# A Correlation Study of Carbohydrate Molecular Structure with Nutritional Value and Rumen Degradation Characteristics in Differently Heat-Treated Okara (Postprint)

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## Abstract

This study aimed to investigate the effects of different heat treatments on the carbohydrate molecular structure of soybean residue and its correlation with nutritional value and rumen degradation characteristics. Conventional chemical analysis methods combined with the Cornell Net Carbohydrate and Protein System (CNCPS) and nylon bag technique were adopted to evaluate the nutritional value of differently heat-treated soybean residue, and Fourier Transform Infrared Spectroscopy (FTIR) was utilized to analyze changes in the carbohydrate molecular structure of soybean residue, thereby exploring the correlations among them. The results showed that: 1) Heat treatment reduced the nutritional value of soybean residue and the rumen degradation rate of neutral detergent fiber (NDF). 2) Different heat treatments had significant effects ($P<0.05$) on the peak areas of structural carbohydrate (STCHO), cellulosic compounds (CELC), and total carbohydrate (CHO) in the carbohydrate molecular structure of soybean residue, as well as the corresponding peak heights in the three peak areas. 3) The peak area ratio of STCHO to CHO, as well as the peak height ratio in total carbohydrate, could effectively estimate the nutritional value of differently heat-treated soybean residue and the parameters of NDF rumen degradation. In conclusion, different heat treatments affected the carbohydrate molecular structure, nutritional value, and NDF rumen degradation rate of soybean residue; there existed a correlation between the carbohydrate molecular structure and its nutritional value and NDF rumen degradation characteristics in differently heat-treated soybean residue, preliminarily demonstrating that the spectral information of soybean residue obtained by FTIR spectroscopy could directly reflect its heat damage extent.

# Full Text

## Study on Correlation of Molecular Structure of Carbohydrate with Its Nutritional Value and Ruminal Degradation Characteristics in Different Heat Treated Okara

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### Abstract

This study aimed to investigate the effects of different heat treatments on the carbohydrate molecular structure of okara and its correlation with nutritional value and ruminal degradation characteristics. The nutritional value of heat-treated okara was evaluated using conventional chemical analysis combined with the Cornell Net Carbohydrate and Protein System (CNCPS) and nylon bag technique, while Fourier transform infrared spectroscopy (FTIR) was employed to analyze changes in carbohydrate molecular structure. The results showed that: (1) Heat treatment reduced the nutritional value of okara and the ruminal degradability of neutral detergent fiber (NDF). (2) Different heat treatments significantly affected the peak areas of structural carbohydrates (STCHO), cellulosic compounds (CELC), and total carbohydrates (CHO), as well as the corresponding peak heights within these three spectral regions ($P < 0.05$). (3) The ratio of STCHO to CHO peak area and the peak height ratios within the total carbohydrate region could effectively estimate the nutritional value and NDF rumen degradation parameters of heat-treated okara. In conclusion, different heat treatments affected the carbohydrate molecular structure, nutritional value, and NDF ruminal degradability of okara. Correlations exist between carbohydrate molecular structure and nutritional value as well as NDF ruminal degradation characteristics, demonstrating that FTIR spectral information can serve as a sensitive indicator to directly and non-destructively assess the degree of heat damage in okara.

**Keywords:** heat treatment; okara; FTIR; carbohydrate molecular structure; correlation

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## Introduction

Okara is a byproduct of soybean processing for tofu and soybean oil. Previous studies have shown that okara contains abundant nutrients including dietary fiber, carbohydrates (CHO), and protein [1]. As an unconventional feed ingredient, the development and utilization of okara can effectively reduce dairy cattle feeding costs and enhance the competitiveness of China’ s dairy industry.

Li et al. [2] evaluated the nutritional value of okara from four regions using the Cornell Net Carbohydrate and Protein System (CNCPS) and in vitro gas production methods, concluding that okara provides high available energy for ruminants and promotes microbial protein synthesis. Zhang et al. [3] demonstrated through CNCPS and in vitro fermentation parameters that okara has superior feeding value compared to rapeseed meal. However, with moisture content exceeding 80%, okara is highly susceptible to spoilage during transport and storage, causing environmental pollution and substantial resource waste [4,5]. To address this issue, dehydration or drying methods are commonly employed to reduce feed moisture content [6]. Appropriate heat treatment can lower okara’ s moisture content for easier transport and storage without compromising nutritional value, but excessive heat treatment may trigger Maillard reactions that reduce nutritional quality [7]. Traditional chemical methods for evaluating heat-treated okara are time-consuming and labor-intensive, necessitating a sensitive indicator that can directly reflect the degree of heat-induced nutritional damage.

