

Detecting Chemical Molecular Structure Differences among Different Iranian Barley Cultivars Using Fourier Transform Infrared Spectroscopy

Hojjat Gholizadeh^{1,2*}, Abbasali Naserian², Reza Valizadeh²,
Abdol Mansour Tahmasbi² and Peiqiang Yu¹

¹Department of Animal and Poultry Science, College of Agriculture and Bioresources, University of Saskatchewan, Saskatoon, S7N 5A8, Canada.

²Department of Animal Science, Faculty of Agriculture, Ferdowsi University of Mashhad, P.O. Box 91775-1163, Mashhad, Iran.

Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

Research Article

Received 11th May 2013
Accepted 26th August 2013
Published 5th October 2013

ABSTRACT

Objectives: To characterize protein molecular structure using Fourier Transform Infrared Spectroscopy (FTIR) with univariate and multivariate molecular spectral analysis and identify the structure differences among barley cultivars.

Place and Duration of Study: Six barley cultivars: (1) cv. Yousef, (2) cv. Nosrat, (3) cv. CB.79.10, (4) cv. Makooie, (5) cv. Abidar, and (6) cv. Sararood were provided from Crop Development Center Karaj, Iran. FTIR spectroscopy was carried out at the University of Saskatchewan (Saskatoon, Canada), between May 2012 to November 2012.

Methodology: The spectral data were collected from feeds using JASCO FTIR-ATR-4200. The molecular structure spectral analyses involved protein amide I, amide II, α -helix, β -sheet, total carbohydrate, the ratio of amide I to amide II, α -helix to β -sheet, and starch to amide I.

Results: Molecular spectral techniques were able to identify spectral differences associated with the molecular structural differences among barley cultivars. It was found that protein molecular structure in terms of amide II, the ratio of amides I to II and the ratio

*Corresponding author: Email: gholizadeh.hojat@gmail.com;

of α -helix to β -sheet significantly differed among cultivars. Makooie had the greatest peak area and height amide II and the ratio of α -helix to β -sheet. It had the lowest ratio of amide I to amide II and the ratio of starch to amide I. Carbohydrate molecular spectral intensities were also varied among barley cultivars, but it was not statistically significant. CB.79.10 had the greatest peak area CHO. The infrared absorbance intensity ratio of starch to amide I was different among barley cultivars. It was higher for CB.79.10 indicating that CB.79.10 is more heterogeneous than those of other barley cultivars.

Conclusion: The biological function differences between barley cultivars may be related to molecular structure difference and biopolymer conformation between the cultivars of barley. Further study is needed to quantify protein and carbohydrate related molecular spectral features of barley in relation to nutrient supply and availability of protein and carbohydrate.

Keywords: Molecular spectral analyses; protein structure; barley cultivar.

1. INTRODUCTION

Barley (*Hordeum vulgare L.*), a member of the grass family, is a principal cereal grain in Iran, due to the limitations of climate and soil fertility. Barley is used as animal fodder, as a source of fermentable material for beer and certain distilled beverages, and as a component of various health foods. In a 2007 ranking of cereal crops in the world, barley was fourth both in terms of quantity produced (136 million tons) and in area of cultivation (566,000 km²) [1]. Iran has locally adapted and registered different barley cultivars depending upon local climate condition. Ghorbani and Hadj-Hussaini [2] showed that barley cultivars growing at different climate condition had different chemical profile and ruminal digestion characteristics. In-vitro gas production results also show there are significant differences in ruminal dry matter disappearance among Iranian barley cultivars [3]. Recent studies reported that protein molecular structure is related to the availability of nutrients in the gastrointestinal tract of animal [4-6]. It has been suggested that changes in protein, carbohydrate and lipid structure can influence the nutritional value, utilization and digestion behavior of a feed [4-6]. The result suggest that the biological function differences are expected to be related to molecular structure difference and biopolymer conformation between the cultivars of barley. Traditional "wet" chemical analyses only considers the total feed composition and does not consider the feed's internal structure, mainly because it rely heavily on the use of harsh chemicals and derivatization which can destroy the native physiochemical and molecular structures during the chemical analysis [7]. The Fourier Transform infrared molecular spectroscopy (FTIR) is able to detect molecular structural features in complex plant systems [8,9]. This molecular spectroscopy technique plus multivariate molecular spectral analyses are capable to distinguish the structural differences on a molecular basis between the treatments and different cereal grain varieties [10]. So far, there has been no study to investigate the structure differences among Iranian barley cultivars. It was expected that differences in the structural conformation and chemical makeup of barley cultivars will help us to understand barley functionality, biodegradation kinetics, nutrient availability and interaction between the structure and biological functions. The objective of this study was to use FTIR molecular spectroscopy with multivariate molecular spectral analysis to detect protein structure differences among barley cultivars.

