Vibrational Spectroscopic Investigation of Biomolecular Responses of Carbohydrate Structure to Moisture and Dry Heating in Soybean Seed *(Glycine max)*

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Abstract. The objective of this experiment was to investigate carbohydrate structures of seed tissue affected by different heat processing methods using infrared vibrational molecular spectroscopy. In this study, soybean seeds (two different harvested years; 2008 and 2010) were used as a model to investigate the alteration of inherent structure carbohydrate due to heat treatments. Structural characteristics of the bands in typical infrared molecular spectrum were studied in the region at ca. 1452-1188 cm^{-1} related to cellulosic and hemicellulosic compounds and the region at ca. 1193-881 cm^{-1}, related to total CHO. Multivariate molecular spectral analyses: Hierarchical cluster analysis (CLA) and principal components analysis (PCA) were applied to identify heat-induced changes of molecular spectral profiles. Treatments used in this study were raw soybean seeds as control, autoclaved soybean seeds at 120°C for 1 h (HT-1: wet heating) and dry roasted soybean seeds at 120°C for 1 h (HT-2: dry heating). The results showed that the heat treatments did not change spectral profiles of cellulosic, hemicellulosic and total CHO. Based on spectral analysis, CLA and PCA also did not produce any alterations among different treatments in original spectra at cellulosic, hemicellulosic and total CHO regions. In conclusion, the molecular spectral technique with multivariate spectral technique can be considered as a research tool to investigate the magnitude of heat-induced change in carbohydrate molecular structure and other biopolymers in feeds, seed and plant tissues. These techniques could be used in the food and feed industry in which, losing or changing carbohydrate molecular chemistry was able to be detected in rapidly without any destruction and chemical hazardous. Further studies are needed to understand the trend in structural changes by heating with increasing temperature and time of exposure.

Keywords: carbohydrate molecular, heat processing, molecular spectroscopy, soybean seed, feeds

Abstrak. Tujuan penelitian ini adalah untuk melihat struktur molekul karbohidrat pada jaringan biji-bijian sebagai akibat dari perlakuan jenis pemanasan berbeda dengan menggunakan *infrared vibrational molecular spectroscopy*. Dalam penelitian ini kacang kedelai yang dipanen pada tahun berbeda (2008 dan 2010) digunakan sebagai model untuk melihat perubahan struktur molekul dari karbohidrat. Struktur molekul karbohidrat ditentukan dengan menggunakan sinar infra merah yang berada pada area kira-kira 1452-1188 cm^{-1} untuk selulosa dan hemiselulosa dan kira-kira 1193-881 cm^{-1} untuk total karbohidrat. Penentuan analisa multivariate dilakukan dengan cara: *Hierarchical cluster analysis (CLA)* and *principal components analysis (PCA).* Perlakuan yang digunakan dalam penelitian ini adalah kacang kedelai sebagai kontrol, kacang kedelai yang di*autoclave* pada suhu 120 °C selama 1 jam (HT-1: *autoclave*) dan pemanasan kering pada suhu 120°C selama 1 jam (HT-2: pemanasan kering). Hasil penelitian menunjukkan bahwa pemanasan tidak menyebabkan perbedaan terhadap komposisi selulosa, hemiselulosa dan total karbohidrat dari biji kacang kedelai. Berdasarkan hasil CLA dan PCA juga menunjukkan bahwa pemanasan baik secara basah maupun kering tidak menunjukkan perbedaan terhadap struktur molekuler dari biji kacang kedelai. Dari hasil penelitian dapat disimpulkan bahwa, teknik molekuler spektra dapat digunakan sebagai satu alat untuk menentukan perubahan dalam struktur molekul pada karbohidrat dan biopolimer lain pada pakan ternak, biji-bijian dan jarangan pakan tanaman. Teknik ini dapat digunakan pada industri pakan ternak dan makanan, dimana perubahan struktur kimia dari suatu bahan dapat diketahui dengan cepat tanpa proses destruksi dan menggunakan bahan kimia yang berbahaya. Studi lanjutan diperlukan untuk mendapatkan bagaimana perubahan dalam biopolimer suatu bahan dengan meningkatkan suhu dan juga waktu pemanasan.

