EFFECT OF CARBOHYDRATE TRAITS ON NUTRITIONAL CHARACTERISTICS AND

SPECTRAL FEATURES OF MOLECULAR STRUCTURE IN HULLESS BARLEY

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By

LING YANG

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ABSTRACT

Four hulless barley varieties (zero-amylose waxy, CDC Fibar; 5%-amylose waxy, CDC Rattan; normal-amylose, CDC McGwire and high-amylose, HB08302) were developed at the Crop Development Centre, University of Saskatchewan with differences in carbohydrates traits on the basis of amylose (1 to 20% DM), amylopectin (34 to 51% DM), amylose to amylopectin ratio (0.02 to 0.59) and β-glucan (5 to 10% DM) content. The objectives of this research were to determine: 1) the effect of the alteration of these carbohydrate traits in hulless barley on nutrient availability in ruminants, and 2) spectral characteristics of molecular structures in comparison with hulled barley-CDC Copeland. Studies on chemical and nutrient profiles, rumen degradation kinetics, *in vitro* intestinal nutrient digestion and potential protein supply estimated by the Dutch model and the NRC Dairy 2001 model were carried out. Fourier Transform Infrared Spectroscopy (FTIR) and advanced synchrotron-based FTIR Microspectroscopy (SR-FTIRM) with univariate and multivariate analysis were applied to investigate the influence of genetic modification of barley cultivars on the molecular structure features at the regions of protein amide I and II, β-glucan, cellulosic compounds and carbohydrates. By quantifying the relationship between the measured parameters and the alteration of carbohydrate traits, the results of studies revealed: 1) the hulless barley lines with altered carbohydrate traits have the potential to increase rumen and intestinal nutrient availability, thus improving the truly absorbed protein supply to ruminants compared to hulled barley; 2) lower amylose and higher β-glucan level in the hulless barley varieties increased estimated energy and metabolizable protein supply to ruminants; 3) molecular structure differences of the hulless barley varieties can be detected by both conventional FTIR spectroscopy and SR-FTIRM; 4) metabolizable protein (MP) was affected significantly by protein molecular structure characteristics in hulless barley.

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LIST OF ABBREVIATIONS

ABCP	Truly absorbed bypass protein in the small intestine
ADF	Acid detergent fibre
ADICP	Acid detergent insoluble crude protein
ADL	Acid detergent lignin
AECP	Truly absorbed rumen endogenous protein in the small intestine
AMCP	Truly absorbed microbial protein in the small intestine
ARUP^{NRC}	Truly absorbed rumen undegradable protein in the small intestine (NRC Dairy 2001 model)
BCHO	Rumen bypass CHO
BCP	Rumen bypass crude protein (DVE/OEB system)
BDM	Rumen bypass dry matter
BDNDF	Rumen bypass neutral detergent fibre
BST	Rumen bypass or undegraded starch
CA	Fast degradable carbohydrates with the degradation rate of 300% /h
CB1	Intermediately degradable CHO (starch) with a degradation rate of 20-50% /h
CB2	Slowly degradable available cell wall with a slow degradation rate 2-10% /h
CC	Unavailable cell wall.
CHO	Carbohydrates
CLA	Hierarchical cluster analysis
CP	Crude protein
D	Potentially degradable fraction
dBCHO	Digestibility of rumen bypass or undegraded CHO

dBST	Digestibility of rumen bypass or undegraded starch
DE_{1x}	Digestible energy
DE_{p3x}	Digestible energy at a production level (3x maintenance)
DM	Dry matter
DOM	Digested organic matter
DVE	Total truly digested absorbed protein in the small intestine
ECP	Rumen endogenous protein
ED	Effective degradability
EDCHO	Effective degradability of CHO
EDCP	Effective degradability of crude protein
EDDM	Effective degradability of dry matter
EDNDF	Effective degradability of neutral detergent fibre
EDST	Effective degradability of starch
EE	Ether extracts (crude fat)
ENDP	Endogenous protein in the small intestine
FOM	Organic matter fermented in the rumen
FTIR	Fourier transform infrared microspectroscopy
IDBCHO	Intestinal digestible rumen bypass CHO
IDBST	Intestinal digestible rumen bypass starch
IDP	Intestinal degradable protein
K_d	The rate of degradation of D fraction (%/h)
K_p	Passage rate (%/h)
MCP	Microbial protein
MCP_{TDN}	Microbial protein synthesized in the rumen based on discounted TDN

ME	Metabolizable energy
ME_{p3x}	Metabolizable energy at a production level (3x maintenance)
MP	Metabolizable protein
N_MCP	Microbial protein synthesized in the rumen based on available nitrogen
NDF	Neutral detergent fibre
NDICP	Neutral detergent insoluble crude protein
NE_g	Net energy for gain
NE_{Lp3x}	Net energy for lactation at a production level (3x maintenance)
NE_m	Net energy for maintainance
NPN	Non-protein nitrogen
NSC	Non-structural carbohydrate
OEB	Degraded protein balance
PA	Non-protein N
PB	True protein
PB1	Rapidly degraded protein or soluble true protein fraction with a degradation rate of 120–400% /h
PB2	Intermediately degraded crude protein with an intermediate degradation rate of 3–16% /h
PB3	Slowly degraded protein or insoluble true protein bound to fibre with a degradation rate of 0.06–0.55% /h
PC	Undegradable and unavailable protein
PCA	Principal component analysis
RUP	Rumen undegradable crude protein (NRC Dairy 2001 model)
S	Soluble fraction in the in situ incubation
SCP	Soluble crude protein

SR-FTIRM	Synchrotron-based Fourier Transform Infrared Microspectroscopy
ST	Starch
T_0	Lag time (h)
TDCHO	Total digestible CHO
tdCP	Total digestible crude protein (NRC chemical approach)
tdFA	Total digestible fatty acid (NRC chemical approach)
TDN_{1x}	Total digestible nutrients
tdNDF	Total digestible neutral detergent fibre (NRC chemical approach)
tdNFC	Total digestible non-fibre carbohydrates (NRC chemical approach)
TDP	Total digestible protein
TDST	Total digestible starch
TPSI	True protein supplied to the small intestine
U	Undegradable degradable fraction
UASH	Undigested inorganic matter
UDM	Undigested dry matter
UOM	Undigested organic matter

1. General Introduction

Barley (*Hordeum vulgare* L.) is one of the major cereal grains in Canada with 8.21million tons of production in 2012 (MacLeod et al., 2012). Ninety percent of the barleys grown in Western Canada, of which eighty percent used in feed production domestically (Ullrich, 2011). Starch is the major storage compound in the barley endosperm, consisting of two polymers: amylose and amylopectin. Amylose normally accounts for 15–25% while amylopectin accounts for 75–85% of barley starch, respectively (Ullrich et al., 1986). Amylose is composed of α–1,4 glucopyranosidic units, with branching of the chain occurring at the sixth carbon in the amylopectin molecule (Zobel, 1988). β-glucans are mainly concentrated in internal aleurone and endosperm cell walls of cereal grains such as barley and oat (Havrlentová and Kraic, 2006). In barley, β-glucan accounts for 2–7% of DM with around 75% in cell wall polysaccharides (Zhang et al., 2000; Ullrich, 2011). Hulless barley differs from hulles barley for its removed hull-coating during mechanical processing of grain (Thomason et al., 2009). Compared to hulled barley, previous studies indicated that hulless barley contained higher energy and had better nutrient availability due to its reduced fibre coating, which increased nutrient digestibility for pigs and digestibility of postruminal organic matter (OM), starch and N for feedlot steers (Bowman et al., 2001; Shon et al., 2007; Pieper et al., 2008; Jha et al., 2010; Lehman et al., 1995; Beams et al., 1996; Zinn et al., 1996). However, the concern of feeding hulless barley to ruminants will be the incidence of digestive disorders owing to fast starch degradation and digestion, resulting in accumulation of acidic products (Yang et al., 1997; Zinn et al., 1996). Recently, four hulless barley varieties and breeding lines with altered carbohydrate composition were developed at the Crop Development Centre, University of Saskatchewan, based on amylose (1 to 20% DM), amylopectin (34 to 51% DM), amylose to amylopectin ratio (0.02 to 0.59), and β-glucan (5 to 10% DM) content.

Prior to applying these hulless barley cultivars into animal diets, understanding their chemical profiles and metabolic characteristics is essential for animal health and nutritionists. The overall objective of this study was to determine the effect of carbohydrate traits of hulless barley [(1) amylose level, (2) amylopectin level, (3) amylose to amylopectin ratio, and (4) β-glucan level)] on nutrient availability in dairy cattle and molecular structural features. The sub-objectives of this study included comparing differences in the four hulless barley lines

(zero-amylose waxy, CDC Fibar; 5%-amylose waxy, CDC Rattan; normal-amylose, CDC McGwire; and high-amylose, HB08302) in chemical and nutrient profiles, rumen degradation kinetics, *in vitro* intestinal nutrient digestion, and potential protein supply estimated by the DVE/OEB system and the NRC Dairy 2001 model, and to investigate whether the alteration of carbohydrate traits will improve nutrient availability and utilization for ruminants, in comparison with hulled barley−CDC Copeland. In addition, two molecular spectroscopy technologies−conventional Fourier Transform Infrared Spectroscopy (FTIR) and advanced Synchrotron-based Fourier Transform Infrared Microspectroscopy (SR-FTIRM) were applied to reveal molecular structure spectral profiles of the four hulless barley varieties and to quantify the molecular structural features in relation to rumen degradation kinetics, intestinal nutrient digestion and potential protein supply.

2. Literature Review

2.1. Barley Utilization in Canada

2.1.1. Barley Varieties

Barley was introduced to western Canada from Europe by the earliest settlers (Juskiw et al., 2011). Early barley production was mainly used by the malting industry. As trade barriers limited the development of the brewering industry, barley production turned to feed (Metcalfe, 1995). Barley now is one of the major cereal grains grown in Canada with 8.1 million tonnes of production in 2012 (MacLeod et al., 2012). Ninety percent of the barley is grown in Western Canada. The vast majority (80%) is used in feed production domestically (Ullrich, 2011).

Barley is superior in growing at the areas with humid climate and variable precipitation than corn (*Zea mays* L.) due to its less water-holding capacity (Thomason et al., 2009). Barley grain mainly consists of a fibrous hull, pericarp, aleurone layer, endosperm and germ (Evers et al., 1999). Pericarp and seed coat both play protective roles by covering the whole seed. Endosperm tissue is the main storage site of starch granules and with the aleurone layer, usually accounts for the major portion of the barley kernal (Black, 2000; Kulp and Ponte, 2000). The aleurone layer is composed of cells which include starch granules. In the aleurone layer, non-starch polysaccharides, β-glucan and arabinoxylan are mainly found in the cell wall (Bacic and Stone, 1981; Newman and Newman, 1992). Thicker cell walls in barley can be found in the varieties that are higher in β-glucan (Oscarsson et al., 1997; Zheng et al., 2000). Overall, there have been 200 barley cultivars registered in Canada, of which over 50 produced in western Canada, including 8 hulless cultivars and 13 malting cultivars (CFIA, 2009; Damiran and Yu, 2012).

