# Characterizations of Structural, Biochemical, and Nutritive Profiles in Silage among Cool-Season Corn Cultivars in Relation to Heat Units (aCHU, dCHU) with Curvilinear Response and Multivariate Analyses

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**ABSTRACT:** Molecular spectroscopy is able to reveal structural features of biomaterials. Corn grown in Canadian prairies is known as cool-season corn, which is different from warm-season corn varieties. To our knowledge, to date, there has been no study on the magnitude difference in structure on a molecular basis among cultivars, no study on biochemical and nutritive profiles associated with heat unit, and no study on how heat unit affects the molecular structure and biochemical and nutritive profiles. This study investigates how corn varieties grown in cooler climates are affected by crop heat units (CHU) in relation to molecular spectral profiles, nutrient storage, biochemical composition, and nutritive value of silage among different cool-season corn cultivars. Corn cultivars (Pioneer and Dekalb) were from seven farm locations, and samples were analyzed for major nutrients (digestible and metabolic energy and protein). The Fourier transform infrared (FT/IR) spectroscopic technique was applied to understand and differentiate molecular structural spectral profiles in silage. A correlation ($P < 0.05$) of CHU with some nutrients (mean $\pm$ SD, %DM) (CP, 8.1 $\pm$ 1.3, $r = 0.56$; NDF, 56.3 $\pm$ 3.5, $r = -0.54$; ADF, 33.6 $\pm$ 2.3, $r = -0.71$; NDICP, 1.6 $\pm$ 0.4, $r = -0.66$; SCP, 4.2 $\pm$ 1.3, $r = 0.61$), protein and carbohydrate fractions (mean $\pm$ SD, %DM) (PB1 (= fast degradable protein fraction), 1.3 $\pm$ 0.4, $r = 0.54$; PB3 (= slowly degradable protein fraction), 1.5 $\pm$ 0.4, $r = -0.74$; CB2 (= medium degradable carbohydrate fraction), 45.1 $\pm$ 2.8, $r = -0.65$; CB3 (= slowly degradable carbohydrate fraction), 13.9 $\pm$ 0.9, $r = -0.54$) and intestinal availability of ruminally degraded fractions (mean $\pm$ SD, %DM) (rdPB1, 1.1 $\pm$ 0.3, $r = 0.54$; rdPB3, 1.0 $\pm$ 0.3, $r = -0.74$; RDP, 6.6 $\pm$ 1.2, $r = 0.59$; rdCB2, 40.0 $\pm$ 2.5, $r = -0.65$; rdCB3, 8.9 $\pm$ 0.6, $r = 0.54$; RDCHO, 50.1 $\pm$ 2.9, $r = -0.65$) was found contentious. Molecular spectral data indicated many similarities and few differences among the cultivars. However, CHU correlated ($r = -0.4$, $P < 0.05$) with molecular spectral intensity ratio of carbohydrate to amide I. This result indicates that molecular structural differences may be influenced by epiphytic bacterial compounds. Cool corn cultivars were grown acceptably well in cooler dry climates, and those silages had acceptable nutrient levels for cattle. Cultivars that reached target CHU were found to be optimal in nutrient and energy synchronization aspect.

**KEYWORDS:** crop heat unit, Canadian Prairies corn silage, structure, forage quality, curvilinear response analysis

## INTRODUCTION

Corn has been used globally as an ensiled forage particularly in Europe, United States, China, and Canada.[1,2] Use of corn as a feed in the recent past is little less than 40% of global corn production.[3] Canadian forage production of corn is widely spread in Eastern provinces: approximately 60% in Ontario and 20% in Quebec.[4] Corn grown in Canadian prairies (Saskatchewan) is known as cool-season corn and is different from conventional corn grown in warm climatic conditions such as in United States.[5] The main differences are due to the growth modifications suited to a shorter growing season and relatively low temperatures (cool) in Saskatchewan compared to the original growth pattern of warm season corn in United States.[6] These growth differences influenced by the weather conditions lead to changes in plant height, stem strength, leaf size, cob size, seed count, and storing compounds of the plant and hence cause changes in chemical profiles and nutrient compositions of silages.[7,8] However, these nutritional changes in terms of chemical profile, nutrient availability, and molecular structural characteristics are not well understood yet. Determining whether a corn silage grown in Saskatchewan (cool climate)[9] can be as nutritionally

effective as the counterpart grown in United states (warm climate) is important because it may be substituted for commonly used but expensive silages such as barley.[10]

In relation to corn cultivation, crop heat units (CHU) are calculated from daytime temperatures above 10 °C and nighttime temperatures above 4.4 °C on a daily basis from seeding to harvest. In general, many corn cultivars require 2000 or more CHU to reach silage harvest stage with kernel maturity of 45% dry matter. A common visual maker used by farmers to identify this stage is a white line extending about halfway down the kernel.[9] This white line appears due to crystallized starch at this stage, and almost all of the potential starch will be in the kernel. However, this is inconclusive information; therefore, there is a need for further information on corn cultivars and heat unit requirements for optimum maturity in Saskatchewan conditions. In this project, an evaluation of corn silage stage of maturity

**Table 1. Crop Heat Units, Chemical Profile and Energy Values in Corn Silage: Comparison of Cultivars[a]**

| item | Pioneer P7213R | Dekalb 2679 | Pioneer 39B61 | Pioneer 39F45 | Pioneer 39M26 | Pioneer 39D95 | Pioneer H39D95 | SD | range |
|---|---|---|---|---|---|---|---|---|---|
| aCHU | 2185 | 2274 | 2301 | 2112 | 2078 | 1849 | 2221 | | |
| dCHU | 135 | 124 | 201 | 112 | −22 | −301 | 71 | | |
| *Chemical Profile, % DM* | | | | | | | | | |
| dry matter, % | 28.26 | 31.01 | 32.65 | 33.72 | 29.48 | 33.59 | 32.76 | 2.12 | 5.47 |
| ash | 5.30 | 5.03 | 5.28 | 5.19 | 6.16 | 5.27 | 4.83 | 0.46 | 1.51 |
| crude protein | 7.98 | 8.34 | 10.04 | 9.19 | 8.01 | 6.39 | 6.53 | 1.27 | 3.96 |
| crude fat | 1.64 | 1.89 | 1.84 | 2.11 | 1.89 | 1.83 | 1.55 | 0.18 | 0.65 |
| NDF | 58.44 | 52.23 | 57.73 | 57.01 | 52.92 | 61.74 | 53.90 | 3.51 | 11.14 |
| ADF | 33.26 | 29.87 | 33.77 | 35.00 | 32.64 | 37.26 | 33.64 | 2.25 | 7.84 |
| ADL | 3.95 | 3.71 | 5.69 | 4.16 | 3.22 | 4.29 | 2.84 | 0.89 | 3.11 |
| NDICP | 1.39 | 1.60 | 1.74 | 1.61 | 1.44 | 2.43 | 1.20 | 0.40 | 1.51 |
| ADICP | 0.19 | 0.22 | 0.22 | 0.25 | 0.06 | 0.06 | 0.02 | 0.10 | 0.30 |
| SCP | 4.29 | 4.78 | 5.51 | 6.02 | 3.78 | 2.18 | 3.07 | 1.30 | 4.02 |
| NPN | 3.17 | 2.89 | 4.04 | 4.56 | 3.07 | 1.06 | 1.66 | 1.20 | 3.63 |
| starch | 8.49 | 15.72 | 6.00 | 10.64 | 15.03 | 5.71 | 11.06 | 3.87 | 10.26 |
| *Truly Digestible Nutrient, % DM* | | | | | | | | | |
| tdNDF | 33.12 | 29.04 | 29.52 | 31.59 | 30.49 | 34.09 | 32.06 | 1.97 | 6.67 |
| tdCP | 0.97 | 0.97 | 0.97 | 0.96 | 0.99 | 0.98 | 1.00 | 0.01 | 0.04 |
| tdFA | 0.64 | 0.89 | 0.84 | 1.11 | 0.89 | 0.83 | 0.55 | 0.18 | 0.65 |
| tdNFC | 27.47 | 33.42 | 26.30 | 27.55 | 31.81 | 26.64 | 33.70 | 3.35 | 10.73 |
| *Total Digestible Nutrients at a Maintenance Level, % DM* | | | | | | | | | |
| TDN | 62.25 | 64.99 | 60.72 | 63.34 | 64.64 | 60.98 | 65.41 | 1.93 | 6.06 |
| *Energy Values for Dairy and Beef, Mcal·kg$^{-1}$* | | | | | | | | | |
| DE$_{3\times}$ | 2.74 | 2.86 | 2.67 | 2.79 | 2.84 | 2.68 | 2.87 | 0.08 | 0.27 |
| ME$_{3\times}$ | 2.52 | 2.63 | 2.45 | 2.56 | 2.61 | 2.46 | 2.64 | 0.07 | 0.24 |
| NEl$_{3\times}$ | 2.09 | 2.20 | 2.03 | 2.13 | 2.19 | 2.04 | 2.22 | 0.07 | 0.25 |
| NEm | 1.37 | 1.47 | 1.33 | 1.41 | 1.46 | 1.34 | 1.48 | 0.06 | 0.19 |
| NEg | 0.80 | 0.88 | 0.75 | 0.83 | 0.87 | 0.76 | 0.89 | 0.05 | 0.17 |

[a]SD, standard deviation; aCHU, achieved crop heat units; dCHU, difference CHU from achieved to target; NDF, neutral detergent fiber; ADF, acid detergent fiber; ADL, acid detergent lignin; NDICP, neutral detergent insoluble crude protein; ADICP, acid detergent insoluble crude protein; SCP, soluble crude protein; NPN, non protein nitrogen; td, total digestible; TDN, total digestible nutrients; DE, digestible energy; ME, metabolisable energy; NE, net energy; 3×, at production level of intake; l, lactation; m, maintenance; g, growth.

with an emphasis on the nutritional characteristics is carried out for ration formulation with advanced models such as National Research Council (NRC) 2001 and Cornell CNCP.[11]

To our knowledge, there has been no study on the magnitude difference in structure on a molecular basis among cultivars, and no study on how heat unit affects the molecular structure and biochemical and nutritive profiles. This study investigated how corn varieties grown in cooler climates are affected by CHU in relation to molecular structural spectral profiles, nutrient storage, biochemical composition, and nutritive value of silage among different cool-season corn cultivars.