Kata kunci: molekul karbohidrat, pemanasan, molekular spektroskopi, kacang kedelai, pakan

Introduction

It is essential to provide slowly degradable nutrients with high potential for rumen escape in high-producing and rapidly growing ruminant animals. Heat treatment is known to be able to improve nutrient utilization and availability (Yu and Nuez-Ortin, 2010; Samadi and Yu, 2011). McKinnon et al. (1985) and Yu et al. (2002) reported that heat treatment can reduce the solubility of nutrients, decrease rumen fermentation and degradability, increase availability by passing the rumen for intestinal digestion and absorption. The positive effect of heat treatment on nutrient utilization and availability on alteration of inherent structure of animal feed has been reported (Yu et al., 2004; Doiron et al., 2010; Samadi and Yu, 2011).

Composition of structural carbohydrates such as cellulosic and hemicellulosic compounds or neutral and acid detergent fibers in ruminant nutrition and non-structural carbohydrate such as starch influences nutrient availability or digestive behavior of animals. Alteration of carbohydrate inherent structures due to feed processing has been reported by Yu et al. (2011) in which the bioethanol processing changes carbohydrate molecular structural profiles. Compared to the original grains, the bioethanol processing increases the molecular spectral intensities for the structural carbohydrates and decreases the intensities for the non-structural carbohydrates. According to various publications from Himmelsbach et al. (1998), Wetzel et al. (1998) and Yu et al., (2005), the CHO related to molecular spectral peak bands included the following parameters: (1) A_Cell (peak area region and baseline ~1485–1188 cm^{-1}), mainly associated with hemicellulosic and cellulosic compounds; (2) A_1240 (peak area centered at ~1240 cm^{-1} with region and baseline ~1292–1198 cm^{-1}), mainly associated with cellulosic compounds; (3) A_CHO (peak region and baseline ~1187–950 cm-1), mainly associated with total CHO; (4) A_928 (peak area centered at ~928 cm with region and baseline ~952–910 cm^{-1}), mainly associated with nonstructural CHO; (5) A_860 (peak area centered at ~860 cm^{-1} with region and baseline ~880–827 cm^{-1}), mainly associated with nonstructural CHO; (6) H_1415 (peak height centered at ~1415 cm-1 with baseline ~1485–1188 cm^{-1}), mainly associated with structural CHO; and (7) H_1370 (peak height at ~1370 cm^{-1} with a baseline ~1485–1188 cm cm^{-1}), mainly associated with structural CHO.

To date, few studies in literature reported the effect of carbohydrate structures and alteration of the inherent carbohydrate due to different heat treatment in relation to nutritive value and digestive behavior of animals. Meanwhile, alteration of inherent carbohydrate structures can influence availability and degradation of feed in digestive tract of ruminant animals. Conventional “wet” chemical analyses have been used for a long time to investigate chemical compositions of feed stuff. But this method is failed to detect structural chemical since it can destroy the native physiochemical and molecular structures during the chemical analysis (Budevska, 2002). FT/IR spectroscopy is known as potential techniques that could be used in rapid, nondestructive, and noninvasive to screen feed molecular chemistry in relation to quality of animal feed. By this technique, the quantity, composition, structure and distribution of chemical constituents and functional groups in a tissue can be recorded. Every biological component of functional group in food and feed tissues has specific molecular chemical feature, therefore IR absorption of biological tissue has a unique IR spectrum. The objective of this experiment was to investigate magnitude of heat-induced changes in carbohydrate structures of seed tissue affected different heat processing methods using infrared vibrational molecular spectroscopy. In this study, soybean seeds were used as a model to investigate the alteration of inherent

structure carbohydrate due to heat treatment. The hypothesis of this study was that the method of heating (wet vs. dry) influenced molecular structures of carbohydrate, nutrient profile and functionality.

Materials and Methods

Soybean processing methods (moisture heating and dry heating). Two different harvested years (2008 and 2010) of soybeans (*Glycine Max)* were used in this study as a modeled CHO molecular investigation. A 2-kg sample of soybeans from each year was heated by either moist heating (autoclaving, heat treatment 1 [HT-1]) or dry heating (Amsco Eagle SG-3031; Steris Corp., Mentor, OH) for 1 h at 120°C (heat treatment 2 [HT-2]). The treatment was done in 1 batch and year as replication. Control samples were kept raw. Heated samples were subsequently cooled at room temperature and then ground (Braun KSM 2; Braun GmbH, Kron- berg, Germany). Ground samples were fitted through a 2-mm screen. The chemical profiles of heat treatment in our study have been published in Journal of Diary Science (Samadi and Yu, 2011).

Carbohydrate molecular structure by 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) 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 region of specific interest in this present study included structure and non-structural carbohydrate is shown in Figure 2.