2.1.1.1. Hulled barley (*Hordeum vulgare* L.): Two-row Barley vs. Six-row Barley

The early barleys were generally two- and six-rowed types (Juskiw et al., 2011). Wild and cultivated barleys have sessile spikelets. Sterile lateral spikelets can be found in two-row barley, whereas fertile ones are found in six-row barley due to a pair of mutations (von Bothmer and Komatsuda, 2011). Both varieties are important for beer production historically that two-row

barley is more widely used in England and German, while six-row barley was predominant in Canada, especially in southern Alberta, until the mid 1990s (Metcalfe, 1995; Juskiw et al., 2011). However, changes began in Saskatchewan when the advent of the two-row malting barley cultivar 'Harrington' replaced six-rowed barley (Harvey and Rossnagel, 1984). As reported by Campbell et al. (1995), two-row barleys grown in Manitoba had higher starch content on average than did six-row cultivars whereas six-row barley had higher protein and less starch than two-row barley, which resulted in wider utilization as animal feed.

2.1.1.2.   Hulled vs. Hulless Barley

Hulled barley has a hull that covers the caryopsis. Hulless barley is superior in nutritional characteristics such as protein, starch, β-glucan, total dietary fibre and limiting amino acids compared with hulled cultivars (Bhatty, 1986; Edney et al., 1992; Boros et al., 1996).

In the early 1970s, investigations on the nutritional quality of barley found the hull content of barley affected the digestible energy in monogastric animal feeding (Bhatty et al., 1975), which led to the registration of some hulless barley cultivars in order to further extend the use of hulless barley in food, malt and brewing (Bhatty, 1999). Hulless barley production was found in Canada with more than 800,000t in 1998 (Bhatty, 1999). However, due to the lack of a hull, hulless barley is more likely affected by mechanical damage and invation by insects compared to hulled barley (Thomason et al., 2009). Thus, there is usually lower grain yield for hulless barley when compared to hulled barley (Choo et al., 2003).

2.2.   Benefits of Hulless Varieties for Animal Nutrition

Barley is widely used as a feed grain for various livestock species such as beef, dairy cattle, goat, swine and poultry (Blake et al., 2011), although feeding barley could result in digestive disorders due to its rapidly degradable carbohydrate content (Yang et al., 1997). Hulless barley has been reported to have higher energy values and better nutrient availability due to its reduced fibre and increased starch content compared to hulled cultivars (Zinn et al., 1996; Bowman et al., 2001; Shon et al., 2007; Pieper et al., 2008; Jha et al., 2010). There is an interest

in increasing the use of hulless barley in ruminant rations due to concerns with animal health, nutrient availability and potential profit in dairy and beef production.

2.2.1. Nutritional Effects on Monogastric Animals

Hulless barley was developed primarily for swine and poultry feeding (Bhatty, 1999). Previous studies indicated that hulless barley had higher energy and digestibility than did hulled barley in pigs (Lehman et al., 1995; Beames et al., 1996). Hulless barley varied in β-glucan and amylose levels had a strong effect on gut microbial profiles of pigs compared with hulled barley (Pieper et al., 2008). In a study on weaned piglets, a hulless barley based diet was found higher in ileal organic matter, crude protein and total non-starch polysaccharide digestibility as well as short-chain fatty acids (SCFA) and lactic acid (LA) compared to hulled barley or oat (Jha et al., 2010). This indicated hulless barley has a potential to improve nutrient digestibility and also gut health in weaned piglets. Hulless barley applied in poultry diets is usually combined with β-glucanase or phytase in order to reduce the viscous condition in the digestive tract due to the high β-glucan level in barley which is considered as an anti-nutrition factor for broiler chickens (White et al., 1983; Hesselman et al., 1986; Missct 1996). Recent study on dilution of whole hulless barley in broiler chicken diets revealed that inclusion of hulless barley in the diet with a dilution level of 7.5% could be beneficial to chicken growth in the grower period and at 15% in the finisher period (Anderson et al., 2012).

2.2.2. Nutritional Effects on Ruminants

Barley is the third most readily degradable cereal for ruminants due to its superior starch and energy content. However, the impact of barley in ruminants includes bloat, acidosis and laminitis when the diet is high in barley starch (Blake et al. 2011). The incidence of digestive disorders could be more severe when ruminants are fed with hulless barley as its lack of a hull coating would expose more adhering area for micro-organisms. This would result in faster starch digestion and accumulation of acidic products (Zinn et al., 1996; Yang et al., 1997). However, in a feedlot steer trial reported by Zinn et al. (1996), cattle fed hulless barley had greater digestibility of postruminal OM, starch and N as well as net energy as compared to hulled barley. This indicates there is potential to use hulless barley to improve cattle performance.

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2.3. Newly Developed Hulless Barley Varieties

2.3.1. Breeding Targets for Newly Developed Hulless Barley

The early registered hulless barley cultivars included two- and six-row, low and high β-glucan, and waxy and normal starch (Bhatty, 1999). New hulless barley varieties used in this project were developed by the University of Saskatchewan's Crop Development Centre with special targets for amylose and β-glucan levels. The four hulless barley lines include: zero-amylose but very high β-glucan level—CDC Fibar; low-amylose but high β-glucan level—CDC Rattan; normal-amylose and normal β-glucan level—CDC McGwire, as well as high-amylose but normal β-glucan level—HB08302.

2.3.2. Barley Starch

2.3.2.1. Starch Digestiblity

Starch is the major strogae carbohydrate in plants (Singh et al., 2010). Starch digestion mainly occurs in the small intestine in the non-ruminant but differs in the ruminants due to the action of microorganisms in the rumen (Cerrilla and Martinez, 2003). Digestion of starch requires enzymes produced by salivary glands, rumen microorganisms or the pancreas (Cerrilla and Martinez, 2003; Singh et al., 2010). Starch or starchy products can be classified by the rates of starch digestion (Singh et al., 2010). Diversified morphological characteristics of starches can be found among botanical sources and vary with the genotype (Singh et al., 2010). These morphological characteristics include the size and shape of the starch granules. Several studies confirmed a negative relationship between granule size and starch digestibility (Langworthy and Deuel, 1922; Lindeboom et al., 2004). The reason for this is that the large granule starches are lower in susceptibility to enzymatic hydrolysis due to smaller granule-specific surface area for enzyme binding, resulting in less hydrolysis than with small granules (Tester et al., 2006). Dreher et al. (1984) suggested that the surface characteristics of starch granules affect enzymatic digestion and were responsible for higher digestibilities for cereal starches than for tuber and legume starches. This may due to pores on the surface which facilitate the entry of the digestive enzymes (Singh et al., 2010). Non-starch substances such as protein and lipid on the granule surface are considered to limit the rate of enzymatic hydrolysis by blocking adsorption sites,

impacting enzyme binding and reducing surface accessibility (Oates, 1997; Singh et al., 2010). The molecular structure of the starch granule influences the hydrolysis pattern by the arrangement of the different polymeric forms of the starch, especially A-type and B-type crystallites, which vary in the packing of the amylopectin double helices (Lehmann and Robin, 2007; Singh et al., 2010). For ruminants, barley starch is easily degraded by rumen microorganisms and considered to be associated with metabolic disorders (Cerrilla and Martinez, 2003). McAllister et al. (1993) reported that ruminal starch digestion was not affected by starch granule size but was a function of the protein and structural carbohydrate matrix within the grain. Therefore, studies to improve barley quality have been concentrated on how to increase by-pass starch and reduce starch degradation in the rumen (Juskiw et al., 2011).

2.3.2.2. Starch components: Amylose and Amylopectin

The main polymers in barley starch granules are amylose and amylopectin, which normally account for 15-25% and 75-85% of the starch, respectively (Ullrich et al., 1986). Amylose is composed of α-1,4 glucopyranosidic units, whereas there is branching at the sixth carbon in amylopectin (Zobel, 1988). Amylopectin has a much larger molecular weight (10^5 to 10^6) than does amylose (10^4) and a much larger surface area per molecule than amylose, which makes it a preferable substrate for amylolytic attack (Singh et al., 2010). Compared to amylopectin, the glucose chains of amylose are more tightly bound to each other by hydrogen bounds, resulting in less availability for enzyme hydrolysis (Singh et al., 2010). In cereal grains, varieties high in amylopectin are termed 'waxy' which originally referred to the translucent property of the endosperm of high amylopectin corn (Dieckmann, 2011). The variation of amylose and amylopectin composition in barley starch will affect functional properties of starch. For example, a higher percentage of amylopectin increase solubility, whereas the higher the amylose content, the lower the starch digestibility due to a positive correlation between amylose content and resistant starch formation (Singh et al., 2010). Variations in starch among different cereal grains, as well as variations within cultivars, are considered to influence starch degradability in the rumen (McAllister and Cheng, 1996; Mills et al., 1999; Offner et al., 2003; Svihus et al., 2005). Genetic modification allows variation of starch composition for targeted functionality (Martin, 2012). The alteration of starch in barley was primarily applied in food barley for humans (Izydorczyk and Dexter, 2008). However, these barley varieties may affect

nutrient availability in animals. Previous studies found that genetic differences in barley varieties in amylose to amylopectin ratios affected starch degradability in the rumen, which was associated with the α-amylase activity of rumen microorganisms. Low amylose to amylopectin ratios in barley starch resulted in higher sensitivity to α-amylase and a higher starch degradation rate (MacGregor and Fincher, 1993; Hristov et al., 2002).

2.3.3. β-Glucan

2.3.3.1.   Physical Properties and Chemical Structures of β-Glucan

As one of the non-starch polysaccharides, β-glucan can be found in different organic sources including cereal grains, bacteria and algae, but is mainly concentrated in the internal aleurone and endosperm cell walls of cereals (Charalampopoulos et al., 2002; Demirbas 2005; Holtekjølen et al., 2006) such as oat and barley (Havrlentová and Kraic, 2006). In cereals, β-glucan consists of β-d-glucopyranose units linked through (1→4) and (1→3) glycosidic bonds (Havrlentová et al., 2011). Molecular weights of β-glucan vary between cultivars. Higher molecular weights of β-glucan will result in higher viscosity of viscous slurries (Juskiw et al., 2011). In barley, β-glucan accounts for 2-7% of DM with around 75% in cell wall polysaccharides (Zhang et al., 2000; Ullrich, 2011). The β-glucans found in yeast and fungi are different from those found in cereals as they consist of a 1,3 β-linked glycopyranosyl backbone with 1,6 β-linked side chains. β-glucan in food grains is important for human health, while in feed grains, glucan is indigestible bymonogastric animals due to a lack of β-glucanases, but digestible by ruminants due to microorganism activities (Ullrich, 2011).

2.3.3.2.   Nutrient Effects of β-Glucan in Humans

Hulless barley has been successfully used for food by humans with the advantage of the higher β-glucan level (Bhatty, 1986). β-glucan plays an important role in maintaining some blood biochemical parameters such as lowering plasma cholesterol, reducing glycaemic index, reducing the risk of colon cancer, and reducing the risk of coronary heart diseases (Maki et al., 2007; Vasiljevic et al., 2007; Izydorczyk and Dexter, 2008). Some studies reported the potential effect of β-glucan in prevention of colonic diseases (Nilsson et al., 2008; Kim et al., 2006). Its significance in improving resistance to infections was reported by Cheol-Heui et al. (2003). Bae

et al. (2009) reported inclusion of β-glucan in the diet could significantly reduce the body weight of model mice. In the food industry, β-glucan was superior in improving sensoric and gustatory properties in beverages or breadmaking due to its high viscosity (Lyly et al., 2003; Gajdošová et al., 2007; Butt et al., 2008; Lazaridou et al., 2004; Lee et al., 2009).

