Irrigated Corn 2015

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

pubs.acs.org/JAFC

Magnitude Differences in Agronomic, Chemical, Nutritional, and Structural Features among Different Varieties of Forage Corn Grown on Dry Land and Irrigated Land

Hangshu Xin,[†,⊥] Samen Abeysekara,[†] Xuewei Zhang,[§] and Peiqiang Yu*[,†,§]

[†]Department of Animal and Poultry Science, College of Agricultural and Bioresources, University of Saskatchewan, Saskatoon, Saskatchewan S7N 5A8, Canada

[§]College of Animal Science and Veterinary Medicine, Tianjin Agricultural University, Tianjin 300384, China

ABSTRACT: In this study, eight varieties of corn forage grown in semiarid western Canada (including Pioneer P2501, Pioneer P39m26, Pioneer P7443, Hyland HL3085, Hyland HLBaxxos, Hyland HLR219, Hyland HLSR22, and Pickseed Silex BT) were selected to explore the effect of irrigation implementation in comparison with nonirrigation on (1) agronomic characteristics, (2) basic chemical profiles explored by using a near-infrared reflectance (NIR) system, and (3) protein and carbohydrate internal structural parameters revealed by using an attenuated total reflectance−Fourier transform infrared spectroscopy (ATR-FTIR) system. Also, principal component analysis (PCA) was performed on spectroscopic data for clarification of differences in molecular structural makeup among the varieties. The results showed that irrigation treatment significantly increased ($P < 0.05$) contents of dry matter (DM) and organic matter (OM) but decreased crude protein (CP) of corn forages. Significant interactions of irrigation treatment and corn variety were observed on most agronomic characteristics (DM yield, T/ha, days to tasseling, days to silking) and crude fiber (CF) and ether extract (EE) contents as well as some spectral data such as cellulosic compounds (CELC) peak intensity, peak ratios of CHO third peak to CELC, α-helix to β-sheet, and CHO third peak to amide I. Additionally, the spectral ratios of chemical functional groups that related to structural and nonstructural carbohydrates and protein polymers in forages did not remain constant over corn varieties cultivated with and without water treatment. Moreover, different cultivars had different growth, structure, and nutrition performances in this study. Although significant differences could be found in peak intensities, PCA results indicated some structural similarities existed between two treated corn forages with the exception of HL3085 and HLBaxxos. In conclusion, irrigation and corn variety had interaction effects on agronomic, chemical, nutritional, and structural features. Further study on the optimum level of irrigation for corn forage cultivation might be helpful in semiarid regions such as western Canada.

KEYWORDS: *corn forage, irrigation, agronomic, nutritive, molecular structure, ruminants*

INTRODUCTION

Corn forage is a common and good fiber source for ruminant animals, and it is widespread in Europe and Canada as ensiled silage, which has more stable performance in feeding values than other forage.[1] The role of fiber in dairy nutrition and health and the nutritive value of corn silage in relation to dairy cow performance and milk quality were reviewed recently.[2,3]. In Canada, >190,000 ha of corn are harvested as forage every year,[4] and this number may be currently higher.

In general, forage yield and quality heavily rely on environmental factors such as temperature, water availability, and other cultural aspects reviewed by Roth et al.[5] One of the most important factors contributing to forage production is water use during growing and filling stages of crops,[6,7] especially in arid or semiarid regions.[8] Previous investigators found that every 1 kg of dry matter (DM) production in corn required 368 kg water.[9] Therefore, agricultural irrigation is always regarded as an efficient measure to avoid detrimental impacts on development of plants due to the deficient precipitation during critical growing and reproductive period. Numerous studies have been devoted to study the irrigation effect on yield and quality of various kinds of forage.[6,7,10,11] The results showed that irrigation implementation mostly enhanced DM yield[7,10] and crude protein accumulation as well as fiber content of forages.[7,11] However, some researchers found little loss in DM yield when irrigation was occasionally limited.[12]

The chemical and nutrition values of corn forage were reported before using traditional "wet" chemical analysis and animal methods.[13−17] However, to our knowledge, no study has been made on the effect of irrigation implementation on the magnitude of changes in inherent molecular structural features of the whole plant.

The objective of this study was to investigate the effect of irrigation treatment on different varieties of corn forage grown in semiarid western Canada, not only on agronomic characteristics and basic chemical profile but also on protein and carbohydrate internal molecular structural parameters revealed by using an attenuated total reflectance−Fourier transform infrared spectroscopy (ATR-FTIR) system[14−17] with chemometrics.

Received: December 6, 2014
Revised: February 11, 2015
Accepted: February 19, 2015
Published: February 19, 2015

DOI: 10.1021/jf505911a
J. Agric. Food Chem. 2015, 63, 2383−2391

Table 1. Agronomic Measures of Different Varieties of Corn Forage Grown with or without Irrigation

	cultivar	variety	plants/ha	DM (%)	moisture (%)	DM yield (T/ha)	days to tasseling	days to silking
irrigation	Pioneer	P2501	79688	40.6	59.5	18.6	74.3	75.8
		P39m26	79948	48.7	51.3	15.6	62.0	71.8
		P7443	78646	47.1	52.9	18.0	68.5	74.3
	Hyland	HL3085	68229	39.8	60.2	13.4	66.5	73.3
		HLBaxxos	74740	40.8	59.3	16.6	73.0	76.5
		HLR219	78646	41.4	58.6	18.3	74.0	76.0
		HLSR22	80209	36.2	63.8	20.0	76.8	78.8
	Pickseed	Silex BT	72656	37.5	62.5	18.3	72.8	76.5
mean			76595	41.5	58.5	17.4	71.0	75.3
dry land	Pioneer	P2501	74219	39.6	60.4	17.2	75.5	77.8
		P39m26	70052	46.2	53.8	13.8	63.8	73.3
		P7443	71354	42.7	57.4	12.5	69.8	75.8
	Hyland	HL3085	68229	38.2	61.8	14.7	74.0	78.8
		HLBaxxos	73438	41.9	58.1	13.9	67.5	73.8
		HLR219	71355	39.8	60.3	15.9	74.8	77.5
		HLSR22	69010	35.0	65.1	14.0	78.3	81.5
	Pickseed	Silex BT	71615	36.3	63.7	15.3	72.5	77.5
mean			71159	39.9	60.1	14.6	72.0	77.0
statistical analysis								
SEM			1991	0.82	0.82	0.55	0.46	0.44
P	irrigation		<0.0001	0.0003	0.0003	<0.0001	<0.0001	<0.0001
	variety		0.004	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
	variety × irrigation		0.045	0.09	0.09	<0.0001	<0.0001	<0.0001
contrast	Pioneer vs Hyland		0.02	<0.0001	<0.0001	0.74	<0.0001	<0.0001
	Pioneer vs Pickseed		0.04	<0.0001	<0.0001	0.06	<0.0001	<0.0001
	Hyland vs Pickseed		0.59	0.001	0.001	0.03	0.20	1.00

