeds with high levels of protein and carbohydrates (Higgs et al., 2012; Van Amburgh et al., 2010, 2013). In soluble pools, to obtain a more appropriate reflection of cattle biology, re-assignment of the liquid passage rate equations to CHO CA, PA, and PB has been made (Van Amburgh et al., 2010). The resultant higher predicted outflow of soluble components would reduce estimated microbial yield and ammonia production (Van Amburgh et al., 2010).

Detection of Molecular Structure Changes

Pelleting-induced changes in the protein and carbohydrate fractions of co-products from bio-oil processing were detected using advanced molecular spectroscopy with attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FT/IR). The ATR-FT/IR analyses with chemometrics were performed at the molecular spectroscopy laboratory of the Department of Animal and Poultry Science, University of Saskatchewan (Saskatoon, SK, Canada) using an AJasco FT/IR-4200 (Jasco Corp., Tokyo, Japan). The ATR-FT/IR machine was equipped with a ceramic infrared light source and a deuterated L-alanine-doped triglycine sulfate detector that contains an MI Racle attenuated total reflectance (ATR) accessory module and outfitted with a ZnSe crystal and pressure clamp (Pike Technologies, Madison, WI). Spectra were collected at the mid-infrared region from approximately 4,000 to 700 cm^{-1} with a spectral resolution of 4 cm^{-1} with 128 scans co-added. To minimize infrared absorption by CO_2 and

water vapor in the ambient air, background spectra were collected with 256 scans. Jasco Spectra manager II software was used to collect spectral data. Spectral data of each sample was collected 5 times. Omnic 7.3 (Thermo-Nicolet, Madison, WI) software was used to identify and collect spectral information of functional groups of protein, cellulosic components, and carbohydrate.

Univariate Molecular Structure Analysis

Spectral bands of functional groups were detected based on the literature (Theodoridou and Yu, 2013a; Xin and Yu, 2013a,b; Yang et al., 2013b; Peng et al., 2014a,b). When identifying the secondary structure of protein, the baseline was set at $\sim 1,718$ to $1,481 \text{ cm}^{-1}$. Amide I consists of 80% C=O stretching vibrations and 20% C-N stretching vibrations; amide II consists of 60% N-H bending vibrations and 40% stretching vibrations (Jackson and Mantsch, 1995, 2000; Yu, 2004). Band absorption selection of amide I, amide II, α -helix, and β -sheet was determined based on previous studies as showed in Figure 1 (Jackson and Mantsch, 1995, 2000; Yu, 2004; Xin and Yu, 2013b; Yang et al., 2013b). Therefore, for protein amide I, peak center ranged from $\sim 1,644$ to $1,625 \text{ cm}^{-1}$ and area ranged from $\sim 1,718$ to $1,567 \text{ cm}^{-1}$. For protein amide II, peak center ranged from $\sim 1,538$ to $1,535 \text{ cm}^{-1}$ and area ranged from $\sim 1,567$ to $1,481 \text{ cm}^{-1}$. Amide I and amide II are 2 major bands associated with the protein secondary structure (Yu, 2004). However, amide I, rather than amide II, is the first band used when studying the protein secondary structure. This is because amide II is affected by multiple functional groups, which lead to complex vibrations; therefore, amide II has a lower sensitivity when predicting protein secondary structure (Jackson and Mantsch, 2000). Amide I can be further divided into several multi-component peaks using the second derivative function in Omnic 7.3. The α -helix and β -sheet were then selected from these multi-components. The α -helix peak center was located at $\sim 1,660$ – $1,650 \text{ cm}^{-1}$ and β -sheet peak center was located at $\sim 1,627$ – $1,618 \text{ cm}^{-1}$.

Statistical Analysis

This study was designed using a randomized complete block design with a 3×2 factorial treatment arrangement. Statistical analyses were performed using the MIXED procedure of SAS 9.3 (SAS Institute Inc., Cary, NC). The following model was used: $Y_{ijrl} = \mu + \alpha_i + \beta_j + \gamma_k + S_r + e_{ijrl}$, in which Y_{ijrl} was an observation of the dependent variable $ijrl$, μ was the population mean for the variable, α_i was the effect of condi-

Figure 1. Typical Fourier transform infrared spectrum for co-products from bio-oil processing at the regions of protein ($\sim 1,718$ – $1,481$ cm^{-1}). Color version available online.

tioning temperature, β_j was the effect of conditioning time, γ_k was the interaction of conditioning time and temperature, S_r was the effect of sources as a random effect ($\gamma_k = \alpha_i \times \beta_j$), and e_{ijrl} was the random error associated with observation $ijrl$. A contrast statement was performed to detect the difference between the unprocessed mash and pellets. Also, polynomial contrasts (linear and quadratic) of conditioning temperature (70, 80, and 90°C) were determined if the linear effect of conditioning temperature was significant. The significant level for all statistical analyses was declared at $P < 0.05$ and trends at $P < 0.10$. Treatment means were compared using the Tukey-Kramer method.