2.3.3.3. Nutritional Effects of β-Glucan in Livestock

β-glucans extracted from the cell wall of baker's yeast (*Saccharomyces cerevisiae*) is reported to simulate the immune system (Miura et al., 1996; Cox and Dalloul, 2010). Chae et al. (2006) reported that broilers fed diets with 0.02% and 0.04% of β-glucan supplementation had improved feed intake and weight gain. In another study, male broilers fed with β-glucan at 50 and 75 mg/kg inclusion rate showed higher intake and weight gain compared with birds fed a normal diet or diet with a higher β-glucan inclusion rate (Zhang et al., 2008). On the effect on the immune response, the proliferating ability of macrophages in chickens was enhanced when chickens were fed a β-glucan supplemented diet (Guo et al., 2003). Moreover, Lowry et al. (2005) reported the enhancement of protection against pathogens when β-glucan was applied as a feed additive.

In pigs, some studies observed that β-glucan supplementation could improve average daily gain (ADG). The optimal inclusion rates of β-glucan in pig diets varied in several reports but all were between 250 and 500 ppm (Dritz et al., 1995; Hiss and Sauerwein, 2003). Being a soluble non-structure polysaccharide (NSP), β-glucan may increase viscosity. Some studies reported an increase in retention time of digesta in gastro-intestinal tract (GIT), which may influence microbial activity in the upper GIT by affecting bacteria growth (Leterme et al., 2000; Charalampopoulo et al., 2002). Pieper et al. (2008) reported that the mixed-link β-glucan content of barley and oat influenced significantly the composition of the microbial community in the intestine. As β-glucan in hulless barley varieties increased, it reduced microbial diversity. Bird et al. (2007) also mentioned that the number of *Lactobacilli.* can be increased by β-glucan and resistant starch.

β-glucan is assumed to be completely digested in the rumen of cattle. As reported by Gruve et al. (2006c), β-glucan digestibility varied between cultivars. β-glucan content has been observed to be positively correlated to barley qualities such as viscosity, gelation, particle size and barley starch cell wall (Bleidere and Gaile, 2012). However, this trait may lower the starch

degradation rate in the rumen (Oscarsson et al., 1997; Zheng et al., 2000; Izydorczyk and Dexter, 2008). The greater bypass β-glucan will then be useful for stimulating the immune system in ruminants (Gruve et al., 2008; Juskiw et al., 2011).

2.4. Conventional Feed Evaluation Methods for Ruminants

2.4.1. Cornell Net Carbohydrate and Protein System for Feed Evaluation

The Cornell Net Carbohydrate and Protein System (CNCPS), was first published by Russell et al. (1992), Sniffen et al. (1992), and Fox et al. (1992). The system was intended to summarize empirical and mechanistic approaches into models and programs in order to estimate feed intake, fermentation, passage and intestinal digestion of feed protein and carbohydrate, nutrient utilization, reserves, and excretion (Chalupa and Boston, 2003; Tylutki et al., 2008).

Carbohydrate and protein fractionation in CNCPS is used to describe feed composition by their variation in digestion rates and passage, and estimate the amount of structural carbohydrate (SC) and non-structural carbohydrate (NSC), metabolizable energy and available protein animal in feed (Sniffen et al., 1992; Tylutki et al., 2008).

The crude protein content of feed is partitioned into three major fractions, including non-protein nitrogen (NPN) such as ammonia, peptides or amino acids (PA), true protein (PB) and unavailable nitrogen or protein (PC). The true protein fraction is furthered divided into three subfractions based on differences in degradation rate in the rumen and which are: PB1, PB2 and PB3. PB1 is known as the rapidly-degraded protein or soluble true protein fraction with a degradation rate of 120−400% /h. Fraction PB2 is true protein with an intermediate degradation rate of 3–16% /h. Fraction PB3 is slowly degraded protein referred to as insoluble true protein bound to fibre with a degradation rate of 0.06–0.55% /h (Van Soest et al., 1981; Krishnamoorthy et al., 1983; Sniffen et al., 1992).

Carbohydrate fractions are computed based on NSC, SC and indigestible fibre content of feed. Carbohydrates are partitioned into five fractions: fraction CHO A (CA), fraction CHO B1 (CB1), fraction CHO B2 (CB2), fraction CHO C (CC) and fraction CNSC (non–starch carbohydrate). CA is sugar with a rapid degradation rate of 300% /h. CB1 is starch and pectin with an intermediately degradable with degradation rate of 20−50% /h. A slowly degradable fraction CB2 is available cell wall with a slow degradation rate of 2−10% /h. An unfermentable

fraction CC is the unavailable cell wall (Sniffen et al., 1992). In CNCPS ver.6, CA is further partitioned into four subfractions (CA1 to CA4) in considering the usage of carbohydrate in microbial activities and rumen fermentation in various feedstuffs (Lanzas et al., 2007; Tylutki et al., 2008).

CNCPS shows advantages in predicting feed ME, rumen N and amino acid availability when developing diets for cattle, thanks to its coverage of effects of feed variation (Lanzas et al., 2008). The system is widely used in farm management for balancing feeds and related costs, optimizing herd size, and improving the annual return, although the system is not ideal for planning feeding strategies for whole herds (Tylutki et al., 2004; Fox et al., 2004; Tylutki et al., 2008).

2.4.2. Energy Value Estimation in Feed Ingredients

The National Research Council (1996) defined energy as 'the potential to do work and can be measured only in reference to defined, standard conditions' and regarded the 'defined units are equally absolute'. Although people are familiar with the energy unit 'Joule', the calory is more welcomed by nutritionists. In animal studies, the megacalorie (1 Mcal=1,000 kcal) is more widely used in energy values of animal requirement standards (National Research Council, 1996). There are two ways to describe the energy content of feed or food: one is the underlying biochemical pathways of nutrient-ATP based modeling while the other is based on energy partitioning (GE/DE/ME/NE) (National Research Council, 1996). The later one is most commonly used in animal studies.

Energy values are estimated differently due to various feed sources as well as the energy requirement of the animal. Gross Energy (GE) is the energy in organic substrates, such as fat, protein and carbohydrate, when oxidized to carbodioxide and water via a series of reactions producing ATP. In animal feeding, precise estimation of the energy value of feed is essential for cost-effective farm management as well as for nutrient availability (National Research Council, 1996). DE, which stands for digestible energy, is the difference between gross energy and fecal energy. In the new edition of NRC Dairy (National Research Council, 2001), DE at 1X maintenance is calculated from the estimated digestible nutrient content instead of 0.04409 times total digestible nutrients (TDN) because of the variation in gross energy values among different feedstuffs. Metabolizable energy is considered as useable energy supply and is the energy from

feed after energy loss from feces, urine and gas are accounted for. It can be described by heat increment and retained energy (National Research Council, 1996), and also calculated from DE with the equation ME=$1.01 \times$DE-0.45 (National Research Council, 2001).

In NRC Dairy 2001, net energy for lactation units (NE_L) is used to describe energy values for feed, diets and the requirements of adult cows, including maintenance, lactation and pregnancy. NE_L of feeds in NRC Dairy 2001 is calculated at 74 percent of a total diet TDN_{1x} with the assumption of intake at 3X and 4X maintenance.

TDN is used to describe feed values and determined via experimental methods. In the previous edition of NRC Dairy (National Research Council, 1989), ME, DE and NE_L were calculated from old TDN at 1X maintenance. However, due to a lack of ME and NE_L values and a direct method to measure TDN of many feeds nowadays, the calculation of TDN is revised by measuring the feed composition in the 7th edition of the Nutrient Requirements of Dairy Cattle (National Research Council, 2001). TDN_{1X} now is calculated from truly digestible non-fibre carbohydrate (NFC), CP, fatty acids (converted from estimates of ether extract) and NDF of each feed (Weiss et al., 1992; National Research Council, 2001).

Tyrrell and Moe (1975) reported the negative relationship between digestibility of diets fed to dairy cattle and feed intake. A discounted TDN value is then introduced using TDN_{1X} of the entire diet instead of an individual feed, along with a discount value in determining TDN_{3X}, further applied in calculation of DE, ME and NE_L at productive levels of intake (National Research Council, 2001).

In the net energy system, NE of feed can be separated based on physiological activities of the animal without considering the influence of the diet. NE_m and NE_g are two net energy values used to estimate the energy requirement of maintenance and growth in the net energy system. They both can be calculated from ME using equations reported by Garrett (1980), in which ME was assumed as DE times 0.82 (National Research Council, 1996).

2.4.3. *In Situ* Technique—Estimation of Rumen Degradability and Kinetics of Feed Nutrients

The *in situ* technique, initially called the 'fibre bag technique', was first reported by Quin et al. (1938) to estimate feed digestion in cannulated sheep by incubating silk bags together with

feed samples in the rumen of sheep. The *in situ* technique was aimed at estimating the degradability of protein (Mehrez and Ørskov, 1977; Ørskov and McDonald, 1979) although this technique is now widely used in studying digestion of feedstuffs within the rumen and considered as a standard tool to obtain the digestion parameters as inputs in models for feed evaluation (Vanzant et al., 1998). A brief procedure is as follows: A feed is milled to pass a 3-mm screen or roller ground depending on feed quality, and then samples are placed into nylon bags with a pore size of 40-60 μm, which allows few particles to escape but does not inhibit the accessibility of microorganisms to the feed in the bags. The tied-up bags and samples are gradually introduced into the rumen of cannulated cattle at different time intervals with no more than 30 bags in each animal. Bags are then withdrawn at certain time points, washed and dried. Degradation characteristics of DM, CP, starch and NDF can be measured by analyzing the residues in bags against time after incubation, while the soluble materials within samples can be obtained by reweighing the bag and samples after washing and drying (Ørskov, 2000). Combined with the retention time effects and degradation characteristics in the rumen, Ørskov and McDonald (1979) developed the first order kinetic nonlinear model to dynamically assess the degradability of nutrients in a feed. The model was then modified by Robinson et al. (1986) and Dhanoa (1988) as the equation below:

$$R(t) = U + (100 - S - U) \times e^{-Kd \times (t - T0)},$$

where R(t) = the residue after t h incubation (%), S = soluble fraction determined from the 0 h incubation (%), U = undegradable fraction (%), T_0 = lag time (h), and K_d = degradation rate (%/h).

Based on the parameters above, the effective degradability (ED), or the extent of degradation of nutrients (Nuez-Ortń and Yu, 2010) is thus estimated according to Tamminga et al. (1994):

$$ED (\%) = S + [(100 - S - U) \times Kd)] / (Kp + Kd),$$

where S = soluble fraction (%) and K_p = estimated passage rate of digesta from the rumen (%/h) and it is assumed to be 2.5%/h for structural carbohydrate and 6%/h for concentrates (Tamminga et al., 1994). These parameters together with incubation time intervals, can further be applied to estimate the hourly effective degradation ratio with the equation reported by Sinclair et al. (1993):

$$\text{Hourly ED (g/kg DM)} = S + [(D \times Kd)/(Kp + Kd)] \times 1 - e^{-t \times (Kd + Kp)},$$

Hourly ED ratio N/CHO$_t$ =

$$1000 \times (HEDN_t - HEDN_{t-1})/[(HEDNDF_t - HEDNDF_{t-1}) + (HEDNFC_t - HEDNFC_{t-1})],$$

where N/CHOt = ratio of N to CHO at time t (g N/kg CHO); HEDN$_t$ = hourly effective degradability of N at time t (g/ kg DM); HEDN$_{t-1}$ = hourly effective degradability of N 1 h before t (g/kg DM); HEDCHO$_t$ = hourly effective degradability of CHO at time t (g/kg DM); HEDNDF$_t$ = hourly effective degradability of neutral detergent fibre at time t (g/kg DM); HEDNDF$_{t-1}$ = hourly effective degradability of neutral detergent fibre at 1 h before t (g/kg DM) HEDNFC$_t$ = hourly effective degradability of non-fibre carbohydrate at time t (g/kg DM); HEDNFC$_{t-1}$ = hourly effective degradability of non-fibre carbohydrate at 1 h before t (g/kg DM).