2. MATERIALS AND METHODS

2.1 Sample Preparation and FTIR Data Collection

Six barley cultivars: (1) cv. Yousef, (2) cv. Nosrat, (3) cv. CB.79.10, (4) cv. Makooie, (5) cv. Abidar, and (6) cv. Sararood were chosen. Barley samples were provided from Crop Development Center Karaj, Iran. For FTIR molecular structural study, all the samples were finely ground in the same way, through 0.5 mm screen (Retsch ZM200, Rose, Scientific Ltd., Canada), and then were used for FTIR spectroscopy. FTIR was carried out at the University of Saskatchewan (Saskatoon, Canada). The spectral data were collected from feeds using JASCO FTIR-ATR-4200 (JASCO Corporation, Tokyo, Japan). Spectra were generated from the mid-infrared range from ca. 4000 to 700 cm^{-1} in transmission mode with 256 scans per spectrum at a spectral resolution of 4 cm^{-1} . Molecular spectral data integration and analyses were done with OMNIC 7.3 software (Spectra Tech, Madison, WI, USA).

2.2 Molecular Spectral Analysis of Protein

Protein molecular structure is usually determined from two primary bands in the spectra, namely the amide I and II regions [11,12]. The amide I region contains 80% C=O stretching, 10% C—N and 10% N—H and was identified in this study in the range of ca. 1716–1574 cm^{-1} . The amide II region consists of 40% C—N stretching and 60% N—H bending vibrations and was found in the range of ca. 1574–1483 cm^{-1} . Amide I was further resolved into several multicomponent peaks where α -helices (centred at ca. 1655 cm^{-1}) and β -sheets (centred at ca. 1630 cm^{-1}) were identified using the secondary derivative functions in the OMNIC software [11,13]. The peak area total carbohydrate (baseline: ca. 1180-950 cm^{-1}) and the peak of carbohydrate in ca. 1017 cm^{-1} region were determined to calculate the ratio of starch to protein. Because all samples used for analysis were dry, there is no or minimal moisture absorption band to affect the protein amide I and II bands.

2.3 Statistical Analysis

Molecular structural spectral analysis was performed using MIXED procedure of SAS (version 9.2). The model used for the analysis was $Y_{ij} = \mu + T_i + e_{ij}$, where, Y_{ij} is an observation of the dependent variable, μ is the population mean of the variable; T_i is the effect of treatments, as a fixed effect, and e_{ij} was the random error associated with the observation ij . For all statistical analyses, significance was declared at $P= 0.05$. Differences among the treatments were evaluated using the Tukey-method.

Agglomerative hierarchical cluster analysis (CLA) (Distance method: Euclidean; Cluster method: Ward'S algorithm) and principal component analysis (PCA) were performed to compare protein molecular spectral region (ca. 1720- 1480 cm^{-1}) of barley cultivars using Statistica 8.0 software (Stat soft, Inc., USA) according to the procedure published by Yu [14]. These analyses are able to classify and distinguish inherent chemical structural differences, and identify the main sources of variation within the protein and CHO fingerprint spectra.

3. RESULTS AND DISCUSSION

3.1 Detecting the Chemical Structure Differences in the Barley Cultivars

Typical molecular spectrum in barley grain (Fig. 1) in the region ca. 4000-800 cm^{-1} shows chemical functional groups in complex plant systems: N-H and O-H stretch, C-H stretch, amides I and II, C=O carbonyl ester, CHO and cellulosic compounds. Within the region of around 1720-1480 cm^{-1} , and 1180-950 cm^{-1} , the dominant IR absorption features are assigned predominantly to protein and carbohydrate (CHO) stretching vibrations of amides I, II and CHO respectively.