The detailed objectives of this project were to evaluate molecular structural, chemical, and nutrient profiles of Saskatchewan cool-season corn silage and their availability to ruminants, mainly to dairy and feedlot cattle. However, the main objective drives many goals: (1) to determine chemical characterizations and nutrient profiles of the cool-season corn cultivars; (2) to measure the relationship between CHU and nutritive values; (3) to assess protein (PA, PB1, PB2, PB3, PC) and carbohydrate (CA, CB1, CB2, CC) subfractions (CNCPS) which are closely related to animal digestive behaviors and ration formulation; (4) to predict nutrient flow to intestine using CNCPS model (such as rumen bypass or undegraded CP based on each fraction PA, PB1, PB2, PB3, PC and degradation rate and passage rate for each fractions, rumen bypass CHO etc); (5) to determine energy values (tdNDF, tdCP, tdFA, tdNFC, TDN1x, DE1x, DE, ME, NE) of the cool-season corn silage samples; (6)

to determine the impact of the cool-season corn on ruminal fermentation characteristics and rumen degradation kinetics (DM, CP, starch, OM, NDF); and (7) to reveal and compare inherent molecular structural spectral features of the cool-season corn as a novel approach. In particular, differences in specific molecular absorption bands associated with compounds interfere with animal digestion and utilization of materials were identified using molecular spectroscopy.

## ■ MATERIALS AND METHODS

**Farm Locations.** In order to study the relationship between CHU and the nutritive and structure profile of cool-season corn silage, in 2010 summer, seven different locations/farms in Saskatchewan were selected for corn silage sample collection. A number of seed companies assisted in finding those dairy farms that planted corn forage cultivars and provided seeding and harvest dates for the 2010 growing season. On the basis of the farm location within one of the 33 weather zones in Saskatchewan, CHUs were calculated from the Weather Farmzone Website: http://www.farmzone.com/index.php?product=farmzone&pagecontent=saskatchewan.

"CHU is an energy term calculated for each day and accumulated from planting to the harvest date. They are calculated daily using the daily maximum and minimum temperature for each area. Source: http://www.ontarioweather.com/industry/agriculture/agrcornheat.asp"

*Sampling from Farm Locations.* Cultivars used in this study for corn silage were Pioneer P7213R, Pioneer 39B61, Pioneer 39F45, Pioneer 39M26, Pioneer 39D95, Pioneer H39D95, and Dekalb 2679. They were in four farmzones: (1) Saskatoon, latitude 52.1°; (2)

**Table 2. Relationship of Crop Heat Units and Chemical Profiles and Energy Estimates in Corn Silage**[a]

| item | mean ± SD | range | r aCHU | P-value | | | | r dCHU | P-value | | | |
| --- | --- | --- | --- | correlation | linear | quadratic | cubic | --- | correlation | linear | quadratic | cubic |
| | | | | Chemical Profile, % DM | | | | | | | | |
| DM | 31.64 ± 2.12 | 5.47 | 0.54 | 0.048 | 0.067 | 0.512 | 0.939 | 0.38 | 0.179 | 0.116 | 0.071 | 0.095 |
| ash | 5.30 ± 0.46 | 1.51 | −0.27 | 0.351 | 0.234 | 0.127 | 0.020 | −0.19 | 0.513 | 0.383 | 0.143 | 0.012 |
| CP | 8.07 ± 1.27 | 3.96 | 0.56 | 0.037 | 0.008 | 0.853 | 0.005 | 0.71 | 0.005 | 0.002 | 0.094 | 0.072 |
| EE | 1.82 ± 0.18 | 0.65 | −0.17 | 0.553 | 0.473 | 0.568 | 0.019 | −0.01 | 0.98 | 0.989 | 0.885 | 0.762 |
| NDF | 56.28 ± 3.51 | 11.14 | −0.54 | 0.048 | 0.049 | 0.181 | 0.601 | −0.43 | 0.125 | 0.052 | 0.007 | 0.628 |
| ADF | 33.63 ± 2.25 | 7.84 | −0.71 | 0.004 | 0.009 | 0.574 | 0.828 | −0.60 | 0.024 | 0.026 | 0.175 | 0.942 |
| ADL | 3.98 ± 0.89 | 3.11 | 0.11 | 0.706 | 0.627 | 0.031 | 0.078 | 0.18 | 0.543 | 0.129 | 0.001 | 0.017 |
| NDICP | 1.63 ± 0.40 | 1.51 | −0.66 | 0.010 | 0.001 | 0.001 | 0.182 | −0.68 | 0.007 | 0.001 | 0.002 | 0.791 |
| ADICP | 0.15 ± 0.10 | 0.30 | 0.41 | 0.144 | 0.174 | 0.886 | 0.496 | 0.55 | 0.041 | 0.037 | 0.131 | 0.512 |
| SCP | 4.23 ± 1.30 | 4.02 | 0.61 | 0.019 | 0.013 | 0.309 | 0.072 | 0.79 | 0.001 | 0.002 | 0.423 | 0.872 |
| NPN | 2.92 ± 1.20 | 3.63 | 0.50 | 0.071 | 0.026 | 0.084 | 0.024 | 0.72 | 0.003 | 0.006 | 0.781 | 0.391 |
| starch | 10.38 ± 3.87 | 10.26 | 0.28 | 0.329 | 0.304 | 0.093 | 0.654 | 0.22 | 0.441 | 0.253 | 0.002 | 0.841 |
| | | | | Truly Digestible Nutrients, % DM | | | | | | | | |
| tdNDF | 31.42 ± 1.97 | 6.67 | −0.64 | 0.012 | 0.004 | 0.673 | 0.013 | −0.57 | 0.034 | 0.036 | 0.799 | 0.162 |
| tdCP | 0.98 ± 0.01 | 0.04 | −0.34 | 0.234 | 0.275 | 0.908 | 0.720 | −0.47 | 0.088 | 0.083 | 0.185 | 0362 |
| tdFA | 0.82 ± 0.18 | 0.65 | −0.17 | 0.554 | 0.473 | 0.568 | 0.019 | −0.01 | 0.988 | 0.989 | 0.885 | 0.762 |
| tdNFC | 29.56 ± 3.35 | 10.73 | 0.31 | 0.279 | 0.303 | 0.465 | 0.521 | 0.13 | 0.670 | 0.594 | 0.014 | 0627 |
| TDN | 63.19 ± 1.93 | 6.06 | 0.29 | 0.319 | 0.283 | 0.079 | 0.479 | 0.20 | 0.488 | 0.223 | 0.001 | 0.198 |
| | | | | Energy Values for Dairy and Beef, Mcal·kg$^{-1}$ | | | | | | | | |
| $DE_{3\times}$ | ±0.08 | 0.27 | 0.29 | 0.320 | 0.283 | 0.079 | 0.479 | 0.20 | 0.488 | 0.223 | 0.001 | 0.198 |
| $ME_{3\times}$ | ±0.07 | 0.24 | 0.29 | 0.320 | 0.283 | 0.080 | 0.480 | 0.20 | 0.488 | 0.223 | 0.001 | 0.198 |
| $NEl_{3\times}$ | ±0.07 | 0.25 | 0.29 | 0.320 | 0.283 | 0.080 | 0.480 | 0.20 | 0.488 | 0.223 | 0.001 | 0.198 |
| NEm | ±0.06 | 0.19 | 0.29 | 0.321 | 0.283 | 0.078 | 0.479 | 0.20 | 0.487 | 0.221 | 0.001 | 0.195 |
| NEg | ±0.05 | 0.17 | 0.29 | 0.322 | 0.284 | 0.077 | 0.478 | 0.20 | 0.487 | 0.219 | 0.001 | 0.193 |

[a]r, Pearson correlation coefficient; aCHU, achieved crop heat units; dCHU, difference CHU from achieved to target; NDF, neutral detergent fiber; ADF, acid detergent fiber; ADL, acid detergent lignin; NDICP, neutral detergent insoluble crude protein; ADICP, acid detergent insoluble crude protein; SCP, soluble crude protein; NPN, non protein nitrogen; td, total digestible; TDN, total digestible nutrients; DE, digestible energy; ME, metabolisable energy; NE, net energy; 3×, at production level of intake; l, lactation; m, maintenance; g, growth.