As reported in previous studies (Tamminga et al., 1990; Sinclair et al., 1993), the ratio of 32 g N/kg CHO truly digested in the rumen is the optimum ratio to balance microbial protein synthesis and energy cost in regards to rumen fermentation.

The advantage of this technique is its cost-effectiveness for less labour or feed required to evaluate feed quality. However, the potential problem with this technique is overestimating the actual digestibility of feed in the diet compared with *in vivo* measurement due to no or a lack of chewing and rumination to break down feed particles (Ørskov et al., 1980). Therefore, it is difficult to estimate the actual feed intake accurately via the *in situ* technique. However, the *in situ* technique is still considered an adequate and cost-effective methodology in assessing degradation characteristics of feed in the rumen environment (Ørskov et al., 1980; Ørskov, 2000).

2.4.4. *In Vitro* Technique—Estimation of Intestinal Digestibility of Feed Nutrients

The *in vitro* technique used to estimate intestinal protein digestion is considered to be cost-effective, rapid and reliable in revealing the characteristics of protein digestion in the rumen environment. A three-step *in vitro* technique was described by Calsamiglia and Stern (1995) to estimate protein digestibility in the small intestine, which aims to further predict the intestinal absorbable dietary protein of each feed. Residues from 12 h or 16 h of pre-ruminal incubation are used in this technique. After exposing the ground residues to HCl solution for 1h and then neutralizing pH with phosphate buffer, the solution is incubated at 38℃ for 24 h. Trichloroacetic acid (TCA) solution is then added to precipitate undigested protein. Intestinal digestibility of

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protein is determined by the percentage of TCA-soluble N in the N of the rumen residue (Calsamiglia and Stern, 1995).

2.4.5. Prediction of the Truly Digestible Protein Supply in the Small Intestine of Dairy Cattle

There are several models used to evaluate truly absorbed protein values for dairy cattle. Two modern protein evaluation systems, DVE/OEB system (Tamminga et al., 1994), known as the truly absorbed protein in small intestine (DVE) and degraded protein balance (OEB), and the NRC Dairy 2001 model (National Research Council, 2001) have been developed, based on previous models (National Research Council, 2001; Tamminga et al., 1994), to estimate the potential protein supply in feeds or diets for dairy cattle. However, the two models are applied differently in different countries, is that the DVE/OEB system is more welcomed in some European countries, while the NRC Dairy 2001 model is widely used for research in North America. Therefore, various studies have been conducted to compare the two models in evaluating feedstuffs in order to extend their application worldwide (Yu et al., 2003 a, b; Yu, 2005; Nuez-Ort ń and Yu, 2011; Damiran and Yu, 2012).

2.4.5.1. DVE/OEB System

Prior to estimating the truly digestible protein in the small intestine, studies on metabolic characteristics of nutrients including rumen degradation and intestinal digestion of feed are vital. In the DVE/OEB system, DVE stands for total truly digested protein in the small intestine. The DVE value of feed is calculated from the sum of digestible rumen bypass true protein (ARUP) and truly absorbed microbial protein synthesized in the small intestine (AMCP) minus a correction of endogenous protein losses in the digestive tract (EDCP). AMCP can be estimated from digestibility correction factors times fermentable organic matter, while ARUP is calculated from the digestibility of rumen undegraded protein in the small intestine, estimated from the *in vitro* technique, in proportion of total rumen undegraded protein. ENDP is estimated as 75 g/kg of undigested dry matter (Tamminga et al., 1994; Yu et al., 2003b). All parameters are in g/kg DM.

The synthesis of microbial protein requires energy supplied from carbohydrate digestion in the rumen. Therefore, the balance between efficient energy and N supply from feed is essential to maximize microbial protein synthesis. The OEB value of each feed in the DVE/OEB system is used to describe the degraded protein balance, also known as the difference between microbial protein synthesis from rumen degradable CP and that synthesized from energy available for anaerobic fermentation in the rumen. A positive OEB value indicates potential loss of energy, while a negative value represents a shortage of N supply, resulting in impaired protein synthesis (Tamminga and Jansman, 1993; Tamminga et al., 1994).

2.4.5.2.   Comparison between DVE/OEB System and NRC Dairy 2001 Model

Both the DVE/OEB and the NRC Dairy 2001 models target two outputs: 1) the truly digested and absorbed protein in the small intestine (DVE) and 2) the degraded protein balance (OEB) (Damiran and Yu, 2012), which are aimed to maximize animal productivity with the minimum amount of dietary CP input and more efficient energy utilization, although in NRC Dairy 2001, microbial protein synthesis in the rumen of a feed is calculated from total digestible nutrients (TDN). In the NRC Dairy 2001 model, the concept of metabolizable protein is introduced as composed of truly absorbed rumen undegraded feed CP (ARUP), truly absorbed microbial CP (AMCP) and truly absorbed rumen endogenous protein in the small intestine (AECP). Differing from the DVE/OEB system, MP is calculated as the sum of $ARUP^{NRC}$, $AMCP^{NRC}$, and $AECP^{NRC}$, which considers endogenous protein as gain instead of losses, in comparing to the DVE/OEB system (Yu et al., 2003a, b; Nuez-Ortń and Yu, 2011).

2.5.   Mid-IR Spectroscopy Techniques in Feed Science

2.5.1. Infrared Spectroscopy

Infrared spectroscopy is one of the spectroscopic techniques used by chemists. It measures the absorption of different IR frequencies by positioning a sample in the path of an IR beam. Previous studies suggest that the absorption of infrared radiation is proportional to energy changes, which also correspond to the various types of vibration of molecules, such as stretching and bending (Hsu, 1997). Due to the different absorption frequencies of different chemical

functional groups, IR spectroscopy can be used to determine the functional groups in the sample based on the frequencies, intensities and patterns of the peaks of functional group bands (Jackson and Mantsch, 2000; Stuart, 2004). IR spectroscopy can be applied to a wide range of sample types such as gases, liquids and solids, which makes IR spectroscopy a popular tool for identifying unknown compounds and elucidating sample structures (Hsu, 1997).

The IR region consists of three smaller areas including near IR, mid IR and far IR. Near IR means the light source region at wave numbers between ca. 13,000-4,000 cm^{-1}, mid IR located in the region of ca. 4,000-200 cm^{-1} while far IR means the region at ca. 200-10 cm^{-1}, among which mid IR is the most commonly used region. Near IR spectroscopy requires minimal or no sample preparation, while far IR requires special optical materials and equipment (Hsu, 1997). The chemical structures of specific compounds are assigned to certain absorption bands within associated infrared radiation regions. However, the regions for certain functional group are not the same among different studies owing to sample types (Yu, 2006a; Griffiths and Haseth, 2007). For example, using synchrotron-based FTIR to detect the protein amide I region of barley varieties, Liu and Yu (2010) detected the protein amide I region of six barley varieties at ca. 1722-1578 cm^{-1}, while Yu (2006a) reported the region of ca. 1710-1576 cm^{-1} as the absorption band for the protein amide I region for Valier and Harrington barley. Most commercial instruments use a dispersive spectrometer or a Fourier transform spectrometers to measure IR radiation.

2.5.2. Fourier Transform Infrared Spectroscopy

Fourier Transform Infrared Spectroscopy (FTIR) is a method of infrared spectroscopy. Compared to a dispersive IR spectrometer, a Fourier transform spectrometer has advantages of speed and sensitivity (Hsu, 1997; McCluskey, 2000). A typical dispersive IR spectrometer generates the electrical signal from the beams that impinge on the detector after passing through the sample and being dispersed by a monochromator. Each result frequency is viewed sequentially in a dispersive IR spectrometer. All frequencies are examined at the same time in Fourier Transform Infrared Spectroscopy (FTIR), which extend the capabilities of infrared spectroscopy in analyzing areas limited by dispersive instruments (Hsu, 1997).

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2.5.2.1.   Basic Principles

There are three fundamental spectrometer components in a Fourier transform system the radiation source, the interferometer and the detector. Although the radiation sources used in Fourier transform spectrometers are the same as in dispersive spectrometers, the source is water-cooled in FTIR instruments for better stability (Hsu, 1997). The key component within a FTIR system is the interferometer, and the most commonly used type is the Michelson interferometer. It consists of three components a beamsplitter, a moving mirror and a fixed mirror (Figure 2.1) (Hsu, 1997). The analysis process begins with radiation from the broadband IR source. The radiation is collimated and directed into the interferometer. Then the semitransparent beamsplitter divides the beam into two parts. Half of the IR beam is transmitted to a fixed mirror and the other half is reflected off a moveable mirror. The divided beams then are combined again at the beamsplitter to pass through the sample and then impinge on a detector which will show the proportional intensity of the interfered beam. The plot of intensity versus optical path difference is called the interferogram, which will be shown as a plot of the spectrum in frequency space when the interferogram is Fourier transformed (McCluskey, 2000). Improved sensitivity and higher optical throughput with FTIR is mainly contributed by its more sensitive detectors. The two most commonly used detectors for FTIR are deuterated triglycine sulfate (DTGS) and mercury cadmium telluride (MCT) (Figure 2.1), which helped increase the response times although MCT detectors need to be maintained at liquid nitrogen temperature to be effective.
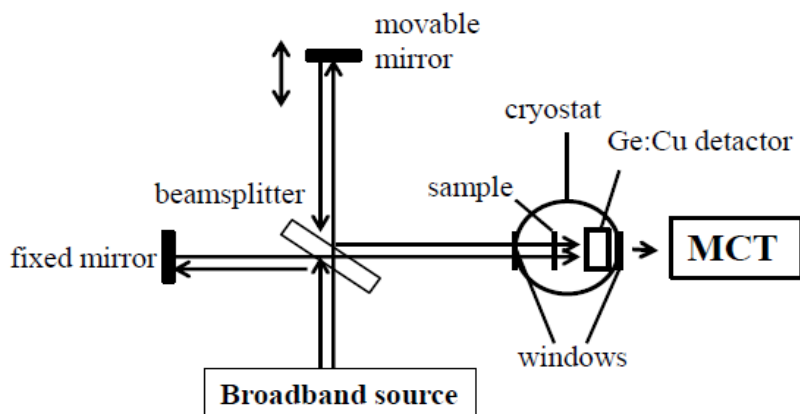
Figure 2.1 Schematic of a Fourier transform infrared (FTIR) spectrometer
(adapt from McCluskey, 2000)

2.5.2.2. Application of FTIR in Feed Analysis

FTIR can be applied in all materials in any forms but is separated into various series according to samples characteristics. For example, known as one of the most versatile sampling techniques, FTIR attenuated total reflectance (FTIR-ATR) accessories are applicable in obtaining IR spectra of difficult samples such as thick or highly absorbing solid and liquid materials in energy-limited situations (Hsu 1997; Gamage et al., 2012). As a non- destructive method, FTIR provides rapid and precise measurement with good signal-to-noise ratios (Hsu 1997; McCluskey, 2000). Vibrational spectroscopic techniques like FTIR are now applied commonly in physics, chemistry and biology, as they require little or no sample preparation, and are reagent-free, high-throughput and cost-effective analysis methods. Examples include investigating molecular changes in crops after genetic modification, or identifying the chemical compositions of microorganisms or unknown compounds in feed or food (Kizil et al., 2002; Zotti et al., 2008; Mauer et al., 2009; Santos et al., 2010). In feed analyses, it is considered that the metabolic characteristics of feed nutrients can be determined by their related molecular structures and biopolymer conformation. However, conventional reagent-based analysis methods are not able to identify the biopolymer conformations of feeds on a molecular basis due to the harsh damage of chemicals to feed samples and related internal structure during chemical reagent-based analysis (Budevska, 2002; Liu and Yu, 2011). Recent studies showed that the FTIR-ATR technique can be used as a powerful molecular means of investigating biomolecular spectral characteristics of animal feeds e.g. changes caused by heat processing and gene transformation, without chemical damage to the feed sample (Jonker et al., 2012; Gamage et al., 2012). By combining results of multivariate analysis [agglomerative hierarchical cluster analysis (CLA) and principal component analysis (PCA), univariate analyses and conventional statistical analysis], the relationship between molecular structure differences in relation to nutrition availability can be detected (Damiran and Yu, 2011; Liu and Yu, 2011; Jonker et al., 2012; Gamage et al., 2012).