Fourier transform infrared spectroscopy (FTIR) can rapidly and directly reveal internal molecular structures of feed without causing destructive effects [8]. The carbohydrate spectral region primarily arises from C-O stretching vibrations in carbohydrates [9]. Characteristic regions for total carbohydrates are located at 1,180–800 cm $^{1}$, while structural carbohydrates appear at 1,486–1,188 cm $^{1}$, providing molecular structural information that influences feed nutritional value and utilization efficiency [10,11]. Xin et al. [8] established correlations between carbohydrate molecular structure and nutritional value/ruminal degradation characteristics in different corn stover fractions using FTIR and developed regression equations. Ji et al. [12] reported similar correlations for alfalfa hay using attenuated total reflectance FTIR (ATR-FTIR). This study investigates the effects of varying heat treatment intensities on okara’ s nutritional value and NDF effective degradability (ED), while employing FTIR to explore how heat treatment affects carbohydrate molecular structure and its relationship with nutritional value and NDF ruminal degradation characteristics. The objective is to validate FTIR spectral information as a simple, rapid, non-destructive sensitive indicator for assessing heat damage in okara.

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## Materials and Methods

### 1.1 Sample Collection and Processing

Fresh wet okara was collected from a feed processing plant in Harbin in May 2017, with measured moisture content of 80%. The fresh okara was subjected to different heat treatments at temperatures of 100, 115, and 130 °C for durations of 2, 4, and 6 hours. The heat treatment method involved spreading fresh okara on filter paper (approximately 1 cm thick) in an oven, with stirring every 0.5 hours [13,14]. After cooling, portions of each sample were ground to pass through a 1 mm sieve for routine chemical analysis, a 2 mm sieve for rumen degradation trials, and a 0.25 mm sieve for spectral analysis.

### 1.2 Experimental Animals and Diet

Three healthy Holstein dairy cows (approximately 600 kg body weight) fitted with permanent rumen fistulas at the Acheng Experimental Base of Northeast Agricultural University were used for rumen degradation trials. Animals were fed twice daily (08:00 and 16:00) with free access to water. Nutritional requirements were formulated according to NRC (2001) [15] standards. The basal diet composition and nutrient levels are presented in Table 1 .

**Table 1** Composition and nutrient levels of the basal diet (air-dry basis)

| Items | Content |
|---|---|
| **Ingredients** | |
| Chinese wildrye | |
| Corn silage | |
| Corn | |
| Wheat bran | |
| Molasses beet | |
| Soybean meal | |
| Dried distillers grain | |
| Cottonseed meal | |
| Corn fiber feed | |
| Corn germ meal | |
| Premix1) | |
| **Total** | |
| **Nutrient levels** | |
| NEL/(MJ/kg)2) | |
| Crude protein CP | |
| Neutral detergent fiber NDF | |
| Acid detergent fiber ADF | |

1) Per kilogram of premix contained: VA 8,000,000 IU, VD 700,000 IU, VE 10,000 IU, Fe 1,600 mg, Cu 1,500 mg, Zn 10,000 mg, Mn 3,500 mg, Se 80 mg, I 120 mg, Co 50 mg.
2) NEL was a calculated value, while other nutrient levels were measured values.

### 1.3 Analytical Methods

**1.3.1 Routine Chemical Composition Analysis** Dry matter (DM), crude ash, and ether extract (EE) were determined according to *Feed Analysis and Feed Quality Detection Technology* [16]. Crude protein (CP) was measured using a FOSS 8400 automatic Kjeldahl nitrogen analyzer. Starch content was determined following Zhang et al. [17]. Neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) were analyzed using the method of Van Soest et al. [18]. Total carbohydrate, non-fibrous carbohydrate (NFC), hemicellulose, and cellulose contents were calculated using NRC (2001) [15] formulas. CNCPS carbohydrate fractions including rapidly degradable carbohydrate (CA), moderately degradable carbohydrate (CB1), slowly degradable carbohydrate (CB2), and unavailable cell wall component (CC) were determined using CNCPS [19] equations.

