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Dry and moist heating-induced changes in protein molecular structure, protein subfraction, and nutrient profiles in soybeans

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ABSTRACT

Heat processing has been used to improve protein utilization and availability of animal nutrition. However, to date, few studies exist on heat-induced protein molecular structure changes on a molecular basis. The aims of this study were to use molecular spectroscopy as a novel approach to determine heat-induced protein molecular structure changes affected by moist and dry heating and quantify protein molecular structures and nutritive value in the rumen and intestine in dairy cattle. In this study, soybean was used as a model for feed protein and was autoclaved at 120°C for 1 h (moist heating) and dry heated at 120°C for 1 h. The parameters assessed in this study included protein structure α-helix and β-sheet and their ratio, protein subfractions associated with protein degradation behaviors, intestinal protein availability, and energy values. The results show that heat treatments changed the protein molecular structure. Both dry and moist heating increased the amide I-to-amide II ratio. However, for the protein α-helix-to-β-sheet ratio, moist heating decreased but dry heating increased the ratio. Compared with dry heating, moist heating dramatically changed the chemical and nutrient profiles of soybean seed. It greatly decreased soluble crude protein, nonprotein nitrogen, and increased neutral detergent insoluble protein. Both dry and moist heating treatments did not alter digestible nutrients and energy values. Heating tended to decrease the nonprotein nitrogen fraction (soluble and rapidly degradable protein fraction) and true protein 1 fraction (fast-degradable protein fraction). Conversely, the true protein 3 fraction (slowly degradable fraction) significantly increased. The in situ rumen study showed that moist heating decreased protein rumen degradability and increased intestinal digestibility of rumen-undegradable protein. Compared with the raw soybeans, dry heating did not affect rumen degradability and intestinal digestibility. In conclusion, compared with dry heating, moist heating dramatically affected the nutrient profile, protein subfractions, rumen degradability, intestinal digestibility, and protein molecular structure (amide I-to-II ratio; α-helix-to-β-sheet ratio). The sensitivity of soybean seed to moist heating was much higher than that to dry heating in terms of the structure and nutrient profile changes.
Key words: protein molecular structure, heat processing method, nutrient availability, intestinal digestibility

INTRODUCTION

An understanding of the molecular structure of the whole protein is often vital to understanding its digestive behavior, nutritive quality, utilization, and availability in animals (Dyson and Wright, 1993; Carey, 1996; Yu et al., 2004, 2005; Doiron et al., 2009a). In ruminants, heat processing has been used to improve the utilization and availability of nutrients (Goelema et al., 1999; Jones et al., 2000; Yu et al., 2001, 2002) and inactivate anti-nutritional factors (van der Poel et al., 1990). Studies on protein structures and the change of their inherent structures because of heat treatment in relation to nutritive value and digestive behavior of protein are relatively rare. This is partially due to the fact that no analytical techniques are available that can be applied to detect inherent molecular changes of protein structures by heating processes (Doiron et al., 2009a,b). Conventional wet chemical analyses fail to detect the molecular structure difference and fail to detect structural chemical makeup, mainly because the conventional wet chemical analyses rely heavily on the use of harsh chemicals and derivatization, which can destroy the native structure (Budevska, 2002; Yu, 2004).

The objective of this study was to reveal protein molecular structures affected by 2 methods of heat processing (dry vs. moist heating) using Fourier transform/infrared-attenuated total reflectance (**FT/IR-ATR**) molecular spectroscopy and to study the sensitivity of feed tissue to heating and heat-induced nutrient profile and availability changes. The hypothesis of this study was that different heating methods result in different

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effects on molecular structure changes and are highly associated with nutrient availability.

MATERIALS AND METHODS

Soybean Processing Method (Moist Heating vs. Dry Heating)

Harvested soybeans *(Glycine Max)* from 2 different years (2008 and 2010) were used in this study as a modeled feed protein source. A 2-kg sample of soybeans from each year was heated by moist heating (autoclaving) and dry heating (Amsco Eagle SG-3031; Steris Corp., Mentor, OH) for 1 h at 120°C. The treatment was done in 1 batch and year as replication. Control samples were kept untreated. Heated samples were subsequently cooled at room temperature (20–22°C) and then ground (Braun KSM 2; Braun GmbH, Kronberg, Germany). Ground samples were fitted through a 2-mm screen.

