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Changes in molecular structure of protein and carbohydrate in soybean products with different processing methods and their effects on nutrient degradation characteristics of the products

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Abstract: In this study, four types of soybean products with different processing methods (soybean meal 1 and soybean meal 2, extruded soybean meal, fermented soybean meal and extruded soybean) were used to examine the effect of fermentation and extrusion on molecular structures of protein and carbohydrate. Extrusion and fermentation significantly decreased ($P < 0.05$) the values of related protein spectral intensities (height and area of amide and secondary structure) and the biggest reduction was found in extruded soybean compared to soybean meal 1 and soybean meal 2. Compared with extruded soybean meal, the area ratio of amide I to amide II and the height ratio of α-helix to β sheet in extruded soybean were significantly reduced ($P < 0.05$), and there was no difference in these spectral values between extruded and fermented soybean. Extrusion and fermentation significantly decreased ($P < 0.05$) the values of carbohydrate spectral intensities, including structural carbohydrate (STCHO) and cellulosic compounds (CELC) and total carbohydrate (CHO), compared to soybean meal 2. The ratio of α-helix to β-sheet was positively related to the DM of soybean degradability in the rumen ($P < 0.05$, $r = 0.590$), so was A-CELC to A-STCHO ($P < 0.05$, $r = 0.747$). A positive relationship was found between CP degradability in the rumen and the area ratios of amide I and amide II, CELC to CHO, and STCHO to CHO. Spectral intensity of CHO area was negatively associated with neutral detergent fibre (NDF) and acid detergent fibre (ADF) degradability in the rumen. The study indicated that extrusion and fermentation could alter the molecular structure of protein and carbohydrate and the degradation characteristics of soybean products in the rumen.

Keywords: fermentation; extrusion; infrared spectroscopy; spectral intensities; rumen degradability

The feed processing method has an important effect on the degradation rate of feed nutrients, and improving the utilization rate of feed through a variety of processing methods, which could help improve the quality of animal products, reduce the feed supply and maximize the economic benefits (Maidala et al. 2014; Bokelman 2015). Extrusion was often used in feed processing and there were differences in feed chemical profiles after processing. Amornthewaphat et al. (2005) found that the content of crude fibre decreased after extrusion. In addition, the extruded feed had good taste, which was beneficial to the digestion and absorption of animals, and had a great effect on improving the production performance of animals. Fermented feeds are widely applied in animal production for the good quality

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protein and abundant amino acids. In recent years, fermented soybean meal has become a very attractive alternative to fish meal in the production of *Alpheus vannamei* (Chiu et al. 2016) and broilers (Lien et al. 2010). Up to now, the quality of fermented and extruded feed has not been evaluated objectively because the traditional analysis method used by researchers would damage the internal structure of feed molecules and also require special sample preparation and a large amount of experimental time. In fact, the inherent molecular makeup differs in feed types and responds differently to the processing methods. Peng et al. (2014b) showed that moist heating could significantly change the protein molecular structures of camelina seed while dry heating could not change any of the spectral characteristics associated with protein molecular structures compared with raw seeds. The degradation characteristics and bioavailability of feed were also related to the internal structures, where existed a great correlation between nutrient degradability and internal structures (Yan et al. 2014). Therefore, it is crucial to conduct a study on feed internal structures with different treatments for making good use of feed in animal production.

Fourier transform infrared spectroscopy (FTIR) using a Michelson interferometer has the advantages of high signal-to-noise ratio, high precision wavelength, less time, and low sample consumption. In general, infrared spectroscopy consists of three regions, which include near infrared, mid-infrared and far-infrared. An infrared region (4 000–400/cm) is used in organic compound structure identification, which could well reflect the internal characteristics of molecular structure according to the absorption frequency of functional groups of the sample being tested (Yu et al. 2005).

There are nine characteristic absorption bands in protein absorption spectrum and amide I and amide II (the spectral region of the molecular structure associated with proteins) are primary bands, in which peak intensities (peak, height and peak area) are correlated with the nutritional value of protein (Jackson and Mantsch 2000).Furthermore, the protein secondary structures (α-helix and β-sheet) in the amide I area also have a strong relationship with the utilization of protein for animal body (Damiran and Yu 2011). Carbohydrates and proteins cannot be separated for consideration as they interfere with each other in the processing of digestion (Kotarski et al. 1992). The main structural carbohydrates in soybean products include neutral detergent fibre (NDF), acid detergent fibre (ADF), lignin and pectin etc. A negative interference was found between some carbohydrate constituents and protein digestion in the rumen (Peng et al. 2014a). However, a few researches on the feedstuff nutrient concerning molecular structure have been found.

In this paper, four types of soybean products with different processing methods were used to find the changes in (1) chemical composition profile, (2) CNCPS (cornell net carbohydrate protein system) protein subfractions, (3) protein molecular structures (amide I, amide II, ratio of amide I to II, α-helix, β-sheet, ratio of α-helix to β-sheet), (4) carbohydrate molecular structures, (5) ruminal degradation characteristics, to figure out (6) the correlation between molecular spectral parameters of protein and carbohydrate and some nutrient degradation in the rumen.

MATERIAL AND METHODS

Samples and processing

In this study, four types of soybean products were used. The first type included two samples named "soybean meal 1" and "soybean meal 2" produced by the same process but from different soybean processing plants of the same company (Harbin Yuanda Animal Husbandry Industry Co., Ltd., P.R. China), and the two samples were residues of soybean after soybean was softened and broken at 60–65 °C for 20 min, rolled and extracted with organic solvent; the second type was "extruded soybean meal" (Ri zhao Bang ji San wei Oil Machinery Co., Ltd., P.R. China), which was a residue of soybean extracted with organic solvent after extrusion at 110 °C using a bulking machine; the third type was "extruded soybean" (Linyi Pufan Feed Industry Co., Ltd., P.R. China), for which whole soybean was extruded at 145 °C and 3.5–5 MPa using a bulking machine; the last type was "fermented soybean meal" (Qingdao Genyuan Biotechnology Industry Co., Ltd., P.R. China), which was obtained by fermenting the first kind of soybean meal with the microorganisms *Lactobacillus acidophilus* and *Saccharomyces cerevisiae*.

Animals and diets

The cows were raised in the barn of Northeast Agricultural University (NEAU) (Harbin, P.R.

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China). Based on the NRC 2001 requirement (NRC 2001), the cows were fed 15.8% maize silage, 42.8% Chinese wild rye and 32% concentrate, which provided 14.4% crude protein (CP), 49.2% NDF, 30.6% ADF, 0.6% Ca, and 0.4% P on the air-dry basis. Besides, the cows were fed twice a day at 8 am and 4 pm with free access to water. Care and treatments for all the animals were approved by the Ethical Committee of the Veterinary Faculty of NEAU.

