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# Spectroscopic impact on protein and carbohydrate inherent molecular structures of barley, oat and corn combined with wheat DDGS

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**Abstract.** The objectives of this experiment were to use non-invasive and non-destructive infrared molecular spectroscopy as a novel approach to explore and identify protein and carbohydrate molecular structure spectral features of DDGS (dried distillers grain solubles from wheat, *Triticum aestivum*) and its combinations with barley (*Hordeum vulgare*), corn (*Zea mays*) and oat (*Avena sativa*). The spectral parameters assessed in this study included amides, protein molecular structures of $\alpha$-helix and $\beta$-sheet, lignin, cellulosic compounds (CeC) and nonstructural carbohydrates (NSC). The results of the study show that the combinations of DDGS with cereal grains significantly changed ($P < 0.05$) protein and carbohydrate structures and protein secondary structure. The use of FT/IR molecular spectroscopy in terms of identification of inherent structural changes was remarkable. The combination of DGGS with different grains alters the constituents and intrinsic molecular structures. This change would improve the nutritional quality and digestive characteristics of the feed. Further studies are recommended to evaluate the effect on digestibility, availability and its structural correlation.

Keywords: Protein and carbohydrate structures, spectroscopic impact, oat, barley, corn, wheat DDGS

## 1. Introduction

A new co-products from bioethanol processing is wheat (*Triticum aestivum*) dried distillers grains with solubles (wheat DDGS) produced in North America [17]. DDGS are a key by-product from ethanol industry, and extensively used in animal feed industry. The nutritive value of dried distillers grain solubles (DDGS) from wheat after ethanol production, makes them an excellent source of protein and energy for dairy and beef cattle [17–19]. Subsequent to the process of bioethanol production, ethanol fermentation utilizes most of the starch from grain kernels hence concentrates the remaining components, mainly protein, fiber, fat and minerals into DDGS [37]. Wheat DDGS provides many compounds which could be categorized as digestible and available nutrients to animals [17–19]. DDGS from wheat contains higher amounts of dry matter (DM, 938 g/kg), crude protein (CP, 393 g/kg) and starch (63 g/kg) [17]. However, high content of lignin (ADL, 43 g/kg) reported by Nuez-Ortin and Yu [17] may lead its nutritional value low. Although it contains high protein, fat and fibre and low starch [12], DDGS were reported to be slow in degradability and digestibility in rumen because ready available fermentable fractions were already

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depleted [18,23]. Grains (barley, oat and corn), on other hand, contain more starch (readily fermentable fractions) hence have higher rumen degradability [3,10]. It is widely accepted that the combination of feeds and ingredients to form a mew mixer of feed improves the chemical and nutrient profile [11,22,27, 32]. Thereby, combination of DDGS and other grains is a suggestive approach which would be a sustainable application as well. Many expect that the chemical profile and nutritional value may be improved by combining with other grains such as barley (*Hordeum vulgare*), corn (*Zea mays*) or oat (*Avena sativa*). Therefore, we expect that a combination of DDGS and grains would manipulate a change in physiochemical profile, and deliver optimal digestive characteristics and rumen degradation kinetics. However, we do not know which ratio of the combination would be best. Therefore, our work investigates this question, using different techniques, FT/IR, *in vitro* and chemical analysis of those combinations.

The nutritive value, degradation characteristics, utilization and availability of protein are not only determined by the nutrient or chemical composition but also affected by intrinsic chemical structures such as protein secondary structures of $\alpha$-helix and $\beta$-sheet ratio and biological component matrix such as protein to starch matrix, protein to carbohydrate matrix [24,31,33,35]. Therefore, an understanding of the internal structure of protein and starch matrix in those feed combinations is very critical for their digestive behavior, nutritive quality, utilization and availability. The influence of such structures, $\alpha$-helix and $\beta$-sheet ratio on the protein quality, utilization, availability and digestive behavior were important in feed evaluation [31,33,35]. It was reported that the protein structure $\alpha$-helix to $\beta$-sheet ratio affect total intestinally absorbed protein supply (protein DVE value) and degraded protein balance (protein OEB value) [6,7,37]. The changes in the protein molecular structure $\alpha$-helix to $\beta$-sheet ratio and the amide I to amide II ratio during bioethanol processing were associated with (estimated protein) intestinal digestibility and degraded protein balance, however, were not associated with total intestinally absorbed protein supply from the DDGS to dairy cattle [37].

During the last decades, molecular and chemical–structural spectroscopy developed as rapid, direct, non-destructive and non-invasive bioanalytical technique [4,35]. Thereby, this technique paves the way to visualize and understand the quantity, composition, structure and distribution of chemical constituents and functional groups in a tissue (feed and ingredients). The objectives of this experiment were to reveal the relationship of feed intrinsic structures pertaining to protein molecular structures, carbohydrate and starch matrices of wheat DDGS combinations with barley, corn and oat using the infrared molecular spectroscopy. We hypothesis that the known change of DDGS from its original grain (wheat) influences the combining grain types; barley, corn or oat at different inclusions of 25–75% to alter their intrinsic ultra-structural components.

## 2. Materials and methods

### *2.1. Feed combinations of cereal grain with DDGS*

Wheat DDGS were obtained from two bioethanol processing plants (Terra Grain Fuels, Regina, and North West BioEnergy, Unity) in Saskatchewan, Canada. Two cultivars of barley (CDC Lophy-1 and CDC Cowboy), corn (from Saskatchewan CO-OP and University Feedlot) and oat (Sea-biscuit and CDC SOI-FI) were used for making combinations with wheat DDGS in this study. Four combinations from each grain were made by DDGS inclusion ratios of 0, 25, 50, 75 and 100. Grain type and their DDGS combination ratios (five treatments) were DDGS alone (100% DDGS + 0% grain), 75% DDGS + 25% grain, 50% DDGS + 50% grain, 25% DDGS + 75% grain, and grain alone (0% DDGS + 100%

grain). All samples were in replicate and undergone molecular spectral analyses. The detailed chemical compositions were reported before.

### 2.2. Molecular spectral data collection

All feed combinations were pulverized to 0.12 mm powder using grinder (Retsch Ultra Centrifugal Rotor Mill ZM 200, Retsch GmbH, Haan, Germany). The molecular spectral data of those samples were collected and corrected with the background spectrum using JASCO FT/IR 4200 (JASCO Corporation, Tokyo, Japan). Spectra were generated in a transmission mode with mid-IR (ca. 4000–700 $cm^{-1}$) and fingerprint region (ca. 1800–800 $cm^{-1}$) with of spectral resolution of 4 $cm^{-1}$. Figures 1–3 show typical spectra of combinations of cereal grains with wheat DDGS at five different ratios (100:0; 75:25; 50:50; 25:75; 0:100). Figures 1–3 show spectral features of combinations of barley, corn and oat with DDGS, respectively.

### 2.3. Protein and carbohydrate molecular structure by molecular spectroscopy

The spectral data of each area were analyzed using OMINIC 7.2 (Spectra-Tech, Madison, WI, USA) software. Chemical functional groups were identified according to previous literature [9,28,31,35]. The regions of specific interest in this present study included the protein amide I, II lignin, secondary protein structures of $\alpha$-helix and $\beta$-sheet, cellulosic compounds (CeC) and nonstructural carbohydrate (NSC with component peaks 1–3). Basically those structures were determined in the IR regions of ca. 1800–700 $cm^{-1}$.

Primary and secondary protein structures spectrum ranged in IR regions of ca. 1800–1400 $cm^{-1}$; amide I (ca. 1750–1550 $cm^{-1}$); amide II (ca. 1600–1450 $cm^{-1}$); $\alpha$-helix at approximately ca. 1656 $cm^{-1}$ with a baseline of ca. 1710–1550 $cm^{-1}$ and $\beta$-sheet, at approximately ca. 16230 $cm^{-1}$; CeC (ca. band 1292–1189 $cm^{-1}$); NSC including peaks 1–3 (ca. band 1190–878 $cm^{-1}$).

### 2.4. Statistical analysis

Statistical analysis of molecular spectral analysis were performed using the Mixed procedure of SAS software (SAS v9.1, SAS Institute Inc., Cary, NC, USA). The model used for analysis was:

$$Y_{ij} = \mu + T_j + e_{ij},$$

where $Y_{ij}$ is an observation on the dependent variable $ij$; $\mu$ is the population mean for the variable, $T_j$ is the effect of inclusion ratio/treatment, as a fixed effect; Grain cultivars/varieties were replications, and $e_{ij}$ is the random error associated with the observation $ij$. Treatment means were compared using Tukey–Kramer test [25]. Statistical significant difference was declared at $P < 0.05$ and trends at $P \leqslant 0.10$.

### 2.5. Multivariate molecular spectral analysis

The multivariate methods of data analysis were used to classify spectral groups by applying the whole spectral information. The multivariate analysis included agglomerative hierarchical cluster analysis (CLA), using Wards' algorithm method without prior to parameterization, and principal component analysis (PCA), which was performed using Statistica software 8.0 (StatSoft Inc., Tulsa, OK, USA). For the purpose and objectives of this study statistical comparisons were performed with a grain type (barley, corn or oat) contrast to DDGS, and results were presented under the same theme.