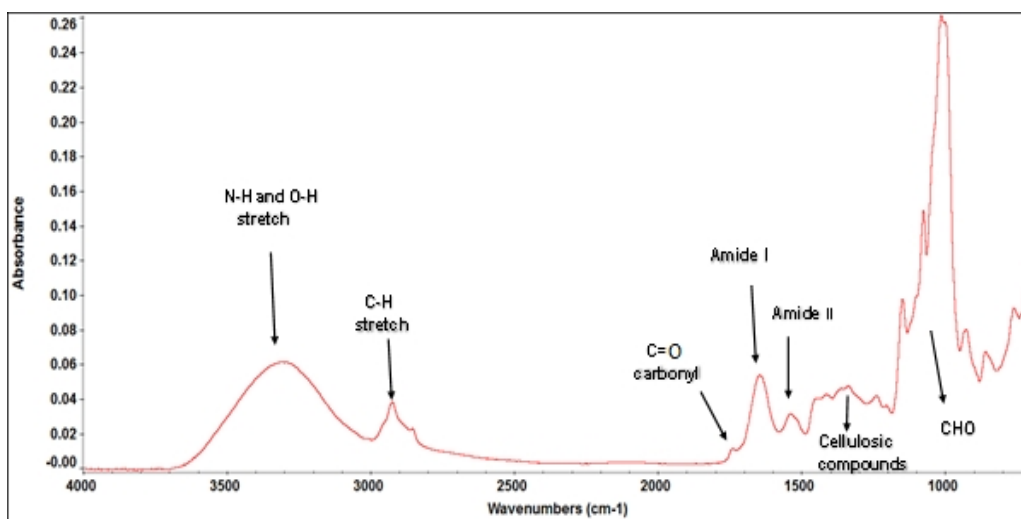


Fig. 1. Typical FTIR molecular spectrum in barley grain in the region ca. 4000- 800 cm^{-1} showed function groups of biopolymers in complex plant system: N-H and O-H stretch, C-H stretch, amide I and II, CHO and cellulosic compounds

The wavenumbers for the above protein and carbohydrate functional groups for barley cultivars are similar to published values from previous studies [9,11,13]. The results suggest that the bands in the IR regions of ca. 1650 cm^{-1} , 1574 cm^{-1} , 1017 cm^{-1} are attributed to protein amide I, amide II and carbohydrate (starch) respectively.

Table 1. Shows the absorbed peak area and height intensities of protein and carbohydrate functional group. Based on the data from each treatment, no differences were found among barley cultivars in terms of peak area and height amide I. The amide I band arises predominantly from the C=O stretching vibration (80%) of the amide C=O group plus C-N stretching vibration [11,12]. The amide II (predominantly an N-H bending vibration coupled to C-N stretching) is also used to assess protein conformation [13]. The result shows that there were significant differences ($P= 0.05$) in the peak area and height intensities of amide II. The peak area and height amide II ranged from 0.610 to 0.402 and 0.012 to 0.008 respectively. Makooie had the greatest peak area and height amide II indicating that barley cultivars had different protein conformation and barley breeding programs change the protein structure in terms of amide II in barley cultivars.

The α -helix and β -sheet were not different among barley cultivars, because amide I was not different among barley cultivars. The vibrational frequency of the protein amide I is particularly sensitive to protein secondary structure [15]. Protein α -helix is typically in the range of approximately 1648-1660 per cm and β -sheet is in the range of approximately 1625-1640 per cm. Results of the current study are not similar to reported values from previous studies [9, 16]. The results suggest that barley cultivars were different in the protein amide I. Peak area amide I, peak height amide I and amides I and II of six barley cultivars ranged from 27.61 to 23.44, 0.30 to 0.35 and 23.72 to 19.85 (IR absorbed intensity unit) respectively [9]. Walker et al, [16] reported that barley cultivars were different in terms of α -helix and β -sheet. The α -helix and β -sheet for four barley cultivars ranged from 0.69 to 0.87 and 0.62 to 0.88 respectively ($P = 0.05$) [16]. The discrepancies between studies may be attributed to the differences between cultivars and growing conditions. The environment and season variations might have impact on the chemical composition and nutrient structure. Further study is needed to understand the effect of climate on the protein and carbohydrate molecular structural makeup of barley cultivars.