In situ incubation

According to the method reported by Xin and Yu (2013a), three Holstein cows (age of 3.5 years, weight 500 ± 10 kg, dry period) with permanent rumen intubation were used in an *in situ* nylon bag technique. Briefly, feed samples were ground through a 2 mm screen (JGJ52-2006; Jingnuo Inc., P.R. China) and about 7 g (DM basis) of feed were weighed per nylon bag (the size was 10 cm × 20 cm, and the holes in the bag were 40 um), and they were put into the rumen for incubation according to the schedule of "gradual addition all out" (Yu et al. 2005) for 48, 24, 16, 12, 8, 4, and 2 h and 4, 4, 3, 3, 2, 2 and 2 nylon bags, respectively, were put into each rumen culture time point. The total number of nylon bags in the rumen at any one time point was not more than 30. All the bags were randomly allocated to three cows during ruminal fermentation in two experimental runs. After incubation, all the bags were removed from the rumen, washed manually with cold tap water until the water was clean (including the samples for 0 h) and then dried at 65 °C for 48 h. The residues in bags were used to analyse chemical components (Yu et al. 2005).

Chemical analysis

All samples of soybean products and their ruminal residues were ground and passed through a 1 mm screen (JGJ52-2006; Jingnuo Inc., P.R. China) for chemical analysis. The procedures of chemical analysis were done according to AOAC (1990) for dry matter (method 930.15), ash (method 942.05), ether extract (EE) (method 920.39), crude protein (CP), acid detergent insoluble protein (ADIP), neutral detergent insoluble protein (NDIP), non-protein N (NPN) and soluble crude protein (SCP) (method 984.13, Kjeltec 2 400; Foss North America, Eden Prairie, USA). The procedure developed by Van Soest et al. (1991) was applied to determine the values of acid detergent fibre (ADF), neutral detergent fibre (NDF), and acid detergent lignin (ADL) using a fibre bag method (China Agricultural University fibre bag; Beijing, P.R. China) with a modified fat extraction procedure by including 2 h ether extract before the standard procedure of using acetone for extraction, which could reduce the error of measurements due to high content of oil in the samples. NPN content was calculated based on the precipitation of true protein in the filtrate with trichloroacetic acid (TCA, final concentration 10%) (Roe et al. 1990). The determination of soluble protein (SCP) was based on the procedure of Roe et al. (1990) by incubating the sample with bicarbonate-phosphate buffer and filtering through Whatman #54 filter paper.

Protein components on the basis of CNCPS

The determination of crude protein fraction was according to the Cornell Net Carbohydrate and Protein System (CNCPS) where fraction PA was considered as NPN, fraction PB1 was a fast degradable part, PB2 was a medium degradable part, PB3 was a slowly degradable part, and PC was an undegradable part.

Collection of information on spectral molecular structure

Molecular spectral data of samples were collected and corrected with the background spectrum using ALPHA FT/IR (Bruker Alpha Inc.; Germany). Spectra were generated in a transmission mode with mid-IR (ca. 4 000–800/cm) and finger print region (ca. 1 800–800/cm) with spectral resolution of 4/cm. More specifically, the samples were ground to pass through a 0.5 mm screen (JGJ52-2006; Jingnuo Inc., P.R. China) and then mixed with potassium bromide at the ratio of 1 : 100 per tablet for analysis. The spectral data were analyzed using Ominic v8.2 software (Spectratech, Madison, WI, USA). The regions of specific interest related to protein include the protein amide I (peak baseline, ca. 1 715–1 576/cm), amide II (peak baseline, ca. 1 576–1 468/cm) and protein secondary structures of α-helix (peak centre at ca. 1 648/cm with the baseline of ca. 1 715–1 468/cm) and β-sheet

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(peak centre at ca. 1 640/cm with the baseline of ca. 1 715–1 468/cm). There were three functional group bands associated with carbohydrate as well as several parameters detected in this study. (1) Structural carbohydrates (STCHO, peak baseline, ca.1 469–1 183/cm, there were three peaks in this region centred at ca. 1 444, 1 386, 1 304/cm, respectively) that are mainly associated with hemi-cellulosic and cellulosic compounds. (2) Cellulosic compounds (CELC, peak baseline, 1 285–1 183/cm) and associated with cellulose compounds. (3) Carbohydrate (CHO, peak baseline, ca. 1 183–860/cm and there were three peaks in this region centred at ca. 1 149, 1 104, 1 041/cm, respectively).

Statistical analysis

All data were analyzed using SAS v9.4 (SAS Institute, Inc., Cary, NC, USA).

PROC MIXED was used to analyse chemical composition, protein subfraction and spectral parameters of different soybean products. The model was:

$$Y_{ij} = \mu + F_i + e_{ij} \quad (1)$$

where:

Y_{ij} – dependent variable *ij;*
μ – population mean for the variable;
F_i – fixed effect of soybean products;
e_{ij} – random error associated with observation *ij*.

PROC MIXED was also used for analysing *in situ* degradation kinetics of soybean products by the model as follows:

$$Y_{ijm} = \mu + P_i + R_j + e_{ijm} \quad (2)$$

where:

Y_{ijm} – observation of the dependent variable *ijm*;
μ – population mean for the variable;
P_i – effect of soybean products (fixed effect);
R_j – *in situ* experimental animals (random effect);
e_{ijm} – random error associated with the observation *ijm*.

PROC CORR was used to perform a correlation analysis between spectral profiles of carbohydrate and protein in soybean products and kinetic parameters of *in situ* degradation. Multiple regression analysis of molecular structure spectral profiles with *in situ* parameters was performed to select the best spectral parameters that explain nutrient values using REG procedure with a revised stepwise option. For all statistical analyses, Duncan's test with multiple comparisons was used and significance was declared at $P < 0.05$.