■ MATERIALS AND METHODS

Corn Cultivation, Experimental Design, and Sampling. Eight varieties were collected from the Canada−Saskatchewan Irrigation Diversification Centre, Agriculture and Agri-Food Canada (Outlook, SK, Canada; 51°30′ N, 107°03′ W). These varieties were Pioneer 2501 (P2501), Pioneer 39m26 (P39m26), Pioneer 7443 (P7443; Pioneer Hi-Bred International Inc., Johnston, IA, USA); Hyland 3083 (HL3083), Hyland Baxxos (HLBaxxos), Hyland R219 (HLR219), Hyland SR22 (HLSR22; Hyland Seeds, Blenheim, ON, Canada); and Silex BT (Pickseed Canada Inc., Nipawin, SK, Canada). This experiment was designed as a randomized complete block design. All of the varieties were arranged with four plots (blocks) with a total of 64 plots. The plot size was 6 m × 1.7 m with seeding rate of 600 kernels per plot on May 20, 2012. For the irrigated plots, irrigation was started at the end of July and ended in mid-September at 25 mm per week according to the practical standard. For the dry land plots, no irrigation was performed. The precipitation from seeding date to harvesting date was 192 mm. When the moisture content of corn forage of the entire plot reached 65−70%, the forage was ready for harvest. Harvests were carried out at the same time (September 28, 2012) with a kemper chopper (Champion 1200, Denmark) for all corn plots. Cutting height was set at 10 cm above the ground.

Agronomic Measurement. The numbers of corn plants in each plot, tassel initiation, and silk initiation were recorded during the experiment. On the harvesting day, the corn forages of the entire plot were chopped and weighed with a built-in scale in the chopper before unloading onto the ground. Approximately 500 g of fresh chopped plant from each plot was sealed in a plastic sample bag for the determination of DM according to AOAC Official Method 930.15.[18] In addition, chopped forage samples of around 1 kg for each treatment were collected, dried in a forced-air oven at 55 °C for 72 h, and ground through 1.0 mm screen (Retsch ZM-1; Brinkmann Instruments, Mississauga, ON, Canada) for further analyses.

Basic Chemical Profile with Near-Infrared Reflectance (NIR) Spectroscopy System. The air-dried forage samples were scanned using a NIR system (Foss InfraXact, Hilleroed, Denmark) at the University of Saskatchewan (SK, Canada) for measurements of DM, organic matter (OM), crude protein (CP), crude fiber (CF), and ether extract (EE) based on internal available calibration curves in the NIR system. Approximately 50 g of forage sample was used under the analysis model of plant (forage) material in NIR system. Spectra were collected in the visible (vis) and NIR regions in reflectance (ca. 1850−570 nm), within 60 s by a monochromator-based NIR system in triplicates. Both spectrophotometer operation and data acquisition were performed by ISIscan Software IS-2200 (FOSS Analytical, Hilleroed, Denmark).

Carbohydrate and Protein Molecular Structures by ATR-FT-MIR Spectroscopy. The spectral scans (within mid-IR region ca. 4000−800 cm^{-1}) of ground forage samples (0.5 mm) were performed using a JASCO FT/IR 4200 with ATR (JASCO Corp., Tokyo, Japan) at the University of Saskatchewan. In the present study, both protein- and carbohydrate-related molecular structures were revealed by 128 co-added scans at a resolution of 4 cm^{-1}. The parameters related to carbohydrate molecular structures included peak heights of total carbohydrate (CHO, peak baseline ca. 1198−800 cm^{-1}; three peaks fell into this region, named CHO first, second, and third peaks centered at 1154, 1103, and 1033 cm^{-1}, respectively), cellulosic compounds (CELC, peak baseline ca. 1290−1180 cm^{-1}) centered at 1240 cm^{-1}, and lignin (peak baseline ca. 1525−1490 cm^{-1}) centered at 1512 cm^{-1}. The protein spectral parameters involved protein amide I, amide II, and its secondary structures such as α-helix and β-sheet; all of the protein peaks were identified within the baseline ca. 1696−1488 cm^{-1} and the peaks centered at 1610, 1538, 1652, and 1629 cm^{-1}, respectively. More detailed information about peak identification can be found in previous publications.[19,20] Then the spectral analysis was carried out using OMNIC 7.2 software (Spectra Tech., Madison, WI, USA).

Principal Component Analysis (PCA). Multivariate spectral analyses−PCA was performed using Statistica 8.0 software (StatSoft Inc., Tulsa, OK, USA) within the NIR fingerprint spectra ca. 1850−570 nm on each corn variety to clarify whether there was any

Table 2. Chemical and Nutrition Profile Derived from NIR Spectral Analysis of Different Varieties of Corn Forage Grown with or without Irrigation

	cultivar	variety	OM (% DM)	protein (% DM)	CF (% DM)	EE (% DM)
irrigation	Pioneer	P2501	94.97	9.51	11.73	1.17
		P39m26	94.59	8.58	10.52	1.10
		P7443	94.60	9.17	10.38	1.18
	Hyland	HL3085	95.00	10.18	11.25	1.28
		HLBaxxos	94.95	8.91	13.54	1.01
		HLR219	95.40	9.76	11.89	1.17
		HLSR22	94.51	10.14	14.78	0.90
	Pickseed	Silex BT	94.93	10.29	12.43	1.16
mean			94.87	9.57	12.07	1.12
dry land	Pioneer	P2501	93.97	9.57	12.67	1.01
		P39m26	94.35	8.95	11.10	1.20
		P7443	93.88	9.36	11.52	0.99
	Hyland	HL3085	95.04	10.00	12.52	1.04
		HLBaxxos	94.08	9.76	10.28	1.27
		HLR219	94.78	9.76	10.97	1.25
		HLSR22	94.04	10.81	14.17	0.86
	Pickseed	Silex BT	95.30	10.70	13.02	1.23
mean			94.43	9.88	12.03	1.10
statistical analysis						
SEM			0.289	0.200	0.459	0.051
P	irrigation		0.002	0.002	0.87	0.52
	variety		0.001	<0.0001	<0.0001	<0.0001
	variety × irrigation		0.19	0.19	<0.0001	<0.0001
contrast	Pioneer vs Hyland		0.03	<0.0001	<0.0001	0.76
	Pioneer vs Pickseed		0.002	<0.0001	<0.0001	0.03
	Hyland vs Pickseed		0.08	0.0004	0.36	0.02

molecular structural relationship between irrigated and nonwatered corn forages. The application of PCA in feedstuff molecular structure research has been described clearly by Yu,[21] which was also referred to in the current study.