The major absorption from carbohydrate are found in the ca. 950- 1180 cm^{-1} region of the spectrum. It arises from C-O, C-C and C-O-H stretching vibrations. There are three major spectral peak bands in this region (ca. 1017, 1074, 1152 cm^{-1}). The region around 1017 cm^{-1} of the spectrum are attributed to C-O and C-C stretching vibrations and C-O-H deformation of starch [11,17]. The result showed that the peak area total carbohydrate (range: 21.266 - 17.166) and starch (range: 10.534 - 8.159) varied considerably between cultivars, but were not statistically significant. CB.79.10 had the greatest peak absorbance intensity of CHO and starch. These results are consistent with previous studies [16,18]. The result suggest that carbohydrate molecular structural makeup was different between different barley cultivars. The results indicated that structural differences in terms of molecular spectral intensities of the functional groups may cause differences in degradation rate and extent among different cultivars of barleys.

3.2 Ratios of the Functional Group of Biopolymers

Table 2 shows the peak area and height ratios of protein functional groups and starch to amide I in the barley cultivars. The peak area and height amides I to II ranged from 5.744-4.613 and 4.322- 3.219 respectively (Table 2). The spectral analysis of amides ratios showed a significant differences between barley cultivars ($P= 0.05$). Yousef and CB.79.10 had the greatest peak area ratio of amides I to II. There were no significant differences between Yousef and CB.79.10. Peak height ratio of amides I to II was higher for Yousef than those of the others. There were significant differences ($P= 0.05$) in the ratio of α - helix to β -sheet.

Table 1. Structural characteristics of the amides, secondary structures and carbohydrate stretching bands in infrared molecular spectrum in barley proteins revealed using FTIR molecular spectroscopy

Item	Cultivars						SEM	P-value
	Yousef	Nosrat	CB.79.10	Makooie	Abidar	Sararood		
Amide I peak area	2.373	2.184	2.312	2.734	2.239	2.181	0.190	0.340
Amide I peak height	0.036	0.033	0.035	0.041	0.034	0.033	0.002	0.406
Amide II peak area	0.414 ^b	0.457 ^{ab}	0.402 ^b	0.610 ^a	0.485 ^{ab}	0.435 ^b	0.038	0.009
Amide II peak height	0.008 ^b	0.009 ^{ab}	0.009 ^b	0.012 ^a	0.010 ^{ab}	0.009 ^{ab}	<0.001	0.010
Amides I,II peak area	2.787	2.642	2.714	3.344	2.724	2.616	0.226	0.247
Alpha helix	0.034	0.032	0.035	0.040	0.034	0.032	0.002	0.403
Beta sheet	0.032	0.027	0.031	0.036	0.029	0.029	0.002	0.225
Total CHO peak area	18.966	17.160	21.266	17.485	19.101	17.716	1.397	0.345
Starch	9.046	8.184	10.534	8.318	9.285	8.159	0.712	0.180

SEM= Standard error of means. Mean separation was done by using the Tukey method

Table 2. Proteins amide I to II, alpha helix to beta sheet, and starch to amide I functional groups ratio of barley revealed using FTIR molecular spectroscopy

Item	Cultivars						SEM	P-value
	Yousef	Nosrat	CB79.10	Makooie	Abidar	Sararood		
Spectral ratio profiles								
Area ratio Amide I:II	5.744 ^a	4.789 ^b	5.744 ^a	4.485 ^b	4.613 ^b	5.003 ^b	0.166	<0.001
Height ratio Amide I:II	4.322 ^a	3.566 ^{bc}	3.970 ^{ab}	3.219 ^c	3.349 ^{bc}	3.505 ^{bc}	0.146	<0.001
Height ratio alpha :beta	0.006 ^b	0.007 ^{ab}	0.006 ^b	0.009 ^a	0.007 ^{ab}	0.006 ^b	<0.001	0.014
Area ratio starch :Amide I	3.802 ^c	3.781 ^c	4.553 ^a	3.042 ^d	4.145 ^b	3.736 ^c	0.073	<0.001

SEM= Standard error of means. Mean separation was done by using the Tukey method

The ratio of α -helix to β -sheet was higher for Makooie than those of other cultivars. These results are consistent with those reported by Yu [15]. The result suggest that the ratio of α -helix to β -sheet were different between barley cultivars. Changes in the protein molecular structure α -helix to β -sheet ratio and the amides I to II ratio during bioethanol processing were highly associated with estimated protein intestinal digestibility and degraded protein balance [15]. High percentage of β -sheet structure may partly cause low access to gastrointestinal digestive enzymes, which may result in a low protein value and low protein availability, such as feather protein. Other studies found that the protein secondary structure profiles influence beef quality, plant desiccation tolerance, long term stability and low temperature tolerance [20-23]. However, Walker et al. [16] reported that the ratio of α -helix to β -sheet was not different between four barley cultivars (range: 1.049 -1.039). The ratio of α -helix to β -sheet in the present study was lower than reported by Walker et al. [16]. This may be related to the differences between cultivars and growing conditions.