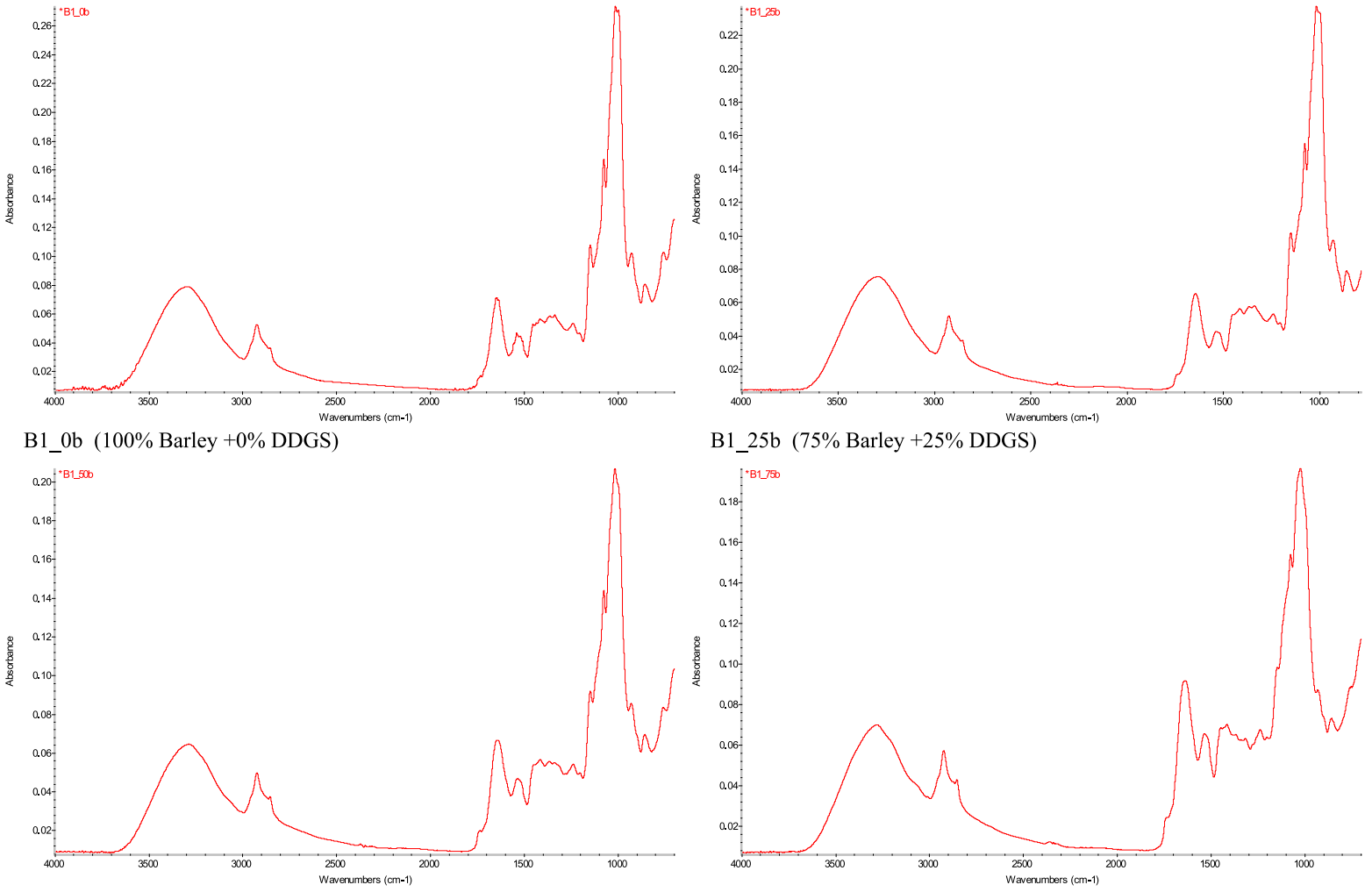



Fig. 1. Typical spectra of five combinations of hulled barely with wheat DDGS. (Colors are visible in the online version of the article; http://dx.doi.org/10.3233/SPE-2011-0546.)

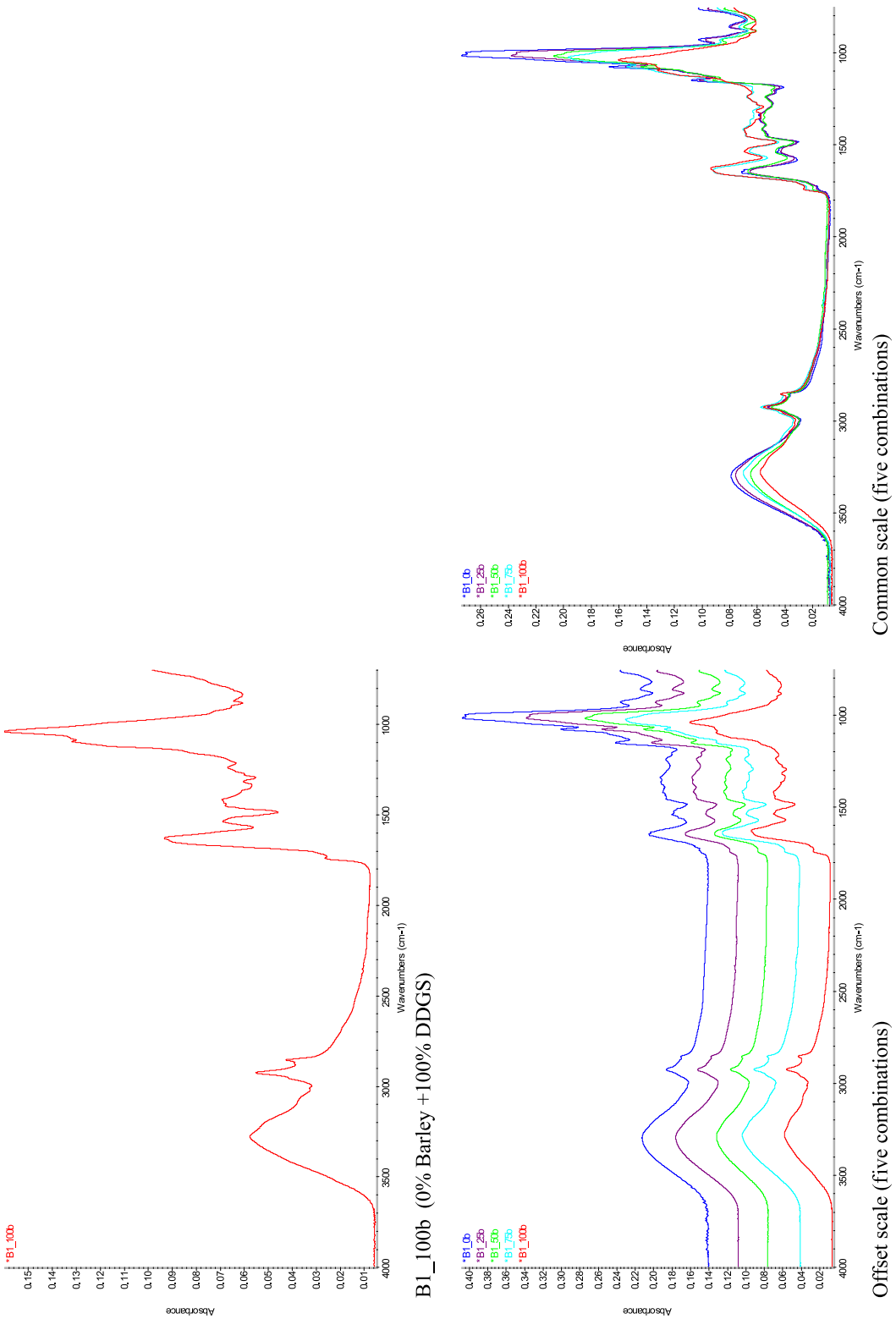



Fig. 1. (Continued.)