2.5.3. Synchrotron-Based Fourier Transform Infrared Microspectroscopy (SR-FTIRM)

2.5.3.1. Why Synchrotron Technology?

The synchrotron is known as a particle accelerator turning electrons into light. The major components of a synchrotron include the electron gun, linear accelerator, booster ring, storage ring, beam lines and end experimental stations (Yu, 2010). Synchrotron light, also known as a full spectrum photon beam, is generated by accelerated high-speed and high-energy electrons. Beam light (100–1000 million times brighter than sunlight) produces synchron-based data at experimental stations where researchers collect molecular structure information to determine the biomolecular characteristics of a sample (Yu, 2010). Compared to globar-sourced FTIR microspectroscopy, synchrotron beam light has the advantages of higher speed and higher spatial resolution, and a smaller effective source size which could be as fine as 3-10 μm (Miller et al., 1998; Holman et al., 2002; Yu et al., 2003c, 2004c). This improves data collection efficiency and accuracy (Yu, 2010).

2.5.3.2. Application of SR-FTIRM in Plant-Based Food and Feed Research

Similar to FTIR, advanced synchrotron-based Fourier Transform Infrared Microspectroscopy (SR-FTIRM) is a non-destructive bioanalytical technique capable of detecting the biomaterial structure of plant-based foods and feeds at molecular and cellular levels with the advantages of brilliant light brightness, fast data collection, higher accuracy and small effective source size (Yu, 2010). SR-FTIRM is used in physics, biology, environmental science and human health research, as in FTIR. SR-FTIRM has been used to probe the structure of model boundary lubricant layers in nanotribology to identify the effect of the chemical structure of a lubricant on the friction and wear characteristics of a system (Beattie et al., 2012). In human health research, SR-FTIRM is used to analyze the biochemical composition of neurons to diagnose pathological changes in human body (Zhu et al., 2012). SR-FTIRM is able to image the molecular chemistry of different botanical parts (Wetzel et al., 2003). Several studies applied advanced SR-FTIRM techniques to evaluate and screen feed quality, detect inherent structure of plant-based feeds, such as dried distillers grains with solubles (DDGS), wheat, triticale, canola

and barley, with processing-induced and treatment-induced changes in relation to rumen degradation characteristics (Yu et al., 2008; Doiron et al., 2009; Yu, 2010; Liu and Yu, 2010; Liu et al., 2012).

2.5.3.3. Spectral Analysis Methods—Univariate and Multivariate Analyses

There are two types of statistical analyses used to interpret spectral information of functional group bands into biological meanings—univariate and multivariate analysis (Yu, 2006b). In univariate analysis, band intensities, integrated intensities, band frequencies and band intensity ratios are available for researchers from spectra images for quantifying spectra intensity information on a mathematical basis in relation to biological significance. Regardless of the mathematical means associated with spectral assignments for each functional group bands, multivariate analysis provides a more convenient means to distinguish differences between samples using entire spectra information with consideration of multiple properties of several objectives (Naumann et al., 2009). Multivariate analyses consist of two methods, hierarchical cluster analysis (CLA) and principal component analysis (PCA). CLA groups samples into cluster classes based on the similarity with other spectra and displays results in dendrograms with a calculated distance matrix (Yu, 2006b). A cluster is formed by the minimal distance between two spectra at the beginning. After that, the distances between all remaining spectra are recalculated and resorted accordingly to an algorithm to generate a tree diagram (Yu, 2006b). PCA focuses on the effect of independent principal components on the spectra characteristics of samples by transforming the original data with interrelated variables into a new dataset with uncorrelated principal components (PCs) in which the first few PCs may account for 95% variance. Results are usually exhibited by two-dimensional (two PCs) or three dimentional (three PCs) scatter plots depending on how many PCs are needed to distinguish the variability. Both CLA and PCA need no prior knowledge about the spectral assignments (Martin et al., 2004).

2.6. Literature Summary, Research Objectives and Hypotheses

Barley is one of the major cereal grains in Canada. There were approximately 8.1 million tonnes of barley was produced in western Canada in 2012 (MacLeod et al., 2012), of which

eighty percent of the western Canadian barley crop was used in feed production domestically (Ullrich 2011). Within barley varieties, hulless barley is a type with little or no hull and considered higher in energy and nutrients than hulled cultivars. Amylose and amylopectin are two major polymers of starch, with 15-25% and 75-85% in barley starch, respectively (Ullrich et al., 1986). As a non-starch polysaccharide, β-glucan can be found in cereal grains such as oat and barley (Havrlentová and Kraic, 2006), and is mainly concentrated in the internal aleurone and endosperm cell walls (Charalampopoulos et al., 2002; Demirbas 2005; Holtekjølen et al., 2006). Hulless barley varing in β-glucan and amylose levels has a strong effect on gut microbial profiles of pigs compared with hulled barley (Pieper et al., 2008). However, feeding barley to ruminants could induce acidosis and laminitis because the diet is high in starch, which is rapidly degraded in the rumen, resulting in accumulation of acid which damages rumen epithelium and inhibits microbial activity (Yang et al., 1997; Blake et al., 2011). The situation could be more severe if ruminants were fed hulless barley due to the absence of hull and greater kernel surface exposed to microorganisms in the rumen.

Recently, four hulless lines have been developed by the Crop Development Centre at the University of Saskatchewan. The four hulless barley lines are: zero-amylose but with very high β-glucan level--CDC Fibar; low-amylose but with high β-glucan level--CDC Rattan; normal-amylose and normal β-glucan level--CDC McGwire; and high-amylose, high β-glucan level--HB08302. Concerning the nutrient impact of hulless barley to ruminants, understanding the properties of the newly developed hulless barleys is essential for animal health and for inclusion in rations. This project aims to investigate the influence of these carbohydrate traits of the new hulless barley cultivars on chemical profiles and nutrient availability to ruminants, using chemical analysis, *in situ* rumen incubation technique, and *in vitro* intestinal nutrient digestion to predict potential protein supply by the models of DVE/OEB system and NRC Dairy 2001. Two non-destructive spectroscopic techniques, Fourier Transformed Infrared Spectroscopy (FTIR) and advanced synchrotron-based FTIR microspectroscopy, also have been used to determine the differences of molecular structure features in the new hulless barley cultivars in relation to metabolic characteristics in dairy cattle.

2.6.1. Objectives

The objectives of this study were to:

- Determine the quantitative effect of altered carbohydrate conformation features (1) amylose level, (2) amylopectin level, (3) amylose to amylopectin ratio, and (4) β-glucan level on the nutrient utilization and availability of newly developed hulless barleys in ruminants;
- Quantify the molecular structure spectral features of hulless barley with altered CHO traits in relation to nutrient availability;
- Extend information on newly developed hulless barley lines from the aspects of nutritional values and molecular structure spectral and chemical characterization.

2.6.2. Hypotheses

The hypotheses of this study were:

- Newly developed hulless barleys with specific CHO traits contain higher nutrient, digestible energy and metabolizable protein content which improves nutrient utilization and availability for ruminants;
- There are structural effects on nutrient availability of different hulless barley cultivars with altered CHO traits, which could be detected by FTIR and SR-FTIR techniques.

3. Effect of Altered Carbohydrate Traits on Chemical Profile and Rumen Degradation, Intestinal Digestion and Nutrient Supply Prediction in Dairy Cattle

3.1. Introduction

In ruminant nutrition, barley is a readily-degraded grain and used as one of the major feed alternatives in place of corn for dairy cows, due to its rapid digestion in the rumen and high energy content (Herrera-Saldana et al., 1990; McAllister and Cheng, 1996). Hulless barley differs from regular barley as it is a barley line with less hull to cover the caryopsis (Thomason et al., 2009). Hulless barley production was found in Canada with around 800,000t in 1998 (Bhatty, 1999). Previous studies on monogastric animals indicated that hulless barley provided higher energy and increased digestion as compared to hulled barley (Pieper et al., 2008; Jha et al., 2010; Anderson et al., 2012). A similar situation was also found in ruminant animals. Hulless barley contained higher available energy when applied to feedlot steers (Beames et al., 1996; Lehman et al., 1995; Zinn et al., 1996). The main polymers in barley starch are amylose and amylopectin, which normally account for 15-25% and 75-85% of starch, respectively (Ullrich et al., 1986). Amylose is composed of α-1,4 glucopyranosidic units, while there is a branching chain occurred at the sixth carbon in the amylopectin stucture (Zobel, 1988). The variation of amylose and amylopectin composition in barley starch will affect the functional properties of starch. For example, a higher percentage of amylopectin in barley starch will increase starch solubility (Zobel 1988). β-glucan is one of the non-starch polysaccharides and can be found in the internal aleurone and endosperm cell walls of cereals, especially oat and barley (Charalampopoulos et al., 2002; Demirbas, 2005; Holtekjølen et al., 2006; Havrlentová and Kraic, 2006). Some studies observed that β-glucan supplementation could improve ADG to pigs (Dritz et al., 1995; Hiss and Sauerwein, 2003). Pieper et al. (2008) revealed that hulless barley varing in β-glucan and amylose levels affected gut microbial profiles of pigs. However, in feeding barley, especially hulless barley, to ruminants, one needs to be concerned with the incidence of digestive disorders such as acidosis and laminitis (Yang et al., 1997; Blake et al., 2011). Recently, four hulless barley breeding lines varying in amylose level, amylopectin level, amylose to amylopectin ratio and β-glucan content have been developed by the Crop Development Centre, University of Saskatchewan. Prior to inclusion of hulless barley cultivars

in rations, understanding their chemical composition and their metabolic characteristics in ruminants is essential for animal health and nutritionists. Hence, the objectives of this study were to compare hulled barley (CDC Copeland) with three hullless barley cultivars varied in amylose levels (zero-amylose waxy, CDC Fibar; waxy, CDC Rattan; and high-amylose, HB08302) and a normal starch hulless barley cultivar (CDC McGwire) in terms of: 1) chemical and nutrient profiles; 2) rumen degradation kinetics; 3) *in vitro* intestinal nutrient digestion; 4) potential protein supply estimated by the DVE/OEB system and NRC Dairy 2001 model; and 5) quantify the relationship between all measured parameters and altered carbohydrate traits in hulless barley cultivars.