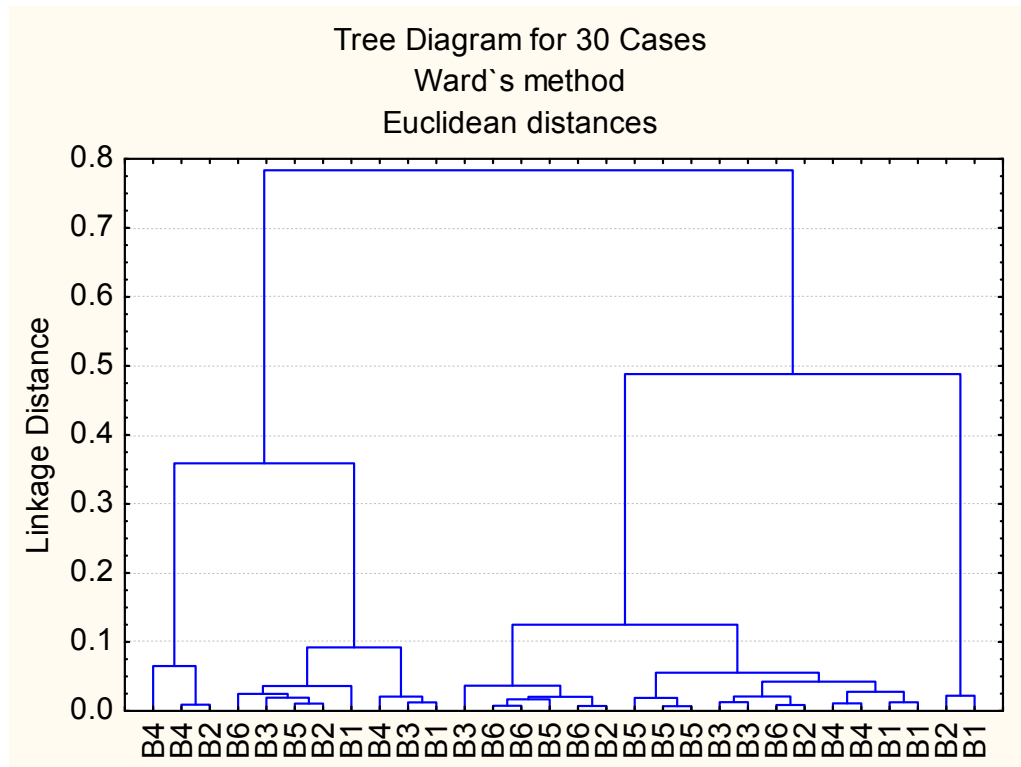
The results also showed that the ratio of starch to amide I was significantly different between barley cultivars ($P= 0.05$). The ratio was ranged from 4.553-3.042. The infrared absorbance intensity of starch to amide I was higher for CB.79.10 than those of other cultivars, indicating that CB.79.10 is more heterogeneous than those of other grains. Makooie had the lowest peak ratio starch to amide I, indicating that starch granules in Makooie are more closely associated with protein matrix. This close association may prevent the starch granules from being rapidly degraded in the rumen [24]. The protein matrix surrounding starch granules in the endosperm serves to hinder bacterial attachment and digestion of starch granules by rumen microorganisms. It has been suggested that this protective action of protein has a greater effect on reducing the bacterial digestion [25]. These results showed that protein and carbohydrate molecular structures were different between barley cultivars.