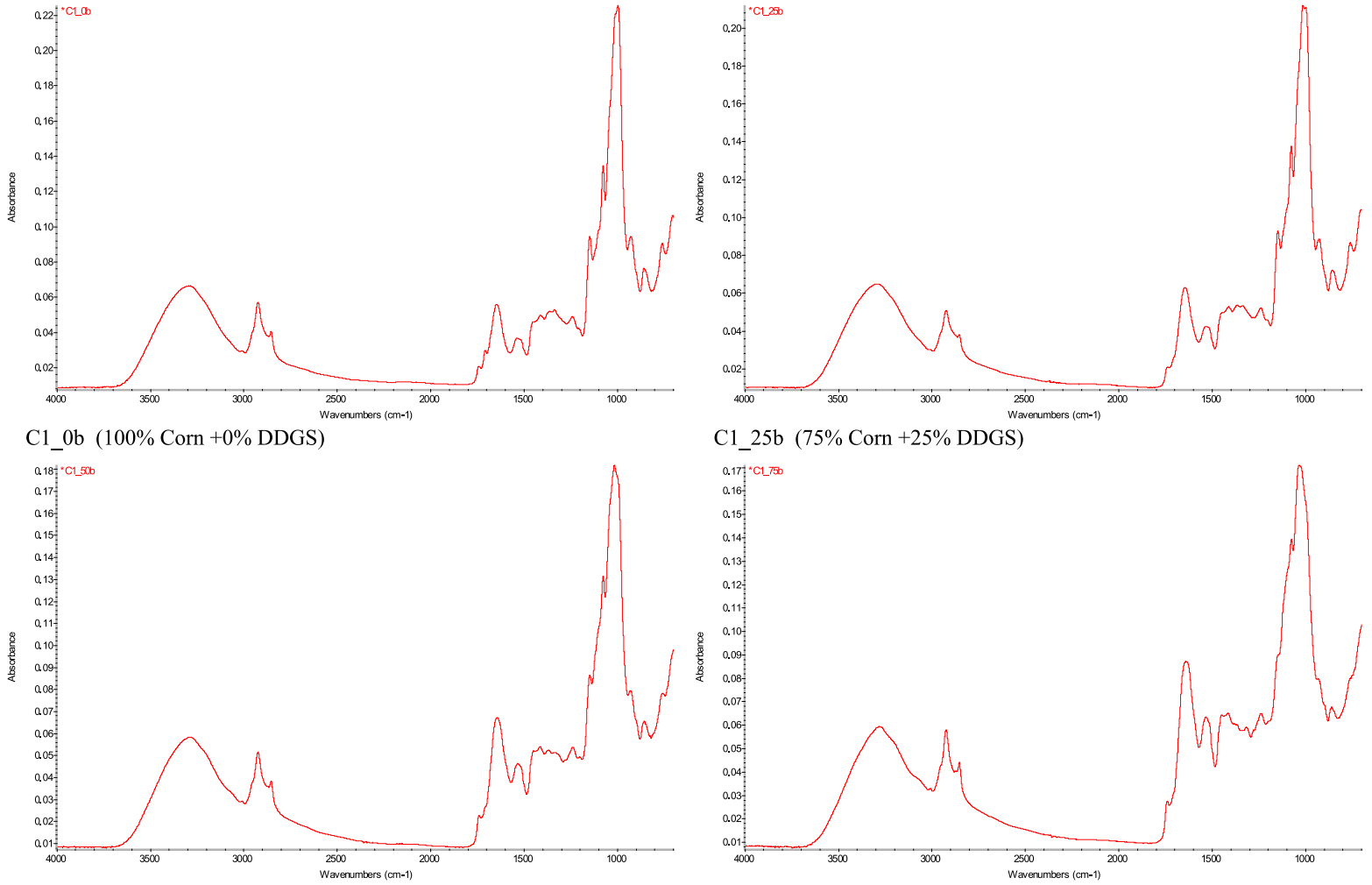



Fig. 2. Typical spectra of five combinations of corn with wheat DDGS. (Colors are visible in the online version of the article; http://dx.doi.org/10.3233/SPE-2011-0546.)

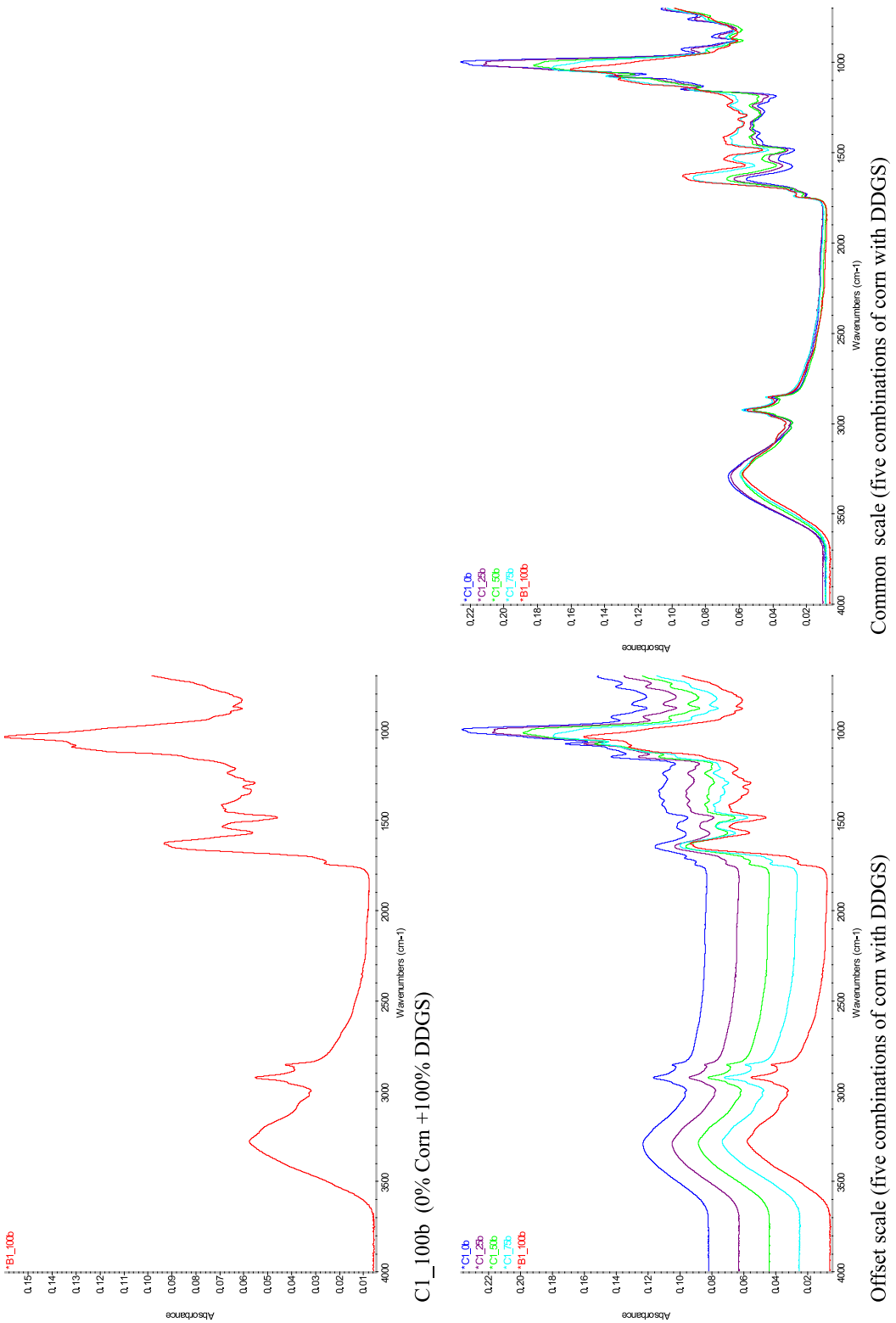



Fig. 2. (Continued.)

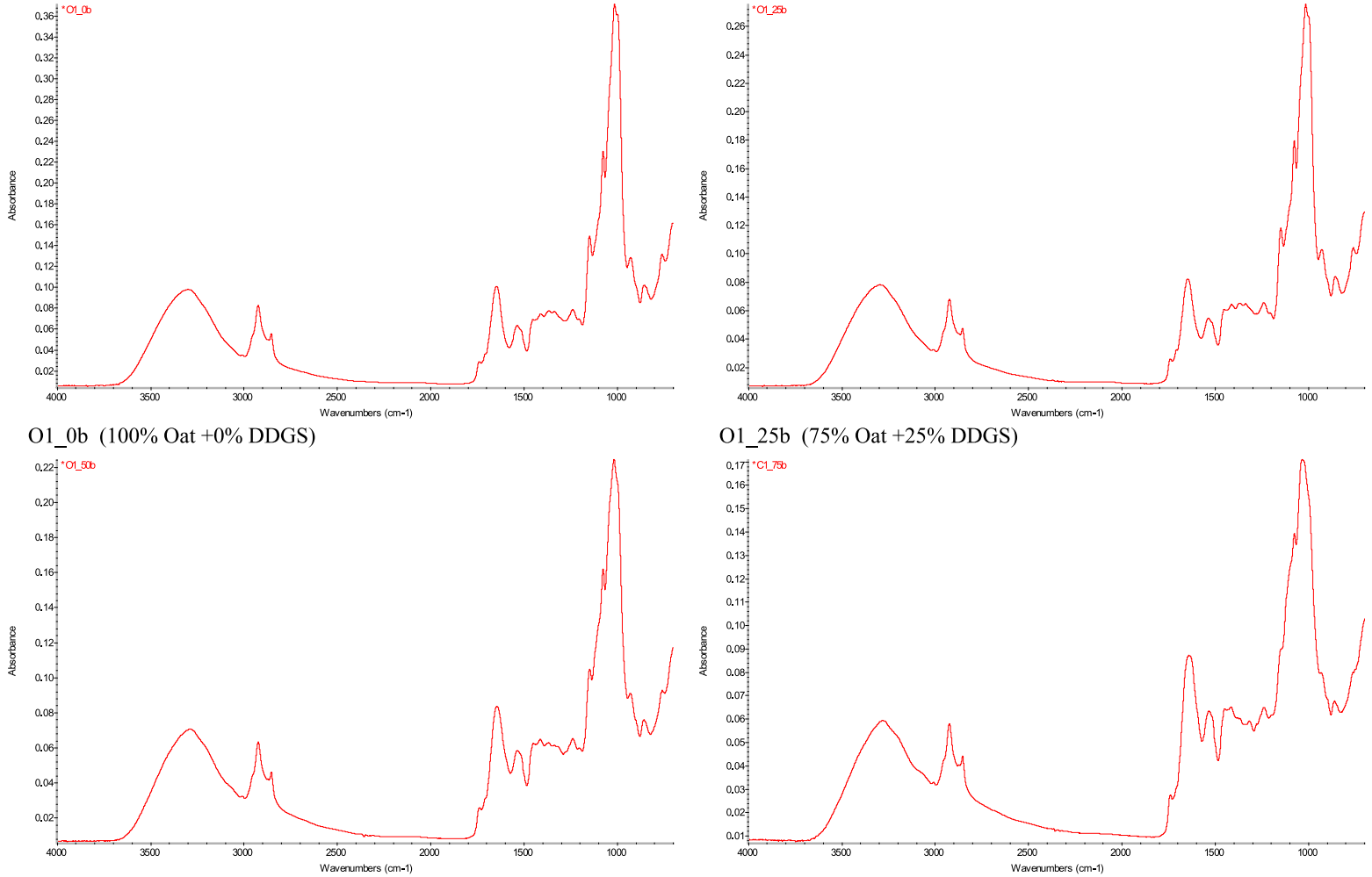



Fig. 3. Typical spectra of five combinations of oat with wheat DDGS. (Colors are visible in the online version of the article; http://dx.doi.org/10.3233/SPE-2011-0546.)