Davidson-Chamberlain, latitude 51.2°; (3) Lake Diefenbaker-Lucky Lake, latitude 50.8°; and (4) Biggar-Unity, latitude 52.4°.

**Sample Analyses.** All the collected samples were analyzed for dry matter (DM), ash, organic matter (OM), crude protein (CP), crude fat (CFat), neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL), neutral detergent insoluble crude protein (NDICP), soluble crude protein (SCP), non protein nitrogen (NPN), and starch. Estimates of nutrient content and intestinal availability were calculated using NRC 2001 and CNCPS. The Fourier transform infrared molecular spectroscopic technique was applied to understand and differentiate molecular structural components. The detailed methodology and setting from the same Prof. Dr. Peiqiang Yu's lab was reported previously.

*Chemical Analyses.* Silage samples were oven-dried (55 °C for 72 h) and ground through a 1 mm screen. Dry matter (AOAC 930.15), ash (AOAC 942.05), CFat (AOAC 954.02), and CP (AOAC 984.13) contents were analyzed according to the procedure of the AOAC. The starch was analyzed using the Megazyme Total Starch Assay kit and by the α-amylase/amyloglucosidase method.[12] The ADF, NDF, and ADL values were analyzed.[13] The acid (ADIN) and neutral detergent insoluble nitrogen (NDIN) values were determined.[14] The NPN content was analyzed by precipitating of true protein with tungstic acid (samples were soaked into water with 0.3 mol L$^{-1}$ sodium tungstate ($Na_2WO_4$) for 30 min) and calculated as the difference between total nitrogen and the nitrogen content of the residue after filtration.[14] Total SCP was determined by incubating the sample with bicarbonate−phosphate buffer and filtering through Whatman #54 filter paper. The nonstructural carbohydrates (NSC) including starch, sugars, organic acids, and other reserve carbohydrates such as fructan were estimated by nonfiber carbohydrates and calculated based on the equation in NRC 2001 dairy.

*Energy Estimate.* Estimated energy contents for total digestible CP (tdCP), fatty acid (tdFA), NDF (tdNDF), NFC (tdNFC), and total digestible nutrient at 1× maintenance ($TDN_{1\times}$), digestible energy at a production level of intake ($DE_{3\times}$), metabolizable energy at a production level of intake ($ME_{3\times}$), and net energy for lactation at a production level of intake ($NEL_{3\times}$) were determined using a summative approach[15] from the NRC 2001 dairy, while net energy for maintenance (NEm) and net energy for growth (NEg) were determined using the NRC 1996. Both NRC dairy and NRC beef used the same formula to estimate NEg and NEm.

*Cornell Net Carbohydrate and Protein System.* The CP and carbohydrate (CHO) subfractions were partitioned according to the Cornell Net Carbohydrate and Protein System (CNCPS).[16] The characterization of the CP fractions as applied in this system is as follows: fraction PA is NPN, fraction PB is true protein (TP), and fraction PC is unavailable protein. Fraction PB is further divided into three fractions (PB1, PB2, and PB3). Buffer-insoluble protein minus fraction PB3 is used to estimate fraction PB2. Fraction PB2 is insoluble in buffer but soluble in neutral detergent, while fraction PB3 is insoluble in both buffer and neutral detergent but is soluble in acid detergent solution. Fraction PB2 is fermented in the rumen at a lower rate than the buffer-soluble fraction, and some PB2 fraction escapes to the lower gut. Fraction PB3 is believed to be more slowly degraded in the rumen than fractions PB1 and PB2 because of its association with the plant cell walls; a large proportion of PB3 is thus believed to escape the rumen. Fraction PC is ADIN, which is highly resistant to breakdown by microbial and mammalian enzymes, and it is assumed to be unavailable to animals.

**Statistical analysis.** Data from chemical analyses and model estimations (NRC and CNCPS) were analyzed using Statistical Analysis Systems (SAS version 9.2, SAS Institute Inc., Cary, NC, USA)

**Table 3. Protein and Carbohydrate Fractions and Intestinal Availability Estimates in Corn Silage: Comparison of Cultivars[a]**

| item | Pioneer P7213R | Dekalb 2679 | Pioneer 39B61 | Pioneer 39F45 | Pioneer 39M26 | Pioneer 39D95 | Pioneer H39D95 | SD | range |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Protein Fraction, % DM | | | | | |
| PA | 3.17 | 2.89 | 4.04 | 4.56 | 3.07 | 1.06 | 1.66 | 1.19 | 3.63 |
| PB1 | 1.12 | 1.89 | 1.47 | 1.46 | 0.71 | 1.12 | 1.41 | 0.37 | 1.45 |
| PB2 | 2.29 | 1.96 | 2.79 | 1.56 | 2.78 | 1.78 | 2.25 | 0.51 | 1.81 |
| PB3 | 1.20 | 1.38 | 1.51 | 1.36 | 1.37 | 2.37 | 1.18 | 0.41 | 1.45 |
| PC | 0.19 | 0.22 | 0.22 | 0.26 | 0.06 | 0.06 | 0.02 | 0.10 | 0.29 |
| | | | | Protein Intestinal Availability, % DM | | | | | |
| rdPA | 3.09 | 2.82 | 3.96 | 4.46 | 3.01 | 1.04 | 1.62 | 1.17 | 3.55 |
| rdPB1 | 0.91 | 1.54 | 1.19 | 1.19 | 0.58 | 0.91 | 1.15 | 0.31 | 1.18 |
| rdPB2 | 1.77 | 1.51 | 2.15 | 1.20 | 2.14 | 1.37 | 1.73 | 0.39 | 1.39 |
| rdPB3 | 0.76 | 0.88 | 0.97 | 0.87 | 0.88 | 1.52 | 0.76 | 0.26 | 0.93 |
| RDP | 6.55 | 6.75 | 8.27 | 7.72 | 6.61 | 4.84 | 5.27 | 1.18 | 3.59 |
| IRDP | 2.26 | 1.98 | 2.76 | 1.70 | 2.83 | 2.66 | 2.19 | 0.43 | 1.49 |
| RUP | 1.43 | 1.58 | 1.77 | 1.47 | 1.40 | 1.55 | 1.26 | 0.17 | 0.63 |
| IAP | 1.24 | 1.36 | 1.55 | 1.22 | 1.34 | 1.49 | 1.24 | 0.14 | 0.50 |
| | | | | Carbohydrate Fraction, %DM | | | | | |
| CA | 19.54 | 18.37 | 20.84 | 17.48 | 17.42 | 21.48 | 23.33 | 2.53 | 8.49 |
| CB1 | 8.49 | 15.72 | 5.99 | 10.64 | 15.03 | 5.71 | 11.06 | 3.87 | 10.26 |
| CB2 | 47.57 | 41.73 | 42.33 | 45.41 | 43.75 | 48.99 | 45.88 | 2.84 | 9.72 |
| CB3 | 14.17 | 12.93 | 14.29 | 14.11 | 13.10 | 15.29 | 13.34 | 0.87 | 2.76 |
| CB total | 70.53 | 70.39 | 62.62 | 70.16 | 71.89 | 69.99 | 70.28 | 3.38 | 13.49 |
| CC | 9.48 | 8.89 | 13.66 | 9.98 | 7.74 | 10.31 | 6.82 | 2.14 | 7.48 |
| | | | | Carbohydrate Intestinal Availability, %DM | | | | | |
| rdCA4 | 1.25 | 1.17 | 1.33 | 1.12 | 1.11 | 1.37 | 1.49 | 0.16 | 0.54 |
| rdCB2 | 42.15 | 36.98 | 37.51 | 40.24 | 38.76 | 43.42 | 40.65 | 2.51 | 8.62 |
| rdCB3 | 9.26 | 8.28 | 9.15 | 9.03 | 8.39 | 9.78 | 8.54 | 0.57 | 1.77 |
| RDCHO | 52.66 | 46.43 | 47.8 | 50.39 | 48.26 | 54.57 | 50.68 | 2.97 | 9.46 |
| RUCHO | 32.42 | 38.30 | 34.84 | 33.12 | 35.67 | 31.94 | 36.41 | 2.47 | 7.98 |
| DRUCHO | 22.94 | 29.41 | 21.18 | 23.14 | 27.94 | 21.62 | 29.58 | 3.74 | 11.85 |
| | | | | Nitrogen to Carbohydrate Ratios | | | | | |
| RDN/RDCHO | 19.90 | 23.29 | 27.69 | 24.54 | 21.90 | 14.19 | 16.63 | 4.55 | 15.59 |
| RDP/RUP | 4.60 | 4.27 | 4.68 | 5.28 | 4.74 | 3.12 | 4.18 | 0.68 | 2.44 |

[a]SD, standard deviation; aCHU, achieved crop heat units; dCHU, difference CHU from achieved to target. Protein subfractions using CNCPS: PA, fraction of crude protein (CP) that is instantaneously solubilized at time zero; PB1, fraction of CP that is soluble in borate-phosphate buffer and precipitated with trichloroacetic acid; PB2, calculated as total CP minus sum of fractions PA, PB1, PB3 and PC; PB3, calculated as the difference between the portions of total CP covered with NDF and ADF; PC, fraction of CP recovered with ADF and is considered to be undegradable; rd, ruminally degraded; RDP, ruminally degraded feed CP; IRDP, insoluble ruminally degraded feed CP; RUP, ruminally undegraded feed CP; IAP, intestinally available (ruminally undegraded) feed CP. Carbohydrate subfractions using CNCPS: CA, sugars/fast degradable; CB1, medium degradable/starch, pectin etc; CB2, useful fiber/useful cell wall/slow degradable; CB3, degradable NDF calculated as aNDF-CC; CC, unuseful fiber/unuseful cell wall fraction/lignin; rd, ruminally degraded; RDCHO, ruminally degraded feed carbohydrate; RUCHO, ruminally undegraded feed carbohydrate; DRUCHO, intestinally digestible and ruminally undegraded feed carbohydrate; RDN, ruminally degraded feed CP; RDP, ruminally degraded feed protein; RUP, ruminally undegradable protein.

for means, range, standard deviations (SD), correlations and polynomial regression.[17]

*Correlations.* Correlation ($r$) between nutrient versus achieved-CHU (aCHU) and difference-CHU (dCHU = achieved − breeding target) were analyzed using the CORR procedure of SAS and $P$-values for Pearson's correlation coefficients.