RESULTS AND DISCUSSION

Changes in the chemical profile of soybean products with different processing methods

Chemical profiles of the soybean products are shown in Table 1. Significant changes were observed for all detected items ($P < 0.05$). The maximum value of DM was observed in extruded soybean (98.49%) because of the high extrusion process temperature (145 °C). However, extruded soybean meal under the same processing method had lower DM content (94.99%), which could be caused by the lower processing temperature (110 °C). Fermented soybean meal had the highest protein content (48.61%) because of the bacterial protein produced during fermentation (Hong et al. 2004). However, the lowest protein content (37.98%) was found in extruded soybean due to the higher oil content of 17.88%, which was so because the processing temperature of the extruded soybean was the highest and there was no oil extraction. Soybean meal 1 had the maximum NDIP content (7.61%) while soybean meal 2 had a lower value (3.46%) ($P < 0.000\ 1$), which was probably caused by the cultivar variations of soybean. In addition, a marked decrease of NDIP in extruded soybean was different from increased NDIP using the autoclave method (Samadi et al. 2013). When the soybean was dry extruded, the extrusion process of the extruder could destroy the fibre in the soybean and release some protein bound to the fibre. The ADIP content of extruded soybean meal, extruded soybean and fermented soybean meal was higher than in two samples of ordinary soybean meal ($P < 0.000\ 1$), which indicated that high temperature and fermentation were able to increase the ADIP. Yang et al. (1992) showed that the right conditions for ADIP production were when feed was dried and fermented with low moisture. Mustafa et al. (2003a,b) reported that NPN content of flaxseed was decreased by autoclave treatment, and on the contrary, NPN content was increased by extrusion. In our study, NPN content was significantly decreased in extruded soybean meal but increased

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Table 1. Chemical profiles of soybean product[1]

Item	Soybean meal 1	Soybean meal 2	Extruded soybean meal	Fermented soybean meal	Extruded soybean	SEM	*P*
DM%	94.94^{c}	95.35^{b}	94.99^{c}	94.47^{d}	98.49^{a}	0.048	< 0.000 1
Ash (%DM)	6.91^{b}	6.98^{b}	9.01^{a}	7.08^{b}	6.68^{b}	0.143	< 0.000 1
EE (%DM)	2.67^{b}	1.94^{c}	1.36^{e}	1.61^{d}	17.88^{a}	0.061	< 0.000 1
CP (%DM)	45.75^{bc}	46.22^{b}	45.06^{c}	48.61^{a}	37.98^{d}	0.368	< 0.000 1
NDICP (%CP)	7.61^{a}	3.46^{c}	3.63^{c}	6.77^{b}	2.41^{d}	0.118	< 0.000 1
ADICP (%CP)	0.15^{d}	0.19^{d}	0.32^{c}	0.56^{b}	0.95^{a}	0.010	< 0.000 1
NPN (%CP)	4.85^{c}	5.26^{c}	2.26^{d}	12.61^{a}	8.66^{b}	0.086	< 0.000 1
SCP (%CP)	5.51^{d}	7.77^{c}	6.19^{d}	22.55^{a}	12.19^{b}	0.098	< 0.000 1
NDF (%DM)	32.82^{a}	25.22^{b}	16.79^{c}	14.26^{d}	11.36^{cd}	0.708	< 0.000 1
ADF (%DM)	11.14^{c}	13.84^{a}	7.71^{b}	5.79^{e}	7.95^{b}	0.251	< 0.000 1
ADL (%DM)	1.75^{b}	2.74^{a}	1.46^{c}	0.47^{d}	0.27^{e}	0.000 3	< 0.000 1

ADF = acid detergent fibre; ADL = acid detergent lignin; ADICP = acid detergent insoluble protein; CP = crude protein; DM = dry matter; EE = ether extract; NDF = neutral detergent fibre; NDICP = neutral detergent insoluble protein; NPN = non-protein N; SCP = soluble crude protein; SEM = standard error of the mean

[1]Means with different letters in the same row are significantly different ($P < 0.05$)

in extruded soybean ($P < 0.000\ 1$). Consistent with other reports, NPN in fermented soybean meal increased significantly (Weng and Chen 2010), which indicated that NPN was increased by fermentation within 36 hours. The fermentation process decomposed the protein macromolecules into small molecules, resulting in a greatly increased SCP value of fermented soybean meal. In addition, extruded soybean also got the higher value of SCP compared to ordinary soybean meal ($P < 0.000\ 1$), which was supported by Samadi et al. (2013) when the whole canola seeds increased SCP to some extent through dry heat. In addition, the contents of NDF and ADF were obviously reduced in extruded soybean meal, fermented soybean meal and extruded soybean compared with soybean 1 and soybean 2 ($P < 0.000\ 1$), which were parallel with the previously published study (Mustafa et al. 2003b; Weng and Chen 2010). The results indicate that extrusion and fermentation were able to decrease the contents of NDF and ADF.

Changes in protein components of CNCPS of soybean products with different processing methods

Protein subfractions for CNCPS are shown in Table 2, which documents that different processing

Table 2. Protein subfraction profiles of soybean and soybean meal using the Cornell Net Carbohydrate and Protein System (CNCPS)[1]

Item	Soybean meal 1	Soybean meal 2	Extruded soybean meal	Fermented soybean meal	Extruded soybean	SEM	*P*
PA (%CP)	4.84^{c}	5.26^{c}	2.26^{d}	12.61^{a}	8.66^{b}	0.184	< 0.000 1
PB1 (%CP)	0.66^{d}	2.51^{c}	3.93^{b}	9.95^{a}	3.54^{b}	0.205	< 0.000 1
PB2 (%CP)	77.86^{d}	84.75^{b}	92.70^{a}	63.51^{e}	81.45^{c}	0.355	< 0.000 1
PB3 (%CP)	16.48^{a}	7.27^{c}	0.79^{e}	13.38^{b}	5.39^{d}	0.243	< 0.000 1
PC (%CP)	0.15^{d}	0.20^{d}	0.32^{c}	0.55^{b}	0.96^{a}	0.021	< 0.000 1

PA = fraction of CP that is instantaneously solubilized at time zero; PB1 = fraction of CP that is soluble in borate-phosphate buffer and precipitated with trichloroacetic acid; PB2 = calculated as total CP minus the sum of fractions PA, PB1, PB3 and PC; PB3 = calculated as the difference between the portions of total CP recovered with NDF and ADF; PC = fraction of CP recovered with ADF and it is considered to be undegradable; SEM = standard error of the mean

[1]Means with different letters in the same row are significantly different ($P < 0.05$)

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methods could change the protein subfractions. PA (NPN) was instantaneously solubilized at time zero while PB1 was fast degraded in the rumen by reducing soluble protein. It is shown that PA was decreased significantly in extruded soybean meal but it increased significantly in fermented soybean meal ($P < 0.05$), which was supported by Samadi et al. (2013) and Chen et al. (2010) when PA was decreased significantly for the whole canola seed processed by autoclave heating and dramatically got an increase by fermentation, which was so because fermentation could decompose large protein molecules in soybean meal into small molecule proteins such as soybean peptides and amino acids, increasing the SCP content. There was no significant difference in PB1 content between extruded soybean meal and extruded soybean but a remarkable increase was found in fermented soybean meal because of the increase in SCP and NPN content as PB1 was calculated as SCP-NPN, which was different from the previous study by Samadi (2013) where PB1 was decreased by autoclave heating. This discrepancy might be related to inherent differences in the sample type or differences in heating temperature and duration. PB3 was considered to be a slowly degraded part in the rumen and most of this fraction escaped ruminal degradation (Peng et al. 2014a,b). Our study showed the notable differences in PB3 content between extruded soybean meal and extruded soybean ($P < 0.000\ 1$) with the higher value in extruded soybean. We inferred that the treatment of extruded soybean by processing at 145 °C without extra moisture denatured the protein and decreased protein solubility in the rumen. It is indicated that the higher temperature treatment could protect the protein from ruminal degradation (Yang et al. 1992). Fraction PC was combined with lignin and it was not degradable in the rumen, so PC was not available to animals, and there was a marked increase in PC ($P < 0.000\ 1$) in our experiment.