Statistical Analysis. The treatment design was 8 × 2 factorial design with 8 corn forage varieties and 2 treatments (irrigation and nonirrigation). The experiment design was randomized complete block design (RCBD). The data of the agronomic measurements and basic chemical profile as well as carbohydrate and protein spectral data in corn forages were statistically analyzed using the Mixed Model procedure of SAS 9.2, and the model was

$$Y_{ijkl} = \mu + F_i + I_j + F_i \times I_j + B_k + e_{ijkl}$$

where Y_{ijkl} is the observation of the dependent variable *ijkl*; μ is the fixed effect of population mean of the variable; F_i is a fixed effect of corn forage variety (i = 8; P2501, P39m26, P7443, HL3085, HLBaxxos, HLR219, HLSR22, and Silex BT), I_j is a fixed effect of irrigation (j = 2; irrigation and dryland), $F_i \times I_j$ is a fixed effect of interaction between factor F at level i and factor I at level j; B_k is a random block effect; and e_{ijkl} is the random error associated with the observation *ijkl*.

The three assumptions of the RCBD were (1) residual errors are normally distributed; (2) there were common variances among the treatments; and (3) block effects are normally distributed. For normality tests, Proc Univariate of SAS with Normal Plot option was used. For the common variance, treatment ID against residue pattern was checked.

Multiple treatment comparisons were performed using the Tukey–Kramar test. Statistical significance was declared and detected at $P < 0.05$, whereas trends were declared at $P \leq 0.10$.

RESULTS AND DISCUSSION

Agronomic Characteristics. Table 1 shows the agronomic features of different varieties of corn forage response to irrigation. Forage plant number was significantly affected ($P < 0.05$) by the interaction of irrigation and variety. The number of HLSR22 plants grown with irrigation was at the highest level, whereas that of HL3085 (with or without irrigation) was at the lowest level. Most of our data fell into the optimum density range of 79,000−143,000 plants per hectare, which is considered to have maximum forage yields.[22,23] A significant difference (P = 0.0003) on DM content of corn forage was found between irrigation and no water use treatments. The plants grown with irrigation treatment had 4% more DM than those grown on dry land. Limited water supply in the growing stage leads to deficient water content in the soil, and this deficit strongly hinders nutrient uptake by the roots and delays physiological processes for the growth and development of plants.[24] Additionally, the drier soil may slow or even block rooting,[25] transpiration, photosynthetic rates, and nutrient transportation from roots to shoots.[26−28] A positive effect of irrigation on crop DM accumulation was also reported by numerous researchers previously.[11,26,29] Cultivar Pioneer had the greatest DM concentration with an average of 45.4%, followed by Hyland (39.5%) and Pickseed (37.5%). In regard to DM yield, it interacted ($P < 0.0001$) with irrigation regimen and corn variety. The DM yield of HLSR22 cultivated on irrigated land reached 20 tons (per hectare), which was at the highest level among treatments. Our result was in agreement with several studies[11,30,31] in which increased DM yield of forage was highly associated with higher level of irrigation. The timings of tassel initiation and silk initiation were also changed among corn varieties grown with and without water treatment. The interaction of irrigation and variety was significant, and the earliest tassel and silk initiations were found in P39m26 grown

DOI: 10.1021/jf505911a
J. Agric. Food Chem. 2015, 63, 2383−2391

Table 3. Peak Intensity Measures from FTIR Univariate Spectral Data Analysis of Different Varieties of Corn Forage Grown with or without Irrigation

	cultivar	variety	CHO first peak	CHO second peak	CHO third peak	cellulosic compounds	lignin	protein amide I	protein amide II	α-helix	β-sheet
	peak position:[a]		~1154 cm^{-1}	~1103 cm^{-1}	~1033 cm^{-1}	~1240 cm^{-1}	~1512 cm^{-1}	~1610 cm^{-1}	~1538 cm^{-1}	~1652 cm^{-1}	~1629 cm^{-1}
irrigation	Pioneer	P2501	0.0220	0.0624	0.1010	0.0076	0.0040	0.0298	0.0205	0.0127	0.0142
		P39m26	0.0212	0.0553	0.0923	0.0067	0.0039	0.0259	0.0195	0.0102	0.0106
		P7443	0.0224	0.0564	0.0946	0.0066	0.0041	0.0268	0.0194	0.0110	0.0113
	Hyland	HL3085	0.0218	0.0579	0.0960	0.0065	0.0045	0.0298	0.0211	0.0132	0.0140
		HLBaxxos	0.0218	0.0585	0.0955	0.0081	0.0039	0.0282	0.0196	0.0115	0.0128
		HLR219	0.0227	0.0610	0.0999	0.0075	0.0042	0.0303	0.0214	0.0124	0.0134
		HLSR22	0.0210	0.0591	0.0954	0.0077	0.0042	0.0296	0.0204	0.0132	0.0146
	Pickseed	Silex BT	0.0212	0.0585	0.0954	0.0082	0.0037	0.0276	0.0189	0.0111	0.0125
mean			0.0218	0.0587	0.0963	0.0074	0.0041	0.0285	0.0201	0.0119	0.0129
dry land	Pioneer	P2501	0.0202	0.0576	0.0936	0.0077	0.0035	0.0238	0.0164	0.0098	0.0106
		P39m26	0.0207	0.0554	0.0924	0.0081	0.0053	0.0264	0.0203	0.0092	0.0093
		P7443	0.0196	0.0543	0.0892	0.0068	0.0037	0.0241	0.0179	0.0086	0.0094
	Hyland	HL3085	0.0206	0.0562	0.0924	0.0075	0.0050	0.0277	0.0203	0.0107	0.0110
		HLBaxxos	0.0195	0.0524	0.0854	0.0061	0.0038	0.0248	0.0179	0.0107	0.0113
		HLR219	0.0206	0.0564	0.0918	0.0075	0.0046	0.0271	0.0196	0.0106	0.0110
		HLSR22	0.0183	0.0549	0.0877	0.0070	0.0042	0.0258	0.0186	0.0110	0.0118
	Pickseed	Silex BT	0.0196	0.0567	0.0898	0.0072	0.0038	0.0279	0.0197	0.0107	0.0120
mean			0.0199	0.0555	0.0903	0.0072	0.0042	0.0259	0.0189	0.0102	0.0108
statistical analysis											
SEM			0.00079	0.00207	0.00345	0.00031	0.00039	0.00114	0.00095	0.00050	0.00057
P	irrigation		<0.0001	0.003	0.001	0.20	0.32	<0.0001	0.004	<0.0001	<0.0001
	variety		0.34	0.23	0.503	0.02	0.003	0.01	0.048	<0.0001	<0.0001
	variety × irrigation		0.84	0.83	0.89	<0.0001	0.07	0.051	0.10	0.11	0.16
contrast	Pioneer vs Hyland		0.60	0.89	0.65	0.58	0.19	0.002	0.06	<0.0001	<0.0001
	Pioneer vs Pickseed		0.34	0.67	0.67	0.09	0.25	0.07	0.64	0.10	0.01
	Hyland vs Pickseed		0.54	0.73	0.89	0.03	0.04	0.82	0.42	0.050	0.60

[a]Protein amide data unit, IR absorbance unit; the protein peak baseline, ca. 1696−1488 cm^{-1}; carbohydrate (CHO) peak baseline, ca. 1198−800 cm^{-1}; cellulosic compounds peak baseline, ca. 1290−1180 cm^{-1}; lignin peak baseline, ca. 1525−1490 cm^{-1}.