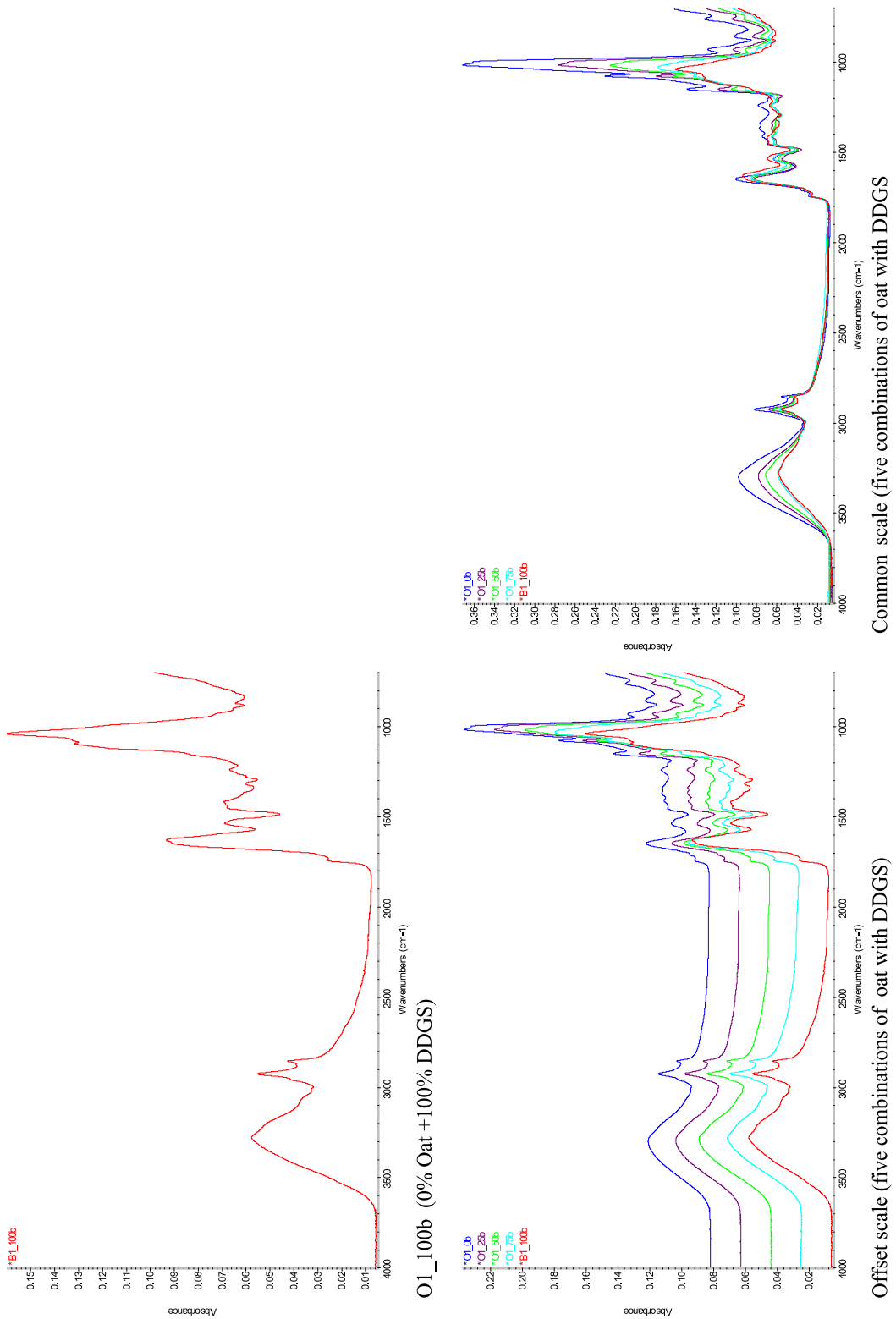



Fig. 3. (Continued.)

## 3. Results and discussion

### *3.1. Characteristics of protein secondary structure and CHO components*

FTIR spectrum and different spectral measurements carried out in our study to reveal inherent molecular characteristics of protein and carbohydrate. Protein and carbohydrate structures of wheat DDGS determined at band ca. 1800–700 $cm^{-1}$. Other grain and combination feeds were also measured at a band similar to the above (Figs 1–3). Primary spectrum (for proteins and carbohydrates) and secondary structure spectrum (for proteins band ca. 1800–1400 $cm^{-1}$) were revealed. The area of amide I stretched out on a baseline band ca. 1750–1550 $cm^{-1}$. The area of amide II lied on a baseline band ca. 1600–1450 $cm^{-1}$. A secondary structural peak for $\alpha$-helix was at approximately 1656 $cm^{-1}$ with a baseline band of ca. 1707–1571 $cm^{-1}$ and $\beta$-sheet was at approximately 1629 $cm^{-1}$. The area of cellulosic compound (CeC) was found at a baseline band ca. 1273–1213 $cm^{-1}$. The area of nonstructural carbohydrates (NSC) including peaks 1–3 were measured at a baseline band ca. 1213–881 $cm^{-1}$.

FTIR spectral intensity characteristics of protein and CHO structure of barley grain and DDGS mixtures are shown in Table 1. DDGS had unique characteristics of both amide I and II which were distinctly different ($P < 0.05$) from that of barley, DDGS and their mixtures (Table 1). Both area and height were higher in DDGS ($P < 0.05$). In addition, the height of $\alpha$-helix and $\beta$-sheet were increased ($P < 0.05$) by DDGS inclusion. CHO structural component (lignin and CeC) characteristics (area and height) increased ($P < 0.05$) by DDGS inclusion (Table 1). However, NSC (peaks 1–3) characteristics (area and height) decreased ($P < 0.05$) by DDGS inclusion. There were no changes in amide I area to II ratio, but found a decrease ($P < 0.05$) in amide I height to II ratio, as a result of DDGS inclusion. Other ratios that indicated an increase ($P < 0.05$) are amide I to NSC, amide I to NSC peak 1, NSC peak 2 to 3, lignin to NSC and CeC to NSC. Ratios of peak 1 to 2, and peak 1 to 3 decreased ($P < 0.05$) by DDGS inclusion. Lignin to CeC ratio was not affected by DDGS inclusion. In general, inherent molecular structural characteristics of barley and their mixtures were affected by the increasing levels of DDGS inclusion.

Table 2 contains protein and CHO structural characteristics of corn feeds and DDGS. Protein (amides I, II, $\alpha$-helix and $\beta$-sheet) intrinsic characteristics (area and height) were changed ($P < 0.05$) similar to barley, and the pattern was a parallel increase to DDGS inclusion. All NSC characteristics were decreased ($P < 0.05$) by DDGS inclusion. However, these differences are not consistent with DDGS concentrations; therefore, it reveals an unpredictability of those characteristic changes. In corn, amide area I to II, lignin to NSC and CeC ratios were not influenced by DDGS inclusion (Table 2). Amide I to NSC and peak 1, peak 2 to 3 ratios were increased ($P < 0.05$) by DDGS inclusion. However, these changes are not continuous and consistent according to DDGD concentration as found in barley.

Protein and CHO structural characteristics of oat, DDGS and their mixtures are shown in Table 3. Protein structure results indicated statistically significant differences ($P < 0.05$) across DDGS inclusions. However, more exuberant effects were on Oat + 75 (75% DDGS), Oat + 50 (50% DDGS) and Oat + 25 (25% DDGS) which had rather distinctive difference from both pure oats and DDGS depending on structural characteristic particularly amide height and $\alpha$-helix height (Table 3). There were no significant effect on structural CHO of oats by DDGS inclusions, other than that of a decrease ($P < 0.05$) in lignin area of oat mixtures (Table 3). NSC were affected similar to barley and corn, therefore, all characteristics of NSC in oat were decreased by DDGS inclusion (Table 3). In contrast, some inherent molecular structural ratios indicated a consistent decrease ($P < 0.05$) by DDGS inclusion. They were $\alpha$-helix to $\beta$-sheet, NSC peak 1 to 2 and 1 to 3 ratios. However, five other ratios were increased ($P < 0.05$); amide area I to II, amide I to NSC, amide I to NSC peak 3, peak 2 to 3, and lignin to NSC. Our structural data for pure wheat DDGS and grains were in agreement with previous work [13,17–19].