Changes in rumen digestibility of soybean products with different processing methods

Significantly different rumen degradation parameters for the samples are presented in Table 3. The CP degradation rate of soybean meal 1 was lower than that of soybean meal 2 ($P < 0.000\ 1$), but there were no differences in the degradation rate of DM, NDF and ADF between the two soybean meals. Compared to soybean meal, extruded soybean meal and extruded soybean showed a reduction in rumen degradable protein ($P < 0.000\ 1$). Zhang and Yu (2012) found that extruded soybeans could reduce the effective degradation rate of protein in the rumen, and the reason for different result might be due to different temperature. In view of NDF and ADF degradability, the study showed that the ED of NDF and ADF of extruded soybean dramatically increased in comparison with soybean meal ($P < 0.000\ 1$). Amornthewaphat et al. (2005) proved that the extrusion process will significantly reduce and soften the fibre. However, we did not observe any difference in NDF between extruded soybean meal and soybean meal, which could be explained by two reasons: (1) The processing temperature of extruded soybean meal did not reach the temperature of extruded soybean to increase the oil yield; (2) extruded soybean meal was made under pressure and the NDF would be pressed tightly so that its degradability was not increased. The fermented soybean meal presented maximum degradability in CP, DM, NDF and ADF. It was reported that there was a decomposition reaction in the fermented feed macromolecule so that the original tight structure became loose and the fibre was translated into glucose and other energy substances that animals could use (Weng and Chen 2010). Fermentation increased the protein and fat content of fermented soybean meal, and the content of essential amino acids was also affected by fermentation, but the amount of carbohydrate was greatly decreased by fermentation (Chen et al. 2010).

Changes in protein amide and protein secondary structures of soybean products with different processing methods

Characteristics of protein spectral structures including amide I, amide II and secondary structures (α-helix and β-sheet) are shown in Table 4. Amide I and amide II, which we detected, were in the region of ca. 1 715–1 576/cm and ca. 1 576–1 468/cm, respectively, and α-helix and β-sheet were covered by amide I and centred at ca. 1 648/cm and 1 640/cm. There were significant differences between soybean meal 1 and soybean meal 2 in all protein spectral characteristics with the exception of amide I height and ratio of amide I to amide II for height and area ($P < 0.05$). Extrusion and fer-

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Table 3. *In situ* ruminal degradation parameters[1] of crude protein (CP), dry matter (DM), neutral detergent fibre (NDF) and acid detergent fibre (ADF) of soybean and soybean meal

Parameter	Soybean meal 1	Soybean meal 2	Extruded soybean meal	Fermented soybean meal	Extruded soybean	SEM	*P*
CP							
a	4.45^{c}	7.03^{b}	6.25^{b}	25.00^{a}	0.6^{d}	0.005	< 0.000 1
b	95.51^{b}	92.97^{c}	93.75^{c}	74.85^{d}	99.40^{a}	0.005	< 0.000 1
c	3.58^{b}	4.24^{ab}	3.58^{b}	4.62^{a}	3.77^{ab}	0.003	< 0.000 1
ed	40.03^{c}	45.43^{b}	38.97^{c}	57.67^{a}	38.93^{c}	0.014	< 0.000 1
DM							
a	20.54^{d}	19.82^{d}	23.43^{c}	35.86^{a}	27.92e	0.006	< 0.000 1
b	79.47^{a}	80.18^{a}	76.58^{b}	64.12^{d}	71.97^{c}	0.006	< 0.000 1
c	4.43^{b}	4.92^{ab}	5.05^{ab}	5.44^{a}	3.49^{c}	0.002	< 0.000 1
ed	54.25^{c}	54.33^{c}	58.34^{b}	66.30^{a}	54.40c	0.009	< 0.000 1
NDF							
a	28.36^{b}	24.17^{c}	27.77^{b}	38.43^{a}	27.8^{b}	0.007	< 0.000 1
b	69.10^{b}	73.27^{a}	68.00^{c}	60.47^{c}	72.1^{a}	0.008	< 0.000 1
c	6.36^{a}	7.17^{a}	6.37^{a}	5.23^{b}	6.70^{a}	0.003	< 0.000 1
ed	63.87^{b}	63.87^{b}	63.98^{b}	66.5^{a}	65.87^{a}	0.639	< 0.000 1
ADF							
a	26.23^{cd}	24.67^{d}	28.6^{c}	38.26^{b}	45.83^{a}	0.011	< 0.000 1
b	73.31^{a}	68.36^{b}	68.83^{b}	56.03^{d}	54.10^{c}	0.007	< 0.000 1
c	5.6^{c}	7.53^{bc}	6.9^{b}	9.93^{a}	4.06^{d}	0.004	< 0.000 1
ed	61.93^{d}	62.57^{d}	65.3^{c}	73.17^{a}	67.7^{b}	0.003	< 0.000 1