DOI: 10.1021/jf505911a
J. Agric. Food Chem. 2015, 63, 2383−2391

in wetter plots, which were around 16 and 10 days prior to the latest one, HLSR22 cultivated on dry land. Such findings partially complied with previous results of NeSmith and Ritchie[32] and Abrecht and Carberry,[33] who reported that delays in crop phenology could be expected with severe water deficits.

Basic Chemical Profile Derived from NIR Spectral Analysis. The contents of OM, CP, CF, and EE in different varieties of corn forage grown with and without irrigation are shown in Table 2. Both growing conditions (irrigated or not; $P = 0.002$) and corn variety ($P = 0.001$) significantly affected OM content. Compared with corn grown on dry land, corn grown with water stress had 0.44% unit higher OM content, which partially corresponded to the recent result of Jahanzad et al.,[5] who found the ash content (=100 − OM) followed a declining trend as irrigation levels increased in two forage sorghum cultivars named Speedfeed and Pegah. Within three cultivars, Pioneer had a higher OM content than Hyland and Pickseed, and the latter two were similar to each other. Also, this result was not far from the observation reported by Abeysekara et al.[34] The concentration of CP plays an important role in forage quality and ruminant feed nutrition.[35] In the current research, the CP contents in different kinds of corn forage were strongly influenced by irrigation conditions ($P = 0.002$). A progressive rise in CP concentration was found in the corn forage grown on dry land. As for the effect of various irrigation regimens on CP accumulation in crops, previous studies showed inconsistent results. Some researchers reported an improved CP content in forage as water deficit intensified, which might result from nitrogen accumulation in drier soil,[7,36,37] the breakdown in plant protein molecules,[36] or the decrease in amino acids incorporated into protein,[38,39] which were also supported by our data. In contrast, certain increased levels of CP concentration in alfalfa were found in wetter cultivated spots.[40,41] Distinctively, Nielsen[11] found that the CP levels showed inconsistent variation from year to year (2001−2008) in soybean forages under four irrigation treatments.[11] The possible reasons for these controversial results might be partly associated with the precipitation conditions as well as nitrogen fertilizer supply as reviewed by Peyraud and Astigarraga.[42] Additionally, the CP concentrations varied ($P < 0.05$) among different cultivars. The averaged CP content of Pickseed was 10.5%, which was at the highest level, whereas that of Pioneer was at the lowest level (9.19%); an intermediate level (9.92%) was observed in the Hyland cultivar. The CP values in Pioneer and Hyland were much greater than those found in a very recent publication[34] in which these two cultivars had similar CP contents (averaged at 7.1% DM). This variation might be related to different growing years between two studies (2011 vs 2012) as discussed by Nielsen.[11] The variation might be also due to the analysis methods: NIR in this study versus wet chemical analysis. As for the carbohydrate composition like CF in the corn forages, there was a significant interaction between irrigation treatment and corn variety. Regardless of irrigated or dry plots, HLSR22 had the greatest the content of CF, whereas HLBaxxos grown on dry land had the lowest concentration. Compared with Hyland and Pickseed, which were similar to each other ($P = 0.36$), cultivar Pioneer had the lowest level of CF content. However, the average value for CF was only 12.1% DM, which was markedly lower than the value in the Dairy One Forage Database.[43] In this database, the ranges of CF content of crops accumulated from 2000 to 2013 are 18.3−23.7%. The possible reason for these inconsistent results might be related to the different growing conditions such as climate, fertilizer application, and water use or different biological stage of corn crops at harvesting time or different chemical analysis methods (NIR vs wet chemical analysis). Additionally, the EE content was highly affected by the interaction of corn variety and irrigation implementation. The average EE concentration in corn forages was 1.11% DM, and this value was slightly beyond the range of 1.4−1.8% DM reported by Abeysekara et al.,[34] who worked on the corn forages obtained from Pioneer and Hyland.

Spectral Characteristics of Corn Forage. Table 3 gives the carbohydrate and protein molecular structural characteristics for eight varieties of corn forage grown in both irrigated and dry plots. The CHO first peak (ca. 1154 cm^{-1}), second peak (ca. 1103 cm^{-1}), and third peak (ca. 1033 cm^{-1}) exhibited significant differences ($P < 0.05$) between corn forages cultivated on wet and dry lands. The CHO peak intensity of crops under irrigation treatment was approximately 1.1-fold that of the plants grown on dry land. No variation could be found among varieties or cultivars ($P > 0.05$). Irrigation treatment interacted with corn variety on mid-IR absorbance of cellulosic compounds (CELC), and the greatest and weakest intensities were observed on Silex BT grown with water stress (0.0082 IR unit) and HLBaxxos cultivated on dry plot (0.0061 IR unit), respectively. All of these chemical functional groups' spectral intensities are related to carbohydrate structure makeup. Our results indicated that irrigations implication and corn forage variety affected carbohydrate molecular structure makeup. The peak intensity of lignin was not altered by irrigation treatment ($P > 0.05$). Moreover, among three cultivars, Pickseed had the highest IR value on CELC and the lowest value on lignin, whereas Hyland displayed the opposite features and Pioneer was intermediate. There is no literature published on the effect of irrigation on spectral characteristics of corn forage, and therefore we did not have any data to compare with. However, our results indicated that carbohydrate structure makeup in corn variety differed and irrigation implication had a significant impact on carbohydrate structure makeup.