3.3 Multivariate Molecular Spectral Analysis

The cluster (CLA) and principal component analyses (PCA) were applied to study molecular structural differences between barley cultivars in the protein molecular spectral region ($1720-1480\text{ cm}^{-1}$). Cluster analysis is a multivariate analysis of which function performs an (agglomerative hierarchical) cluster analysis of an infrared spectra data set and displays the results of cluster analysis as dendrograms [14]. The principal component analysis is a statistical data reduction method. It transforms the original set of variables to a new set of uncorrelated variables called principal components. The purpose of PCA is to derive a small number of independent linear combinations (principal components) of a set of variables that retain as much of the information in the original variables as possible [14]. Fig. 2 showed the results of the multivariate molecular spectral analyses of the protein among the barley cultivars for the ca. $1720-1480\text{ cm}^{-1}$. The CLA analysis (Fig. 2a) shows that all cluster contain combinations of spectra from the other barley cultivars, and no unique or distinct cluster could be formed for each cultivar. This implies that cluster analysis unable to distinguish barley cultivars from each other. The CLA comparison failed to demonstrate any distinct groupings among all the cultivars.

PCA spectral comparisons are shown in Fig. 2b. The results are similar to those with CLA analysis. The spectral comparisons by PCA were not able to show any clear group of the spectra among barley cultivars, indicating that barley cultivars did not completely differ from each other or PCA analysis is unable to detect the differences in protein conformation as shown in Table 2. Analysis of peak area and height provides a measure of component concentration and distribution. However, the multivariate methods of data analysis create spectral corrections by utilizing the entire spectral information [14]. Univariate molecular

spectral analysis (Table 2) showed that barley cultivars were not completely different in protein structure. Peak area and height amide I, α -helix, β -sheet, and peak area amide I and II were similar among barley cultivars. These results are consistent with multivariate results. Liu and Yu [9] used both PCA and CLA molecular spectral analyses to compared barley varieties in both hull and seeds and found out that there were no differences in the hull among the barley varieties, but there were dramatic differences in the seeds. Similar results were reported by Yu [15]. In the present study, both CLA and PCA multivariate molecular spectral analysis were not able to show any clear group of the spectra among barley cultivars indicating that the barley breeding program does not dramatically change inherent structure between barley cultivars.



(a)