3.2. Materials and Methods

3.2.1. Sample Preparation

There were five barley cultivars used in this study including one hulled barley cultivar (CDC Copeland) as a reference control and four hulless barley cultivars which have been developed by Crop Development Centre, University of Saskatchewan. The four hulless cultivars were distinguished by their amylose and β-glucan content: zero-amylose, waxy, very high β-glucan CDC Fibar; low-amylose waxy, high β-glucan CDC Rattan; normal-amylose and normal β-glucan CDC McGwire; and high-amylose and high β-glucan HB08302. All cultivars were planted and grown at the University of Saskatchewan, Saskatoon, Saskatchewan, and then harvested in three consecutive years (2008, 2009, 2010), except for HB08302 (grown in 2009, 2010) for experimental purposes (Table 3.1). For chemical profile analysis, samples were ground in a Retsch mill [Retsch ZM-1, Brinkmann Instruments (Canada) Ltd., Mississauga, ON, Canada] through a 0.5 mm screen for starch, amylose, amylopectin and β-glucan analysis, and through a 1 mm screen for other chemical analysis. In preparation for *in situ* rumen incubation, 1 kg samples were coarsely ground through a 0.203 mm roller gap (Sven Grain Mill, Apollo Machine and Products Ltd., Saskatoon, SK, Canada) at the Chemical and Biological Engineering Laboratory, University of Saskatchewan (Saskatoon).

Table 3.1 Breeding targets and sampling years of four CDC hulless barleys which varied in amylopectin, amylose and β-glucan levels (comparison of the zero-amylose waxy, waxy, high-amylose and normal starch cultivars) and CDC hulled barley

Type	Lines or variety	Sample year	Amylose (% of Starch)	Amylopectin (% of Starch)	β-glucan (% DM)
			------------------Breeding targets------------------		
Hulless	CDC Fibar	2010, 2009, 2008	0	100	Very high
	CDC Rattan	2010, 2009, 2008	5	95	High
	CDC McGwire	2010, 2009, 2008	25	75	Normal
	HB08302	2010, 2009	40	60	High
Hulled	CDC Copeland	2010, 2009, 2008	25-29	71-75	3.5-4

3.2.2. Animals and Diets

Three dry Holstein cows, each fitted with a rumen cannula with an internal diameter of 10 cm (Bar Diamond, Parma, ID), were used for estimating the rumen degradation characteristics of barley cultivars. Care for the animals was taken following the guidelines of the Canadian Council on Animal Care (1993). The cows were given ad libitum access to water and individually fed 15 kg (as fed basis) of a totally mixed ration (TMR) twice daily at 0800 and 1600. TMR was formulated as a 50:50 barley silage to concentrate diet which consisted of barley, wheat, oats, dairy supplement pellets and molasses) according to the NRC maintenance requirement (National Research Council, 2001; Damiran and Yu, 2012).

3.2.3. Chemical Analysis

Dry matter (DM) (AOAC official method 930.15), ash (AOAC official method 942.05), crude fat (EE) (AOAC official method 954.02), and crude protein (CP) (AOAC official method 984.13) contents were analyzed according to the procedure of the AOAC (1990). The acid detergent fibre (ADF), neutral detergent fibre (NDF), and acid detergent lignin (ADL) values were analyzed following the procedures reported by Van Soest et al. (1991) combined with the ANKOM A200 filter bag technique (ANKOM Technology Corp., Fairport, NY). The Megazyme starch, amylose/amylopectin and β-glucan assay procedures, utilizing Megazyme test kits

(Catalogue No. K-TSTA, K-AMYL, K-BGLU; Megazyme International Ltd., Wicklow, Ireland) were followed according to the manufacturer's recommendation. The non-protein nitrogen (NPN) content was estimated by precipitation of true protein in the filtrate with trichloroacetic acid and calculated as the difference between total N and the N content in the residue after filtration. Total soluble crude protein (SCP) was determined according to the procedure of Roe et al. (1990) that included incubating the sample with bicarbonate-phosphate buffer and filtering through Whatman #54 filter paper. The amount of neutral detergent insoluble CP (NDICP) was determined by analyzing the NDF residues for CP (AOAC 1990). Non-structural carbohydrate was calculated as $100 - (ash + CP + Fat + NDF-NDICP)$ while the total carbohydrate (CHO) and true protein were calculated according to the formulas of NRC Dairy (National Research Council, 2001).

3.2.4. Protein and Carbohydrate Fractions (CNCPS) and Energy Values

The crude protein fractions were partitioned according to the Cornell Net Carbohydrate Protein System Version 5 model (Sniffen et al., 1992). The characterizations of the protein fractions analyzed in this system were divided as: fraction PA is non-protein N, fraction PB is true protein and PC is unavailable protein. Three subfractions PB1, PB2 and PB3 were divided from PB due to differences in degradation rates in the rumen. PB1 is known as rapidly degraded protein or the soluble true protein fraction with a degradation rate of 120-400% /h. Fraction PB2 stands for intermediately degraded crude protein with an intermediate degradation rate of 3-16% /h. Fraction PB3 is slowly degraded protein referring to insoluble true protein bound to fibre with a degradation rate of 0.06-0.55% /h. Fraction PC is undegradable, unavailable protein (Van Soest et al., 1981; Krishnamoorthy et al., 1983; Sniffen et al., 1992).

Carbohydrate was partitioned into: fraction CHO A (CA), fraction CHO B1 (CB1), fraction CHO B2 (CB2) and CHO C (CC). CA is sugars with a rapid degradation rate of 300% /h. CB1 is starch and pectin with an intermediate degradation rate of 20-50% /h. A slowly degradable fraction CB2 is available cell wall with a slow degradation rate of 2-10% /h. An unfermentable fraction CC is the unavailable cell wall (Sniffen et al., 1992).

The energy values at the production level of total digestible nutrients (TDN_{1X}), digestible energy (DE_{1X}), digestible energy at 3X maintainance (DE_{p3X}), metabolizable energy (MEp_{3X}) and net energy at 3X maintainance (NE_{L3X}) were determined using the summative chemical

approach from NRC Dairy 2001. Metabolizable energy (ME), net energy for maintainance (NE_m) and net energy for gain (NE_g) were estimated from NRC (1996, 2001).

3.2.5. Rumen Incubation and Rumen Degradation Kinetics

The rumen degradation characteristics of DM, CP, starch, NDF and CHO were determined by the *in situ* rumen incubation method. Seven grams of coarsely-ground samples were weighed into each nylon bag having pore size of 40 μm. All samples bags were tightened and randomly placed into the rumens of three cannulated, dry Holstein cows for incubation for 0, 2, 4, 8, 12, 24 and 48 h according to the "gradual addition/all out" schedule (Yu et al., 2000). The bags were removed from the rumen after incubation and washed with cool water without detergent to rinse off ruminal contents. The bags were dried at 55℃ for 48 h. Dry residues in bags after incubation were weighed and reserved for chemical analysis. The residues were ground through a 1mm screen for DM, NDF and CP analysis and through a 0.5mm screen for starch analysis [Retsch ZM-1, Brinkmann Instruments (Canada) Ltd., Mississauga, ON, Canada]. Dry matter and crude protein levels in the dry residue were analyzed according to the AOAC procedure (1990) (Leco Protein/N Analyser FP-528, Leco Corp., St Joseph, MI, USA). NDF values were analyzed with the ANKOM A200 filter bag technique (ANKOM Technology Corp., Fairport, NY, USA) following the procedures reported by Van Soest et al. (1991). Starch analyzed by the manufactures' procedure using the Megazyme Total Starch Assay kit (Catalogue No. K-TSTA; Megazyme International Ltd., Wicklow, Ireland).

Degradation characteristics of DM, CP, starch (ST), NDF and CHO were determined using the first-order kinetics degradation model described by Ørskov and McDonald (1979) and modified by Robinson et al. (1986) and by Tamminga et al. (1994). The results were calculated using the non-linear (NLIN) procedure of SAS and iterative least-squares regression (Gauss-Newton method):

DM, CP, NDF and CHO: $\quad R(t) = U + D \times e^{-Kd \times (t-T0)}$,

Starch: $\quad R(t) = D \times e^{-Kd \times (t-T0)}$,

where R(t) stands for the residue of incubated material after t h incubation in the rumen (%); U and D stand for the undegradable and potentially degradable fractions, respectively (%); T_0 is lag time (h); and Kd is the degradation rate (h^{-1}).

The bypass (B) or rumen undegradable (R) values of nutrients on a percentage basis were calculated according to NRC Dairy (2001):

$$\%BDM, BCP, BNDF \text{ or } BCHO = U + D \times K_p/(K_p+K_d)$$

$$\%BST = 0.1 \times S + D \times K_p/(K_p+K_d)$$

where, S stands for soluble fraction (%); K_p stands for estimated passage rate from the rumen (h^{-1}) and was assumed to be 6% /h for DM, CP, ST and CHO, but 2.5% /h for NDF. The factor 0.1 in the formula represents that 100 g/kg of soluble fraction (S) escapes rumen fermentation (Tamminga et al., 1994).

The rumen undegradable or bypass DM, starch (ST), NDF and CHO in g/kg DM were calculated as:

$$BDM (BST \text{ or } BNDF \text{ or } BCHO) (g/kg DM) =$$

$$DM (ST \text{ or } NDF \text{ or } CHO) (g/kg DM) \times \%BDM (BST \text{ or } NDF \text{ or } CHO),$$

except the rumen undegradable protein (RUP) and rumen bypass protein (BCP) were calculated differently in the Dutch model (Tamminga et al., 1994) and NRC Dairy 2001 model (National Research Council, 2001):

$$BCP^{DVE} (g/kg DM) = 1.11 \times CP (g/kg DM) \times RUP (\%),$$

$$RUP^{NRC} (g/kg DM) = CP (g/kg DM) \times RUP (\%),$$

where 1.11 refers to the regression coefficient between *in situ* RUP and *in vivo* RUP (Verite and Geay, 1987).

The effective degradability (ED) values were calculated as:

$$\%EDDM (EDCP \text{ or } EDNDF \text{ or } EDST \text{ or } EDCHO) = S + D \times K_d/(K_p + K_d),$$

$$EDDM (CP, ST, NDF \text{ and } CHO) (g/kg DM) = DM (CP, ST, NDF \text{ and } CHO) (g/kg DM)$$

$$\times \%EDDM (CP, ST, NDF \text{ and } CHO).$$

3.2.6. Intestinal Digestion of Crude Protein, Starch and Carbohydrates

The estimation of intestinal protein digestion was determined using the three-step *in vitro* procedure described by Calsamiglia and Stern (1995). Residue samples from 12 h rumen incubation were ground and exposed to 10 mL 1 N HCl containing 1 g/L of pepsin for 1 h. The pH was neutralized with 0.5 mL 1 N NaOH and 13.5 mL phosphate buffer (pH 7.8) which contained 37.5 mg pancreatin, and then incubated at 38 ℃ for 24 h. Three mL 100% trichloriacetic acid solution was added after incubation to precipitate undigested protein. Samples

were centrifuged and the supernatant (soluble N) was analyzed by the Kjeldahl method for N (AOAC 984.13). Intestinal digestion of carbohydrate, starch and crude protein were estimated from rumen degradation kinetics of residues after incubation as described by Calsamiglia and Stern (1995) and Nuez-Ort ń and Yu (2010).