SEM = standard error of the mean

[1]Means with different letters in the same row are significantly different ($P < 0.05$); Degradation parameters: a = fast degradable part, b = slowly degradable part, c = degradation rate constant of part b (%/h), ed = effective degradation (consider the rumen outflow rate 0.06/h)

mentation decreased the related spectral value (height and area of amide and secondary structure) of soybean products compared with soybean meal 1 and soybean meal 2 ($P < 0.05$). In addition, the spectral intensities of extruded soybean that was processed at 145 °C were reduced compared with extruded soybean meal that was processed at 110 °C ($P < 0.05$) while no great differences were found between extruded soybean meal and fermented soybean meal in all protein spectral characteristics. The results indicated that changes in the internal structure of protein might be related to the temperature and processing method of the feed (Yu et al. 2015). The extruded soybean meal and the fermented soybean meal did not show any significant changes in the area and height ratios of amide I to amide II or α-helix to β-sheet in comparison with soybean meal 1 and soybean meal 2 ($P > 0.05$). But there was a dramatic decrease in the area ratio of amide I to amide II and the height ratio of α-helix to β-sheet in extruded soybean compared with other samples ($P < 0.05$). Samadi et al. (2013) reported a significant increase in amide I to II area ratios for dry roasting of soybean. However, the experiment of Peng et al. (2014a,b) indicated that there was no significant difference in amide I to II area ratios for flaxseed roasting. Our data showed that there were no significant changes in amide I to II area ratios of soybean meal 1, soybean meal 2, extruded soybean meal and fermented soybean meal, but an obvious decrease was found in extruded soybean. Yu et al. (2015) found a decrease in amide I and amide II intensities in fermented soybean meal as well as in the height of α-helix and β-sheet during ruminal incubation, which was similar to our finding, indicating that

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Table 4. Protein molecular structural characteristics[1] of soybean and soybean meal revealed using molecular spectroscopy

Item	Soybean meal 1	Soybean meal 2	Extruded soybean meal	Fermented soybean meal	Extruded soybean	SEM	*P*
Amide							
Amide I (height)	1.103^{ab}	1.262^{a}	0.666^{bc}	0.782^{bc}	0.385^{d}	0.080	0.000 1
Amide II (height)	0.568^{b}	0.762^{a}	0.471^{bc}	0.390^{cd}	0.233^{d}	0.056	0.000 1
Amide I (area)	81.036^{b}	106.452^{a}	53.394^{c}	60.918^{c}	8.825^{d}	6.374	< 0.000 1
Amide II (area)	32.495^{b}	43.991^{a}	23.849^{bc}	22.140^{c}	27.416^{bc}	2.933	0.000 7
α-helix (height)	0.982^{b}	1.318^{a}	0.698^{c}	0.820^{bc}	0.029^{d}	0.073	< 0.000 1
β-sheet (height)	1.023^{b}	1.312^{a}	0.698^{c}	0.824^{bc}	0.632^{c}	0.087	0.001 6
Ratio							
Amide I : amide II (height)	1.692	1.767	1.974	1.938	1.809	0.154	0.675 6
Amide I : amide II (area)	2.324^{a}	2.536^{a}	2.539^{a}	2.669^{a}	0.332^{b}	0.184	0.000 1
α-helix : β-sheet	0.964^{b}	1.005^{a}	1.000^{ab}	0.994^{ab}	0.045^{c}	0.011	< 0.000 1

SEM = standard error of means

[1]Means with different letters in the same row are significantly different ($P < 0.05$)

Peak baseline region for amide I and II together ca. 1 715–1 468/cm, protein amide I region ca. 1 715–1 576/cm, protein amide II region ca. 1 576–1 468/cm, the peak height and area are measured in infrared absorbance units; Peak baseline for protein secondary structure ca. 1 715–1 468/cm, protein α-helix and β-sheet were modelled in amide I region and centred at ca. 1 648 and 1 640/cm, respectively

the internal structure change of protein was related to the processing methods. Compared with soybean meal 1 and soybean meal 2, the remaining samples were reduced significantly in the peak height of α-helix and β-sheet, but there were no significant differences in the height ratio of α-helix to β-sheet without considering extruded soybean. Discrepancies in response to heat treatment could be related to different sample types and processing additions.

Overall, the protein molecular structures would be changed when processed with external conditions, and the content of α-helix and β-sheet would influence the quality and degradation rate of protein.

Changes in carbohydrate molecular structures of soybean products with different processing methods

The carbohydrate spectral data on STCHO (structural carbohydrates) and CHO (carbohydrate) are shown in Table 5. Soybean meal 2 still had the highest values among most STHO and CELC spectral characteristics, which was similar to the spectral features of protein absorption peaks to a great extent.

There were no differences in STHO and CELC spectral characteristics between extruded soybean meal and fermented soybean meal except the peak 3 height of STCHO. However, a remarkable decrease was found in extruded soybean compared to extruded soybean meal ($P < 0.05$). Because the STCHO and CELC regions are associated with hemicellulose and cellulose, this result indicated that the crude fibre was dramatically decreased by extrusion at a high temperature (Zhang and Yu 2012). With regard to the absorbance peak height and area intensities of CHO, the highest value of total area was observed in soybean meal 2 followed by extruded soybean meal, soybean meal 1, extruded soybean and fermented soybean meal. Extruded soybean had the lower value of CHO than extruded soybean meal ($P < 0.05$), which suggested that a higher temperature could definitely contribute to the reduction of total carbohydrate (Yu et al. 2015). Fermented soybean meal had the lowest values in CHO area as well as peak 1 and peak 3 areas, because the crude fibre and insoluble sugar were degraded during fermentation (Xin and Yu 2013a). Yu et al. (2015) reported that almost all carbohydrate spectral parameters were significantly reduced with increasing ruminal incubation time, which suggested that the carbohydrate components in the rapeseeds were digested during ruminal fermentation.

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Table 5. Carbohydrate structure profiles[1] of soybean and soybean meal revealed using molecular spectroscopy

Item	Soybean meal 1	Soybean meal 2	Extruded soybean meal	Fermented soybean meal	Extruded soybean	SEM	*P*
STCHO							
Total area	24.617^{b}	34.872^{a}	17.815^{b}	22.021^{b}	9.775^{c}	2.531	0.000 5
Peak 1 height	0.140^{ab}	0.180^{a}	0.096^{b}	0.129^{ab}	0.125^{b}	0.016	0.041 0
Peak 2 height	0.216^{b}	0.324^{a}	0.149^{b}	0.149^{b}	0.046^{c}	0.022	< 0.000 1
Peak 3 height	0.030^{b}	0.042^{a}	0.017^{c}	0.041^{a}	0.009^{c}	0.003	< 0.000 1
CELC							
Peak height	0.24^{b}	0.34^{a}	0.180^{b}	0.223^{b}	0.087^{c}	0.024	0.000 3
Peak area	12.096^{b}	17.174^{a}	9.249^{b}	11.662^{b}	3.629^{c}	1.206	0.000 2
CHO							
Total peak area	26.500^{b}	37.510^{a}	29.113^{b}	16.557^{c}	22.087^{b}	2.519	0.001 8
Peak 1 height	0.104^{ab}	0.137^{a}	0.088^{bc}	0.056^{c}	0.149^{a}	0.014	0.006 5
Peak 2 height	0.002^{c}	0.002^{c}	0.006^{b}	0.053^{a}	0.003^{bc}	0.001	< 0.000 1
Peak 3 height	0.469^{b}	0.622^{a}	0.371^{bc}	0.341^{cd}	0.227^{d}	0.038	0.000 3
Peak 1 area	2.573^{b}	3.456^{b}	2.399^{b}	0.623^{c}	4.989^{a}	0.452	0.000 7
Peak 2 area	0.020^{c}	0.052^{bc}	0.119^{b}	0.771^{a}	0.011^{c}	0.023	< 0.000 1
Peak 3 area	23.907^{b}	34.002^{a}	26.596^{b}	15.163^{c}	17.088^{c}	2.127	0.000 6
Area ratio of CELE : STCHO	0.491^{c}	0.493^{c}	0.518^{b}	0.530^{a}	0.372^{d}	0.002	< 0.000 1
Area ratio of CELC : CHO	0.454^{bc}	0.466^{b}	0.320^{c}	0.711^{a}	0.164^{d}	0.043	< 0.000 1
Area ratio of STCHO : CHO	0.925^{b}	0.946^{b}	0.617^{c}	1.343^{a}	0.442^{c}	0.088	0.000 3