Multivariate molecular spectral analyses. Two multivariate molecular spectral analyses, hierarchical cluster analysis (CLA) and principal components analysis (PCA) were applied to compare the spectra of the different treatments to the control to determine if there were some primary structural differences. The detailed principles of this method were explained by Yu (2005). CLA results were presented as dendograms while PCA results were plotted based on the two highest factor scores and plotted as a function of those scores. The multivariate analysis included agglomerative hierarchical cluster analysis (CLA), using Wards's algorithm method without prior to parameterization, and principal component analysis (PCA), which was performed using Statistica software 8.0 (StatSoft Inc., Tulsa, OK). In each comparison the eigenvector for factor 1 was plotted against that for factor 2 which accounted for over 99% of the variability in the data.

Structural characteristics of the bands in typical infrared molecular spectrum in cellulosic and himicelullosic with the area under ca. 1452-1188 cm^{-1} of soybean seed were revealed using infrared molecular spectroscopy (Figure 1). Total CHO of soybean with area under ca. 1193-881 cm^{-1} were also examined. CLA and PCA spectral analysis of the carbohydrate both structural and non-structural CHO related to spectral regions were analyzed. Scatter plot of the 1st principal component vs. the 2nd principal component of PCA analysis of spectrum were performed. Multivariate molecular spectral analyses on a molecular basis between the raw, moisture heated and dry heated soybean seeds were shown.

Statistical analysis. Statistical analyses of the functional groups spectral intensities and ratios were performed using the MIXED procedure of SAS (version 9.1.3). The model used for the analysis was: $Y_{ij} = \mu + T_i + e_{ij}$, where, Y_{ij} was an observation of the dependent variable ij; μ was

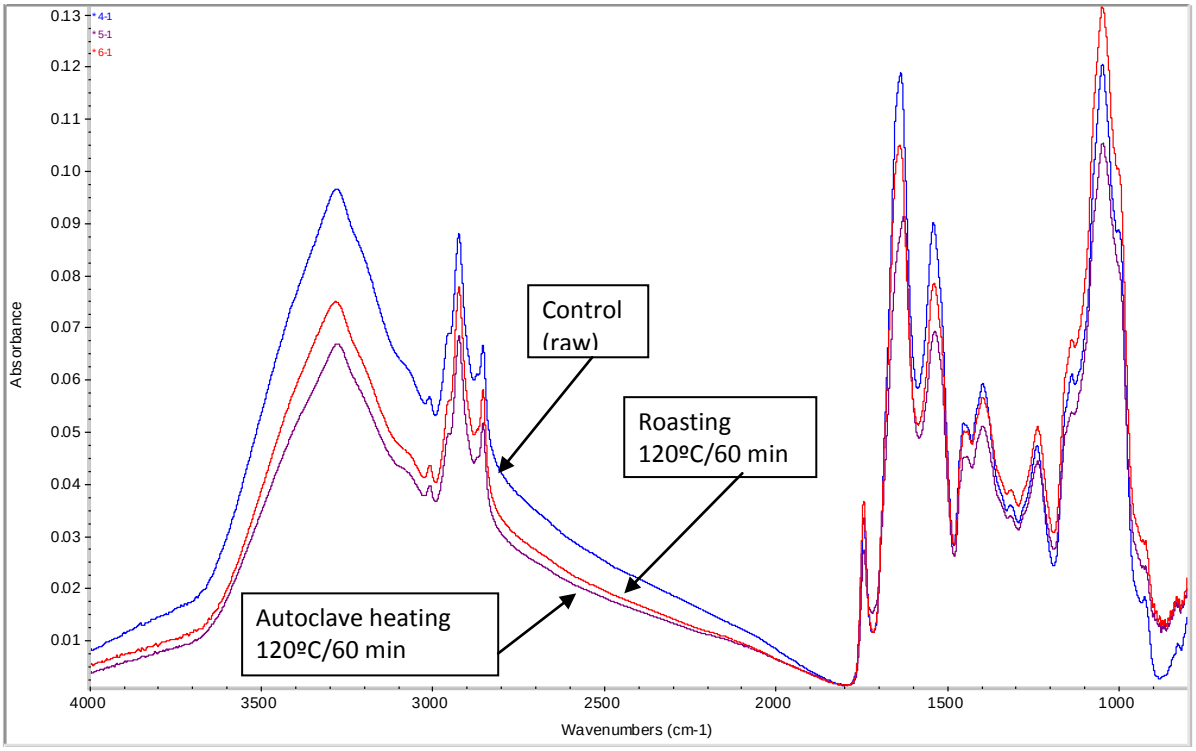


Figure 1. Typical FT/IR molecular spectrum in soybean (raw, finely ground) in the region ca. 4000-800 cm^{-1} showing chemical function groups**.**

the population mean for the variable; T_i was the fixed effect of the heating treatments (moisture and dry heating), and e_{ij} was the random error associated with the observation ij. Statistical significance was declared and detected at $P<0.05$ while trends were declared at $P\leq0.10$.