*Multiple Regression Analysis.* In order to determine which nutrient or parameter in corn silage cultivar increases or decreases with aCHU or dCHU level, a multiple regression analysis was carried out using the "PROC REG" procedure of SAS with a model as follows: $y = x\ x^2\ x^3$ to evaluate the role of linear, quadratic, or cubic correlation. Here, $y$ represents nutrient or estimate, and $x$ represents aCHU or dCHU. Statistical significance was declared at $P < 0.05$.

The same principles (mean, correlation, and regression) were applied for univariate data derived from FT/IR spectrum basically examining fingerprint regions: amide and carbohydrate. Those measurements were amide I, amide II, $\beta$ sheet, $\alpha$ helix, nonstructural carbohydrate peaks, cellulosic compound, and lignin.

*Multivariate Analysis.* The multivariate (exploratory) methods of data analysis were used to classify spectral groups by applying the whole spectral information.[18] The multivariate exploratory techniques included agglomerative hierarchical cluster analysis (HCA), using Wards's algorithm method without prior to parametrization, and principal component analysis (PCA), which was performed by Statistica software 8.0 (StatSoft Inc., Tulsa, OK).

## ■ RESULTS

**Crop Heat Units.** Cultivars from two locations were harvested before reaching breeding target crop heat units by those farmers; therefore, they owe negative dCHU values (Table 1). Pioneer 39M26 and Pioneer 39D95 were the cultivars that did not receive the target CHU because of unfavorable weather fluctuations in those locations.

**Chemical Profile.** Chemical profiles for each location and cultivar are shown in Table 1. As an indication of this under-CHU achievement, cultivar (Pioneer 39D95) grown in Biggar-Unity Farmzone had low CP, SCP, and starch content (Table 1); however, NDF content, a parameter for useful fiber, was

**Table 4. Relationship of Crop Heat Units versus Protein and Carbohydrate Fractions, and Their Intestinal Availability Estimates in Corn Silage**[a]

| item | mean ± SD | range | r aCHU | P-value correlation | linear | quadratic | cubic | r dCHU | P-value correlation | linear | quadratic | cubic |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Protein Fraction, %DM | | | | | | |
| PA | 2.92 ± 1.19 | 3.63 | 0.50 | 0.071 | 0.026 | 0.084 | 0.024 | 0.72 | 0.003 | 0.006 | 0.781 | 0.391 |
| PB1 | 1.31 ± 0.37 | 1.45 | 0.54 | 0.048 | 0.037 | 0.096 | 0.371 | 0.44 | 0.111 | 0.060 | 0.131 | 0.041 |
| PB2 | 2.20 ± 0.51 | 1.81 | 0.36 | 0.206 | 0.211 | 0.988 | 0.194 | 0.28 | 0.339 | 0.175 | 0.699 | 0.003 |
| PB3 | 1.48 ± 0.41 | 1.45 | −0.74 | 0.003 | 0.001 | 0.001 | 0.245 | −0.80 | 0.001 | 0.001 | 0.002 | 0.532 |
| PC | 0.15 ± 0.10 | 0.29 | 0.41 | 0.144 | 0.174 | 0.886 | 0496 | 0.55 | 0.041 | 0.038 | 0.131 | 0.512 |
| | | | | | | Protein Intestinal Availability, %DM | | | | | | |
| rdPA | 2.85 ± 1.17 | 3.55 | 0.50 | 0.071 | 0.026 | 0.084 | 0.024 | 0.72 | 0.003 | 0.006 | 0.781 | 0.391 |
| rdPB1 | 1.07 ± 0.31 | 1.18 | 0.54 | 0.048 | 0.037 | 0.096 | 0.371 | 0.44 | 0.111 | 0.060 | 0.131 | 0.041 |
| rdPB2 | 1.69 ± 0.39 | 1.39 | 0.36 | 0.206 | 0.212 | 0.988 | 0.194 | 0.28 | 0.339 | 0.175 | 0.699 | 0.003 |
| rdPB3 | 0.95 ± 0.26 | 0.93 | −0.74 | 0.003 | 0.001 | 0.001 | 0.245 | −0.80 | 0.001 | 0.001 | 0.002 | 0.532 |
| RDP | 6.57 ± 1.18 | 3.59 | 0.59 | 0.028 | 0.006 | 0.464 | 0.005 | 0.75 | 0.002 | 0.001 | 0.203 | 0.086 |
| IRDP | 2.34 ± 0.43 | 1.49 | −0.25 | 0.398 | 0.364 | 0.261 | 0.134 | −0.35 | 0.21 | 0.041 | 0.307 | 0.001 |
| RUP | 1.49 ± 0.17 | 0.63 | 0.15 | 0.614 | 0.437 | 0.006 | 0.023 | 0.12 | 0.672 | 0.508 | 0.002 | 0.113 |
| IAP | 1.35 ± 0.14 | 0.50 | −0.13 | 0.659 | 0.419 | 0.001 | 0.016 | −0.26 | 0.366 | 0.161 | 0.005 | 0.016 |
| | | | | | | Carbohydrate Fraction, %DM | | | | | | |
| CA | 19.78 ± 2.53 | 8.49 | −0.01 | 0.972 | 0.967 | 0.103 | 0.105 | −0.17 | 0.553 | 0.572 | 0.369 | 0.754 |
| CB1 | 10.38 ± 3.87 | 10.26 | 0.28 | 0.329 | 0.304 | 0.093 | 0.654 | 0.22 | 0.441 | 0.253 | 0.002 | 0.841 |
| CB2 | 45.10 ± 2.84 | 9.27 | −0.65 | 0.011 | 0.004 | 0.677 | 0.014 | −0.57 | 0.033 | 0.034 | 0.777 | 0.155 |
| CB3 | 13.94 ± 0.87 | 2.76 | −0.54 | 0.048 | 0.049 | 0.181 | 0.601 | −0.43 | 0.125 | 0.052 | 0.008 | 0.628 |
| CB total | 69.41 ± 3.38 | 13.49 | −037 | 0.198 | 0.127 | 0.038 | 0.166 | −0.33 | 0.244 | 0.086 | 0.004 | 0.061 |
| CC | 9.56 ± 2.14 | 7.48 | 0.11 | 0.706 | 0.628 | 0.032 | 0.078 | 0.18 | 0.54 | 0130 | 0.001 | 0.017 |
| | | | | | | Carbohydrate Intestinal Availability, % DM | | | | | | |
| rdCA4 | 1.26 ± 0.16 | 0.54 | −0.01 | 0.972 | 0.967 | 0.103 | 0.105 | −0.17 | 0.553 | 0.572 | 0.369 | 0.754 |
| rdCB2 | 39.96 ± 2.52 | 8.62 | −0.65 | 0.011 | 0.004 | 0.677 | 0.014 | −0.57 | 0.032 | 0.034 | 0.778 | 0.155 |
| rdCB3 | 8.92 ± 0.56 | 1.77 | −0.54 | 0.048 | 0.049 | 0.181 | 0.601 | −0.43 | 0.125 | 0.052 | 0.008 | 0.628 |
| RDCHO | 50.14 ± 2.97 | 9.46 | −0.65 | 0.011 | 0.005 | 0.893 | 0.025 | −0.57 | 0.033 | 0.032 | 0.418 | 0.185 |
| RUCHO | 34.67 ± 2.47 | 7.98 | 0.56 | 0.037 | 0.056 | 0.816 | 0.697 | 0.36 | 0.206 | 0.218 | 0.248 | 0.745 |
| DRUCHO | 25.12 ± 3.74 | 11.85 | 0.31 | 0.285 | 0.302 | 0.352 | 0.357 | 0.136 | 0.642 | 0.534 | 0.007 | 0.595 |
| | | | | | | Nitrogen to Carbohydrate Ratios | | | | | | |
| RDN/RDCHO | 21.16 ± 4.55 | 15.59 | 0.64 | 0.013 | 0.001 | 0.613 | 0.001 | 0.75 | 0.002 | 0.001 | 0.323 | 0.054 |
| RDP/RUP | 4.41 ± 0.68 | 2.44 | 0.53 | 0.052 | 0.005 | 0.003 | 0.040 | 0.74 | 0.003 | 0.003 | 0.150 | 0.481 |