SEM = standard error of the mean

[1]Means with different letters in the same row are significantly different ($P < 0.05$)

Carbohydrate data unit, IR absorbance unit; structural carbohydrate (STCHO) peak baseline ca. 1 469–1 183/cm and there were three peaks in this region with the first, second and third peak centred at ca. 1 444, 1 386 and 1 304/cm, respectively. The first, second and third peak regions were ca. 1 469–1 418, 1 418–1 316 and 1 316–1 285/cm, respectively. Structural carbohydrates are associated with major hemicellulosic and cellulosic compounds; The cellulosic compound (CELC) peak baseline ca. 1 285–1 183/cm and they are mainly associated with cellulose compounds; The carbohydrate (CHO) peak baseline ca. 1 183–860/cm and there were three peaks in this region with the first, second and third peak at ca. 1 149, 1 104 and 1 041/cm, respectively. The first, second and third peak regions were ca. 1 183–1 128, 1 128–1 090 and 1 090–860/cm, respectively

Functional group ratios were considered to be associated with feed quality and nutritive values (Yu et al. 2005). There were significant differences between the samples in area ratios of CELC : STCHO, CELC : CHO and STCHO : CHO ($P < 0.05$). Maximum values of area ratios of CELC : STCHO, CELC : CHO and STCHO : CHO were found in fermented soybean meal because of the lower CHO intensity. Area ratio values of CELC : STCHO and CELC : CHO were lower in extruded soybean compared to extruded soybean meal ($P < 0.000\ 1$). This result was in line with the changes in STCHO and CELC. Spectral ratios varied among the soybean products, which indicated that these samples had different internal structural conformation after different processing, and the peak area of CHO was negatively correlated with the rapid degradation part of NDF, positively correlated with the slow degradation part, and negatively correlated with the effective degradation rate. The results were supported by published studies for crop residues (Yu et al. 2015) and oil seed, bio-fuel and bio-ethanol co-products (Yu et al. 2005).

Correlations and regression of protein spectrum with nutrient degradability

The results of correlations and regression of protein spectral features with nutrient digestibility are pre-

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sented in Table 6. The spectra of H_1 (r = –0.596, P = 0.019), H_2 (r = –0.593, P = 0.020), A_1(r = –0.417, P = 0.016), α (r = –0.596, P = 0.019) and β (r = –0.539, P = 0.038) were weakly negatively associated with DM fast degradable part and positively associated with DM slowly degradable part. There was a significant positive correlation between DM degradability and the ratio of α-helix to β-sheet. In terms of CP, the ratio of α-helix to β-sheet was positively correlated with CP fast degradable part and negatively correlated with CP slowly degradable part, and a positive relationship was found between CP digestibility and the area ratio of amide I and amide II. However, there was no relationship between α-helix to β-sheet and CP degradation. The higher the α-helix content in the protein structure, the more easily it would be degraded, and the higher the protein quality would be; the more the β-sheets in the protein structure, the harder it would be for animals to use (Xin and Yu 2013a). However, Liu et al. (2017) reported that the changes in the ratio of α : β during thermal processing were not significantly correlated with the corresponding changes in protein digestibility across the pea types, which was similar to our findings. All these findings indicated that the characteristics of DM and CP degradation were influenced by feed protein internal structures.

Correlations and regression of carbohydrate spectrum with nutrient degradability

Structural carbohydrates were biopolymers composed of various biomolecules, which were not easily degraded by ruminants in the rumen and intestines, and had a low nutritional value (Zhang and Yu 2012). The soybean carbohydrates mainly included structural carbohydrates such as cellulose, hemicellulose and pectin, which were not only difficult to be used by animals but also affected degradation of other nutrients like DM and CP (Yu et al. 2005). Meanwhile, non-structural carbohydrates such as starch and soluble sugar in soybean products were low. Table 7 shows that there was

Table 6. Correlations between protein molecular structural spectral profiles[1] and *in situ* ruminal degradation parameters[2] of dry matter (DM) and crude protein (CP) of soybean and soybean meal

Item		H_1	H_2	A_1	A_2	α	β	$A_1H : A_2H$	$A_1A : A_2A$	α : β
DM										
a	*r*	–0.596	–0.593	–0.607	–0.417	–0.596	–0.539	0.175	–0.161	–0.175
	P	0.019	0.020	0.016	0.076	0.019	0.038	0.532	0.567	0.533
b	*r*	0.595	0.592	0.606	0.470	0.599	0.543	–0.177	0.159	0.173
	P	0.019	0.020	0.017	0.077	0.018	0.036	0.528	0.571	0.537
c	*r*	0.188	0.186	0.179	–0.356	0.218	0.054	–0.118	0.532	0.783
	P	0.503	0.507	0.524	0.193	0.435	0.850	0.675	0.041	0.000 6
ed	*r*	–0.132	–0.132	–0.150	–0.556	–0.082	–0.225	0.075	0.361	0.590
	P	0.638	0.638	0.594	0.031	0.771	0.420	0.75	0.186	0.021
CP										
a	*r*	0.368	0.349	0.351	–0.113	0.358	0.234	0.041	0.597	0.748
	P	0.176	0.203	0.200	0.689	0.190	0.400	0.884	0.019	0.001
b	*r*	–0.368	–0.349	–0.351	0.113	–0.358	–0.234	–0.042	–0.597	–0.748
	P	0.176	0.202	0.200	0.689	0.190	0.400	0.884	0.019	0.01
c	*r*	0.261	0.288	0.256	0.036	0.322	0.288	–0.142	0.435	0.356
	P	0.347	0.297	0.357	0.899	0.241	0.297	0.612	0.105	0.192
ed	*r*	0.404	0.407	0.400	0.209	0.466	0.464	0.014	0.533	0.331
	P	0.135	0.132	0.139	0.454	0.079	0.081	0.959	0.040	0.229