RESULTS AND DISCUSSION

Effects of Conditioning Temperature and Time on PDI

Shearing and abrasion happen during pellet manufacturing. This can damage pellets, potentially turning them into mash. Hence, pellets must have sound resistance against stress during transportation and distribution (Thomas and van der Poel, 1996). The results of pellet durability tests are shown in Table 1. The interaction of conditioning temperature and time on PDI of canola meal pellets was not significant. The PDI of canola meal pellets was not affected by conditioning temperature but was positively affected by conditioning time ($P < 0.05$). Samples conditioned for 75 s had a higher PDI than those conditioned for 50 s (91.32

vs. 73.37%). Previous studies indicated that increasing conditioning time would improve PDI (Thomas and van der Poel, 1996; Thomas et al., 1997; Abdollahi et al., 2013). Abdollahi et al. (2013) indicated that pellet quality would be improved if diets contained a high level of protein. Increasing the retention time of mash feed in the conditioner, along with injection of steam, contributed to the forming of hydrogen bonds between hydrophilic portions of protein and water molecules (Abdollahi et al., 2013). In addition, longer conditioning time could take advantage of the increased thermo-mechanical nutrient interaction and enhanced feed adhesion to improve pellet durability (Fairchild and Greer, 1999; Moritz and Lilly, 2010; Abdollahi et al., 2013).

Effects of Conditioning Temperature and Time on Chemical Profiles

Table 2 shows the effects of pelleting under different conditions on the chemical profiles of canola meal. The interaction of conditioning temperature and time was not significant on the basic chemical profiles. The conditioning temperature and time did not exhibit a significant effect on basic chemical profiles (ash, DM, and EE). For carbohydrate profiles, NDF (30.5% DM), ADF (19.4% DM), and ADL (8.1% DM) were not affected by conditioning temperature, time, or their interaction. Total CP content of canola meal pellets with an average 39.9% DM was not affected by conditioning temperature and time. Samadi and Yu (2011) reported

Table 1. Effects of temperature and time of conditioning during the pelleting process on pellet durability index (PDI) in co-products (canola meal) from bio-oil processing

Item	Temperature (T)			SEM	Time (t)		SEM	P-value			Polynomial contrast for T (P-value)	
	70°C	80°C	90°C		50 s	75 s		T	t	T × t	Linear	Quadratic
PDI (%)	85.3	81.2	80.3	5.45	73.4 ^x	91.3 ^y	4.49	0.80	0.03	0.83	0.54	0.85

^{x,y}Means with different letters in the same row are significantly different ($P < 0.05$).

that heating methods would not influence total CP content. McKinnon et al. (1995) indicated that the total CP content did not change when processing under different heating temperatures and time. However, we observed that conditioning time had significant effects on SCP ($P < 0.05$) and NDICP ($P < 0.01$). Samples conditioned for 75 s had a lower SCP content and a higher NDICP content than those conditioned for 50 s. However, the unprocessed mash was different from the pelleted samples ($P < 0.05$) with higher NDICP. Goelema (1999) reported that heating could stabilize protein structure and create crosslinks between proteins and carbohydrates. In addition, protein is very sensitive to environmental temperature. Any change in temperature that is able to affect the noncovalent interaction in protein structure may induce changes in the quaternary, tertiary, or secondary structures of the protein (Goelema, 1999). Both processing temperature and processing time during heat treatment play important roles in determining the level of denaturation and degradation of protein (Goelema, 1999). During the conditioning stage, increasing conditioning time not only hydrates the materials but also reduces friction when they are passed through the pellet die (Thomas et al., 1997; Abdollahi et al., 2013). The ideal heating treatment was able to increase NDICP, reduce SCP of feed, and minimize the increase in ADICP at the same time (Van Soest, 1989). McKinnon et al. (1995) observed that reduced SCP and increased NDICP contents were associated with increased NDF content when processing time was increased during dry heating of canola meal at 125 and 145°C. In the current study, samples conditioned for 50 s tended to have a lower NDF content than those conditioned for 75 s ($P < 0.10$). Reduced SCP and increased NDICP contents were likely due to the formation of crosslinks between protein and fiber during the pelleting process (Goelema, 1999).