[a]SD, standard deviation; r, Pearson correlation coefficient; aCHU, achieved crop heat units; dCHU, difference CHU from achieved to target. Protein subfractions using CNCPS: PA, fraction of crude protein (CP) that is instantaneously solubilized at time zero; PB1, fraction of CP that is soluble in borate-phosphate buffer and precipitated with trichloroacetic acid; PB2, calculated as total CP minus sum of fractions PA, PB1, PB3, and PC; PB3, calculated as the difference between the portions of total CP covered with NDF and ADF; PC, fraction of CP recovered with ADF and is considered to be undegradable; rd, ruminally degraded; RDP, ruminally degraded feed CP; IRDP, insoluble ruminally degraded feed CP; RUP, ruminally undegraded feed CP; IAP, intestinally available (ruminally undegraded) feed CP. Carbohydrate subfractions using CNCPS: CA, sugars/fast degradable; CB1, medium degradable/starch, pectin etc; CB2, useful fiber/useful cell wall/slow degradable; CB3, degradable NDF calculated as aNDF-CC; CC, unuseful fiber/unuseful cell wall fraction/lignin.; rd, ruminally degraded; RDCHO, ruminally degraded feed carbohydrate; RUCHO, ruminally undegraded feed carbohydrate; DRUCHO, intestinally digestible and ruminally undegraded feed carbohydrate; RDN, ruminally degraded feed CP; RDP, ruminally degraded feed protein; RUP, ruminally undegradable protein.

comparatively high. High starch level was found in the Lake Diefenbaker-Lucky Lake Farmzone (Pioneer 39M26) cultivar even though it is (near) under-CHU levels. The farm background information revealed that fertilizer management in this plantation was advanced and methodical. The land is irrigated and fertilized with barn manure. High CP (10%) was found in the Saskatoon plantation (Pioneer 39B61). Better TDN estimation (65.4%) was found for Pioneer H39D95 from the Saskatoon farmzone. However, in general, many nutrient values and estimations are not very different among those cultivars.

Dry matter, CP, NDF, ADF (−0.71), NDICP (−0.66), and SCP correlated ($P < 0.05$) with both aCHU and dCHU (Table 2). In addition, ADICP correlated ($P < 0.05$) with dCHU. Quadratic relationships were found in aCHU with ADL and NDICP. There were additional quadratic relationships in dCHU with NDF and starch. Digestible NDF linearly ($r = −0.64$, $P = 0.01$) decreased with increasing CHU. This negative relationship was supported

by a quadratic ($P < 0.05$) component in both aCHU and dCHU. Estimated TDN, tdNFC, and energy did not have a correlation with CHU; however, there was a quadratic relationship ($P = 0.001$) with dCHU (Table 2).

*Cornel Net Carbohydrate and Protein System and Intestinal Availability.* According to CNCPS protein fractions, Pioneer 39B61 had an edge over others; however, intestinally available protein estimates favor Pioneer 39F45 (Table 3). CNCPS carbohydrate fractions and intestinal availability estimates strengthen the high nutritional availability of the Pioneer 39B61 cultivar (Table 3). However, Pioneer 39F45 has healthy ratios of ruminally degraded protein (RDP) to ruminally undegraded protein (RUP). The relationship of CNCPS protein fractions versus crop heat units is shown in Table 4. CNCPS protein fraction PB3, ruminally degraded protein (RDP), rdPB3 had strong correlations ($P < 0.05$), with both aCHU and dCHU. There was a strong quadratic relationship ($P < 0.05$)
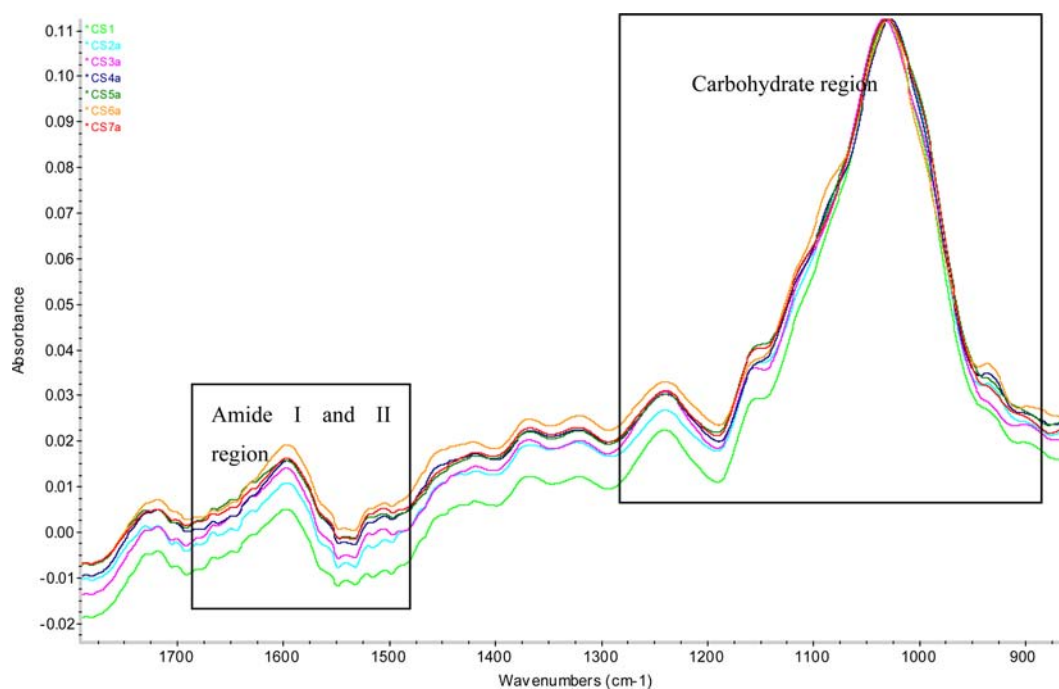
**Figure 1.** Inherent molecular structural spectrum for regions of amides (ca. 1700.91−1500.35 cm$^{-1}$) and carbohydrate (ca. 1299.79−800.31 cm$^{-1}$) using FT/IR spectroscopy. Typical representation of seven cultivars is illustrated in colors. CS1, Pioneer P7213R; CS2a, Dekalb 2679; CS3a, Pioneer 39B61; CS4a, Pioneer 39F45; CS5a, Pioneer 39M26; CS6a, Pioneer 39D95; CS7a, Pioneer H39D95.

with dCHU versus ruminally undegraded protein (RUP) and intestinally available protein (IAP) estimates. Table 4 also shows how CHU would affect CNCPS carbohydrate fractions and intestinally available carbohydrates. Fraction CB2, CB3, rdCB2, and rdCB3 were strongly but negatively correlated with both aCHU and dCHU. Ruminally degraded carbohydrate (rdCHO) also showed a strong correlation to CHU.

Ratio of ruminally degraded nitrogen (RDN) to ruminally degraded carbohydrate (RDCHO) is correlated to CHU with a strong linear correlation ($P$ = 0.001) to dCHU. A similar correlation was found with the ratio of ruminally degraded protein (RDP) to RUP (Table 4). A strong quadratic relationship was found in dCHU versus rdCB1, rdCB3, CB total, CC, and intestinally digestible ruminally degraded carbohydrate (DRUCHO).

**Inherent Molecular Spectral Characteristics.** There were differences detected in inherent molecular characteristics measured related to amide and carbohydrate regions from the spectrum of corn silages using univariate measures (Figure 1 and Table 5). In Figure 1, it was noticeable that a similarity between carbohydrate regions (ca. 1299.79−800.31 cm$^{-1}$) is high although there were some deviations in amide regions (ca. 1700.91−1500.35 cm$^{-1}$). This deviation was illustrated by increased variation in molecular structural ratios: amide I to II and carbohydrate to amide I (with a SD of 20 and 49 respectively, Table 5). The correlation between univariate inherent molecular structural characteristics and crop heat units is shown in Table 6. Crop heat units did not correlate with any of molecular structural characteristics; however, the carbohydrate to amide I ratio correlated with CHU with a correlation coefficient of −0.4 ($P$ < 0.05, Table 7).

Molecular structural differences were further detected by multivariate (exploratory techniques) comparisons of spectral fingerprint data derived from carbohydrate (Figure 2) and amide regions (Figure 3). Ward's method Euclidean distances

by Hierarchical cluster (HCA, i) analysis and principle components and classification (PCA, ii) analysis were illustrated in Figures 2 and 3. Multivariate analysis conducted simultaneously for all cultivars did not facilitate visual separation of cultivars molecular characteristics. However, the detection of inherent molecular structural differences was possible, when the analysis was conducted pairwise (Table 7).

Although HCA detected minimal differences, PCA detected differences in both carbohydrate and amide regions of many cultivars (Table 7). Carbohydrate regions differed as follows: Pioneer P7213R (A) from Dekalb 2679 (B), Pioneer 39F45 (D), Pioneer 39M26 (E), Pioneer 39D95 (F) and Pioneer H39D95 (G); Pioneer 39B61 (C) from Pioneer 39D95 (F) and Pioneer H39D95 (G); Pioneer 39F45 (D) from Pioneer 39M26 (E), Pioneer 39D95 (F) and Pioneer H39D95 (G); Pioneer 39M26 (E) from Pioneer 39D95 (F); and Pioneer 39D95 (F) from and Pioneer H39D95 (G).