Table 1
Structural characteristics of protein and carbohydrates by using FT/IR spectroscopy: comparison of hulled barley versus DDGS[1] combinations

| Item | Treatment (barley with DDGS combinations)[2] | | | | | SEM | Contrast | |
|---|---|---|---|---|---|---|---|---|
| | DDGS | Barley + 75 | Barley + 50 | Barley + 25 | Barley | | Barley vs. DDGS | $P$-value |
| Molecular structural components | | | | | | | | |
| Protein: amide | | | | | | | | |
| Amide I area | 5.00[a] | 3.41[b] | 2.97[c] | 2.88[c] | 2.80[c] | 0.11 | <0.001 | 0.638 |
| Amide I height | 0.05[a] | 0.05[a] | 0.04[b] | 0.04[b] | 0.04[b] | 0.002 | 0.027 | <0.001 |
| Amide II area | 0.95[a] | 0.76[b] | 0.64[b] | 0.62[b] | 0.63[b] | 0.04 | 0.012 | <0.001 |
| Amide II height | 0.020[a] | 0.016[b] | 0.013[b] | 0.013[b] | 0.013[b] | 0.001 | 0.012 | <0.001 |
| Amide I and II area | 5.05[a] | 4.17[b] | 3.61[b,c] | 3.50[c] | 3.44[c] | 0.14 | <0.001 | <0.001 |
| Protein: secondary molecular structures, $\alpha$-helix and $\beta$-sheet | | | | | | | | |
| $\alpha$-helix (height) | 0.051[a] | 0.044[b] | 0.040[b,c] | 0.038[c] | 0.037[c] | 0.002 | <0.001 | <0.001 |
| $\beta$-sheet (height) | 0.052[a] | 0.043[b] | 0.036[b] | 0.038[b] | 0.036[b] | 0.002 | 0.002 | <0.001 |
| Carbohydrates: structural and nonstructural components | | | | | | | | |
| Lignin area | 0.078[a] | 0.070[a,b] | 0.051[c] | 0.051[c] | 0.054[b,c] | 0.005 | 0.123 | <0.001 |
| Lignin height | 0.006[a] | 0.004[b] | 0.003[c] | 0.003[c] | 0.003[c] | 0.0002 | <0.001 | <0.001 |
| CeC[3] area | 0.41[a] | 0.23[b] | 0.19[b] | 0.24[b] | 0.17[b] | 0.04 | 0.027 | <0.001 |
| CeC height | 0.009[a] | 0.007[a,b] | 0.006[b] | 0.007[a,b] | 0.006[b] | 0.0006 | 0.088 | 0.003 |
| NSC[4] area | 14.28[a] | 17.05[b] | 20.78[c] | 23.40[d] | 25.86[e] | 0.58 | <0.001 | <0.001 |
| NSC height | 0.115[a] | 0.128[a] | 0.161[b] | 0.188[c] | 0.216[d] | 0.005 | <0.001 | <0.001 |
| NSC peak 1 area | 0.097[a] | 0.254[b] | 0.461[c] | 0.623[d] | 0.773[e] | 0.018 | <0.001 | <0.001 |
| NSC peak 1 height | 0.0050[a] | 0.0120[b] | 0.0215[c] | 0.0279[d] | 0.0341[e] | 0.0008 | <0.001 | <0.001 |
| NSC peak 2 area | 0.3337[a,b] | 0.3311[a,b] | 0.2764[b] | 0.3526[a,b] | 0.4333[a] | 0.0348 | 0.007 | 0.051 |
| NSC peak 2 height | 0.0104[a] | 0.0118[a] | 0.0190[b] | 0.0249[c] | 0.0301[d] | 0.0008 | <0.001 | <0.001 |
| NSC peak 3 area | 3.4491[a] | 4.7674[b] | 6.6557[c] | 8.0384[d] | 9.5553[e] | 0.2032 | <0.001 | <0.001 |
| NSC peak 3 height | 0.0552[a] | 0.0700[b] | 0.1010[c] | 0.1244[d] | 0.1502[e] | 0.0032 | <0.001 | <0.001 |

Table 1
(Continued)

| Item | Treatment (barley with DDGS combinations)[2] | | | | | SEM | Contrast | |
|---|---|---|---|---|---|---|---|---|
| | DDGS | Barley + 75 | Barley + 50 | Barley + 25 | Barley | | Barley vs. DDGS | $P$-value |
| Ratios of inherent molecular structures | | | | | | | | |
| Area amide I : II | 4.34 | 4.50 | 4.71 | 4.67 | 4.55 | 0.14 | 0.995 | 0.185 |
| Height amide I : II | 2.765[a] | 3.003[a] | 3.243[a] | 3.336[a,b] | 3.337[b] | 0.087 | 0.014 | <0.001 |
| Amide I : NSC | 0.29[a] | 0.20[b] | 0.14[c] | 0.12[c,d] | 0.11[d] | 0.005 | <0.001 | <0.001 |
| Amide I : NSC peak 3 | 1.20[a] | 0.72[b] | 0.45[c] | 0.36[c,d] | 0.29[d] | 0.02 | <0.001 | <0.001 |
| $\alpha$-helix : $\beta$-sheet | 0.99 | 1.04 | 1.10 | 1.00 | 1.04 | 0.03 | 0.817 | 0.509 |
| NSC peak 1:2 | 0.44[a] | 1.20[b] | 1.66[b,c] | 1.77[c] | 1.79[c] | 0.13 | 0.001 | <0.001 |
| NSC peak 1:3 | 0.027[a] | 0.053[b] | 0.069[c] | 0.078[c,d] | 0.081[d] | 0.003 | <0.001 | <0.001 |
| NSC peak 2:3 | 0.101[a] | 0.071[a,b] | 0.042[b] | 0.044[b] | 0.045[b] | 0.01 | 0.077 | <0.001 |
| Lignin : NSC | 0.006[a] | 0.004[a] | 0.002[b] | 0.002[b] | 0.002[b] | 0.001 | <0.001 | <0.001 |
| CeC : NSC | 0.028[a] | 0.014[b] | 0.009[b] | 0.010[b] | 0.007[b] | 0.002 | 0.0015 | <0.001 |
| Lignin : CeC | 0.29 | 0.33 | 0.28 | 0.22 | 0.32 | 0.04 | 0.357 | 0.621 |

[a–e] Means within a row with different superscript differ ($P < 0.05$). Mean separation was done by using Tukey–Kramer test. SEM = pooled standard error of means.

[1]DDGS = dried distillers grains with solubles from wheat.

[2]Treatments: (1) DDGS = barley 0% and DDGS 100%; (2) Barley + 75 = barley 25% and DDGS 75%; (3) Barley + 50 = barley 50% and DDGS 50%; (4) Barley + 25 = barley 75% and DDGS 25%; (5) Barley = barley 100% and DGGS 0%.

[3]CeC = cellulosic compounds.

[4]NSC = nonstructural carbohydrates.

Table 2
Structural characteristics of protein and carbohydrates by using FT/IR spectroscopy: comparison of corn versus DDGS[1] combinations

| Item | Treatment (corn with DDGS combinations)[2] | | | | | SEM | Contrast | |
|---|---|---|---|---|---|---|---|---|
| | DDGS | Corn + 75 | Corn + 50 | Corn + 25 | Corn | | Corn vs. DDGS | $P$-value |
| Inherent molecular structural components | | | | | | | | |
| Protein: amide | | | | | | | | |
| Amide I area | 4.10[a] | 3.15[b] | 2.68[c] | 2.57[c] | 1.85[d] | 0.11 | <0.001 | <0.001 |
| Amide I height | 0.054[a] | 0.043[b] | 0.036[c] | 0.036[c] | 0.028[d] | 0.002 | <0.001 | <0.001 |
| Amide II area | 0.95[a] | 0.71[b] | 0.54[c] | 0.53[c] | 0.42[d] | 0.03 | <0.001 | <0.001 |
| Amide II height | 0.020[a] | 0.015[b] | 0.011[c] | 0.011[c] | 0.009[d] | 0.001 | <0.001 | <0.001 |
| Amide I and II area | 5.05[a] | 3.89[b] | 3.23[c] | 3.10[c] | 2.26[d] | 0.13 | <0.001 | <0.001 |
| Protein: secondary molecular structures, $\alpha$-helix and $\beta$-sheet | | | | | | | | |
| $\alpha$-helix (height) | 0.051[a] | 0.041[b] | 0.033[c] | 0.035[c] | 0.026[d] | 0.002 | <0.001 | <0.001 |
| $\beta$-sheet (height) | 0.052[a] | 0.040[b] | 0.032[c] | 0.030[c] | 0.023[d] | 0.001 | <0.001 | <0.001 |
| Carbohydrates: structural and nonstructural components | | | | | | | | |
| Lignin area | 0.078[a,b] | 0.121[c] | 0.097[b,c] | 0.094[b] | 0.057[a] | 0.006 | <0.001 | 0.001 |
| Lignin height | 0.0052[a] | 0.0055[a] | 0.0043[b] | 0.0043[b] | 0.0029[c] | 0.0002 | <0.001 | <0.001 |
| CeC[3] area | 0.41 | 0.32 | 0.27 | 0.35 | 0.27 | 0.05 | 0.219 | 0.097 |
| CeC height | 0.009 | 0.008 | 0.007 | 0.008 | 0.007 | 0.001 | 0.221 | 0.095 |
| NSC[4] area | 14.28[a] | 14.72[a] | 16.00[a] | 21.04[b] | 20.97[b] | 0.63 | <0.001 | <0.001 |
| NSC height | 0.115[a] | 0.109[a] | 0.121[a] | 0.165[b] | 0.170[b] | 0.005 | <0.001 | <0.001 |
| NSC peak 1 area | 0.097[a] | 0.195[b] | 0.342[c] | 0.614[d] | 0.771[e] | 0.018 | <0.001 | <0.001 |
| NSC peak 1 height | 0.005[a] | 0.009[b] | 0.015[c] | 0.025[d] | 0.031[e] | 0.001 | <0.001 | <0.001 |
| NSC peak 2 area | 0.33 | 0.37 | 0.32 | 0.34 | 0.38 | 0.04 | 0.268 | 0.556 |
| NSC peak 2 height | 0.010[a] | 0.009[a] | 0.014[b] | 0.022[c] | 0.025[c] | 0.001 | <0.001 | <0.001 |
| NSC peak 3 area | 3.45[a] | 3.95[a] | 5.03[b] | 7.42[c] | 8.31[d] | 0.21 | <0.001 | <0.001 |
| NSC peak 3 height | 0.055[a] | 0.056[a] | 0.074[b] | 0.112[c] | 0.124[c] | 0.003 | <0.001 | <0.001 |