**1.3.2 Rumen Nylon Bag Technique** Following Peng et al. [20], approximately 7 g of sample was weighed into pre-weighed nylon bags (10 cm × 20 cm, 40  m pore size) and sealed with rubber bands. The bags were randomly placed in rumen mesh bags (45 cm × 45 cm) secured to the fistula with a 90 cm rope. Incubation times were 0, 2, 4, 8, 12, 16, 24, 36, and 48 hours, with three bags per time point for each fistulated cow. After removal, bags were rinsed with cold tap water until the rinse water was clear, then dried at 65 °C for 48 hours to constant weight. Residues were weighed, ground to pass through a 1 mm sieve, and stored in sealed bags for analysis.

**1.3.3 Spectral Data Collection and Analysis** Under infrared lamp illumination, 2 mg of dried sample was mixed with 200 mg potassium bromide (KBr) in an agate mortar. The mixture was ground thoroughly and pressed into translucent pellets using an infrared pellet press (Model: 769YP-15A). Fourier transform infrared spectroscopy (Model: Shimadzu FTIR-8400S) was used to scan samples from 4,000 to 400 cm $^{1}$ at 4 cm $^{1}$ resolution with 128 scans per sample. Five replicates were performed for each sample to collect carbohydrate molecular structure spectra.

### 1.4 Calculations and Statistical Analysis

**1.4.1 Rumen Degradation Parameters** The disappearance rate of a component (%) was calculated as:
100 × (component mass - component mass in residue) / component mass.

Rumen degradation kinetics were calculated using the exponential model of Ørskov et al. [21]:

$$Y = a + b(1 - e^{-ct})$$

where:
- $Y$ = disappearance rate (%) after incubation time $t$
- $a$ = rapidly degradable fraction (%)
- $b$ = slowly degradable fraction (%)
- $c$ = degradation rate of slowly degradable fraction (%/h)
- $t$ = rumen incubation time (h)

Effective degradability (ED) was calculated as:

$$\mathrm{ED}(\%) = a + \frac{b \times c}{c + k}$$

where $k$ = rumen outflow rate (0.046/h) [22].

**1.4.2 FTIR Spectral Analysis** OMNIC 8.2 software was used to process and analyze infrared spectra. Baseline positions were identified to define structural carbohydrate region (baseline: ca. 1,475–1,187 cm $^{1}$), cellulosic compounds region (baseline: ca. 1,287–1,187 cm $^{1}$), and total carbohydrate region (baseline: ca. 1,187-898 cm $^{1}$). Fourier self-deconvolution (FSD) identified three peak positions within the total carbohydrate region at 1,155, 1,109, and 1,046 cm $^{1}$. Peak heights and areas were recorded for statistical analysis.

**1.4.3 Statistical Analysis** Data were initially processed using Excel 2010. SAS 9.4 PROC MIXED was used to analyze carbohydrate composition, rumen degradation parameters, and spectral values with the model:

$$Y_{ijk} = \mu + F_i + B_j + F_i \times B_j + e_{ijk}$$

where:
- $Y_{ijk}$ = dependent variable
- $\mu$ = overall mean
- $F_i$ = temperature effect (i = 3; 100, 115, 130 °C)
- $B_j$ = time effect (j = 3; 2, 4, 6 h)
- $F_i \times B_j$ = temperature × time interaction (fixed effect)
- $e_{ijk}$ = residual error

Tukey-Kramer test was used for mean separation (P  0.05). PROC CORR was used for correlation analysis ($P < 0.01$ = highly significant; P  0.05 = significant; $0.05 < P < 0.10$ = tendency). Regression models between molecular structure and nutritional/ruminal degradation characteristics were developed using PROC REG with stepwise selection.

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# Results

## 2.1 Effects of Heat Treatment on Carbohydrate Composition and CNCPS Fractions

Heat treatment temperature and duration significantly affected carbohydrate composition and CNCPS carbohydrate fractions ($P < 0.05$), with significant interaction effects between the two factors (P  0.05). As temperature and heating time increased, NDF, ADF, ADL, hemicellulose, and cellulose contents increased, peaking in the 130 °C, 6 h group and reaching minimum values in the 100 °C, 2 h group. Conversely, NFC content decreased with increasing temperature and heating time, while total carbohydrate content showed minimal change. In CNCPS fractions, CA content decreased with higher temperatures and longer durations (lowest in 130 °C, 6 h; highest in 100 °C, 2 h), whereas CB2 and CC contents increased under the same conditions .

### 2.2 Effects of Heat Treatment on NDF Rumen Degradation Kinetics

Temperature and duration significantly affected the rapidly degradable fraction of NDF (NDFa) ($P < 0.05$) with significant interaction effects ($P < 0.05$). However, no interaction effects were observed for slowly degradable NDF (NDFb), degradation rate (NDFc), or effective degradability (NDFED) ($P > 0.05$). Temperature alone significantly affected NDFb and NDFED ($P < 0.05$). NDFa and NDFED decreased with increasing temperature (highest in 100 °C, 2 h), while NDFb increased with temperature (lowest in 100 °C, 2 h) .