PCA indicated differences of amide regions were found as follows: Pioneer P7213R (A) from Pioneer 39B61 (C), Pioneer 39F45 (D), Pioneer 39M26 (E), Pioneer 39D95 (F) and Pioneer H39D95 (G); Dekalb 2679 (B) from Pioneer 39F45 (D), Pioneer 39M26 (E) and Pioneer 39D95 (F) and Pioneer H39D95 (G); Pioneer 39B61 (C) from Pioneer 39F45 (D), Pioneer 39M26 (E), Pioneer 39D95 (F); Pioneer 39F45 (D) from Pioneer 39M26 (E); and Pioneer 39M26 (E) from Pioneer 39D95 (F) and Pioneer H39D95 (G). Overall there were 15 pairs of cultivars showed differences in amid regions, while only 12 pairs of cultivars showed differences in carbohydrate regions by PCA analysis.

## ■ DISCUSSION

**Chemical Profile and Estimated Digestive Characteristics.** Current results indicate that cool corn cultivars grown in Canadian prairie climatic conditions have a nutrient composition similar to those grown in warm weather.[19,20] However, the

**Table 5. Inherent Molecular Characteristics in Cool-Season Corn Silage: Comparison of Cultivars[a]**

| item | Pioneer P7213R | Dekalb 2679 | Pioneer 39B61 | Pioneer 39F45 | Pioneer 39M26 | Pioneer 39D95 | Pioneer H39D95 | SD | range |
|---|---|---|---|---|---|---|---|---|---|
| | | | Protein and Amide Region Intensity (IR Absorbance Unit) | | | | | | |
| amide I | 0.009 | 0.005 | 0.007 | 0.004 | 0.007 | 0.005 | 0.005 | 0.004 | 0.019 |
| amide II | 0.003 | 0.002 | 0.001 | 0.001 | 0.001 | 0.001 | 0.002 | 0.003 | 0.017 |
| $\beta$ sheet height | 0.013 | 0.008 | 0.011 | 0.007 | 0.010 | 0.008 | 0.008 | 0.004 | 0.021 |
| $\alpha$ helix height | 0.009 | 0.004 | 0.006 | 0.002 | 0.005 | 0.003 | 0.003 | 0.003 | 0.081 |
| | | | Carbohydrate Region Intensity (IR Absorbance Unit) | | | | | | |
| CHO peak 1 | 0.041 | 0.036 | 0.038 | 0.037 | 0.038 | 0.033 | 0.040 | 0.007 | 0.034 |
| CHO peak 2 | 0.077 | 0.068 | 0.071 | 0.072 | 0.069 | 0.070 | 0.073 | 0.012 | 0.057 |
| CHO peak 3 | 0.114 | 0.103 | 0.107 | 0.109 | 0.108 | 0.101 | 0.116 | 0.018 | 0.083 |
| cell-com | 0.035 | 0.027 | 0.031 | 0.029 | 0.028 | 0.029 | 0.030 | 0.006 | 0.032 |
| lignin | 0.005 | 0.002 | 0.004 | 0.002 | 0.003 | 0.003 | 0.003 | 0.003 | 0.016 |
| | | | Ratio | | | | | | |
| amide I/II | 0.039 | 1.315 | 5.865 | 12.26 | 2.431 | 7.059 | 6.285 | 19.95 | 130.3 |
| $\alpha$ helix/$\beta$ sheet | 0.656 | 2.866 | 0.524 | 0.371 | 0.520 | 0.305 | 0.884 | 2.314 | 18.46 |
| CHO/amide I | 13.62 | 10.71 | 15.77 | 27.83 | 14.82 | 33.78 | 8.006 | 39.32 | 130.2 |

[a]SD, standard deviation; aCHU, achieved crop heat units; dCHU, difference CHU from achieved to target. Inherent molecular characteristics were given as peak intensity for protein/amide (ca. 1700.91−1500.35 cm$^{-1}$) and carbohydrate (ca. 1299.79−800.31 cm$^{-1}$) regions. CHO, carbohydrate; Cell-com, cellulosic compounds.

**Table 6. Relationship of Crop Heat Units versus Inherent Molecular Structural Characteristics in Cool-Season Corn Silage: A Curvelinear Response Analysis[a]**

| item | mean ± SD | r aCHU | P-value | | | | r dCHU | P-value | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | correlation | linear | quadratic | cubic | | correlation | linear | quadratic | cubic |
| | | | Protein Amide Region Intensity (IR absorbance Unit) | | | | | | | | |
| amide I | 0.006 ± 0.004 | 0.12 | 0.475 | 0.487 | 0.662 | 0.850 | 0.15 | 0.385 | 0.389 | 0.878 | 0.232 |
| amide II | 0.001 ± 0.003 | 0.04 | 0.811 | 0.815 | 0.745 | 0.498 | 0.10 | 0.554 | 0.563 | 0.459 | 0.826 |
| $\beta$ sheet height | 0.009 ± 0.004 | 0.11 | 0.543 | 0.555 | 0.779 | 0.994 | 0.13 | 0.458 | 0.462 | 0.742 | 0.285 |
| $\alpha$ helix height | 0.005 ± 0.003 | 0.17 | 0.335 | 0.349 | 0.668 | 0.829 | 0.20 | 0.244 | 0.254 | 0.729 | 0.439 |
| CHO peak 1 | 0.038 ± 0.007 | 0.23 | 0.179 | 0.182 | 0.273 | 0.474 | 0.25 | 0.147 | 0.156 | 0.455 | 0.966 |
| CHO peak 2 | 0.071 ± 0.012 | 0.04 | 0.838 | 0.841 | 0.599 | 0.381 | 0.08 | 0.665 | 0.674 | 0.978 | 0.604 |
| CHO peak 3 | 0.108 ± 0.018 | 0.14 | 0.431 | 0.435 | 0.346 | 0.433 | 0.16 | 0.359 | 0.371 | 0.520 | 0.788 |
| cell com | 0.030 ± 0.006 | 0.09 | 0.588 | 0.594 | 0.833 | 0.323 | 0.14 | 0.423 | 0.434 | 0.513 | 0.740 |
| lignin | 0.003 ± 0.003 | −0.01 | 0.935 | 0.937 | 0.936 | 0.780 | 0.02 | 0.923 | 0.925 | 0.575 | 0.600 |
| | | | Ratio | | | | | | | | |
| amide I/II | 1.146 ± 19.95 | −0.03 | 0.854 | 0.856 | 0.302 | 0.872 | −0.12 | 0.509 | 0.517 | 0.681 | 0.449 |
| $\alpha$ helix/$\beta$ sheet | 0.622 ± 2.314 | 0.12 | 0.496 | 0.506 | 0.540 | 0.631 | 0.09 | 0.603 | 0.613 | 0.738 | 0.741 |
| CHO/amide I | 20.65 ± 39.32 | −0.37 | 0.028 | 0.029 | 0.221 | 0.845 | −0.39 | 0.022 | 0.024 | 0.298 | 0.797 |

[a]SD, standard deviation; r, Pearson correlation coefficient; aCHU, achieved crop heat units; dCHU, difference CHU from achieved to target. Inherent molecular characteristics were given as peak intensity for protein/amide (ca. 1700.91−1500.35 cm$^{-1}$) and carbohydrate (ca. 1299.79−800.31 cm$^{-1}$) regions. CHO, carbohydrate; Cell-com, cellulosic compounds.

starch values were close to the lower end of the standard range, 10−30%.[19,20] According to our results, the low CHU access has affected CP, SCP, even though NDF was not affected among location or cultivar. Low starch content did not directly correlate with CHU, and the reason for low starch may not be related to weather but some other factor.[6,21,22] The land irrigation and fertilization with barn manure would override the nutritional quality of silage by enhanced plant growth and maturation.[7,8,23] It was obvious that the constant supply of CHU in the summer would increase NDF, CP, and SCP because they have a positive correlation with CHU. Interestingly, less digestible components (ADICP and ADL) correlated linearly or quadratically with CHU. A few other quadratic or curvilinear relationships were found with CHU versus NDF and starch and it explains that the nutrient content will be stable or decrease or increase regardless of the CHU level, after reaching a certain level of CHU supply.[1] This paradox was proven by reduction of digestible NDF with increasing CHU. Estimated TDN, tdNFC,

and energy values did not change because during the process of plant maturation one nutrient may convert to another.[1,7,8] Among sufficient CHU, low starch content would be a result of other factors such as radiant sun-energy theory which depends on latitude.[22,24] In addition, a low moisture level could cause such changes.[1]

Protein fractions of CNCPS results are in agreement with previous work.[16,25] A strong correlation of CHU with protein fractions (PB1) and ruminally degradable proteins suggests that a sunny environmental condition favors better nutritional quality of a forage.[7,8] Slowly degradable protein fractions are negatively correlated to CHU, and perhaps it occurs with changes in protein molecular structure with maturity.[26,27] Further, an increase rumen degradability and intestinal availability of these proteins was shown with a strong curvilinear relationship to CHU. Ruminaly degraded carbohydrate (rdCHO) also correlated with CHU. However, negative relationships were indicated by carbohydrate availability (CB2, CB3, rdCB2, and rdCB3)