Results and Discussion

The effects of heat processing methods on chemical and nutrient profiles and nutrient availability of soybeans have been published in Journal of Diary Science (Samadi and Yu, 2011).

Multivariate spectral analyses of carbohydrate structure

Multivariate molecular spectral analysis of cellulosic and hemicellulosic

To date, no publish results have been concluded to discriminate internal structure of carbohydrate structure in soybean due to heat treatment. This study indicated that the molecular spectroscopy with multivariate molecular analyses can be utilized to study carbohydrate molecular structure or biopolymer conformation in soybean seeds. Wetzel et al. (1998) stated that the IR spectrum involving the fundamental vibration from ca. 4,000–800 cm^{-1} has been important tool to describe the molecular structure of biological compounds. In addition, FT/IR spectroscopy can be used to increase the basic understanding of inherent chemical structures of plant, food and feed tissues.

CLA spectral analysis of cellulosic, hemicellulosic regions (1452-1188 cm^{-1}) obtained from the raw (control) and the autoclaved canola (HT-1) samples. [Note: CLA: (1) Region of cellulosic and hemicellulosic compounds ca. 1452-1188 cm^{-1}; (2) Distance method: Euclidean; (3) Cluster method: Ward's algorithm] are shown in Figure 3; It includes and represents (I) 1 = raw vs. 2 = H-1, (II) 1 = raw vs. 3 = HT-2, and (III) 2 = HT-1 vs. 3= HT-2. Figure 3 (Ia, IIa and IIIa) indicated that all clusters contained combinations of spectra did not produce a distinct cluster. Research

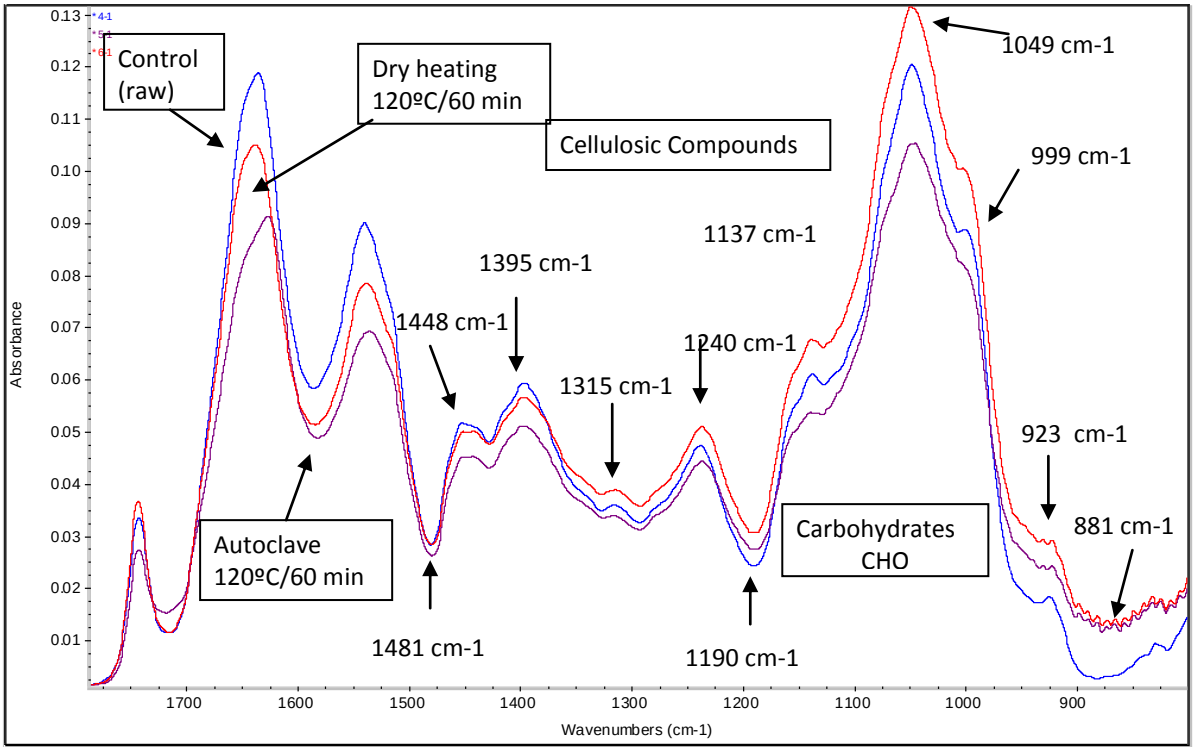
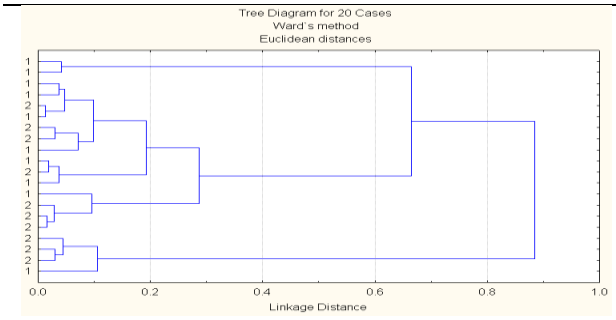