### 2.3 Effects of Heat Treatment on Carbohydrate Molecular Structure

Temperature significantly affected peak areas of structural carbohydrates (A_STCHO), cellulosic compounds (A_CELC), total carbohydrates (A_CHO), and corresponding peak heights [H_1155, H_1109, H_1046] ($P < 0.05$), with significant temperature × time interactions ($P < 0.05$). No interactions were detected for peak area ratios (A_STCHO/A_CHO, A_CELC/A_CHO, A_CELC/A_STCHO) or peak height ratios (H_1155/H_1109, H_1155/H_1046, H_1109/H_1046) ($P > 0.05$), though temperature significantly affected A_CELC/A_STCHO and all peak height ratios ($P < 0.05$). A_STCHO, A_CELC, A_CHO, and the three peak heights decreased with increasing temperature, with the 100 °C, 2 h group showing maximum values .

### 2.4 Correlations Between Carbohydrate Molecular Structure, Nutritional Value, and NDF Rumen Degradation

Significant correlations existed between spectral parameters and nutritional value . Total carbohydrate was highly positively correlated with A_STCHO/A_CHO ($r = 0.62$, $P < 0.01$). NFC was highly positively correlated with A_STCHO/A_CHO, H_1155/H_1046, and H_1109/H_1046 ($r = 0.56$–$0.67$, $P < 0.01$). NDF, ADF, and ADL were highly negatively correlated with A_STCHO, A_CELC, A_CHO, A_STCHO/A_CHO, H_1155/H_1046, and H_1109/H_1046 ($r = -0.50$ to $-0.63$, $P < 0.01$). Hemicellulose and cellulose showed similar negative correlations with these spectral parameters ($r = -0.50$ to $-0.64$, $P < 0.01$).

In CNCPS fractions, CA and CB1 were highly positively correlated with A_STCHO/A_CHO, H_1155/H_1046, and H_1109/H_1046 ($r = 0.54$–$0.65$, $P < 0.01$), while CB2 and CC were highly negatively correlated with these parameters ($r = -0.51$ to $-0.69$, $P < 0.01$).

For NDF degradation parameters, NDFa was highly positively correlated with A_STCHO/A_CHO, H_1155/H_1046, and H_1109/H_1046 ($r = 0.59$-$0.62$, $P < 0.01$). NDFb was highly negatively correlated with A_STCHO/A_CHO ($r = -0.65$, $P < 0.01$). NDFd was significantly positively correlated with H_1155/H_1109, H_1155/H_1046, and H_1109/H_1046 ($r = 0.43$-$0.50$, $P < 0.05$). NDFc and NDFED were highly positively correlated with A_STCHO/A_CHO ($r = 0.55$-$0.64$, $P < 0.01$).

### 2.5 Regression Relationships Between Molecular Structure and Nutritional/Ruminal Parameters

A_STCHO/A_CHO and peak height ratios (H_1155/H_1046, H_1109/H_1046) were the best predictors for estimating nutritional components and NDF degradation parameters . These spectral parameters effectively predicted starch ($R^2 = 0.55$, $P < 0.01$), NFC ($R^2 = 0.63$, $P < 0.01$), NDF ($R^2 = 0.60$, $P < 0.01$), ADF ($R^2 = 0.63$, $P < 0.01$), ADL ($R^2 = 0.49$, $P < 0.01$), hemicellulose ($R^2 = 0.54$, $P < 0.01$), and cellulose ($R^2 = 0.64$, $P < 0.01$). CNCPS fractions CA, CB1, CB2, and CC were also well predicted ($R^2 = 0.50$-$0.63$, $P < 0.01$). For NDF degradation parameters, the models predicted NDFa ($R^2 = 0.60$, $P < 0.01$), NDFb ($R^2 = 0.42$, $P < 0.01$), NDFc ($R^2 = 0.30$, $P < 0.01$), NDFd ($R^2 = 0.23$, $P < 0.01$), and NDFED ($R^2 = 0.41$, $P < 0.01$).