[1]H_1 = amide I height; H_2 = amide II height; A_1 = amide I area; A_2 = amide II area; α = α-helix height, β = β-sheet height; $A_1A : A_2A$ = area ratio of amide I and amide II, α : β = height ratio of α and β; $A_1H : A_2H$ = height ratio of amide I and amide II; [2]Degradation parameters: a = fast degradable part, b = slowly degradable part, c = degradation rate constant of part b (%/h), ed = effective degradation (consider the rumen outflow rate 0.06/h)

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Table 7. Correlations between carbohydrate molecular structural spectral profiles[1] and *in situ* ruminal degradation parameters[2] of dry matter (DM), crude protein (CP), neutral detergent fibre (NDF) and acid detergent fibre (ADF) of soybean and soybean meal

Item		S_1H	S_2H	S_3H	S_1A	S_2A	S_3A	H-CELC	A-STCHO	A-CELC	A-CHO	A-CELC : A-STCHO	A-CELC : A-CHO	A-STCHO : A-CHO
DM														
a	*r*	−0.336	−0.722	−0.166	−0.393	−0.639	−0.103	−0.528	−0.568	−0.503	−0.746	0.325	0.034	−0.032
	P	0.221	0.002	0.554	0.147	0.010	0.713	0.043	0.027	0.056	0.001	0.237	0.904	0.906
b	*r*	0.327	0.721	0.164	0.384	0.638	0.101	0.527	0.566	0.502	0.740	−0.323	−0.037	0.028
	P	0.234	0.002	0.558	0.157	0.010	0.718	0.043	0.028	0.056	0.002	0.239	0.897	0.919
c	*r*	−0.098	0.256	0.543	−0.218	0.302	0.592	0.372	0.345	0.379	−0.193	0.772	0.628	0.617
	P	0.727	0.357	0.036	0.435	0.274	0.020	0.172	0.208	0.164	0.491	0.0007	0.012	0.014
ed	*r*	−0.316	−0.126	0.342	0.416	0.054	0.407	0.041	0.011	0.052	−0.440	0.747	0.457	0.420
	P	0.251	0.671	0.212 1	0.122	0.849	0.132	0.884	0.970	0.854	0.101	0.001	0.087	0.119
CP														
a	*r*	0.079	0.311	0.698	−0.055	0.379	0.749	0.475	0.442	0.497	−0.084	0.835	0.716	0.698
	P	0.780	0.258	0.004	0.844	0.163	0.001	0.073	0.099	0.059	0.766	0.0001	0.003	0.004
b	*r*	−0.079	−0.311	−0.698	0.055	−0.379	−0.750	−0.476	−0.442	−0.497	0.084	−0.835	−0.716	−0.698
	P	0.780	0.258	0.004	0.844	0.163	0.001	0.073	0.099	0.059	0.766	0.0001	0.003	0.008
c	*r*	0.252	0.294	0.629	0.162	0.371	0.716	0.410	0.390	0.424	−0.249	0.464	0.677	0.677
	P	0.364	0.288	0.012	0.562	0.174	0.003	0.129	0.150	0.115	0.371	0.082	0.005	0.006
ed	*r*	0.224	0.268	0.646	0.154	0.357	0.743	0.357	0.339	0.400	−0.214	0.422	0.678	0.666
	P	0.379	0.333	0.009	0.584	0.191	0.001	0.191	0.215	0.139	0.442	0.117	0.005	0.006
NDF														
a	*r*	−0.196	−0.370	0.125	−0.211	−0.314	0.093	−0.239	−0.246	−0.225	−0.703	0.396	0.407	0.336
	P	0.483	0.175	0.657	0.451	0.254	0.742	0.390	0.376	0.320	0.003	0.143	0.132	0.221
b	*r*	0.382	0.320	−0.145	0.416	0.273	−0.121	0.214	0.225	0.197	0.566	−0.629	−0.326	−0.334
	P	0.159	0.245	0.606	0.123	0.324	0.666	0.443	0.420	0.483	0.028	0.012	0.113	0.223
c	*r*	0.054	−0.023	−0.455	0.082	−0.064	−0.434	−0.136	−0.132	−0.143	0.477	−0.502	−0.647	−0.627
	P	0.849	0.934	0.088	0.770	0.819	0.106	0.628	0.637	0.610	0.072	0.057	0.009	0.012
ed	*r*	0.200	−0.413	0.069	0.136	−0.290	0.095	−0.224	−0.259	−0.177	−0.602	0.018	0.219	0.154
	P	0.473	0.126	0.807	0.628	0.294	0.736	0.423	0.350	0.527	0.017	0.950	0.434	0.584
ADF														
a	*r*	−0.293	−0.804	−0.393	−0.282	−0.750	−0.368	−0.675	−0.696	0.668	−0.75	−0.161	−0.218	−0.243
	P	0.290	0.0003	0.147	0.308	0.001	0.177	0.006	0.004	0.004	0.001	0.567	0.435	0.383
b	*r*	0.086	0.663	0.280	0.111	0.589	0.214	0.503	0.535	0.485	0.550	0.146	0.236	0.250
	P	0.761	0.007	0.311	0.694	0.021	0.443	0.056	0.040	0.066	0.034	0.602	0.397	0.369
c	*r*	0.138	0.323	0.703	−0.002	0.416	0.752	0.509	0.474	0.534	−0.030	0.842	0.680	0.634
	P	0.625	0.240	0.003	0.995	0.123	0.001	0.052	0.074	0.040	0.914	< 0.000 1	0.005	0.011
ed	*r*	−0.224	−0.686	−0.104	−0.309	−0.528	−0.059	−0.424	−0.472	−0.405	−0.643	0.391	0.011	−0.072
	P	0.421	0.005	0.712	0.263	0.023	0.834	0.115	0.075	0.133	0.010	0.149	0.100	0.799

[1]S_1H = structural carbohydrate peak 1 height, S_2H = structural carbohydrate peak 2 height, S_3H = structural carbohydrate peak 3 height, H-CELC = cellulosic compound height; S_1A = structural carbohydrate peak 2 area, S_2A= structural carbohydrate peak 2 area, S_3A= structural carbohydrate peak 3 area; A-CELC = cellulosic compound area, A-STCHO = structural CHO area, A-CHO = total carbohydrate area, A-CELC : A-STCHO = area ratio of CELC to STCHO; A-CELC : A-CHO = area ratio of CELC to CHO, A-STCHO : A-CHO = area ratio of STCHO to CHO; [2]Degradation parameters: a = fast degradable part, b = slowly degradable part, c = degradation rate constant of part b (%/h), ed = effective degradation (consider the rumen outflow rate 0.06/h)