The ADICP content of samples, with an average of 4.5% CP, was not affected in the current study. McKinnon et al. (1995) observed increased ADICP and NDICP when canola meal was heated from 125 to 145°C. They assumed that increased processing temperature was responsible for this increase. Nia and

Ingalls (1992) observed that ADICP and NDICP were increased by increasing heating time during autoclaving at 127°C. Mustafa et al. (2000) indicated that NDICP and ADICP were increased and SCP was decreased after desolventizing-toasting (103 to 107°C). In addition, ADICP content can reflect heat-damaged indigestible protein in feed materials (McKinnon et al., 1995). McKinnon et al. (1995) also observed decreased intestinal CP digestibility because of increased ADICP in canola meal. During canola processing, canola meal is heated up to 120°C, which results in protein denaturation and the Maillard reaction (Newkirk, 2009). Canola protein was not damaged further in our study.

Effects of Conditioning Temperature and Time on Energy Values

Effects of temperature and time of conditioning during the pelleting process on energy values of canola meal are presented in Table 3. To summarize, energy values were not affected by conditioning temperature and time. No significant effect of conditioning time, temperature, or their interaction on TDN (tdNFC, tdCP, tdNDF, and tdFA) was found. In addition, the unprocessed mash was not significantly different in TDN from pellets processed under different conditioning temperatures and time ($P > 0.05$). Total digestible nutrients (TDN_{1x}) and predicted energy values (DE_{1x}, DE_{p3x}, ME_{p3x}, NEL_{p3x}, ME, NE_M, and NE_G) were not affected by the pelleting process in the current study ($P > 0.05$). Additionally, the unprocessed mash had similar estimated energy values to the pellets. Therefore, pelleting under different conditioning temperatures and time in our study did not change TDN or estimated energy values of canola meal.

Effects of Conditioning Temperature and Time on Protein and Carbohydrate Fraction Profiles

Table 4 shows the CHO and protein fractions of all samples. Conditioning time had a negative effect on PA2 fraction ($P < 0.05$). Samples conditioned for 50 s exhibited a higher PA2 content than those conditioned for 75 s (21.7 vs. 20.1% CP). Samples conditioned for

Table 2. Effects of temperature and time of conditioning during the pelleting process on processing-induced changes in chemical profiles of co-products (canola meal) from bio-oil processing

Item ¹	Temperature (T)			Time (t)			P-value			Polynomial contrast for T (P-value)			Control vs. processed	
	70°C	80°C	90°C	SEM	50 s	75 s	SEM	T	t	T × t	L ²	Q ²		
Basic chemical profile														
Ash (% of DM)	7.19	7.19	7.22	0.074	7.20	7.20	0.073	0.70	0.82	0.73	0.50	0.64	7.26	0.18
DM (%)	87.03	89.23	87.33	1.459	87.61	88.12	1.191	0.55	0.78	0.25	0.89	0.30	91.03	0.20
OM (% of DM)	92.81	92.81	92.78	0.074	92.80	92.80	0.073	0.70	0.82	0.73	0.50	0.64	92.75	0.18
EE (% of DM)	1.67	1.77	1.73	0.200	1.65	1.79	0.187	0.85	0.35	0.44	0.75	0.66	1.52	0.30
Carbohydrate profile														
NDF (% of DM)	30.54	30.53	30.52	0.887	30.49	30.57	0.878	1.00	0.77	0.93	0.95	0.99	30.24	0.48
ADF (% of DM)	19.66	19.42	19.39	1.116	19.38	19.59	1.113	0.43	0.28	0.73	0.25	0.59	19.14	0.14
ADL (% of DM)	8.19	8.40	8.25	0.356	0.17	8.39	0.351	0.37	0.10	0.18	0.68	0.19	8.14	0.38
ADL (% of NDF)	26.81	27.51	27.02	0.492	26.78	27.45	0.435	0.50	0.20	0.32	0.72	0.28	26.95	0.82
CHO (% of DM)	51.05	51.17	51.20	1.084	51.34	50.95	1.069	0.94	0.33	0.81	0.75	0.92	51.87	0.15
CP profile														
CP (% of DM)	40.09	39.88	39.86	1.187	39.83	40.06	1.172	0.86	0.56	0.98	0.64	0.82	39.35	0.24
SCP (% of CP)	20.65	21.05	20.97	0.568	21.67 ^x	20.11 ^y	0.509	0.79	0.03	0.40	0.62	0.67	20.98	0.89
NPN (% of SCP)	81.88	78.82	81.28	3.627	79.02	82.31	3.462	0.52	0.19	0.35	0.83	0.28	80.11	0.85
NDICP (% of CP)	16.21	16.18	16.35	0.462	16.03 ^y	16.47 ^x	0.457	0.63	0.03	0.38	0.48	0.55	17.16	0.02
NDICP (% of DM)	6.49	6.45	6.51	0.032	6.38 ^y	6.59 ^x	0.026	0.42	<0.01	0.14	0.68	0.22	6.74	0.03
ADICP (% of DM)	1.77	1.72	1.79	0.639	1.76	1.80	0.057	0.58	0.20	0.09	0.71	0.35	1.79	0.77
ADICP (% of CP)	4.41	4.31	4.50	0.279	4.32	4.50	0.265	0.70	0.34	0.19	0.70	0.48	4.56	0.59