Table 2
(Continued)

| Item | Treatment (corn with DDGS combinations)[2] | | | | | SEM | Contrast | |
|---|---|---|---|---|---|---|---|---|
| | DDGS | Corn + 75 | Corn + 50 | Corn + 25 | Corn | | Corn vs. DDGS | $P$-value |
| Ratios of inherent molecular structures | | | | | | | | |
| Area amide I : II | 4.34 | 4.53 | 5.19 | 4.87 | 4.44 | 0.23 | 0.269 | 0.464 |
| Height amide I : II | 2.77[a] | 2.92[a,b] | 3.16[b,c] | 3.38[c] | 3.28[c] | 0.07 | 0.0050 | <0.001 |
| Amide I : NSC | 0.289[a] | 0.216[b] | 0.174[c] | 0.122[d] | 0.088[e] | 0.008 | <0.001 | <0.001 |
| Amide I : NSC peak 3 | 1.20[a] | 0.81[b] | 0.55[c] | 0.35[d] | 0.22[e] | 0.03 | <0.001 | <0.001 |
| $\alpha$-helix : $\beta$-sheet | 0.99[a] | 1.04[b] | 1.05[b] | 1.15[c] | 1.16[c] | 0.01 | <0.001 | <0.001 |
| NSC peak 1:2 | 0.44[a] | 0.69[a] | 1.16[b] | 1.83[c] | 2.01[c] | 0.10 | <0.001 | <0.001 |
| NSC peak 1:3 | 0.027[a] | 0.049[b] | 0.068[c] | 0.083[d] | 0.093[d] | 0.003 | <0.001 | <0.001 |
| NSC peak 2:3 | 0.10[a] | 0.10[a] | 0.06[a,b] | 0.05[b] | 0.05[b] | 0.01 | 0.0091 | <0.001 |
| Lignin : NSC | 0.0055[b,c] | 0.0082[a] | 0.0062[b] | 0.0044[c] | 0.0027[d] | 0.0003 | <0.001 | <0.001 |
| CeC : NSC | 0.028[a] | 0.022[a,b] | 0.017[a,b] | 0.017[a,b] | 0.013[b] | 0.003 | 0.019 | <0.001 |
| Lignin : CeC | 0.29[a,b,c] | 0.45[a] | 0.40[a,b] | 0.27[b,c] | 0.21[c] | 0.04 | 0.003 | 0.012 |

[a–e] Means within a row with different superscript differ ($P < 0.05$). Mean separation was done by using Tukey–Kramer test. SEM = pooled standard error of means.

[1]DDGS = dried distillers grains with solubles from wheat.

[2]Treatments: (1) DDGS = corn 0% and DDGS 100%; (2) Corn + 75 = corn 25% and DDGS 75%; (3) Corn + 50 = corn 50% and DDGS 50%; (4) Corn + 25 = corn 75% and DDGS 25%; (5) Corn = corn 100% and DGGS 0%.

[3]CeC = cellulosic compounds.

[4]NSC = nonstructural carbohydrates.

Table 3
Structural characteristics of protein and carbohydrates by using FT/IR spectroscopy: comparison of oat versus DDGS[1] combinations

| Item | Treatment (oat with DDGS combinations)[2] | | | | | SEM | Contrast | |
|---|---|---|---|---|---|---|---|---|
| | DDGS | Oat + 75 | Oat + 50 | Oat + 25 | Oat | | Oat vs. DDGS | $P$-value |
| Inherent molecular structural components | | | | | | | | |
| Protein: amide | | | | | | | | |
| Amide I area | 4.10[a] | 3.44[b] | 3.32[b] | 3.16[b] | 3.47[b] | 0.14 | 0.811 | 0.001 |
| Amide I height | 0.054[a,b] | 0.048[b] | 0.050[a,b] | 0.049[a,b] | 0.057[a] | 0.002 | 0.006 | 0.281 |
| Amide II area | 0.95[a,b] | 0.83[b] | 0.88[b] | 0.88[b] | 1.07[a] | 0.05 | <0.001 | 0.057 |
| Amide II height | 0.020[a,b] | 0.017[b] | 0.018[b] | 0.017[b] | 0.021[b] | 0.001 | 0.003 | 0.269 |
| Amide I and II area | 5.05[a] | 4.28[b] | 4.20[b] | 4.048[b] | 4.53[a,b] | 0.14 | 0.480 | 0.031 |
| Protein: secondary molecular structures, $\alpha$-helix and $\beta$-sheet | | | | | | | | |
| $\alpha$-helix (height) | 0.051[a,b] | 0.046[b] | 0.048[a,b] | 0.047[a,b] | 0.054[a] | 0.002 | 0.002 | 0.256 |
| $\beta$-sheet (height) | 0.052[a] | 0.043[b] | 0.041[b] | 0.039[b] | 0.043[b] | 0.002 | 0.845 | <0.001 |
| Carbohydrates: structural and nonstructural components | | | | | | | | |
| Lignin area | 0.078[a] | 0.079[a,b] | 0.076[a] | 0.070[a] | 0.098[b] | 0.005 | <0.001 | 0.046 |
| Lignin height | 0.005 | 0.005 | 0.005 | 0.004 | 0.005 | 0.001 | 0.217 | 0.205 |
| CeC[3] area | 0.41 | 0.40 | 0.43 | 0.43 | 0.49 | 0.05 | 0.163 | 0.202 |
| CeC height | 0.009 | 0.009 | 0.010 | 0.010 | 0.011 | 0.001 | 0.043 | 0.043 |
| NSC[4] area | 14.28[a] | 16.07[a] | 20.79[b] | 22.89[b] | 28.52[c] | 0.88 | <0.001 | <0.001 |
| NSC height | 0.115[a] | 0.123[a] | 0.166[b] | 0.187[b] | 0.239[c] | 0.007 | <0.001 | <0.001 |
| NSC peak 1 area | 0.097[a] | 0.24[a] | 0.46[b] | 0.70[c] | 0.95[d] | 0.04 | <0.001 | <0.001 |
| NSC peak 1 height | 0.005[a] | 0.012[b] | 0.021[c] | 0.028[d] | 0.038[e] | 0.001 | <0.001 | <0.001 |
| NSC peak 2 area | 0.33 | 0.39 | 0.40 | 0.33 | 0.44 | 0.04 | 0.065 | 0.182 |
| NSC peak 2 height | 0.010[a] | 0.011[a] | 0.019[b] | 0.024[b] | 0.037[c] | 0.001 | <0.001 | <0.001 |
| NSC peak 3 area | 3.45[a] | 4.49[a] | 6.65[b] | 7.86[b] | 10.41[c] | 0.32 | <0.001 | <0.001 |
| NSC peak 3 height | 0.055[a] | 0.067[a] | 0.103[b] | 0.123[b] | 0.167[c] | 0.005 | <0.001 | <0.001 |

Table 3
(Continued)

| Item | Treatment (oat with DDGS combinations)[2] | | | | | SEM | Contrast | |
|---|---|---|---|---|---|---|---|---|
| | DDGS | Oat + 75 | Oat + 50 | Oat + 25 | Oat | | Oat vs. DDGS | $P$-value |
| Ratios of inherent molecular structures | | | | | | | | |
| Area amide I : II | 4.34[a] | 4.16[a,b] | 3.80[b,c] | 3.65[c,d] | 3.24[d] | 0.11 | <0.001 | <0.001 |
| Height amide I : II | 2.77 | 2.83 | 2.82 | 2.87 | 2.72 | 0.05 | 0.064 | 0.689 |
| Amide I : NSC | 0.29[a] | 0.22[b] | 0.16[c] | 0.14[c,d] | 0.12[d] | 0.01 | <0.001 | <0.001 |
| Amide I : NSC peak 3 | 1.20[a] | 0.78[b] | 0.50[c] | 0.41[c,d] | 0.34[d] | 0.03 | <0.001 | <0.001 |
| $\alpha$-helix : $\beta$-sheet | 0.99[a] | 1.08[b] | 1.16[c] | 1.22[d] | 1.26[e] | 0.01 | <0.001 | <0.001 |
| NSC peak 1:2 | 0.44[a] | 0.79[a] | 1.21[b] | 2.12[c] | 2.14[c] | 0.10 | <0.001 | <0.001 |
| NSC peak 1:3 | 0.027[a] | 0.053[b] | 0.069[c] | 0.089[d] | 0.091[d] | 0.003 | <0.001 | <0.001 |
| NSC peak 2:3 | 0.10[a] | 0.09[a,b] | 0.07[b,c] | 0.04[c] | 0.04[c] | 0.01 | 0.007 | <0.001 |
| Lignin : NSC | 0.0055[a] | 0.0050[a] | 0.0037[b] | 0.0031[b] | 0.0034[b] | 0.0003 | <0.001 | <0.001 |
| CeC : NSC | 0.028 | 0.025 | 0.021 | 0.019 | 0.017 | 0.003 | 0.070 | 0.004 |
| Lignin : CeC | 0.29 | 0.22 | 0.18 | 0.16 | 0.20 | 0.03 | 0.651 | 0.012 |

[a–e] Means within a row with different superscript differ ($P < 0.05$). Mean separation was done by using Tukey–Kramer test. SEM = pooled standard error of means.