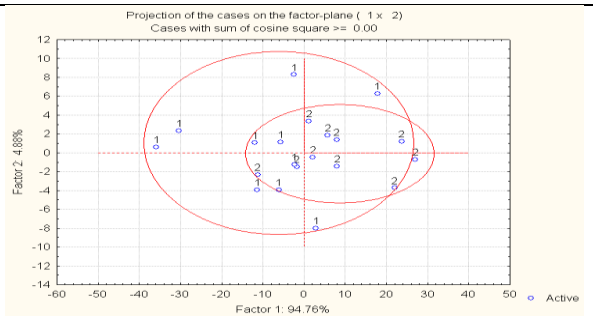
Figure 2. Spectra of soybean (raw, finely ground) determined by FT/IR spectroscopy, cellulose and hemicellulose presented under the area ca. 1452-1188 cm^{-1} and total CHO regions presented under the area ca. 1193-881.

conducted by Liu and Yu (2010) succeed to identify molecular structures of different genotypes of six spring barley varieties by using DRIFT spectroscopy. In this study, they conclude that this potential new technique can be used as bioanalytical technique for plant/seed/feed/food structural molecular structures in relation to functionality, biodegradability, and nutrient availability.

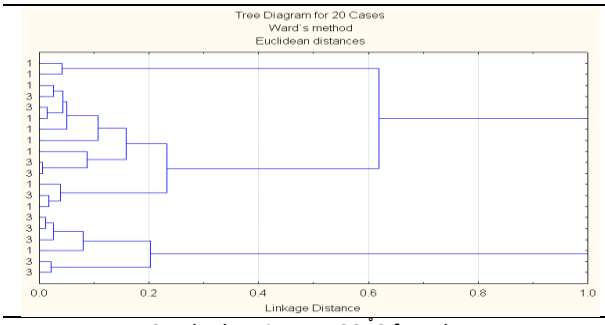
The scatter plot of the 1st principal component vs. the 2nd principal component (a) of PCA analysis of spectrum obtained from the raw and the autoclaved soybean samples is presented in Figure 3, Ib, IIb, and IIIb. In Figure 3, Ib, the 1st and 2nd principal component explained 94.76% and 4.88% of the total variance, respectively. In Figure 3, IIb, the 1st and 2nd principal components explained 95.08% and 4.61% of the total variance, respectively. Then the 1st and 2nd principal components explained 97.07% and 2.55% in Figure 3, IIIb. All treatments of clusters were overlapping each other, hence no difference as indicated by the molecular spectral analysis of cellulosic, hemicellulosic (Figure 3 Ib, IIb and IIb). Review research by Yu (2011) showed that A_Cell, A_CHO, H_1415, and H_1370 had no correlation with CHO chemical and nutrient profiles in the bioethanol co-products. The conclusion of this review was that the changes in CHO molecular structures during the processing for bioethanol production were highly associated with carbohydrate degradable subfractions in ruminants. In this research, DRIFT spectroscopy used to identify molecular structures of carbohydrate from bioethanol production. The successful application of multivariate CLA analysis has been reported by Doiron et al.(2009 and 2010) for flaxseed samples with heat treatment and Liu and Yu (2005) for different genotypes of barley (Yu, 2005; Doiran et al., 2010; Liu and Yu, 2010).