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a strong positive relationship (r = 0.747, P = 0.001) between DM degradability and area ratio of CELC and STCHO. The area ratios of CELC to CHO, STCHO to CHO were positively associated with CP degradability and there was also a positive correlation between STCHO area and CP degradability. The results suggested that carbohydrate had different effects on the digestibility of DM and CP (Peng et al. 2014a), but more specific information still needs to be discussed. Heating and fermentation would break the chemical bonds in the fibre molecules, resulting in a decrease in fibre content and an increase in the rate of degradation (Mustafa et al. 2003a). There was a positive relationship (r = 0.062, P = 0.017) between the CHO area and *in situ* NDF digestibility and a negative relationship was found among STCHO peak 2 height, STCHO peak 2 area, CHO area and ADF digestibility in rumen. Our study results were consistent with the study for common prairie grass nutrient digestion behaviour when the protein molecular structure, protein subfractions, and nutrient profiles in camelina seeds changed (Peng et al. 2014a). Table 8 shows the multiple regression analyses to screen the best spectral parameter of protein and carbohydrate to predict ruminal nutrient degradability of soybean products by the tested model as follows. The results showed that the area ratio of CELC : CHO and amide II

Table 8. Linear regression analysis between *in situ* rumen dry matter (DM), crude protein (CP), neutral detergent fibre (NDF) and acid detergent fibre(ADF) degradation parameters[1] and protein, carbohydrate molecular structural spectral profiles[2]

Prediction equation test model	R^2	RSD	P
DM			
a = −0.006 A-CHO + 0.414	0.597 4	0.041	0.000 7
b = 0.006 A-CHO + 0.586	0.596 4	0.042	0.000 7
c = 0.119 A-CELC : A-STCHO − 0.01	0.744 1	0.004	< 0.000 1
ed = 0.253 A-CELC : A-CHO − 0.138 Amide II height + 0.541	0.869 8	0.019	< 0.000 1
CP			
a = 0.502 A-CELC : A-CHO − 0.189 Amide II height − 0.035	0.893 6	0.033	< 0.000 1
b = −0.502 A-CELC : A-CHO + 0.189 Amide II height + 1.035	0.893 6	0.033	< 0.000 1
c = 0.014 A-STCHO : A-CHO + 0.028	0.533 6	0.005	0.002
ed = 0.676 A-CELC : A-CHO + 0.004 A-CHO −0.056 A-STCHO + 0.353 Structural CHO peak 2 area − 8.405 Structural CHO peak 2 height + 0.215	0.979 5	0.015	< 0.000 1
NDF			
a = 0.167 A-CELC : A-CHO + 0.016 A-CELC − 1.046 Structural CHO peak 2 height + 0.238	0.946 6	0.013	< 0.000 1
b = −0.042 A-CELC + 0.058 Structural CHO peak 2 area + 0.721	0.876 2	0.018	< 0.000 1
c = −0.064 A-CELC : A-CHO + 0.037 Structural CHO peak 3 area − 10.075	0.701 8	0.005	0.000 7
ed = −0.001 A-CHO + 0.067	0.379 6	0.012	0.011 7
ADF			
a = −0.003 A-CHO − 0.923 Structural CHO peak 2 height + 1.742 Structural CHO peak 1 height + 0.352	0.897 0	0.031	< 0.000 1
b = 0.006 A-CHO + 0.475	0.416 9	0.063	0.009 3
c = 0.283 A-CELC : -A-STCHO − 0.068	0.606 6	0.014	0.000 6
ed = −0.004 A-CHO + 0.760	0.514 5	0.031	0.002 6

[1]Degradation parameters: a = fast degradable part, b = slowly degradable part, c = degradation rate constant of part b (%/h), ed = effective degradation (consider the rumen outflow rate of 0.06/h); [2]Amide II height = height of protein amide II, A-CELC = cellulosic compound area, A-STCHO = structural carbohydrate area, A-CHO = total carbohydrate area; A-CELC : A-STCHO = area ratio of CELC to STCHO; A-CELC : A-CHO = area ratio of CELC to CHO, A-STCHO : A-CHO = area ratio of STCHO to CHO

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height were better indices to predict ruminal DM digestibility of soybean feeds by a prediction equation and accuracy (R^2 = 0.869 8). The area ratio of CELC : CHO, CHO area, STCHO area, STCHO peak 2 area and height were better to predict ruminal CP digestibility of soybean feeds by a prediction equation and accuracy (R^2 = 0.979 5). CHO area parameters could predict ADF degradation characteristics in rumen (R^2 = 0.514 5) while they weakly fit with NDF degradation behaviour in rumen (R^2 = 0.379 6). These results indicated that DM and CP utilization in rumen was significantly affected by carbohydrate and protein structures. Therefore, protein and carbohydrate internal molecule structures could be used to predict nutrient digestibility in rumen when fed to animals.

In conclusion, it was observed that chemical structures of protein and carbohydrate were changed differently by extrusion and fermentation. There was a significant decrease in the eigenvalue of the molecular structure of amide I, amide II, α-helix and β-sheet by extrusion and fermentation with the greatest degree of their reduction in extruded soybean that was processed at a higher temperature (145 °C). Meanwhile, the peak intensity of STCHO, CELC and CHO decreased significantly with the extrusion and fermentation of soybean products. Moreover, changes in molecular structures of protein and carbohydrate influenced the digestibility of nutriments like DM and CP, NDF and ADF of soybean products in the rumen. The *in situ* study showed that ruminal protein degradation was reduced by extrusion and increased by fermentation. The degradation of NDF and ADF in extruded soybean meal and fermented soybean meal was significantly improved. In terms of the correlation analysis, the ratios of α-helix to β-sheet and A-CELC to A-STCHO were positively related to the DM degradation rate of soybean products in the rumen. The degradation characteristics of CP were not only related to the molecular structure of proteins in the rumen, but also to the total carbohydrate in the rumen. The results also showed that the degradation of NDF was negatively correlated with total carbohydrates, and the degradation of ADF was negatively correlated with structural carbohydrates and total carbohydrates. Because the conclusion originated from a small scale of soybean feed data, more accurate regression equations could be developed on the basis of a larger amount of data accumulation.

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Conflict of interest

The authors declare no conflict of interest.

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