^{x,y}Means with different letters in the same row are significantly different ($P < 0.05$).¹EE = ether extract; CHO = carbohydrate; SCP = soluble CP; NDICP = neutral detergent insoluble CP; ADICP = acid detergent insoluble CP.²L = linear; Q = quadratic.

Table 3. Effects of temperature and time of conditioning during the pelleting process on changes in energy values of co-products (canola meal) from bio-oil processing for dairy and beef cattle

Item ¹	Temperature (T)			Time (t)			P-value			Polynomial contrast for T (P-value)			Contrast P-value
	70°C	80°C	90°C	SEM	50 s	75 s	SEM	T	t	T × t	L ²	Q ²	Control (raw) vs. Control processed
Truly digestible nutrients (% of DM)													
tdNFC	26.47	26.55	26.64	0.429	26.68	26.42	0.350	0.96	0.63	0.91	0.78	0.99	27.81
tdCP	39.39	39.19	39.15	1.211	39.14	39.34	1.194	0.88	0.63	1.00	0.64	0.87	38.64
tdNDF	6.10	5.95	6.03	0.216	6.15	5.90	0.197	0.80	0.21	0.67	0.77	0.57	5.85
tdFA	0.67	0.77	0.73	0.200	0.65	0.79	0.187	0.85	0.35	0.44	0.75	0.66	0.52
Total digestible nutrients (% of DM)													
TDN _{1x}	66.44	66.41	66.44	0.465	66.42	66.45	0.450	0.99	0.91	0.51	1.00	0.89	66.46
Predicted energy values (Mcal/kg)													
DE _{1x} , dairy	3.33	3.33	3.33	0.036	3.33	3.34	0.037	1.00	0.51	0.23	1.00	1.00	3.33
DE _{p3x} , dairy	3.17	3.17	3.16	0.027	3.16	3.17	0.027	0.78	0.58	0.39	0.50	1.00	3.16
ME _{p3x} , dairy	2.75	2.75	2.75	0.026	2.75	2.75	0.026	0.71	0.34	0.40	0.69	0.49	2.74
NE _{Lp3x} , dairy	1.74	1.74	1.74	0.019	1.74	1.75	0.018	0.86	0.17	0.40	1.00	0.60	1.74
ME, beef	2.74	2.73	2.73	0.029	2.73	2.74	0.029	0.77	0.71	0.44	0.50	0.89	2.73
NE _M , beef	1.81	1.80	1.81	0.025	1.81	1.81	0.025	0.59	1.00	0.27	0.74	0.35	1.80
NE _G , beef	1.18	1.18	1.18	0.021	1.18	1.18	0.021	0.93	0.79	0.61	1.00	0.71	1.18

¹tdNFC = total digestible NFC; tdCP = total digestible CP; tdNDF = total digestible NDF; tdFA = total digestible FA; TDN_{1x} = total digestible nutrients at maintenance; DE_{1x} = digestible energy; DE_{p3x} = digestible energy at a production level (3 × maintenance); ME_{p3x} = ME at a production level (3 × maintenance); NE_{Lp3x} = net energy at a production level (3 × maintenance).

²L = linear; Q = quadratic.

75 s tended toward a higher PB1 content than those conditioned for 50 s (63.4 vs. 62.3% CP, $P < 0.10$). The PB2 fraction was significantly affected by the interaction of conditioning time and temperature ($P < 0.05$). At a conditioning time of 50 s, samples conditioned at 70°C were not different from those conditioned at 80°C in PB2, but samples conditioned 90°C had higher PB2 than those conditioned at 80°C. At a conditioning time of 75 s, samples conditioned at 70°C were not different from those conditioned at 80 or 90°C in PB2. However, samples conditioned at 80°C were higher in PB2 than those conditioned at 90°C (Figure 2). The unprocessed mash had a higher PB2 content than pelleted samples ($P < 0.05$), demonstrating that a pelleting process with relatively low temperatures negatively affected PB2. We detected no difference in PC content among all samples.