3.2.7. Hourly Effective Rumen Degradation Ratios/Potential N-to-Energy Synchronization

The effective rumen degradation ratios of N and energy were calculated hourly as modified from Sinclair et al. (1993) as below:

$$\text{Hourly ED ratio N/CHO}_t =$$

$$1000 \times (\text{HEDN}_t - \text{HEDN}_{t-1})/[(\text{HEDNDF}_t - \text{HEDNDF}_{t-1}) + (\text{HEDNFC}_t - \text{HEDNFC}_{t-1})],$$

where N/CHOt = ratio of N to CHO at time t (g N/kg CHO); HEDN_t = hourly effective degradability of N at time t (g/ kg DM); HEDN_{t-1} = hourly effective degradability of N 1 h before t (g/kg DM); HEDCHO_t = hourly effective degradability of CHO at time t (g/kg DM); HEDNDF_t = hourly effective degradability of neutral detergent fibre at time t (g/kg DM); HEDNDF_{t-1} = hourly effective degradability of neutral detergent fibre at 1 h before t (g/ kg DM); HEDNFC_t = hourly effective degradability of non-fibre carbohydrate at time t (g/kg DM); HEDNFC_{t-1} = hourly effective degradability of non-fibre carbohydrate at 1 h before t (g/kg DM).

As reported in previous studies (Tamminga et al., 1990; Sinclair et al., 1993), 32 g N/kg CHO truly digested in the rumen is the optimum ratio to balance microbial protein synthesis and energy cost in regard to rumen fermentation.

3.2.8. Prediction of the Protein Supply and Availability: DVE/OEB System and NRC Dairy 2001

The DVE/OEB system (Tamminga et al., 1994) and NRC Dairy (2001) are two useful models in predicting the protein supply to ruminants, in which DVE/OEB system is used in several European countries, while NRC Dairy (2001) is more popular with scientists in North America (Nuez-Ort ń and Yu, 2011). The principles of the two models are similar except for some differences in concepts and factors. All comparable protein parameters were calculated following the equation details reported by Yu et al. (2003) and Damiran and Yu (2012).

3.2.8.1. DVE/OEB System: Truly Digested and Absorbed Protein in the Small Intestine (DVE) and Degraded Protein Balance (OEB)

The results from studies on the metabolic characteristics of nutrients, including chemical profiles, rumen degradation and intestinal digestion of feed, were used to estimate the truly digestible protein in the small intestine. In the DVE/OEB system, DVE is summarized with the truly absorbed rumen-synthesized microbial protein in the small intestine (AMCP), the truly absorbed rumen undegraded feed protein in the small intestine (ARUP) and the endogenous protein (ENCP). OEB refers to the balance between available N and energy in the rumen (Tamminga et al., 1994). A positive OEB value indicates potential loss of energy, while a negative value represents a shortage of N supply resulting in impaired protein synthesis (Tamminga and Jansman, 1993; Tamminga et al., 1994; Yu et al., 2003a).

Estimation of microbial protein synthesis in the rumen (MCP) and truly absorbable rumen-synthesized microbial protein in the small intestine (AMCP) starts from estimating microbial protein synthesis based on organic matter fermented in the rumen (FOM) (N_MCP):

$$MCP_{FOM} \text{ (g/kg DM)} = 0.15 \times FOM \text{ (g/kg DM)},$$

where 0.15 refers to 150 g of microbial protein is assumed to be synthesized per kg FOM (Tamminga et al., 1994).

$$N\_MCP \text{ (g/kg DM)} = RDP \text{ (g/kg DM)} = CP \text{ (g/kg DM)} \times [1 - (1.11 \times BCP \text{ (\%CP)})/100],$$

where RDP refers to rumen degradable protein, 1.11 refers to the regression coefficient between *in situ* BCP and *in vivo* BCP (Verite and Geay, 1987).

Truly absorbable rumen synthesized microbial protein in the small intestine (AMCP) was estimated as:

$$AMCP^{DVE} \text{ (g/kg DM)} = 0.75 \times 0.85 \times MCP_{FOM} \text{ (g/kg DM)},$$

where 0.75 means 75% of microbial N is present in amino acid, the remaining part of N being nucleic acids. 0.85 means that 85% is assumed as the true digestibility of microbial protein (Egan et al., 1985; Yu et al., 2003d).

The truly absorbed rumen-undegraded feed protein in the small intestine (ARUP^{DVE}) was calculated based on the content and digestibility of BCP^{DVE}:

$$BCP^{DVE} \text{ (g/kg DM)} = 1.11 \times [CP \text{ (g/kg DM)} \times BCP \text{ (\%CP)})/100],$$

Therefore, $ABCP^{DVE}$ was calculated as:

$$ABCP^{DVE} \text{ (g/kg DM)} = [dBCP(\%) \times BCP^{DVE} \text{ (g/kg DM)}]/100,$$

Endogenous protein losses in the small intestine (ENDP) were estimated from undigested dry matter (UDM) as below:

$$UDM \text{ (g/kg DM)} = (Ash \times 0.35) + [OM - ((OM \times dOM)/100)],$$

Then ENDP was estimated as:

$$ENDP \text{ (g/kg DM)} = 0.075 \times UDM \text{ (g/kg DM)},$$

where 0.35 is the factor assuming that 35% of ash is not digested and dOM=OM digestibility after 120 h rumen incubation (Tamminga et al., 1994). 0.075 means the requirement of absorbed protein per kg DM in fecal excretion to compensate for endogenous losses is 75 g.

The total truly digested protein in the small intestine (DVE) and the degraded protein balance (OEB) were estimated as:

$$DVE \text{ (g/kg DM)} = AMCP^{DVE} + ABCP^{DVE} - ENDP,$$
$$OEB^{DVE} \text{ (g/kg DM)} = N\_MCP - MCP_{FOM}.$$

3.2.8.2.   NRC Dairy 2001 Model

The concept of metabolizable protein is considered to be the sum of truly absorbed rumen undegraded feed CP (ARUP), truly absorbed microbial CP (AMCP) and truly absorbed rumen endogenous protein in the small intestine (AECP) (National Research Council, 2001). Microbial protein synthesis in the rumen is estimated from total digestible nutrients (TDN) and dependent on available rumen degradable protein (RDP):

$$MCP_{TDN} \text{ (g/kg DM)} = 0.13 \times TDN_{3X} \text{ (g/kg DM)},$$

where 0.13 means 130 g of microbial protein per kg TDN_{3X} is assumend to be synthesized (National Research Council, 2001).

$EDCP^{NRC}$ is calculated as:

$$EDCP^{NRC} \text{ (g/kg DM)} = CP \text{ (g/kg DM)} \times [100 - (RUP (\%CP))/100],$$

when $EDCP^{NRC} > 1.18 \times MCP_{TDN}$, the MCP_{TDN} value is used as MCP^{NRC} for the final $AMCP^{NRC}$ calculation (National Research Council, 2001). However, in this study, RDP was less than $1.18 \times MCP_{TDN}$, MCP^{NRC} was calculated as:

$$MCP^{NRC} \text{ (g/kg DM)} = MCP_{EDCP} \text{ (g/kg DM)} = 0.85 \times RDP^{NRC},$$

where 1.18 refers to the ratio of mean RDP to microbial N and 0.85 is calculated from 1/1.18

(National Research Council, 2001).

Therefore, truly absorbed rumen synthesized microbial protein in the small intestine ($AMCP^{NRC}$) was estimated as:

$$AMCP^{NRC} \text{ (g/kg DM)} = 0.80 \times 0.80 \times MCP^{NRC},$$

where one 0.80 means that 80% of ruminally synthesized microbial CP was assumed to be true protein while the other means that 80% of true protein was assumed to be digested in the small intestine.

The estimation of rumen undegraded feed CP ($ARUP^{NRC}$) is determined by the content and digestibility of RUP^{NRC}, the calculation was:

$$RUP^{NRC} \text{ (g/kg DM)} = CP \text{ (g/kg DM)} \times RUP \text{ (\%CP)/100,}$$
$$ARUP^{NRC} \text{ (g/kg DM)} = \%dRUP \times RUP^{NRC}.$$

According to NRC model (2001), rumen endogenous CP (ECP) was calculated as:

$$ECP \text{ (g/kg DM)} = 6.25 \times 1.9 \times DM \text{ (g/kg),}$$

where 6.25 represents the conversion factor of protein to N, while 1.9 means that 1 kg DM is assumed to produce 1.9 g of N. Assuming 50% of total rumen ECP passes to the small intestine of which 80% is true protein, $AECP^{NRC}$ was calculated as:

$$AECP^{NRC} \text{ (g/kg DM)} = 0.50 \times 0.80 \times ECP \text{ (g/kg DM)}$$

Therefore, metabolizable protein (MP) was estimated as:

$$MP \text{ (g/kg DM)} = AMCP^{NRC} + ARUP^{NRC} + AECP^{NRC},$$

while protein degraded balance OEB^{NRC} was estimated as:

$$OEB^{NRC} \text{ (g/kg DM)} = EDCP^{NRC} \text{ (g/kg DM)} - 1.18 \times MCP_{TDN} \text{ (g/kg DM).}$$

3.2.9. Statistical Analysis

Due to uneven sampling of barley cultivars across years, the experimental design for this study was a Randomized Incomplete Block Design. The statistical analyses were performed using the MIXED procedure of SAS 9.2 (SAS Institute, Inc., Cary, NC, USA). For chemical and nutrient profiles, the model used for the analysis was $Y_{ij} = \mu + \rho_i + \alpha_j + e_{ij}$; for the *in situ* rumen degradation kinetics study, intestinal digestion and nutrient prediction, the model used for analysis was $Y_{ijk} = \mu + \rho_i + \alpha_j + \gamma_k + e_{ijk}$, where Y_{ijk} is an observation of the dependent variable *ijk*, μ is the population mean for the variable, ρ_i is the effect of harvest year as a random effect, α_j is the effect of the barley cultivars as a fixed effect, γ_k is *in situ* run as a random effect, and e_{ijk} is

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the random error associated with observation *ijk*. Contrast statement was used to compare the difference between hulled and hulless barley cultivars. Means were compared using the Tukey–Kramer method and the significance was declared at P<0.05.

Because the data used for the correlation study were not normally distributed, rank correlations were performed using the PROC CORR of SAS with a SPEARMAN option to investigate the relationship between altered carbohydrate traits (amylose, amylopectin, ratio of amylose to amylopectin and β-glucan) and 1) chemical profiles, protein and carbohydrate subfractions (CNCPS) and energy values; 2) rumen degradation kinetics; 3) intestinal digestion of protein, starch and carbohydrate; and 4) prediction parameters of protein supply in dairy cattle using two models within hulless barley cultivars.