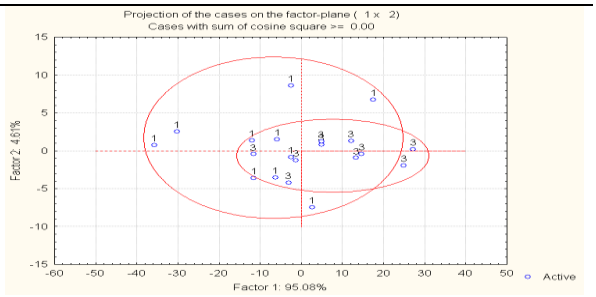
I: 1= raw vs. 2 = wet heating at 120 °C for 1 h
a) CLA spectral analysis of cellulosic and hemicellulosic stretching regions (1452-1188 cm^{-1}) obtained from the raw and the wet heating (autoclaved) soybean samples [Note: CLA: (1) Region of cellulosic and hemicellulosic compounds ca. 1452-1188 cm^{-1}; (2) Distance method: Euclidean; (3) Cluster method: Ward's algorithm]



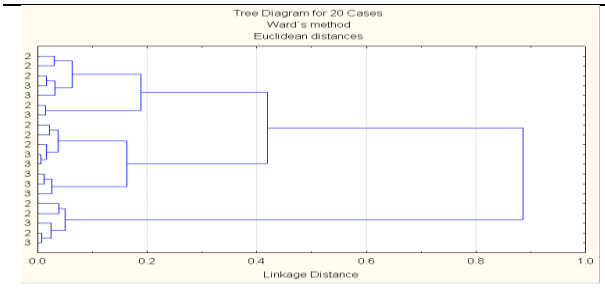
I: 1 = raw vs. 2 = wet heating at 120 °C for 1 h.
b) Scatter plot of the 1st principal component vs. the 2nd principal component (a) of PCA analysis of spectrum obtained from the raw and the autoclaved soybean samples: The 1st and 2nd principal component explains 94.76 % and 4.88 % of the total variance, respectively.



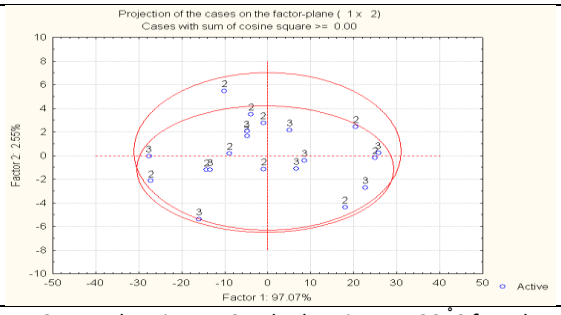
II: 1= raw vs. 3 = dry heating at 120 °C for 1 h
a) CLA spectral analysis of cellulosic and hemicellulosic stretching regions (1452-1188 cm^{-1}) obtained from the raw and the wet heating (autoclaved) soybean samples [Note: CLA: (1) Region of cellulosic and hemicellulosic compounds ca. 1452-1188 cm^{-1}; (2) Distance method: Euclidean; (3) Cluster method: Ward's algorithm]



II: 1 = raw vs. 3 = dry heating at 120 °C for 1 h.
b) Scatter plot of the 1st principal component vs. the 2nd principal component (a) of PCA analysis of spectrum obtained from the raw and the autoclaved soybean samples: The 1st and 2nd principal component explains 95.08 % and 4.61 % of the total variance, respectively.

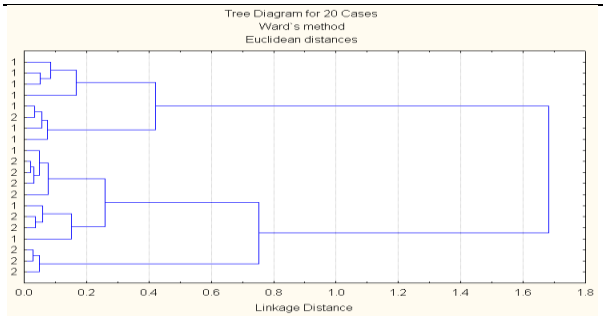


III: 2= wet heating vs. 3 = dry heating at 120 °C for 1 h
a) CLA spectral analysis of cellulosic and hemicellulosic stretching regions (1452-1188 cm^{-1}) obtained from the raw and the wet heating (autoclaved) soybean samples [Note: CLA: (1) Region of cellulosic and hemicellulosic compounds ca. 1452-1188 cm^{-1}; (2) Distance method: Euclidean; (3) Cluster method: Ward's algorithm]