Studying the structure of proteins leads to an understanding of the components that make up a whole protein. An understanding of the structure of the whole protein is often vital to understanding its digestive behavior and nutritive quality. Protein secondary structures include mainly α-helix and β-sheet. The percentage of these two structures in protein secondary structures influences protein nutritive value because protein structure affects access to gastrointestinal digestive enzymes, which affect protein value. All of the protein spectral parameters were significantly influenced by both irrigation treatment and corn forage variety but without any interaction. The crops treated with water stress had 6.5−19.7% stronger peak intensities ($P < 0.05$) on protein amide I, amide II, α-helix, and β-sheet. These differences in FTIR spectral traits indicated that water use during growing period did influence structural conformations of corn forage, which might be highly related to nutritive values as shown in Table 2. Also, the protein structural features varied among corn varieties or cultivars. P3085 and HLR219 showed the highest amide I peak height, whereas P7443 exhibited the lowest peak height and the remaining five were in the middle. This might be one possible explanation of the variation in CP content (Table 2). If these eight varieties of corn forage were ranked on the basis of protein secondary structural profiles (α-helix and β-sheet), HL3085, HLR219, and HLSR22 showed the greatest values of α-helix; besides these

DOI: 10.1021/jf505911a
J. Agric. Food Chem. 2015, 63, 2383−2391

Table 4. Peak Intensity Ratios of Different Varieties of Corn Forage Grown with or without Irrigation

	cultivar	variety	CHO third peak: CELC	CHO third peak: lignin	CELC: lignin	amide I:II	α-helix:β-sheet	CHO third peak: amide I	amide I: lignin	amide II: lignin
irrigation	Pioneer	P2501	13.54	25.74	1.94	1.45	0.90	3.39	7.56	5.20
		P39m26	13.83	25.08	1.81	1.33	0.96	3.59	6.95	5.21
		P7443	14.55	27.17	1.88	1.39	0.98	3.61	7.29	5.19
	Hyland	HL3085	14.98	22.85	1.53	1.42	0.94	3.22	7.07	4.96
		HLBaxxos	11.91	25.24	2.18	1.44	0.90	3.41	7.38	5.12
		HLR219	13.49	24.06	1.79	1.42	0.93	3.29	7.28	5.12
		HLSR22	12.75	22.83	1.85	1.45	0.91	3.22	7.08	4.89
	Pickseed	Silex BT	11.79	26.30	2.26	1.46	0.88	3.46	7.58	5.19
mean			13.35	24.91	1.91	1.42	0.92	3.40	7.27	5.11
dry land	Pioneer	P2501	12.29	27.37	2.24	1.45	0.92	3.94	6.94	4.77
		P39m26	11.59	22.77	1.94	1.34	1.06	3.61	6.07	4.43
		P7443	13.17	24.98	1.90	1.34	0.90	3.71	6.75	5.00
	Hyland	HL3085	12.52	20.08	1.60	1.37	0.99	3.36	5.88	4.24
		HLBaxxos	13.97	22.71	1.64	1.38	0.96	3.45	6.60	4.77
		HLR219	13.63	22.79	1.71	1.39	0.98	3.41	6.52	4.63
		HLSR22	12.68	21.06	1.67	1.39	0.93	3.39	6.22	4.48
	Pickseed	Silex BT	12.52	47.53	3.99	1.43	0.90	3.24	15.56	9.94
mean			12.80	26.16	2.09	1.39	0.96	3.51	7.57	5.28
statistical analysis										
SEM			0.406	5.049	0.427	0.019	0.027	0.077	1.719	0.997
P	irrigation		0.01	0.60	0.38	0.0002	0.003	0.001	0.73	0.72
	variety		0.0002	0.045	0.01	<0.0001	<0.0001	<0.0001	0.046	0.055
	variety × irrigation		<0.0001	0.19	0.23	0.31	0.01	<0.0001	0.10	0.08
contrast	Pioneer vs Hyland		0.72	0.28	0.36	0.01	0.39	<0.0001	0.85	0.72
	Pioneer vs Pickseed		0.003	0.004	0.001	<0.0001	0.001	<0.0001	0.001	0.001
	Hyland vs Pickseed		0.001	0.0002	<0.0001	0.01	0.004	0.94	0.0003	0.0003

three varieties, P2501 and Silex BT were also at the greatest level for β-sheet intensity. Then, P39m26 exhibited the smallest peak intensity for both α-helix and β-sheet. Hyland had greater IR absorbance of amide I, α-helix, and β-sheet than Pioneer. Such results were not completely coincident with recent findings of Abeysekara et al.,[34] who reported that only protein amide II peak height significantly differed between Pioneer and Hyland. In fact, varieties P7443 and HLBaxxos were also used in their study. Even so, they displayed quite different profiles of protein structure. This interesting phenomenon might be attributed to different growing years between these two research studies (2011 vs 2012).

Peak height ratios of eight varieties of corn forage in irrigated and dry lands are presented in Table 4. Irrigation treatment and corn varieties interacted significantly to affect peak height ratios of CHO third peak to CELC, α-helix to β-sheet, and CHO third peak to amide I. Among different cultivars, the ratios of CHO third peak to lignin, CELC to lignin, and amide I to lignin as well as amide II to lignin showed a similar trend that Pickseed had much higher values ($P < 0.05$) than the other two cultivars ,which were not far from each other. Height ratio of amide I to amide II was remarkably modified ($P = 0.0002$) by irrigation treatment. Compared with the crops under water stress (1.42), corn forage on dry land had a smaller ratio value (1.39). These results implied that molecular structures in corn forages did not remain constant, and they could be altered by growing conditions such as irrigation implementation. Again, few studies were directed to the effect of different irrigation conditions on spectral ratio profiles of crops, so no results we can be referred to. However, as we discussed earlier, during the growing period, the plants need to take up nutrients and essential elements by ion transportation from soil to roots, from roots to stems, and from stems to leaves for their growth and development. Water is the best transmission medium for the whole process, so the soil water deficit could cause reduced transpiration, poor efficiency of nutrient transportation, and delayed cell elongation,[28,44,45] which might lead to a poor development of molecular conformation in the plants.

Additionally, although differences in peak intensity were statistically significant among treatments, the variation ranges were generally narrow. According to our knowledge, Fourier transform infrared (FTIR) spectroscopy has already been successfully applied to detect structural differences induced by transgenic modification,[46] heat treatment,[14,15] bioethanol processing,[16,17,47] and climate changes such as frost damage.[48] Then on the basis of our findings mentioned above, even small variations existed in structural makeup of corn forages grown on wet and dry soil could also be detected by this powerful and accurate spectroscopic technique.

Information on molecular structural makeup in forage in relation to nutrient availability is very limited or unknown. Future study is needed to see how structural changes and how structural makeup are affected by irrigation and corn variety and how structural changes affect nutrient utilization and availability. This is a novel research area in feed research.