The total CHO content of canola meal was not affected when pelleting under different conditioning temperatures and time. We found no significant effect of conditioning temperature, time, or their interaction on carbohydrate fractions, in terms of CA4, CB2, CB3, and CC. Pelleted samples were not different from the unprocessed mash in regards to carbohydrate fractions either. Theodoridou and Yu (2013b) observed that CA and CB were decreased but CC was increased when canola presscake was desolventizing-toasted. However, pelleting under different conditioning temperatures and time had no effect on carbohydrate fractions of canola meal in our study.

Figure 2. Effect of interaction of conditioning temperature and time on PB2 content of co-product (canola meal) pellets. Bars with different letters are significantly different ($P < 0.05$).

Table 4. Effects of temperature and time of conditioning during pelleting process on protein and carbohydrate (CHO) subfractions in co-products (canola meal) from bio-oil processing using Cornell Net Carbohydrate and Protein System (CNCPS version 6.1)

Item ¹	Temperature (T)			Time (t)			P-value			Polynomial contrast for T (P-value)			Control vs. processed
	70°C	80°C	90°C	SEM	50 s	75 s	SEM	T	t	T × t	L ²	Q ²	
Protein subfractions													
PA2 (% of CP)	20.65	21.05	21.05	0.568	21.67 ^x	21.11 ^y	0.509	0.79	0.03	0.39	0.62	0.67	0.89
PB1 (% of CP)	63.15	62.77	62.68	0.457	62.30	63.43	0.373	0.76	0.09	0.53	0.50	0.81	0.22
PB2 (% of CP)	11.80	11.87	11.85	0.217	11.71	11.97	0.210	0.87	0.08	0.03	0.72	0.72	<0.01
PC (% of CP)	4.41	4.31	4.50	0.279	4.32	4.50	0.265	0.70	0.34	0.19	0.70	0.48	0.59
Carbohydrate subfractions													
CHO (% of DM)	51.05	51.17	51.20	1.084	51.34	50.95	1.069	0.94	0.33	0.81	0.75	0.92	0.15
CA4 (% of CHO)	23.13	21.39	21.20	0.654	2.14	21.67	0.534	0.16	0.56	0.30	0.09	0.38	0.84
CB2 (% of CHO)	17.43	17.32	20.27	1.731	20.04	16.64	1.413	0.45	0.15	0.22	0.30	0.50	0.17
CB3 (% of CHO)	20.96	21.89	19.88	1.111	19.65	22.17	0.907	0.49	0.11	0.47	0.52	0.33	0.13
CC (% of CHO)	38.49	39.40	38.66	0.968	38.17	39.53	0.898	0.58	0.12	0.37	0.85	0.33	0.25

^{xy}Means with different letters in the same row are significantly different ($P < 0.05$).

¹PA2 = rapidly degradable true protein; PB1 = moderately degradable true protein; PB2 = slowly degradable true protein; PC = undegradable protein; CA4 = sugars; CB2 = soluble fibers; CB3 = available nNDF (nitrogen-free NDF); CC = unavailable cell walls.

²L = linear; Q = quadratic.

Effects of Conditioning Temperature and Time on Rumen Degradable and Undegradable Contents of Protein and Carbohydrate

Effects of temperature and time of conditioning during the pelleting on rumen degradable (RD) and rumen undegradable (RU) fractions of protein and carbohydrate are shown in Table 5. The samples conditioned for 50 s differed from those conditioned for 75 s ($P < 0.05$): higher RDPA2 and RUPA2 (6.6 vs. 6.2% DM, 2.0 vs. 1.9% DM, respectively). Although altering conditioning conditions did not change RDPB2 and RUPB2 in canola meal pellets, the unprocessed mash tended to have higher RDPB2 and RUPB2 contents than the pellets ($P < 0.10$). We observed no significant effect of conditioning temperature, time, or their interaction on rumen degraded and escaped fractions of carbohydrates. None of the samples differed significantly from each other in fractions of rumen degradable and undegradable CHO, in terms of RDCA4, RDCA2, RDCB3, RUCA4, RUCA2, RUCB3, as well as total rumen degraded and escaped CHO (TRDC and TRUC).