[1]DDGS = dried distillers grains with solubles from wheat.

[2]Treatments: (1) DDGS = oat 0% and DDGS 100%; (2) Oat + 75 = oat 25% and DDGS 75%; (3) Oat + 50 = oat 50% and DDGS 50%; (4) Oat + 25 = oat 75% and DDGS 25%; (5) Oat = oat 100% and DGGS 0%.

[3]CeC = cellulosic compounds.

[4]NSC = nonstructural carbohydrates.

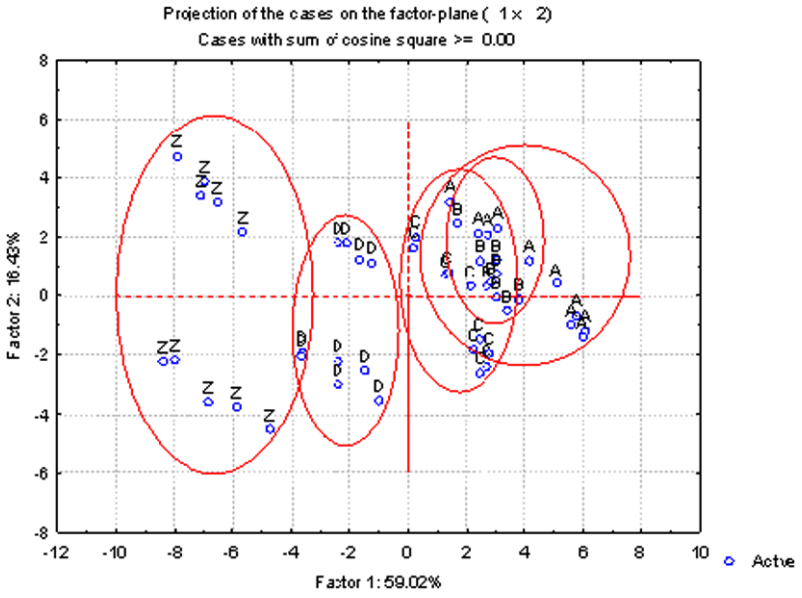
### 3.2. Multivariate molecular spectral analysis of protein spectra

Treatment comparison by principle components and classification (PCA) analysis of FT/IR spectroscopy based on spectra (ca. 1800–700 $cm^{-1}$) obtained from barley, DDGS and their mixtures (Fig. 4a). Hierarchical cluster (CLA) analysis of FTIR spectroscopy based on the same spectra (ca. 1800–700 $cm^{-1}$) obtained from same mixtures (Fig. 4b). The CLA and PCA revealed the molecular structure difference between the feeds and feed combinations. Figure 4a indicated that there were significant molecular structural differences in protein and CHO inherent molecular structures between barley, DDGS and the mixtures (25, 50 and 75%), because they were grouped in separate ellipses (Fig. 4a), and also formed separate classes (Fig. 4b). However, those A, B and C ellipses are heavily overlapping; therefore, barley (A), 25 (B) and 50% (C) are not projected distinct separation, but DDGS (Z) and 75% (D). Separation has broadened with increasing level of DDGS among those mixtures.

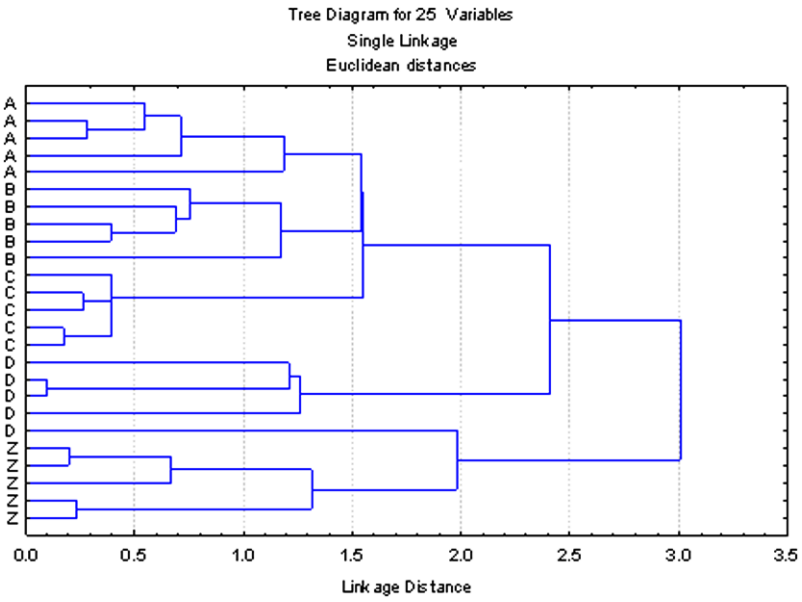
The comparison by PCA (Fig. 4c) and CLA (Fig. 4d) of FT/IR spectroscopy based on spectra (1800–700 $cm^{-1}$) was obtained from corn, DDGS and their mixtures. According to the Fig. 4c, corn (E), 25% (F), 50% (G), were well separated each other and from 75% (H) and DDGS (Z), although DDGS and 75% were not. This finding suggests there would be inherent molecular changes even with lower DDGS inclusions, and DDGS has a strong influence on changing corn intrinsic chemical characteristics [3,17]. PCA and CLA for oat, DDGS and their mixtures were shown in Fig. 4e and f. No distinct separation was projected as many groups were overlapping (Fig. 4e). Only DDGS (Z) separated from oat (I) and 25% (J) showing differences in intrinsic chemical characteristics. Linkage distance only confirms poor linkage or mismatch between DGGS (Z) and others (Fig. 4f). DDGS is not that influential on making detectable changes on oat protein and CHO structures as it was on other two grains; barley and corn.

The multivariate molecular spectral analyses (CLA and PCA) were applied when we study the molecular structure difference. The success to apply multivariate CLA analysis has been reported by Yu [32, 33] for the three feed inherent structures (structure 1 – feed pericarp; structure 2 – feed aleurone; structure 3 – feed endosperm) and different varieties of canola, and Liu and Yu [13] for different genotypes of barley. Being the primary objective of this study, characterization of protein molecular structure using non-invasive and non-destructive FTIR spectroscopy as a novel approach with univariate and multivariate molecular spectral analysis yielded positive results of proving the ability of DDGS to impose intrinsic molecular and structural chemical changes in other grains; barley, corn and oat. In this study, wheat DDGS was combined with other grains in different ratios to evaluate the impact of inclusion. In general protein and carbohydrate inherent molecular characteristics and protein structure $\alpha$-helix and $\beta$-sheet were affected with DDGS inclusion.

The chemical profile study results (data are reported separately) are in agreement with fact that DDGS losses starch and other soluble sugars during the process of bioethanol production for ethanol fermentation, therefore, protein, fibre, fat and minerals are expected to be concentrated in DDGS [37]. In support of previous reports, the results confirm that wheat DDGS provide many compounds in nutritional importance to animals particularly ruminants [17–19]. The changes we found in intrinsic molecular chemical structures such as protein secondary structures of $\alpha$-helix and $\beta$-sheet ratio and biological component matrix such as protein to starch matrix, protein to carbohydrate matrix would improve the nutritional quality and digestive characteristics, once combined with grains [31,32,35]. Intrinsic secondary structures such as $\alpha$-helix, $\beta$-sheet and their ratio on the protein quality, utilization, availability and digestive behavior are useful in modern nutritional evaluation [31,32,35]. The chemical and structural changes we observed in this study were favoring the combinations of barley or corn with DDGS in comparison to the combinations of oat with DDGS. The preferable ratios of combinations found to be 75–50%, 50% or




(a)

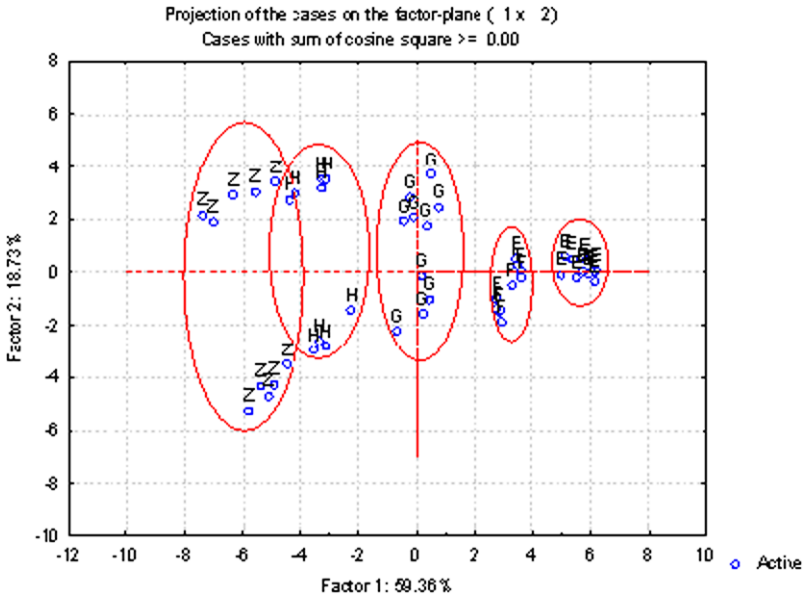



(b)