Principal Component Analysis within the Near-Infrared Region (Figure 1). The detailed principal, variable selections, variable reduction, distance method, matrix method, principal components selection, and explanation of hierarchical cluster analysis and principal component analysis in feed structural research have been explained previously.[21] In the study, we used synchrotron-based FTIR microspectroscopy as an example. The main purpose of PCA and CLA analyses is to detect treatment differences based on structural spectral data at

DOI: 10.1021/jf505911a
J. Agric. Food Chem. 2015, 63, 2383−2391

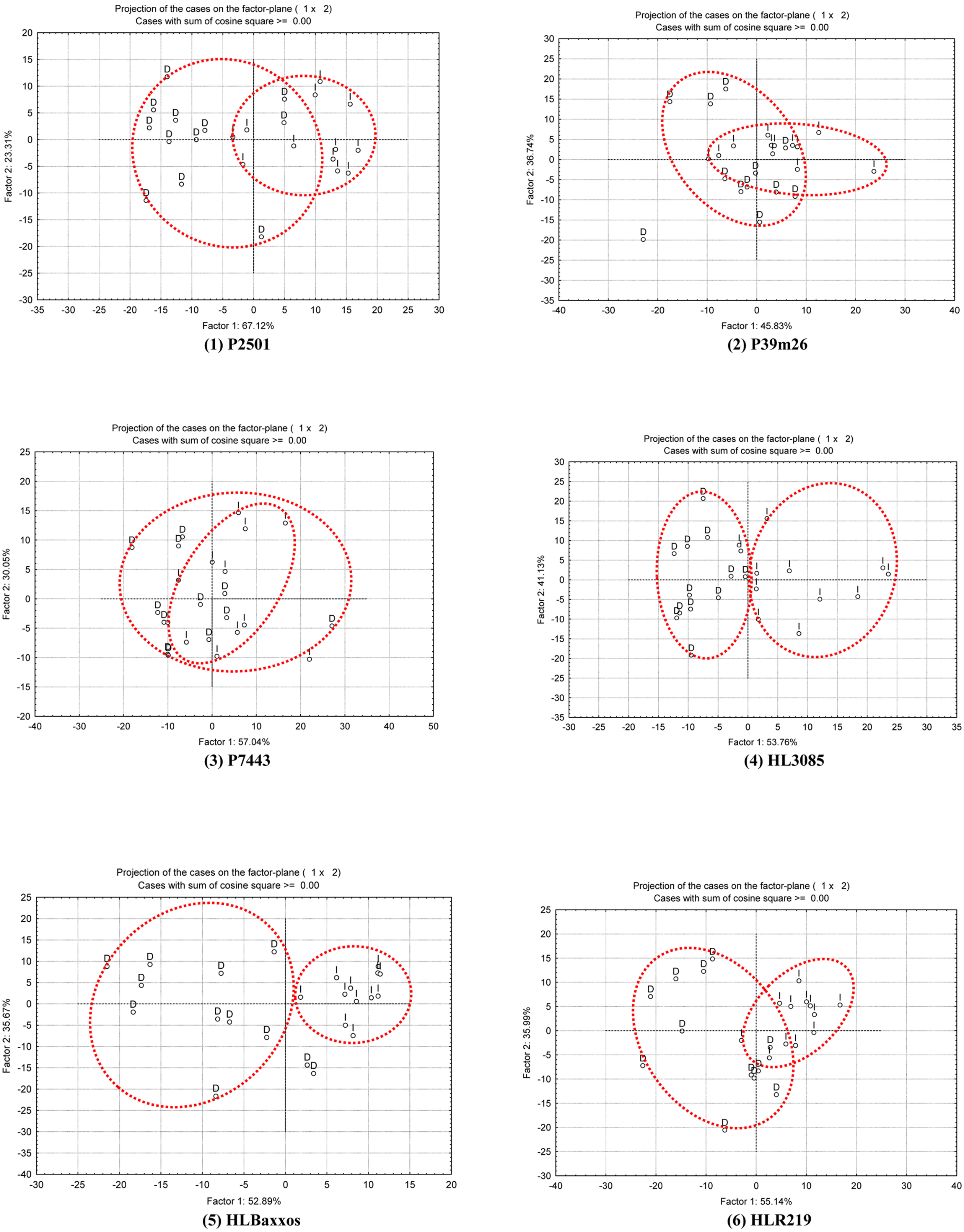
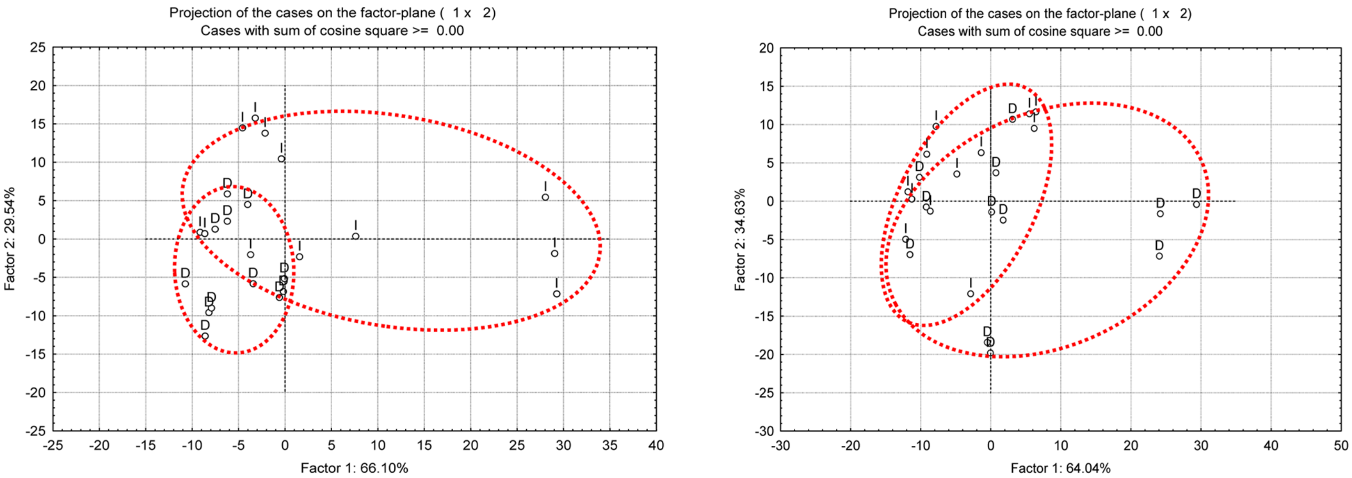


Figure 1. continued

DOI: 10.1021/jf505911a
J. Agric. Food Chem. 2015, 63, 2383−2391



(7) HLSR22 (8) Silex BT

Figure 1. Principal component analysis within the NIR region (1842−570 nm) between irrigated (I) and dry (D) land corn forages.

fingerprint regions. If varieties form different clusters and groups, this indicated the structural difference between varieties.