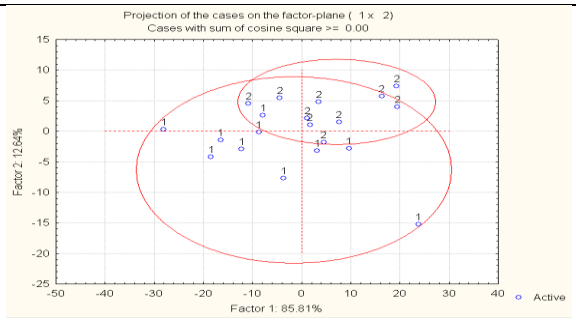


III: 2 = wet heating vs. 3 = dry heating at 120 °C for 1 h.
b) Scatter plot of the 1st principal component vs. the 2nd principal component (a) of PCA analysis of spectrum obtained from the raw and the autoclaved soybean samples: The 1st and 2nd principal component explains 97.07 % and 2.55% of the total variance, respectively.

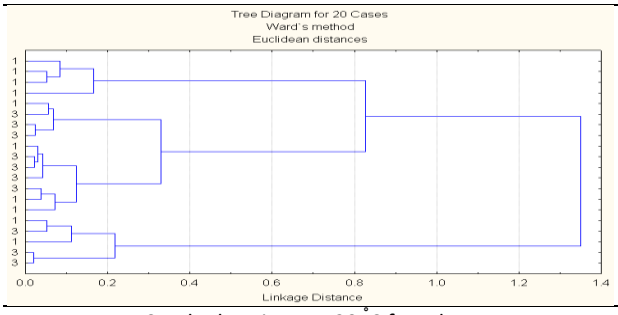
Figure 3. Multivariate molecular spectral analyses of hemicellulosic stretching regions (Region: 1452-1188 cm^{-1}) on a molecular basis between the raw, wet heated and dry heated soybean seeds: **(I)** 1 = raw (control) vs. 2 = wet heating 120 °C/60 min (HT-1); **(II)**: 1= raw vs. 3 = dry heating 120 °C/60 min (HT-2); (**III**): 2 = wet heating 120 °C/60 min vs. 3 = dry heating 120 °C/60 min).



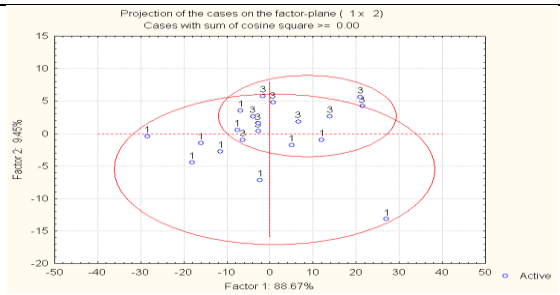
I: 1= raw vs. 2 = wet heating at 120 °C for 1 h
a) CLA spectral analysis of total CHO regions (1193-881 cm^{-1}) obtained from the raw and the wet heating (autoclaved) soybean samples [Note: CLA: (1) Region of total CHO compound ca. 1193-881 cm^{-1}; (2) Distance method: Euclidean; (3) Cluster method: Ward's algorithm]



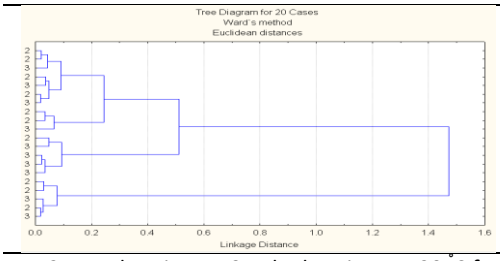
I: 1 = raw vs. 2 = wet heating at 120 °C for 1 h.
b) Scatter plot of the 1st principal component vs. the 2nd principal component (a) of PCA analysis of spectrum obtained from the raw and the autoclaved soybean samples: The 1st and 2nd principal component explains 85.81 % and 12.64 % of the total variance, respectively.