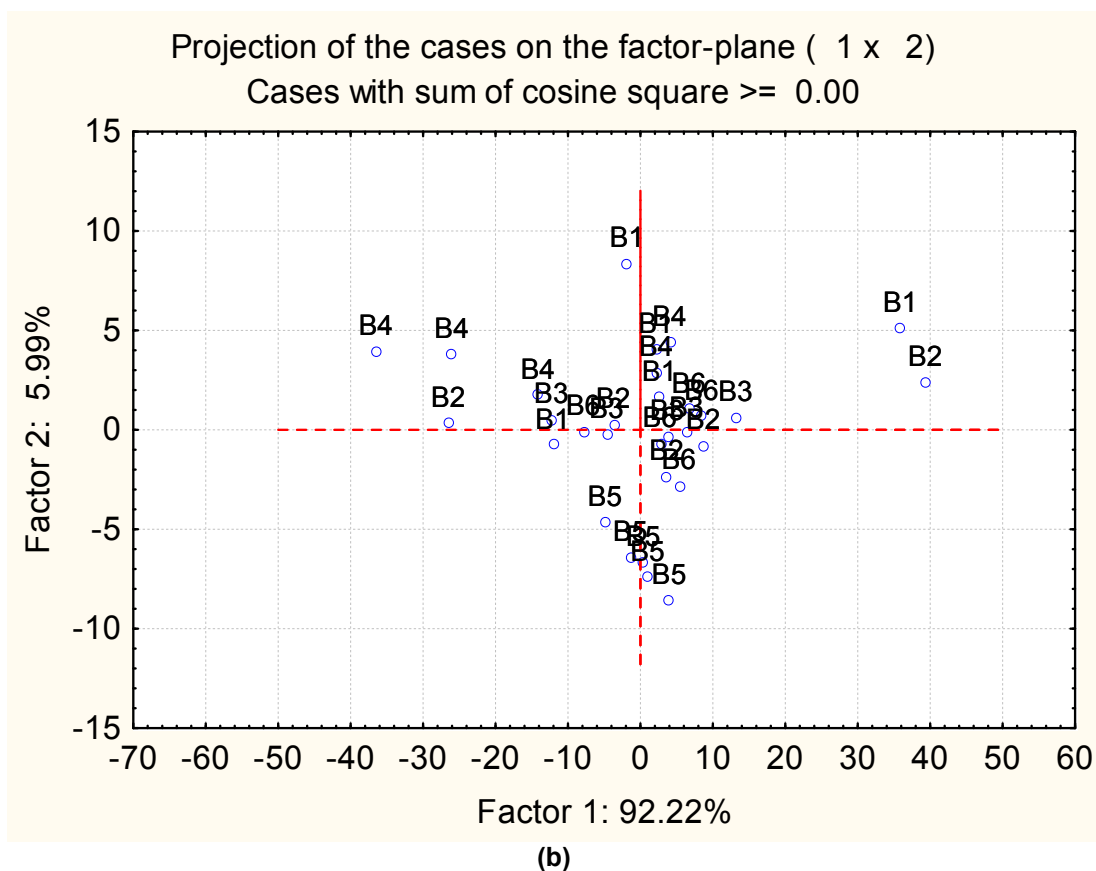


Fig. 2. Multivariate molecular spectral analysis (CLA (2a) and PCA (2b)) of barley cultivars (Yousef (B1), Nosrat (B2), CB.79.10 (B3), Makooie (B4), Abidar (B5), Sararood (B6)) in protein region ca. 1720-1480 cm^{-1}

4. CONCLUSION

The FTIR technique was able to identify spectral features associated with molecular structural differences in barley cultivars. The molecular spectral analyses at the protein region (ca. 1720-1480 cm^{-1}) and carbohydrate region (ca. 1180-950 cm^{-1}) was able to show that the structural makeup of the barley exhibited distinguishing differences among the barley cultivars. The results showed that the infrared absorbance intensity of amide II (both area and height), carbohydrate, the ratio of the amides I to II (both area and height), the ratio of α -helix to β -sheet and the ratio of starch to amide I varied considerably among barley cultivars. Makooie had the greatest peak area and height amide II and the ratio of α -helix to β -sheet. It had the lowest ratio of amides I to II and the ratio of starch to amide I. The results indicated relative differences in protein structures among the barley cultivars, which may partly explain the nutritional differences among the barley cultivars. Further study is needed to understand the effect of climate on chemical structure of barley and know the relationship between barley chemical structure and nutrient availability and digestive behavior.

ACKNOWLEDGMENT

The authors wish to thank the financial support from the Ministry of Agriculture Strategic Research Chair (P.Y.) at the University of Saskatchewan, Saskatoon, Canada and Ferdowsi University of Mashhad, Iran to provide a joint PhD student training (H.G.).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. FAO. Food and Agriculture Organization of the United Nations; 2009. Available: <http://www.faostat.fao.org/>.
2. Ghorbani GR, Hadj-Hussaini A. In situ degradability of Iranian barley grain cultivars. *Small Ruminant Research*. 2002;44(3):207-212.
3. Ghezjeljeh AE, Mesgaran DM, Moghaddam NH, Vakili A. Bulk density, chemical composition and in vitro gas production parameters of Iranian barley grain cultivars grown at different selected climates. *African Journal of Agricultural Research*. 2011;6(5):1226-1232.
4. Yu P. Protein secondary structures (alpha-helix and beta-sheet) at a cellular level and protein fractions in relation to rumen degradation behaviours of protein: a new approach. *The British journal of nutrition*. 2005;94(5):655-65.
5. Yu P. Synchrotron IR microspectroscopy for protein structure analysis: Potential and questions. *Spectroscopy*. 2006;20(5,6):229-251.
6. Yu P. Plant-based food and feed protein structure changes induced by gene-transformation, heating and bio-ethanol processing: a synchrotron-based molecular structure and nutrition research program. *Molecular nutrition & food research*. 2010;54(11):1535-45.
7. Yu P, Block H, and Doiron K. Understanding the differences in molecular conformation of carbohydrate and protein in endosperm tissues of grains with different biodegradation kinetics using advanced synchrotron technology. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*. 2009;71(5):1837-1844.
8. Yu P. Synchrotron-based microspectroscopic analysis of molecular and biopolymer structures using multivariate techniques and advanced multi-components modeling. *Canadian Journal of Analytical Sciences and Spectroscopy*. 2008;53:220-231.
9. Liu N, Yu P. Using DRIFT molecular spectroscopy with uni- and multivariate spectral techniques to detect protein molecular structure differences among different genotypes of barley. *Journal of agricultural and food chemistry*. 2010;58(10):6264-9.
10. Damiran D, Yu P. Structural makeup, biopolymer conformation, and biodegradation characteristics of a newly developed super genotype of Oats (CDC SO-I versus conventional varieties): A Novel Approach. *Journal of Agricultural and Food Chemistry*, 2010;58(4):2377-2387.
11. Wetzel DL, Eilert AJ, Pietrzak LN, Miller SS, Sweat JA. Ultraspatially-resolved synchrotron infrared microspectroscopy of plant tissue in situ. *Cellular and molecular biology*. 1998;44(1):145-68.
12. Marinkovic NS, Huang R, Bromberg P, Sullivan M, Toomey J, Miller LM, Sperber E, Moshe S, Jones KW, Chouparova E, Lappi S, Franzen S, Chnce MR. Center for synchrotron biosciences' U2B beamline: an international resource for biological infrared spectroscopy. *Journal of synchrotron radiation*. 2002;(9):189-97.