NIR spectroscopy is performed on the basis of molecular information and combination vibrations, and every item should have its particular scanning spectrum within the detection region. In our study, to clarify whether irrigation treatment brings out any internal changes in molecular structural conformation, we selected all of the spectral data (ca. 1842−570 nm) of each corn variety obtained from the NIR system for PCA. For this multivariate analysis, the first and second principal components explained 45.83−67.12 and 23.31−41.13%, respectively, of the total variance in PCA figures. As can be seen from the figures, only for the varieties HL3085 and HLBaxxos could the structural makeup of the forage cultivated under water stress be almost distinguished from that grown on dry land. For the remaining six varieties, targeted plants from wet and dry experimental plots could not be grouped into separate ellipses owing to the produced heavy overlapping of groups. These results indicated that for most varieties, internal structural relationships in molecular makeup were exhibited between irrigated corn forage and that grown on dry land.

All of the results shown above might lead one to conclude that irrigation treatment was an influential factor in terms of agronomic measures and chemical compositions as well as molecular structural parameters over different kinds of corn forage. As for DM, OM, and CP contents as well as most spectral parameters, the well-watered plant was quite different from that cultivated on dry land. Moreover, water use interacted with corn variety on most agronomic features and CF and EE contents as well as some spectral data such as CELC peak intensity, peak ratios of CHO third peak to CELC, α-helix to β-sheet, and CHO third peak to amide I. Also, significant differences were often observed among three cultivars on these measured parameters. Multivariate results from PCA indicated some structural similarities existed between two treated corn forages with the exception of HL3085 and HLBaxxos. Further study on the optimum level of irrigation for corn forage cultivation might be helpful in semiarid regions such as western Canada.

■ AUTHOR INFORMATION

Corresponding Author

*(P.Y.) Phone: (306) 966-4132. Fax: (306) 966-4151. E-mail: peiqiang.yu@usask.ca.

Present Address

[⊥]College of Animal Sciences and Technology, Northeast Agricultural University, Harbin China.

Funding

Research projects of the feed research chair group of Professor Dr. Peiqiang Yu are supported by grants from the Ministry of Agriculture Strategic Research Chair Program, the Saskatchewan Agricultural Development Fund (ADF), the Natural Sciences and Engineering Research Council of Canada (NSERC), SaskCanola, the Thousand-Talent-People Research Program and University Innovation Team "Veterinary Biotechnology" (TD12-5019, Tianjin), Saskatchewan Forage Network (SNK), SaskMilk, and Western Grain Research Foundation (WGRF).

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We gratefully acknowledge the vital assistance in corn forage sample supply by Garry Hnatowich at Canada−Saskatchewan Irrigation Diversification Centre (Outlook, SK, Canada) and valuable advice and guidance from Dr. David Christensen. We thank Zhiyuan Niu for kind assistance in sampling and laboratory analysis in this study.

■ REFERENCES

(1) Coors, J. G.; Lauer, J. G. Silage corn. In *Specialty Corns*, 2nd ed.; Hallauer, A. R., Ed.; CRC Press: Boca Raton, FL, USA, 2001; pp 347−392.

(2) Khan, N.; Theodoridou, K.; Yu, P. Chapter 5. Role of fiber in dairy nutrition and health. In *Dietary Fiber: Production Challenges, Food Sources and Health Benefits*; Clemens, M. E., Ed.; Nova Science Publishers: New York, 2015; pp 69−91, ISBN 978-1-63463-655-1.

(3) Khan, N. A.; Yu, P.; Ali, M.; Cone, J. W.; Hendriks, W. H. Invited Review: Nutritive value of maize silage in relation to dairy cow performance and milk quality. *J. Food Agric. Sci. (England)* **2015**, 95, 238−252.

(4) Statistics Canada, Historical Overview of Canadian Agriculture, 1976−1996, Statistics Canada, Catalog 93-359-XPB, 1997.