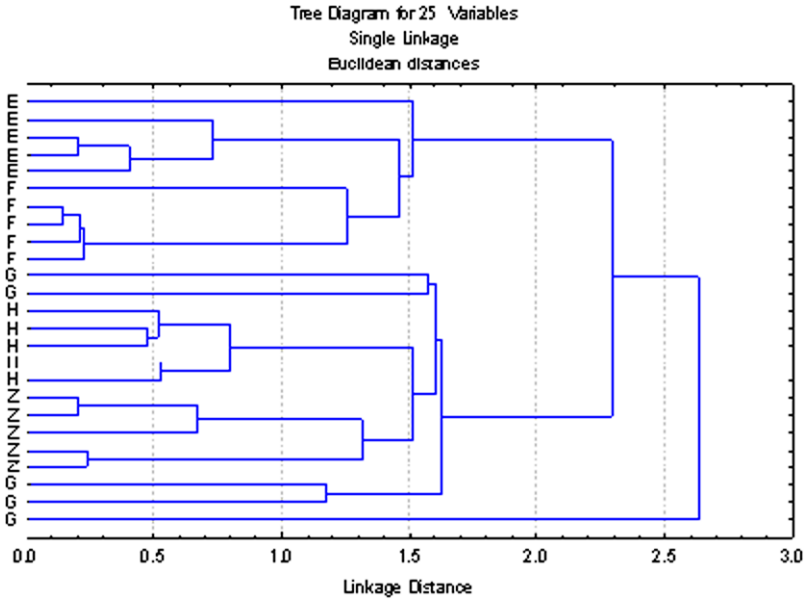
Fig. 4. (a) Treatment comparison by principle components and classification (PCA) analysis of FT/IR spectroscopy based on spectra (1800–700 $cm^{-1}$) obtained from feed samples of barley with DDGS 0 (A), 25 (B), 50 (C), 75 (D) and 100% (Z). (b) Hierarchical cluster (CLA) analysis of FTIR spectroscopy based on spectra (1800–700 $cm^{-1}$) obtained from feed samples of A, B, C, D and Z. (Colors are visible in the online version of the article; http://dx.doi.org/10.3233/SPE-2011-0546.)

50–25% of DDGS with barley, corn or oat respectively. A mixture of those grains combined with DDGS may pave way for better results [3].

Before these novel chemical structural evaluation techniques [34] came into practice, cereal nutrient (mainly protein) evaluations were performed by chemical analysis, NIR (near-infrared reflectance spec-



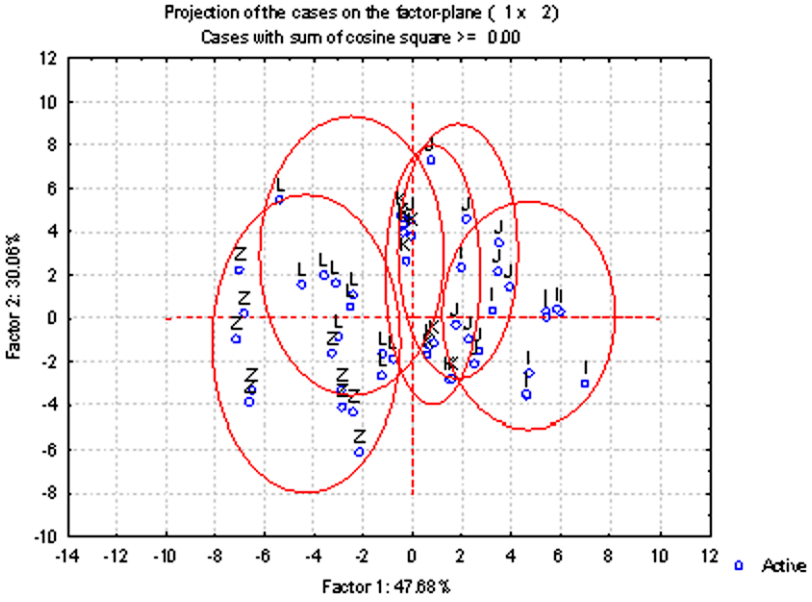

(c)



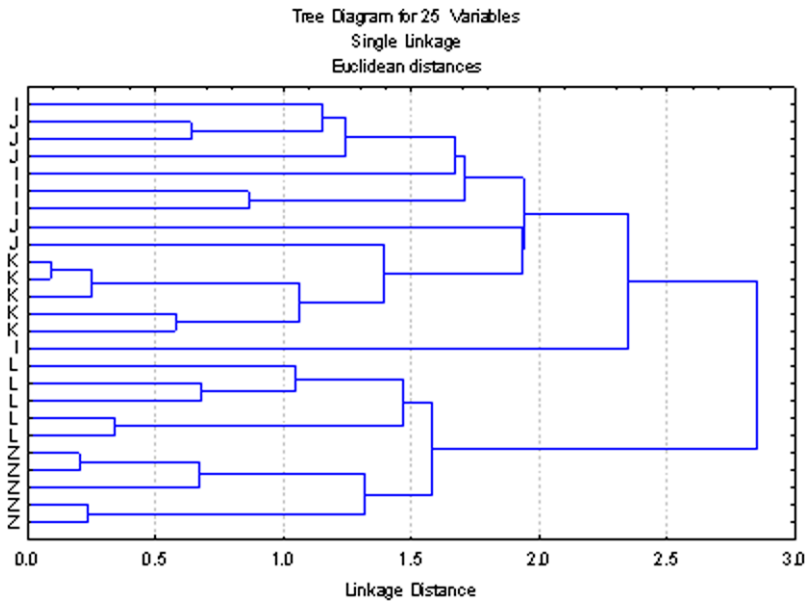

(d)

Fig. 4. (c) Treatment comparison by principle components and classification (PCA) analysis of FT/IR spectroscopy based on spectra (1800–700 $cm^{-1}$) obtained from feed samples of corn with DDGS 0 (E), 25 (F), 50 (G), 75 (H) and 100% (Z). (d) Hierarchical cluster (CLA) analysis of FTIR spectroscopy based on spectra (1800–700 $cm^{-1}$) obtained from feed samples of E, F, G, H and Z. (Colors are visible in the online version of the article; http://dx.doi.org/10.3233/SPE-2011-0546.)

troscopy) or SDS-PAGE (polyacrylamide gel electrophoresis) [2,21,24,29]. NIR techniques have been used for assessment of protein in barley and NIR techniques seemed to be promising in plant or in the food and feed industry for the rapid and accurate analysis of amino acids, decades ago [29]. Cereal




(e)




(f)

Fig. 4. (e) Treatment comparison by principle components and classification (PCA) analysis of FT/IR spectroscopy based on spectra (1800–700 $cm^{-1}$) obtained from feed samples of oat with DDGS 0 (I), 25 (J), 50 (K), 75 (L) and 100% (Z). (f) Hierarchical cluster (CLA) analysis of FTIR spectroscopy based on spectra (1800–700 $cm^{-1}$) obtained from feed samples of I, J, K, L and Z. PCA = principle components and classification analysis; CLA = Hierarchical cluster analysis; FT/IR = Fourier Transform Infrared; DDGS = dried distillers grains with soluble from wheat; I = Oat (oat 100% and DGGS 0%); J = Oat + 25 (oat 75% and DDGS 25%); K = Oat + 50 (oat 50% and DDGS 50%); L = Oat + 75 (oat 25% and DDGS 75%); Z = DDGS (oat 0% and DDGS 100%). (Colors are visible in the online version of the article; http://dx.doi.org/10.3233/SPE-2011-0546.)

seed storage proteins and its chemical constituents, structure were assessed mainly for globulins and prolamine, using SDS-PAGE [24]. Nitrogen content and protein fractions were regularly assessed using procedures prescribed by AOAC [2] and Roe et al. [21]. However, cereal seed compounds were yet to be investigated by techniques such as FTIR [8,31,34]. Starch was the major constituent in many gains and it has been measured by different methods for decades [2,16,26]. Starch, soluble sugars, and carbohydrate fiber were categorically assessed by enzymatic assays [16] or combined procedures [26]. However, there is a need for further understanding of carbohydrate, its structures and intrinsic arrangement which determines nutritional value mainly to ruminants [1,15], and certain extent even to monogastrics [5,14]. This need is further driven by expanding ethanol industry and massive DDGS production [20].

Application of FTIR microspectroscopy provides many information of carbohydrates and its intrinsic structures that is definitely useful in predicting degradability and digestibility of feed at different levels of the gastrointestinal tract including rumen, small intestines or colon [30,31,36]. Our findings on the structural differences happen with combination of grains and grain-distillers-dried soluble gives a background to predict digestibility of those feed combinations. However, correlation of digestive study data may need for a lucid understanding of the connection with the influence on digestibility.

## 4. Conclusions

A combination of DDGS and grains has changed its physiochemical profile, and would deliver better digestive characteristics and rumen degradation kinetics in the form of a concentrate feed. Although it was a physical combination of DDGS and grains, this combination has lead to changes in inherent molecular structures; changes in functional-compound profiles and electromagnetic/molecular/functional-group interactions on each other as a mixture in different ratios would lead to changes in fermentation characteristics or digestive kinetics. Usefulness of FT/IR molecular spectroscopy in terms of identification of inherent structural changes was immense. These results heavily support the fact that the combination of DGGS with different grains chemically and structurally alters the constituents of the new mixture in many ways. This change would improve the nutritional quality of the feed. Further studies are warranted to understand the effect on digestibility and availability status pertaining to structural changes.

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