13. Jackson M, Mantsch HH. The use and misuse of FTIR spectroscopy in the determination of protein structure. *Critical reviews in biochemistry and molecular biology*. 1995;30(2):95-120.
14. Yu P. Applications of Hierarchical Cluster Analysis (CLA) and Principal Component Analysis (PCA) in feed structure and feed molecular chemistry research, using synchrotron-based Fourier Transform Infrared (FTIR) Microspectroscopy. *Journal of Agricultural and Food Chemistry*. 2005b;53(18):7115-7127.
15. Yu P. Molecular chemical structure of barley proteins revealed by ultra-spatially resolved synchrotron light sourced FTIR microspectroscopy: Comparison of barley varieties. *Biopolymers*. 2007;85(4):308-317.
16. Walker AM, Yu P, Christensen CR, Christensen DA, Mckinnon JJ. Fourier transform infrared microspectroscopic analysis of the effects of cereal type and variety within a type of grain on structural makeup in relation to rumen degradation kinetics. *Journal of agricultural and food chemistry*. 2009;57(15):6871-8.
17. Mathlouthi M, Koenig JL, *Vibrational Spectra of Carbohydrates*. in: Tipson RS, Derek H, Editors. *Advances in Carbohydrate Chemistry and Biochemistry*, Academic Press; 1987.
18. Liu N, Yu P. Molecular clustering, interrelationships and carbohydrate conformation in hull and seeds among barley cultivars. *Journal of Cereal Science*. 2011;53(3):379-383.
19. Yu P, Nuez-Ortin WG. Relationship of protein molecular structure to metabolisable proteins in different types of dried distillers grains with solubles: a novel approach. *The British journal of nutrition*. 2010b;104(10):1429-37.
20. Golovina EA, Wolkers WF, Hoekstra FA. Long-Term Stability of Protein Secondary Structure in Dry Seeds. *Comparative Biochemistry and Physiology Part A: Physiology*. 1997;117(3):343-348.
21. Kirschner C, Ofstad R, Skarpeid HJ, Host V, Kohler A. Monitoring of denaturation processes in aged beef loin by Fourier transform infrared microspectroscopy. *Journal of Agricultural and Food Chemistry*. 2004;52(12):3920-3929.
22. Sreedhar L, Wolkers W, Hoekstra F, Bewley D. In vivo Characterization of the Effects of Abscisic Acid and Drying Protocols Associated with the Acquisition of Desiccation Tolerance in Alfalfa (*Medicago sativa* L.) Somatic Embryos. *Annals of Botany*. 2002;89(4):391-400.
23. Wolkers WF, Hoekstra FA. Aging of dry desiccation-tolerant pollen does not affect protein secondary structure. *Plant physiology*. 1995;109(3):907-915.
24. Yu P, Christensen DA, Christensen CR, Drew MD, Rossnagel BG, Mckinnon JJ. Use of synchrotron FTIR microspectroscopy to identify chemical differences in barley endosperm tissue in relation to rumen degradation characteristics. *Canadian journal of animal science*. 2004;84(3):523-527.
25. McAllister T, Phillippe RC, Rode LM, Cheng KJ. Cereal grain digestion by selected strains of ruminal fungi. *Canadian journal of microbiology*. 1993;39(4):367-376.

© 2014 Gholizadeh et al.; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/3.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:

The peer review history for this paper can be accessed here:
<http://www.sciencedomain.org/review-history.php?iid=287&id=32&aid=2189>