Effects of Conditioning Temperature and Time on Protein Molecular Structure Changes

Effects of conditioning temperature and time on protein molecular structure in co-products from bio-oil processing are shown in Table 6. In the protein amide spectral region ($\sim 1,481\text{--}1,718\text{ cm}^{-1}$), the amide I height and area in canola meal pellets were not affected by conditioning temperature, time, or their interaction. In addition, the unprocessed mash was not significantly different from pellets in terms of amide I height and area. Conditioning time and conditioning temperature showed significant effects on amide II area, but the interaction was not significant. Samples conditioned at 70°C had the highest amide II area among samples conditioned at different temperatures. Samples conditioned for 50 s were lower in amide II area than those conditioned for 75 s (1.92 vs. 2.10; $P < 0.05$). The interaction of conditioning temperature and time on amide II peak height was not significant ($P > 0.05$). Conditioning temperature had a negative effect on amide II height so that the samples conditioned at 70°C were highest in amide II peak ($P < 0.01$). Conditioning time had a positive effect on amide II height ($P < 0.01$): the samples conditioned for 75 s had a higher amide II peak height than those conditioned for 50 s (0.041 vs. 0.036). In infrared spectroscopy, obtaining precise values of protein secondary structural profiles for every sample is impossible. However, spectral data from different samples are comparable if samples are processed in similar ways (Yu, 2006). In addition, the

sensitivity of mid-infrared spectroscopy will result in differences in spectra measurement due to differences in methodologies, selected regions, and baselines among studies (Yu, 2006; Doiron et al., 2009).

The ratios of amide I height to amide II height and amide I area to amide II area were positively affected by conditioning temperature ($P < 0.05$) but negatively affected by conditioning time ($P < 0.001$). The interaction was not significant. Samples conditioned at 90°C had the highest ratios of amide I height to amide II height (2.54, $P < 0.05$) and amide I area to amide II area (1.45, $P < 0.05$) compared with those conditioned at 70°C and 80°C. Samples conditioned for 50 s were higher than those conditioned for 75 s in ratio of amide I height to amide II height (1.56 vs. 1.41) and ratio of amide I area to amide II area (2.48 vs. 2.35). Compared with the unprocessed mash, pellets exhibited higher values of these 2 ratios ($P < 0.05$). The ratio of amide I to amide II can be significantly affected by feed materials, heat processing, and gene transformation (Liu et al., 2012). Theodoridou and Yu (2013b) observed that canola meal had a lower amide I to amide II area ratio than canola presscake, indicating that the area ratio was reduced after desolventizing-toasting during canola processing. However, Samadi et al. (2013) indicated that no difference in the area ratio of amide I to amide II was observed when autoclaved (121°C; 1 h) and roasted (120°C; 1 h) canola seeds were compared with unprocessed canola seeds.

No significant effect of conditioning temperature, time, or their interaction on α -helix height and β -sheet height was observed. The difference between the unprocessed mash and the pellets was not significant. With respect to the ratio of α -helix to β -sheet, conditioning temperature, time, or their interaction did not exhibit a significant effect ($P > 0.05$). However, the unprocessed mash was significantly different from pellets with a lower ratio of α -helix to β -sheet ($P < 0.05$). Doiron et al. (2009) indicated that changes in nutrient values of feeds were associated with changes in the ratio of α -helix to β -sheet. Yu (2005b) indicated that a lower protein value was associated with the increased content of β -sheet structure, which reduces accessibility of gastrointestinal enzymes for protein. Yu et al. (2004) observed that raw feather with a high protein content of 84% had a low protein digestibility of 5%. This was partially because the high level of β -sheet was negatively correlated with protein digestibility (Yu et al., 2004). Theodoridou and Yu (2013a) found that canola meal had lower α -helix and β -sheet heights than canola presscake. In their study, α -helix height was positively correlated with ADICP, indicating that a higher α -helix height may result in a lower protein digestibility. They also found that the ratio of α -helix

Table 5. Effects of temperature and time of conditioning during the pelleting process on rumen degradable (RD) and undegradable (RU) contents (% of DM) of protein and carbohydrates in co-products (canola meal) from bio-oil processing for dairy cattle