DOI: 10.1021/jf505911a
J. Agric. Food Chem. 2015, 63, 2383−2391

(5) Roth, G.; Undersander, D.; Allen, M.; Ford, S.; Harrison, J.; Hunt, C.; Lauer, J.; Muck, R.; Soderlund, S. *Corn Silage Production, Management and Feeding*; American Society of Agronomy: Madison, WI, USA, 1995; p 21, ISSN 0021-8561.
(6) Nielsen, D. C.; Vigil, M. F.; Benjamin, J. G. Forage yield response to water use for dryland corn, millet, and triticale in the central great plains. *Agron. J.* **2006**, *98*, 992−998.
(7) Janhanzad, E.; Jorat, M.; Moghadam, H.; Sadeghpour, A.; Chaichi, M.-R.; Dashtaki, M. Response of a new and a commonly grown forage sorghum cultivar to limited irrigation and planting density. *Agric. Water Manage.* **2013**, *117*, 62−69.
(8) Tahir, S. M. Fertilizers alleviate moisture stress. In *17th Coll. Int. Potash Institute, Farm Mechanization for Profit*; Worth, B., Xin, J., Eds.; Granada Publishing: London, UK, 1983; 269 pp.
(9) House, L. R. *A Guide to Sorghum Breeding*; International Crops Research Institute for Semiarid Tropics: Patancheru, India, 1985; pp 344−502.
(10) Saeed, J. A. M.; El-Nadi, A. H. Forage sorghum yield and water use efficiency under variable irrigation. *Irrig. Sci.* **1998**, *18*, 67−71.
(11) Nielsen, D. C. Forage soybean yield and quality response to water use. *Field Crop Res.* **2011**, *124*, 400−407.
(12) Fereres, E.; Soriano, M. A. Deficit irrigation for reducing agricultural water use. *J. Exp. Bot.* **2006**, *58*, 147−159.
(13) Van Soest, P. J. Environment and forage quality. In *Proceedings, Cornell Nutrition Conference for Feed Manufacturers*, Rochester, NY, 1996; pp 224−238.
(14) Doiron, K. J.; Yu, P.; McKinnon, J. J.; Christensen, D. A. Heat-induced protein structures and protein subfractions in relation to protein degradation kinetics and intestinal availability in dairy cattle. *J. Dairy Sci.* **2009**, *92*, 3319−3330.
(15) Abeysekara, S.; Yu, P. Response and sensitivity of lipid related molecular structure to wet and dry heating in canola tissue. *Spectrochim. Acta Part A: Mol. Biomol. Spectrosc.* **2012**, *90*, 63−71.
(16) Zhang, X.; Yu, P. Using ATR-FT/IR molecular spectroscopy to detect effects of blend DDGS inclusion level on the molecular structure spectral and metabolic characteristics of the proteins in hulless barley. *Spectrochim. Acta Part A: Mol. Biomol. Spectrosc.* **2012**, *95*, 53−63.
(17) Zhang, X.; Yu, P. Relationship of carbohydrate molecular spectroscopic features in combined feeds to carbohydrate utilization and availability in ruminants. *Spectrochim. Acta Part A: Mol. Biomol. Spectrosc.* **2012**, *92*, 225−233.
(18) AOAC. *Official Methods of Analysis*; Association of Official Analytical Chemists: Washington, DC, USA, 1990.
(19) Xin, H.; Falk, K. C.; Yu, P. Studies on *Brassica carinata* seed. 1. Protein molecular structure in relation to protein nutritive values and metabolic characteristics. *J. Agric. Food Chem.* **2013**, *61*, 10118−10126.
(20) Liu, N.; Yu, P. Molecular clustering, interrelationships and carbohydrate conformation in hull and seeds among barley cultivars. *J. Cereal Sci.* **2011**, *53*, 379−383.
(21) 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. *J. Agric. Food Chem.* **2005**, *53*, 7115−7127.
(22) Graybill, J. S.; Cox, W. J.; Otis, D. J. Yield and quality of forage maize as influenced by hybrid, planting date, and plant density. *Agron. J.* **1991**, *83*, 559−564.
(23) Jones, R. M.; Sanderson, M. A.; Read, J. C.; Lovell, A. C. Management of corn for silage production in south central USA. *J. Prod. Agric.* **1995**, *8*, 175−180.
(24) Payero, J. O.; Tarkalson, D. D.; Irmak, S.; Davison, D.; Petersen, J. L. Effect of timing of a deficit-irrigation allocation on corn evapotranspiration, yield, water use efficiency and dry mass. *Agric. Water Manage.* **2009**, *96*, 1387−1397.
(25) Bathke, G. R.; Gassel, D. K.; Hargrove, W. L.; Porter, P. M. Modification of soil physical properties and root growth response. *Soil Sci.* **1992**, *154*, 316−329.
(26) Turner, N. C. Stomatal behavior and water status of maize, sorghum, and tobacco under field conditions. II. At low soil water potential. *Plant Physiol.* **1974**, *55*, 360−365.
(27) Masle, J.; Passiour, J. B. The effect of soil strength on the growth of young wheat plants. *Aust. J. Plant Physiol.* **1987**, *14*, 643−656.
(28) Kramer, O. J. K.; Boyer, J. S. *Water Relations of Plant and Soils*; Academic Press: New York, 1995.
(29) Ertek, A.; Kara, B. Yield and quality of sweet corn under deficit irrigation. *Agric. Water Manage.* **2013**, *129*, 138−144.
(30) Berenguer, M. J.; Faci, J. M. Sorghum (*Sorghum bicolor* L. Moench) yield compensation processes under different plant densities and variable water supply. *Eur. J. Agron.* **2001**, *15*, 43−55.
(31) Vasilakoglou, I.; Dhima, K.; Karagiannidis, N.; Gatsis, T. Sweet sorghum productivity for bio-fuels under increased soil salinity and reduced irrigation. *Field Crop Res.* **2011**, *120*, 38−46.
(32) NeSmith, D. S.; Ritchie, J. T. Effects of soil water-deficits during tassel emergence on development and yield component of maize (*Zea mays*). *Field Crop Res.* **1992**, *28*, 251−256.
(33) Abrecht, D. G.; Carberry, P. S. The influence of water deficit prior to tassel initiation on maize growth, development and yield. *Field Crop Res.* **1993**, *31*, 55−69.
(34) Abeysekara, S.; Christensen, D. A.; Niu, Z.; Theodoridou, K.; Yu, P. Molecular structure, chemical and nutrient profiles, and metabolic characteristics of the proteins and energy in new cool-season corn varieties harvested as fresh forage for dairy cattle. *J. Dairy Sci.* **2013**, *96*, 6631−6643.
(35) Assefa, G.; Ledin, I. Effect of variety, soil type and fertilizer on the establishment, growth, forage yield, quality and voluntary intake by cattle of oats and vetches cultivated in pure stands and mixtures. *Anim. Feed Sci. Technol.* **2001**, *92*, 95−111.
(36) Haberle, J.; Svoboda, P.; Raimanova, I. The effect of post-anthesis water supply on grain nitrogen concentration and grain nitrogen yield of winter wheat. *Plant Soil Environ.* **2008**, *54*, 304−312.
(37) Pessarakli, M. M.; Morgan, P. V.; Gilbert, J. Dry-matter yield, protein synthesis, starch, and fiber content of barley and wheat plants under two irrigation regimes. *J. Plant Nutr.* **2005**, *28*, 1227−1241.
(38) Von Mothes, K. *Encyclopedia of Plant Physiology*; Springer Verlag: Berlin, Germany, 1956; pp 656−664.
(39) Kahane, I.; Poljakoff-Mayber, A. Effect of substrate salinity on the ability for protein synthesis in pea roots. *Plant Physiol.* **1968**, *43*, 1115−1119.
(40) Halim, R. A.; Buxton, D. R.; Hattendorf, M. J.; Carlson, R. E. Crop water stress index and forage quality relationships in alfalfa. *Agron. J.* **1990**, *82*, 906−909.
(41) Deetz, D. A.; Jung, H. G.; Buxton, D. R. Water-deficit effects on cell-wall composition and in vitro degradability of structural polysaccharides from alfalfa stems. *Crop Sci.* **1996**, *36*, 383−388.
(42) Peyraud, J. L.; Astigarraga, L. Review of the effect of nitrogen fertilization on the chemical composition, intake, digestion and nutritive value of fresh herbage: consequences on animal nutrition and N balance. *Anim. Feed Sci. Technol.* **1998**, *72*, 235−259.
(43) Dairy One; http://www.dairyone.com/forage/feedcomp/Main_GetResults.asp.
(44) Heyn, A. N. J. The physiology of cell elongation. *Bot. Rev.* **1940**, *6*, 515−574.
(45) Vaadia, Y.; Rane, F. C.; Hagen, R. M. Plant water deficits and physiological process. *Annu. Rev. Plant Physiol.* **1961**, *12*, 265−292.
(46) Yu, P.; Jonker, A.; Gruber, M. Molecular basis of protein structure and nutritive value in proanthocyanidin-enhanced Lc-transgenic alfalfa using synchrotron-radiation FTIR microspectroscopy. *Spectrochim. Acta A: Mol. Biomol. Spectrosc.* **2009**, *73*, 846−853.
(47) Yu, P.; Niu, Z.; Damiran, D. Protein molecular structures and protein fraction profiles of new co-products of bioethanol production: a novel approach. *J. Agric. Food Chem.* **2010**, *58*, 3460−3464.
(48) Xin, H.; Zhang, X.; Yu, P. Using synchrotron radiation-based infrared microspectroscopy to reveal microchemical structure characterization: frost damaged wheat vs. normal wheat. *Int. J. Mol. Sci.* **2013**, *14*, 16706−16718.

DOI: 10.1021/jf505911a
J. Agric. Food Chem. 2015, 63, 2383−2391