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## Discussion

### 3.1 Effects on Carbohydrate Composition and CNCPS Fractions

Samadi et al. [23] reported that dry heating (120 °C, 1 h) reduced NDF content in rapeseed without significantly affecting ADF or ADL, which contradicts our findings. This discrepancy may be attributed to differences in heating intensity or method affecting fiber content changes. Fales [24] demonstrated that increased temperature raised NDF, ADF, hemicellulose, and cellulose contents in tall fescue pasture, consistent with our results. The reduction in NFC content with heating aligns with findings from Peng et al. [20] and Samadi et al. [23]. From a CNCPS perspective, plant-based carbohydrates are primary energy sources for ruminants. Higher CA, CB1, and CB2 contents with lower CC indicate faster ruminal degradation and higher nutritional value [2,3]. Our study shows that heat treatment increased CC fraction and reduced okara' s nutritional value.

### 3.2 Effects on NDF Rumen Degradation Kinetics

Changes in nutrient composition altered NDF degradation parameters. The CNCPS system reflects feed digestion and utilization in the rumen. Increased heating intensity reduced soluble carbohydrate content, decreasing NDFa, while elevated CB2 content increased NDFb. Heat treatment also increased indigestible fiber fractions that remain undegraded in the rumen [19]. Previous reports [25] indicate that reduced NDFa, increased NDFb, and higher indigestible fractions lower NDFED. Heat treatment may form neutral detergent insoluble crude protein (NDICP) complexes, reducing crude protein degradability [26]. In our study, reduced NDFED may result from: (1) conversion of soluble to insoluble fiber, and (2) NDICP formation between NDF and crude protein. Further research is needed to elucidate the mechanisms.

### 3.3 Effects on Carbohydrate Molecular Structure

FTIR spectroscopy examines structural carbohydrates, cellulosic compounds, and total carbohydrates, with internal molecular features affecting NDFED [27]. Heat treatment alters feed molecular structure, and our study demonstrated that heating reduced A_STCHO, A_CELC, A_CHO, and peak heights. While limited research exists on heat treatment effects on carbohydrate molecular structure, Yu et al. [28] reported that heating during bioethanol production altered grain carbohydrate molecular structure, affecting DDGS utilization value. Thus, heat-induced changes in okara' s carbohydrate molecular structure likely impact its feeding value.

### 3.4 Correlations Between Molecular Structure and Nutritional/Ruminal Parameters

Numerous studies have documented correlations between carbohydrate molecular structure and nutritional value [8,12,28,29]. Xin et al. [29] reported significant negative correlations between A_CELC and NDF, ADF, ADL ($r = -0.73$ to $-0.97$), and between A_CELC and NDFc ($r = -0.84$), but positive correlation with NDFED ($r = 0.84$). In our study, A_CELC was significantly negatively correlated with NDF and ADF ($r = -0.50$ to $-0.52$), showed no correlation with NDFc, and was positively correlated with NDFED ($r = 0.40$). These differences may stem from variations in feed types and processing methods, which alter carbohydrate molecular structure and its relationship with nutritional value [30].

### 3.5 Regression Models for Prediction

Xin et al. [8] demonstrated that A_CELC/A_CHO and peak height ratios could rapidly predict nutritional composition and NDFED in corn stover fractions, with the best fit for crude fiber: -195.227 × R_CHO_1_3 - 129.344 × R_CHO_2_3 + 183.411 ($R^2 = 0.66$, $P < 0.01$). Our study shows that A_STCHO/A_CHO and peak height ratios effectively predict nutritional components and NDFED in heat-treated okara. The best-fitting model was for cellulose: 63.81 - 79.38 × A_STCHO/A_CHO - 13.45 × H_1109/H_1046 ($R^2 = 0.64$, $P < 0.01$). Although $R^2$ values for NDF degradation parameters were relatively low, significant correlations remained with A_STCHO/A_CHO and peak height ratios.

## Conclusions

1. Heat treatment affected okara' s nutritional value and NDFED, with the 100 °C, 2 h treatment maintaining the highest nutritional value and NDFED.
2. Heat treatment altered carbohydrate molecular structure, with the 100 °C, 2 h group showing maximum A_STCHO, A_CELC, A_CHO, and peak heights.
3. Significant correlations exist between carbohydrate molecular structure and nutritional value/NDF ruminal degradation characteristics, enabling construction of regression equations. The A_STCHO/A_CHO ratio and total carbohydrate peak height ratios effectively estimate nutritional components and NDF degradation parameters in heat-treated okara.
4. FTIR spectral information can serve as a sensitive, simple, rapid, and non-destructive indicator for assessing heat damage in okara.

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*Note: Figure translations are in progress. See original paper for figures.*