Item ¹	Temperature (T)			Time (t)			P-value			Polynomial contrast for T (P-value)			Contrast vs. processed P-value
	70°C	80°C	90°C	SEM	50 s	75 s	SEM	T	t	T × t	L ²	Q ²	
RD protein fractions													
RDPA2	6.38	6.46	6.44	0.339	6.64 ^x	6.20 ^y	0.329	0.91	0.04	0.38	0.77	0.77	0.73
RDPB1	17.72	17.53	17.49	0.545	17.37	17.78	0.530	0.75	0.17	0.85	0.50	0.79	0.15
RDPB2	1.89	1.89	1.89	0.031	1.87	1.92	0.027	0.99	0.16	0.17	0.90	0.94	0.06
TRDP	25.98	25.87	25.82	0.878	25.88	25.91	0.867	0.89	0.92	0.99	0.65	0.93	0.19
RU protein fractions													
RUPA2	1.91	1.94	1.93	0.102	1.99 ^x	1.86 ^y	0.098	0.90	0.04	0.39	0.75	0.75	0.74
RUPB1	7.59	7.51	7.49	0.234	7.44	7.62	0.227	0.75	0.17	0.85	0.49	0.79	0.15
RUPB2	2.84	2.84	2.83	0.047	2.80	2.88	0.041	0.99	0.15	0.17	0.93	0.96	0.06
TRUP	14.11	14.05	14.00	0.309	13.95	14.15	0.305	0.70	0.09	0.73	0.64	0.50	0.54
RD carbohydrate fractions													
RDCA4	9.08	8.42	8.33	0.230	8.74	8.48	0.188	0.13	0.37	0.26	0.07	0.36	0.56
RDCB2	7.40	7.40	8.67	0.749	8.56	7.08	0.611	0.45	0.15	0.24	0.28	0.52	0.15
RDCB3	4.29	4.48	4.06	0.215	4.04	4.51	0.175	0.46	0.11	0.46	0.50	0.30	0.14
TRDC	20.76	20.30	21.07	0.510	21.34	20.08	0.416	0.59	0.08	0.40	0.69	0.37	0.10
RU carbohydrate fractions													
RUCA4	2.73	2.53	2.50	0.069	2.62	2.55	0.057	0.13	0.38	0.27	0.07	0.36	0.57
RUCB2	1.48	1.48	1.73	0.150	1.71	1.42	0.123	0.45	0.15	0.24	0.29	0.51	0.16
RUCB3	6.43	6.71	6.09	0.324	6.05	6.77	0.264	0.46	0.11	0.46	0.49	0.31	0.15
RUCC	19.66	20.15	19.81	0.856	19.60	20.14	0.846	0.38	0.10	0.19	0.67	0.20	0.39
TRUCC	30.29	30.87	30.13	0.988	29.99	30.87	0.971	0.31	0.06	0.17	0.74	0.15	0.20

^{x,y}Means with different letters in the same row are significantly different ($P < 0.05$).

¹RDPA2 = rumen degradable PA2 (rapidly degradable true protein); RDPB1 = rumen degradable PB1 (moderately degradable true protein); RDPB2 = rumen degradable PB2 (slowly degradable true protein); TRDP = total rumen degradable CP; RUPA2 = rumen undegradable PA2; RUPB1 = rumen undegradable PB1; RUPB2 = rumen undegradable PB2; TRUP = total rumen undegradable CP; RDCA4 = rumen degradable CA4 (sugars); RDCB2 = rumen degradable CB2 (soluble fiber); RDCB3 = rumen degradable CB3 (available nNDF, nitrogen-free NDF); TRDC = total rumen degradable CHO (carbohydrate); RUCA4 = rumen undegradable CA4; RUCB2 = rumen undegradable CB2; RUCB3 = rumen undegradable CB3; RUCC = rumen undegradable CC (unavailable cell walls); TRUCC = total rumen undegradable CHO.

²L = linear; Q = quadratic.

Table 6. Effects of temperature and time of conditioning during the pelleting process on protein molecular structure changes in co-products (canola meal) from bio-oil processing