**Table 7. Multivariate (Exploratory Technique) Comparison of Fingerprint Spectral Data from Corn Silage Based on Cluster Analysis (CLA)[a]**

| nutrient of interest | cultivar | | linkage distance | dendrogram | Factor 1, % | Factor 2, % |
|---|---|---|---|---|---|---|
| | | Spectral Region, ca. 1299−800 cm$^{-1}$ | | | | |
| carbohydrate | Pioneer P7213R (A) | vs Dekalb 2679 (B) | 1.2 | BBBABAAABA | 98.12 | 1.34 |
| | | vs Pioneer 39B61 (C) | 0.7 | CCACCAACAA | 96.04 | 2.88 |
| | | vs Pioneer 39F45 (D) | 0.7 | DDADDDAAAA | 95.8 | 3.37 |
| | | vs Pioneer 39M26 (E) | 0.7 | EEAEEEAAAA | 93.63 | 5.23 |
| | | vs Pioneer 39D95 (F) | 0.7 | FFAFFFAAAA | 98.14 | 1.24 |
| | | vs Pioneer H39D95 (G) | 1.1 | GGGAAAAGGA | 94.47 | 5.1 |
| | Dekalb 2679 (B) | vs Pioneer 39B61 (C) | 0.9 | BBCCBCCBCB | 97.85 | 1.69 |
| | | vs Pioneer 39F45 (D) | 0.9 | BDDDBBBDDB | 98.93 | 0.72 |
| | | vs Pioneer 39M26 (E) | 0.9 | BBEEEBBEEB | 98.77 | 0.59 |
| | | vs Pioneer 39D95 (F) | 1.3 | FBBFBFFBFB | 97.95 | 1.75 |
| | | vs Pioneer H39D95 (G) | 1.6 | GBGGBGGBBB | 98.3 | 1.33 |
| | Pioneer 39B61 (C) | vs Pioneer 39F45 (D) | 0.6 | DDCCDCCDDC | 94.79 | 3.39 |
| | | vs Pioneer 39M26 (E) | 0.6 | EECCECCEEC | 91.21 | 7.02 |
| | | vs Pioneer 39D95 (F) | 0.6 | FCFCFFFCCC | 96.67 | 2.19 |
| | | vs Pioneer H39D95 (G) | 0.8 | GGGCCGCCGC | 96.98 | 2.48 |
| | Pioneer 39F45 (D) | vs Pioneer 39M26 (E) | 0.7 | EEDDEDEEDD | 94.7 | 3.23 |
| | | vs Pioneer 39D95 (F) | 0.7 | FFDDFFDFDD | 96.31 | 2.99 |
| | | vs Pioneer H39D95 (G) | 1 | GDDGGGDGDD | 97.77 | 1.74 |
| | Pioneer 39M26 (E) | vs Pioneer 39D95 (F) | 0.7 | FFEEEEFFFE | 92.47 | 6.72 |
| | | vs Pioneer H39D95 (G) | 1 | GEEGGGEEGE | 98.23 | 1.16 |
| | Pioneer 39D95 (F) | vs Pioneer H39D95 (G) | 1.3 | GFGGFFFGGF | 96.32 | 3.35 |
| | | Spectral Region, ca. 1701−1500 cm$^{-1}$ | | | | |
| amides (protein) | Pioneer P7213R (A) | vs Dekalb 2679 (B) | 0.3 | BBBBABAAAA | 99.33 | 0.38 |
| | | vs Pioneer 39B61 (C) | 0.2 | CCACCCAAAA | 97.89 | 1.44 |
| | | vs Pioneer 39F45 (D) | 0.25 | DDDADDAAAA | 97.72 | 1.5 |
| | | vs Pioneer 39M26 (E) | 0.21 | EEEAEEAAAA | 97.8 | 1.66 |
| | | vs Pioneer 39D95 (F) | 0.2 | FFAFFFAAAA | 97.51 | 2.05 |
| | | vs Pioneer H39D95 (G) | 0.23 | GGAGGGAAAA | 97.95 | 1.53 |
| | Dekalb 2679 (B) | vs Pioneer 39B61 (C) | 0.24 | CBBCCCBBCB | 99.09 | 0.64 |
| | | vs Pioneer 39F45 (D) | 0.18 | DDBBDDDBBB | 97.58 | 1.96 |
| | | vs Pioneer 39M26 (E) | 0.2 | BEEBEEBEBB | 99.16 | 0.65 |
| | | vs Pioneer 39D95 (F) | 0.25 | FBFFFBFBBB | 97.64 | 2.13 |
| | | vs Pioneer H39D95 (G) | 0.35 | GBGGGBGBBB | 98.78 | 1.02 |
| | Pioneer 39B61 (C) | vs Pioneer 39F45 (D) | 0.16 | DDDCCDDCCC | 95.17 | 3.21 |
| | | vs Pioneer 39M26 | 0.14 | EECECEECCC | 96.9 | 1.75 |
| | | vs Pioneer 39D95 (F) | 0.18 | FFCCFFFCCC | 96.54 | 2.69 |
| | | vs Pioneer H39D95 (G) | 0.2 | GGCCGGGCCC | 98.55 | 0.97 |
| | Pioneer 39F45 (D) | vs Pioneer 39M26 (E) | 0.14 | EEEDDDEEDD | 87.52 | 10.82 |
| | | vs Pioneer 39D95 (F) | 0.2 | FDFDDFFDFD | 98.3 | 0.95 |
| | | vs Pioneer H39D95 (G) | 0.2 | GGDDDGGDGD | 98.51 | 0.78 |
| | Pioneer 39M26 (E) | vs Pioneer 39D95 (F) | 0.16 | FFFEEFFEEE | 91.43 | 7.96 |
| | | vs Pioneer H39D95 (G) | 0.2 | GGGGEEEGEE | 96.83 | 2.83 |
| | Pioneer 39D95 (F) | Pioneer H39D95 (G) | 0.3 | GFGFGGFGFF | 99.03 | 0.56 |

[a]Dendrogram and plots are for 10 cases of each comparison; hierarchical cluster analysis (HCA) Ward's method Euclidean distances, and principle components and classification analysis (PCA) projection of the cases on the factor-plane.

with CHU. Nutrient composition of corn silage seemed to have an ideal ratio of carbohydrate and protein.[15,28] It was proven by our calculated ratio of RDN/RDCHO and RDP/RUP by strongly correlating to CHU. As a forage, this is a potential advantage internal protein availability and hence energy synchronization.[25] An additional advantage for this optimal synchronization is that corn stitch has a moderate degree (29.1%) of hydrolysis.[29]

**Univariate and Multivariate Changes of Molecular Structure.** Univariate data derived from the spectra of these silages proved how their inherent molecular structures are related. A uniform pattern on the spectra was obvious even