Molecular Spectroscopy

The molecular spectral data of soybean seed was collected and corrected with the background spectrum using Jasco FT/IR-ATR 4200 (Jasco Inc., Easton, MD). The spectra were generated in transmission mode with mid-IR (ca. 4,000–800 cm^{-1}; Figure 1) and fingerprint region (ca. 1,800–800 cm^{-1}; Figure 2) with spectral resolution of 4 cm^{-1}. The FT/IR spectral data of each area was collected using Ominic 7.2 (Spectra-Tech Inc., Madison, WI) software. Chemical functional groups were identified according to published reports (Kemp, 1991; Himmelsbach et al., 1998; Wetzel, et al., 1998; Miller et al., 2000; Wetzel, 2001). The regions of specific interest in this present study included the protein amide I, II, and protein structure of α-helix and β-sheet in the IR regions of approximately 1,715 to1,480 cm^{-1} (Figures 3 and 4). The ratios of amide I and II and α-helix and β-sheet spectral intensities were calculated (Figure 4).

Animals and Diets

To study in situ protein rumen degradation, 2 lactating Holstein cows were used in this study with flexible rumen cannulas (10-cm i.d.; Bar Diamond Inc., Parma, ID). Cows were housed in pens of approximately 1.5 × 3 m in the research barn at the University of Saskatchewan. The diets of the cows consisted of 51% barley silage, 15% chopped alfalfa hay, and 33% concentrates (24% DAC-525; 9% DAC-449; formulated by David A. Christensen, University of Saskatchewan, Saskatoon, Canada) according to NRC requirements (NRC, 2001). Water was supplied ad libitum and the cows were fed twice daily at 0800 and 1600 h with half the ration fed at each time. All animal care and handling used in this study was in accordance with the guideline protocols approved by the Canadian Council on Animal Care (1993).

Rumen Incubation

Ruminal degradation characteristics were determined using an in situ method. Sample treatments were incubated for 16 h in 2 runs by using 2 lactating cows. The method of rumen incubation followed the department standard (Yu et al., 2003a). Approximately 7 g of sample was placed into coded nylon bags (10 × 12 cm) with a pore size of 41 μm (Screentec Corp., Mississauga, ON, Canada). All samples were placed in a polyester mesh bag (45 × 45 cm) with a 90-cm length of rope to anchor it to the cannula. A plastic bottle (250 mL) filled with gravel was also placed in the polyester mesh bag to maintain the samples in the liquid levels of the rumen. After 16 h of incubation, the bags were removed from the rumen and rinsed with cold water to remove excess ruminal contents. The bags were then washed in groups of 10 with 2 L of cool water without detergent 5 times. The last rinse cycle of water had to be clear. Samples in washed bags were dried at 55°C using a forced-air oven for 48 h and then stored at 4°C until analysis. The residues were pooled according to treatment, in situ run, cows, and year and stored for chemical analysis.

Chemical Analysis

Laboratory samples of the soybean and rumen residues were prepared by grinding to pass through a 1-mm screen using a Retsch ZM 100 mill (Retsch Inc., Newtown, PA). All samples were then analyzed for DM (AOAC, 1990; method 930.15), ash (AOAC, 1990; method 942.05), ether extract (**EE**; AOAC, 1990; method 920.39), and CP (AOAC, 1990; method 984.13; Kjeltec 2400; Foss North America, Eden Prairie, MN) content. The ADIN and NDIN values were determined according to the procedures of Lacitra et al., (1996). The NPN content was determined by the precipitation of true protein in the filtrate with TCA (final concentration 10%) and determined as the difference between total N and the N content of the residue after filtration (Roe et al., 1990). The starch was analyzed by using the Megazyme Total Starch Assay Kit (Megazyme International Ireland, Bray, Co. Wicklow, Ireland; AOAC, 1990; method 996.11). Acid detergent insoluble protein (**ADIP**) and neutral detergent insoluble protein (**NDIP**) were calculated as ADIP =