Item	Temperature (T)			Time (t)			P-value			Polynomial contrast for T (P-value)			Contrast P-value
	70°C	80°C	90°C	SEM	50 s	75 s	SEM	T	t	T × t	L	Q	
Amide I area	5.02	4.71	4.78	0.128	4.76	4.91	0.105	0.21	0.32	0.18	0.19	0.23	0.56
Amide II area	2.14 ^a	1.95 ^b	1.95 ^b	0.060	1.92 ^y	2.10 ^x	0.048	0.04	0.01	0.44	0.03	0.18	0.13
Amide I peak height	0.059	0.055	0.056	0.0015	0.055	0.057	0.0013	0.15	0.29	0.22	0.21	0.13	0.72
Amide II peak height	0.041 ^a	0.037 ^b	0.037 ^b	0.0012	0.036 ^y	0.041 ^x	0.0009	<0.01	<0.001	0.69	<0.01	0.17	0.26
α-Helix height	0.056	0.052	0.054	0.0015	0.053	0.055	0.0012	0.19	0.19	0.30	0.30	0.13	0.85
β-Sheet height	0.056	0.052	0.053	0.0014	0.053	0.054	0.0012	0.25	0.40	0.20	0.28	0.20	0.69
Amide I:amide II height	1.43 ^b	1.49 ^b	1.54 ^a	0.024	1.56 ^x	1.41 ^y	0.020	0.01	<0.001	0.09	<0.01	0.83	0.02
Amide I:amide II area	2.35 ^b	2.44 ^a	2.45 ^a	0.035	2.48 ^x	2.35 ^y	0.030	0.04	<0.001	0.17	0.02	0.29	<0.001
α-Helix:β-sheet height	1.01	1.00	1.01	0.015	1.00	1.01	0.015	0.38	0.05	0.50	0.88	0.17	0.03

^{a,b} or ^{x,y} Means for temperature (a,b) or time (x,y) with different letters in the same row are significantly different ($P < 0.05$).

¹The unit for spectral data is infrared absorbance unit. Baseline for protein spectral peak: ~1,481–1,718 cm^{-1} ; protein amide I region: ~1,718–1,567 cm^{-1} ; protein amide II region: ~1,567–1,481 cm^{-1} ; center range of amide I peak: ~1,644–1,625 cm^{-1} ; center range of amide II peak: ~1,538–1,535 cm^{-1} ; center range for α-helix: ~1,660–1,650 cm^{-1} ; center range for β-sheet: ~1,627–1,618 cm^{-1} .

²L = linear; Q = quadratic.

to β-sheet played an important role in determining ruminal and intestinal digestion of protein. Yu (2005a) indicated that an increase in β-sheet height after heat treatment was likely due to the protein aggregation required to form intermolecular β-sheet structures. Yu (2005b) reported that reduced α-helix and increased β-sheet were observed in roasted golden flax seeds. In addition, the ratio of α-helix to β-sheet was decreased from 1.28 to 0.73. In Doiron et al. (2009), an increased ratio of α-helix to β-sheet was observed in the endosperm area of autoclaved flaxseed (120°C; 20, 40, and 60 min). In addition, the height ratio of α-helix to β-sheet correlated with protein profiles and digestive characteristics. Samadi and Yu (2011) reported that autoclaving (120°C; 1 h) reduced α-helix and β-sheet heights of soybean seeds; therefore, the ratio of α-helix to β-sheet in soybean seed was reduced. Samadi et al. (2013) observed no difference in α-helix and β-sheet heights in raw, autoclaved, and roasted canola seeds. However, the ratio of α-helix to β-sheet, which correlated with rumen degradation and intestinal digestion characteristics of protein, decreased with autoclaving at 121°C for 60 min and increased with roasting at 120°C for 60 min. Peng et al. (2014b) reported that the ratio of α-helix to β-sheet in camelina seeds was decreased with autoclaving at 120°C for 60 min, and this was associated with decreased rumen degradation but increased intestinal digestion of protein. In our study, the lower ratio of α-helix to β-sheet was observed in the unprocessed mash, which was in accordance with the higher rumen degradation and lower intestinal digestion of protein found in the pelleted samples. Again, the structural stability of a protein is sensitive to its environmental conditions (Goelema, 1999). It should be kept in mind that the combination of processing conditions such as temperature, time, and pressure could affect the protein's inherent structure.

In term of industry implications in this study, changes in the detailed data on chemical and nutrient profiles, TDN, energy values, CNCPS protein and CHO fractions, and nutrient supply of canola meal to dairy cattle induced by pelleting conditioning temperature and time are important in ration formulation in feed mills. This will improve the accuracy in predicting nutrient supply and utilization when diets contain the co-product pelleted under different conditions.

CONCLUSIONS

Different conditioning temperatures (70, 80, and 90°C) and time (50 and 75 s) affected nutrient profiles and protein fractions of co-products from bio-oil processing (canola meal). Increasing conditioning time reduced SCP and increased NDICP contents of the pel-

leted co-products. However, the total protein content was not changed. In regards to protein fraction profiles, PA2 decreased with increasing conditioning time during the pelleting process. Conditioning temperature and time had an interaction effect on PB2 content. However, changes in temperature and time during the conditioning stage did not alter CHO profiles, TDN, or predicted energy profiles. Univariate molecular structure analyses showed that pelleting altered inherent protein molecular structures of co-products from bio-oil processing.

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