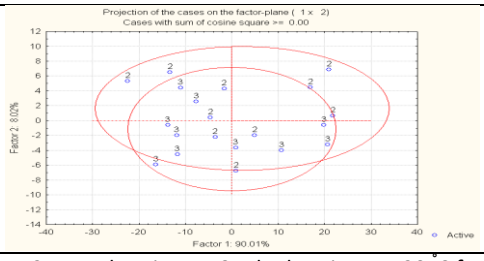
II: 1= raw vs. 3 = dry heating at 120 °C for 1 h
a) CLA spectral analysis of total CHO regions (1193-881 cm^{-1}) obtained from the raw and the wet heating (autoclaved) soybean samples [Note: CLA: (1) Region of total CHO compound ca. 1193-881 cm^{-1}; (2) Distance method: Euclidean; (3) Cluster method: Ward's algorithm]



II: 1 = raw vs. 3= dry heating at 120 °C for 1 h.
b) Scatter plot of the 1st principal component vs. the 2nd principal component (a) of PCA analysis of spectrum obtained from the raw and the autoclaved soybean samples: The 1st and 2nd principal component explains 88.67 % and 9.45 % of the total variance, respectively.



III: 2= wet heating vs. 3 = dry heating at 120 °C for 1 h
a) CLA spectral analysis of total CHO regions (1193-881 cm^{-1}) obtained from the raw and the wet heating (autoclaved) soybean samples [Note: CLA: (1) Region of total CHO compound ca. 1193-881 cm^{-1}; (2) Distance method: Euclidean; (3) Cluster method: Ward's algorithm]



III: 2 = wet heating vs. 3= dry heating at 120 °C for 1 h.
b) Scatter plot of the 1st principal component vs. the 2nd principal component (a) of PCA analysis of spectrum obtained from the raw and the autoclaved soybean samples: The 1st and 2nd principal component explains 90.01 % and 8.02 % of the total variance, respectively.

Figure 4. Multivariate molecular spectral analyses of total CHO (Region: 1193-881 cm^{-1}) on a molecular basis between the raw, wet heated and dry heated soybean seeds: **(I)** 1 = raw (control) vs. 2 = wet heating 120 °C/60 min (HT-1); **(II)**: 1= raw vs. 3 = dry heating 120 °C/60 min (HT-2); (**III**): 2 = wet heating 120 °C/60 min vs. 3 = dry heating 120 °C/60 min).

Multivariate molecular spectral analysis of total CHO

Multivariate molecular spectral analyses of total CHO (ca. 1193-881 cm^{-1}) on soybean seeds; (I) 1 = raw vs. 2 = HT-1, (II) 1 = raw vs. 3 = HT-2, and (III) 2 = HT-1 vs. 3 = HT-2 are presented at Figure 4. Based on the Figure 4 (Ia, IIa, and IIIa) heat treatments in general shown that heat treatment all clusters contained combinations of spectra did not produce a distinct cluster (CLA analyses). In Figure 4, Ib, the 1st and 2nd principal components explained 85.81% and 12.64% of the total variance, respectively. The next, in IIb, the 1st and 2nd principal components explained 88.67% and 9.45% of the total variance, respectively. Then the 1st and 2nd principal components explained 90.01% and 8.02% in IIIb. The figures explained that there was no significant difference among all treatment clusters from total CHO as indicated that overlapping each other of spectral cluster (Figure 4). The research conducted by Yu et al. (2011) shown that there were no differences (P> 0.05) in the peak area intensities of A_CHO (total CHO) at 1187–950 cm^{-1} with average molecular infrared intensity KM unit of 508.1. In this study Yu et al. (2011) used DRIFT spectroscopy to detect molecular features of spectra mainly associated with carbohydrate in the co-products (wheat DDGS, corn DDGS, blend DDGS) from bioethanol processing in comparison with original feedstock (wheat (*Triticum*), corn (*Zea mays*)). From this research Yu et al., (2011) concluded that molecular spectral analytical technique-DRIFT could be used to reveal differences in carbohydrate molecular structures of grains affected by bioethanol processing.

Conclusions

As indicated from the experiment, it could be concluded that FTIR molecular spectroscopy can be used as a tool to investigate alteration of molecular structure due to heat treatments. In our study, the heat treatments did not change molecular of cellulosic, hemicellulosic and total CHO. Based on spectral analysis, CLA and PCA also did not produce any alterations among different treatments in original spectra at cellulosic, hemicellulosic and total CHO regions. But, this result is important to the food and feed industry in the sense that heat processing could be used without losing or changing carbohydrate molecular structure. This technique could be used in rapid screening of feed/food intrinsic structures and feed molecular chemistry in relation to the quality and nutritive value of feeds. Generally, the results of the study showed that our heat treatment studies did not effectively alter the carbohydrate structure in soybean seed. However, further studies are needed to understand the trend in structural changes by heating with increasing temperature and time of exposure.

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