though those cultivars are grown in different locations under different growing conditions (CHU, moisture, and fertilizer), and further, the harvest has undergone the process of ensiling in different situations. Regardless of having all these variations and variability in growth and ensiling, many inherent molecular characteristics were analogous, particularly in the spectral regions of interest: amide I, amide II, and carbohydrate regions. In addition, the carbohydrate region (ca. 1299.79−800.31 cm$^{-1}$) seemed identical, although the amide region was not that uniform among cultivars.[30] A reason for this finding may be an accumulation of new components after the process of ensiling depending on the type of inoculants, or/and representation of
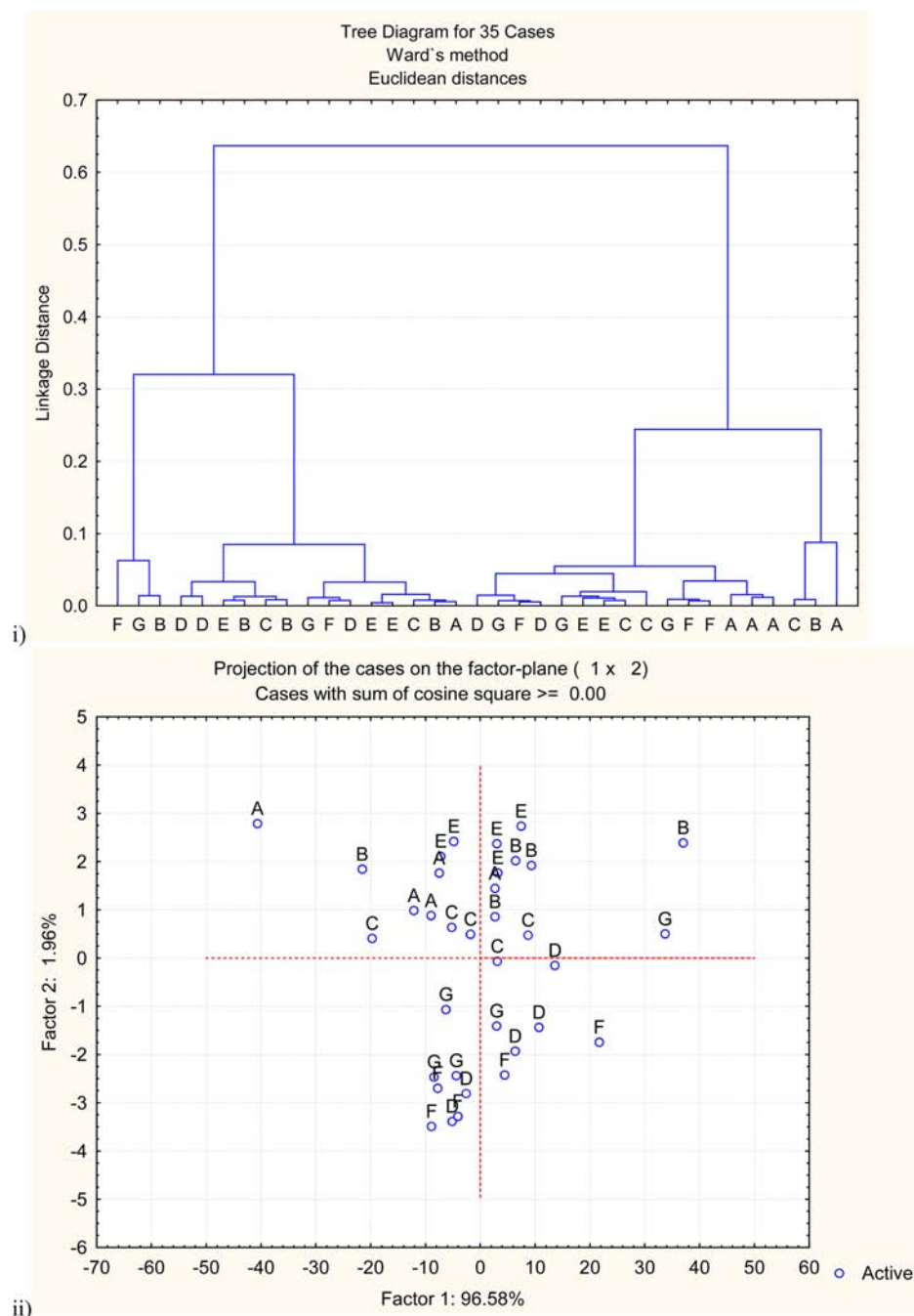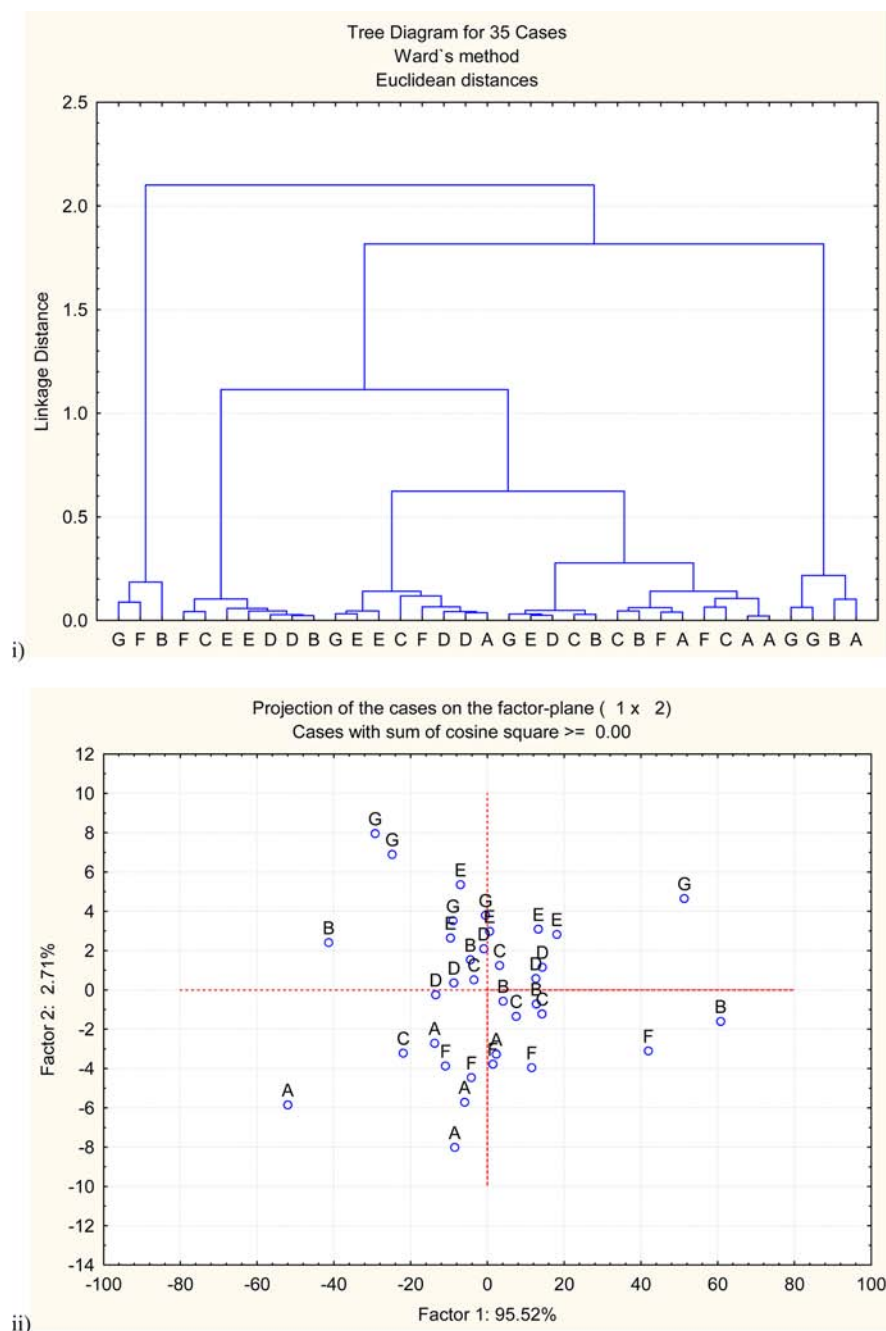
**Figure 2.** (i) PCA Multivariate (exploratory techniques) comparison of fingerprint spectral data derived from carbohydrate (ca. 1299.79–800.31 cm$^{-1}$) region of seven corn silages. A, Pioneer P7213R; B, Dekalb 2679; C, Pioneer 39B61; D, Pioneer 39F45; E, Pioneer 39M26; F, Pioneer 39D95; G, Pioneer H39D95. a, Hierarchical cluster analysis (HCA). (ii) Principle components and classification analysis (PCA).

the components from epiphytic bacteria mixed in the dried silage.[31−33] Epiphytic bacterial proteins may provide components to make changes in amide regions.[31,33] Bacterial contribution of nutrients is a significant event because there is an approximately 300 fold increase of anaerobic bacteria (lactic acid forming bacteria) during the process of ensiling in corn forage.[34,35] Depending on the cultivar and environmental factors, other contaminated microbes would contribute as well.[34,35]

Multivariate comparisons of spectral fingerprint data derived from amide and carbohydrate region were separable by hierarchical cluster (HCA) analysis and principle components and classification (PCA) analysis. With regard to amide region spectral fingerprint data, the Pioneer P7213R cultivar differs

from all cultivars except Dekalb 2679. It is interesting to note that the cultivars different from Pioneer P7213R cultivar belong to Pioneer varieties. These differences may be due to the effect of the environment and sunlight on plant growth rather than the effect of genetic composure.[7,8,22,24] The effect of cultivar on inherent molecular characteristics was reported on different plant materials.[36] These differences are attributed to many different factors.[37] In our findings, the correlation of CHU with the ratio of carbohydrate to amide-I, is interesting to note because it proves that there is an effect of CHU on inherent molecular characteristics.[37] Epiphytic bacterial nutrients may have a role in this ratio change (carbohydrate to amide I) as it increases protein factions.[32,34,35]

**Figure 3.** (i) PCA Multivariate (exploratory techniques) comparison of fingerprint spectral data derived from amide region (ca. 1700.91−1500.35 cm$^{-1}$) of seven corn silages. A, Pioneer P7213R; B, Dekalb 2679; C, Pioneer 39B61; D, Pioneer 39F45; E, Pioneer 39M26; F, Pioneer 39D95; G, Pioneer H39D95. I, Hierarchical cluster analysis (HCA). (ii) Principle components and classification analysis (PCA).

Ruminal fermentation characteristics, nutrient availability, and energy synchronization depend on many factors: composition of feed (total mixed ration), composition of ration ingredients, and predictable fractionation characters of these ingredients.[25] However, a forage in a ruminant diet plays a major role in this process.[9,28] Our findings showed that cool corn silage is not only comparable to other conventional silages in nutrient content, but also it has potential rumen digestibility and intestinal availability of those nutrients with optimal energy synchronization to animal.[38] Although all cultivars are good in silage quality, cultivar of Pioneer 39D95 or its farm conditions seemed slightly ahead in nutritional quality compared to others.

In conclusion, cool season corn silages are a substitute to replace other forages in a cattle operation. Nutrient compositions and potential nutrient supply to the animal showed a relationship to CHUs. Molecular structural characteristics vary with cultivars grown in different CHU areas. Those reached target CHU levels would be apparently optimal in nutrient availability and energy synchronization. Cool corn cultivars would be an alternate source of forage to grow in (cool climates) Canada for feeding cattle.

## ■ AUTHOR INFORMATION

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12326

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