3.3. Results and Discussion

3.3.1. Effect of Altered CHO Traits on Nutrient Profiles, Protein and Carbohydrate Fractions, Energy Values

3.3.1.1. Effect of Altered CHO traits on Nutrient Profiles

Amylose, amylopectin and β-glucan levels of barley varieties observed in this study agreed with breeding targets (Table 3.1) received from the Crop Development Centre, University of Saskatchewan (Table 3.2.1). In agreement with Amans (1985), the starch content of barley cultivars in this study ranged from 53 - 67% of dry matter, whereas β-glucan level fell in the range between 2.5% to 13.2% of DM in wild barley (Henry and Brown, 1987), though the β-glucan level level in normal barley varieties was reported between 3.3% to 6.3% w/w by Izydorczyk et al. (2000). Hulled barley (59.7% DM) was similar to normal-amylose hulless barley CDC McGwire (61.5% DM), (P>0.05), both were higher than the other hulless barley cultivars (P<0.05). CDC Fibar was highest (P<0.05) in amylopectin (50.6% DM) and in β-glucan (10.0% DM) but had the lowest ratio of amylose to amylopectin (Ay:Ap= 0.02) due to its low amylose level. On the contrary, HB08302 was lowest (P<0.05) in amylopectin, highest in Ay:Ap (0.59) but relatively low in β-glucan (7.5% DM) compared to CDC Fibar. For other chemical profiles, the five barley cultivars showed no significant difference in dry matter (DM) (P>0.05) although hulled barley CDC Copeland was significantly higher in ash than the hulless cultivars

(P<0.05) (Table 3.2.2). Carbohydrate profiles, including total carbohydrate (CHO), were similar (P>0.05). NDF, ADF and ADL observed in the hulless barley cultivars of the present study fell in the range reported by Yang et al. (1997). Acid detergent fibre (ADF) was greater in hulled barley than in hulless barley (P<0.05), but no significant difference in total CHO content was observed between hulled barley (CDC Copeland: 84% DM) and CDC Rattan (83% DM) (P>0.05). HB08302, the high amylose hulless barley, had no significant difference (P>0.05) in NDF (15.7% DM) compared to the hulled cultivar (13.8% DM). According to Damiran and Yu (2010), high-amylose hulless barley contained the highest starch and ADL level, however, there was no significant difference in ADL compared to the other hulless barley cultivars, but was significantly lower in starch compared to the normal-amylose HB found in this study. A possible reason could be in the difference in the harvest years of the barley samples between the two studies. The average chemical composition for two years of high-amylose barley was reported in present study, while only hulless barley harvested in 2008 was analyzed by Damiran and Yu (2010).

The fibre contents of the hulless cultivars were significantly lower than that of the hulled barley, resulting in increased percentages of nutrients on a dry matter basis, such as crude protein and crude fat (Table 3.2.2). Crude protein levels agreed with the range of 13-17% reported by Edney et al. (1992) for the hulless barley cultivar Condor. Hulled barley showed significantly higher (P<0.05) soluble protein content compared to CDC Fibar and CDC Rattan (52.5% vs. 48.5% and 48.7% CP). Among hulless barley varieties, the waxy cultivar, CDC Fibar, was significantly higher (P<0.05) in crude fat (2.7% DM), crude protein (16.2% DM) and soluble crude protein on a dry matter basis (7.8% DM) but lower in soluble crude protein (48.5% CP) than the normal-amylose cultivar, CDC McGwire. CDC McGwire was similar in β-glucan and soluble crude protein levels for the hulled control (P>0.05). According to Jagtap et al. (1993), low amylose content in barley grain was associated with higher grain hardness. Bowman et al. (2001) and Svihus et al. (2005) reported greater binding of protein to starch in barley, greater grain hardness and lower starch digestion. Therefore, the low amylose content in barley grain has the potential to improve the availability of protein and energy content, but reduce starch digestion, for ruminants.

3.3.1.2. Effect of Altered CHO Traits on Protein and Carbohydrate Fractions

Protein fractions were observed to be similar among hulless barley varieties (P>0.05) except CDC Fibar which was higher (P<0.05) in intermediately degradable protein compared to the high amylose hulless cultivar HB08302 (PB2: 40.9% vs. 30.5% CP) (Table 3.3). CDC Fibar was significantly lower in total CHO (78.8% vs. 81.0%, 84.0% DM; P<0.05) and intermediately degradable carbohydrate (CB1: 51.9% vs. 56.0%, 59.7% CHO; P<0.05) than the normal-amylose hulless cultivar CDC McGwire and hulled barley CDC Copeland. Hulled barley was lower (P<0.05) in fast degradable sugars (CA: 23.6% CHO) but relatively greater in CB1 (59.7% CHO) and slowly degradable carbohydrate (CB2: 13.1% CHO) and significantly higher in unavailable cell wall (CC: 3.6% CHO) compared to the hulless cultivars. Hulled barley CDC Copeland was also significantly higher (P<0.05) in rapidly degraded protein compared to CDC Fibar (PB1: 45.9% vs. 42.4% CP).

3.3.1.3. Effect of Altered CHO Traits on Energy Values

As shown in Table 3.4, hulless barley cultivars were significantly greater in energy content compared to the hulled cultivar (P<0.05), while there was no difference in total digestible nutrient among the hulless barley cultivars (P>0.05). In hulless barley cultivars, CDC Fibar showed greater digestible energy, metabolizable energy and net energy at 3X maintenance (DE_{p3x}, ME_{p3x} and NE_{Lp3x}) than the other hulless varieties (P<0.05).

3.3.1.4. Correlation Analysis between Altered Carbohydrate Traits and Chemical and Nutrient Profiles of Hulless Barley Cultivars

Among hulless barley varieties, the altered carbohydrate traits were observed with positive and negative effects on tested chemical profiles and energy values (Table 3.5). The total CHO level in hulless barley cultivars was positively correlated to amylose (r=0.77, P<0.01) and amylose to amylopectin ratio (Ay: Ap, r=0.77, P<0.01) in starch but negatively correlated toβ-glucan level (r=−0.82, P<0.001). However, starch level in hulless barley were tended to be positively correlated to amylose (P<0.10) but strongly negatively correlated to β-glucan levels (r=−0.93, P<0.001). This may be an explaination for chemical analysis results that normal

β-glucan hulless barley CDC McGwire was observed similar starch content as hulled barley (61.5% vs. 59.7% DM) which were both higher than other hulless cultivars (P<0.05)(Table 3.2.1). Crude protein and soluble crude protein levels in hulless barley were negatively correlated to amylose and Ay:Ap ratio (P<0.05) but positively correlated to amylopectin and β-glucan level (P<0.05). However, soluble crude protein on crude protein basis was positively correlated to amylose and ratio of Ay:Ap (P<0.05), but negatively correlated to amylopectin and β-glucan levels in hulless barley (P<0.05). In protein subfractions, intermediately degradable protein fraction (PB2) and slowly degradable protein (PB3) were strongly correlated with altered starch traits (P<0.05) instead of β-glucan level (P>0.05), in which PB2 was negatively correlated to amylose (r=−0.85, P<0.01) and Ay:Ap (r=−0.84, P<0.001) but positively correlated to amylopectin (r=0.75, P<0.01), whereas PB3 was positively correlated to amylose (r=0.84, P<0.01) and Ay:Ap (r=0.82, P<0.01) but negatively correlated to amylopectin (r=−0.80, P<0.01).

Altered starch traits were found to be correlated to CHO in carbohydrate profiles. For carbohydrate subfractions, fast degradable sugar (CA) was shown to be negatively correlated to Ay:Ap (r=−0.61, P<0.05) but positively correlated to β-glucan level (r=0.84, P<0.001). Intermediately degradable carbohydrate (CB1) was negatively correlated to β-glucan level (r=-0.93, P<0.001).

Digestible energy (DE_{1x}: r=−0.62, DE_{p3x}: r=−0.83), metabolizable energy (ME_{p3x}: r=−0.83) and net energy for lactation at 3x maintainance (NE_{Lp3x}: r=−0.79) had negative correlation to amylose and Ay:Ap levels in barley starch (P<0.05). These energy values were also found to be positively correlated to β-glucan level (P<0.01) except for DE_{1x} (Table 3.5). For DE_{p3x} and ME_{p3x}, amylose level and Ay:Ap ratio accrounted for over 60% of the variation while β-glucan levels accounted for approximately 50%. This indicates that these energy values had a stronger relationship with altered starch traits compared to β-glucan level. With respect to overall energy contents in hulless barley varieties, lower amylose and amylose to amylopectin ratio and a high β-glucan level were associated with higher energy.

Hulless barleys contained higher soluble protein and energy than the hulled cultivar. Alteration of carbohydrate traits affected total CHO, soluble protein and energy level in hulless barley. The lower amylose and higher β-glucan level in hulless barley, along with the higher protein and energy contents, improving nutrient composition and available energy to ruminants.

Table 3.2.1 Differences in β-glucan levels and starch composition of hulless barleys with altered carbohydrate traits and hulled barley

| Item | Hulled | Hulless | | | | SEM | P value | Contrast |
	Copeland (n=3)	Fibar (n=3)	Rattan (n=3)	McGwire (n=3)	HB08302 (n=2)			Hulled vs. Hulless P value
Breeding Targets								
Amylose Level (% ST)	25-29	0	5	25	40	-	-	-
Amylopectin Level (% ST)	71-75	100	95	75	60	-	-	-
β-Glucan Level (% DM)	3.5-4.0	Very High	High	Normal	High	-	-	-
β-glucan (%DM)	3.8^c	10.0^a	7.4^b	4.7^c	7.5^b	0.40	<0.0001	<0.0001
Starch (%DM)	59.7^a	51.9^c	56.0^b	61.5^a	55.0^{bc}	1.19	0.0002	0.0021
Amylose (%DM)	16.1^b	1.3^d	4.3^c	15.9^b	20.2^a	0.24	<0.0001	<0.0001
Amylopectin (%DM)	43.6^b	50.6^a	51.7^a	45.7^b	33.9^c	1.24	<0.0001	0.0775
Amylose (% of Starch)	27.0^b	2.5^d	7.7^c	25.8^b	36.9^a	0.56	<0.0001	<0.0001
Amylopectin (% of Starch)	73.0^c	97.5^a	92.3^b	74.2^c	63.1^d	0.56	<0.0001	<0.0001
Ratio Amylose:Amylopectin	0.37^b	0.02^d	0.08^c	0.35^b	0.59^a	0.01	<0.0001	<0.0001

Note: ST: starch

SEM= Standard error of mean; Means with different letters within the same row differ (P<0.05).

Multi-treatment comparison: Tukey-Kramer method.

Table 3.2.2 Effect of altered carbohydrate traits on chemical profiles of hulless barleys in comparison with hulled barley

| Item | Hulled | Hulless | | | | SEM | P value | Contrast |
	Copeland (n=3)	Fibar (n=3)	Rattan (n=3)	McGwire (n=3)	HB08302 (n=2)			Hulled vs. Hulless P value
Amylose Level (% of ST)	27.0[b]	2.5[d]	7.7[c]	25.8[b]	36.9[a]	0.56	<0.0001	<0.0001
Amylopectin Level (% of ST)	73.0[c]	97.5[a]	92.3[b]	74.2[c]	63.1[d]	0.56	<0.0001	<0.0001
β-Glucan Level (% DM)	3.8[c]	10.0[a]	7.4[b]	4.7[c]	7.5[b]	0.40	<0.0001	<0.0001
Basic Chemical								
DM (%)	91.1	91.4	91.2	91.0	91.4	0.20	0.3190	0.2911
Ash (% DM)	2.6[a]	2.4[b]	2.2[bc]	2.0[c]	2.1[bc]	0.09	0.0001	<0.0001
EE (% DM)	2.1[b]	2.7[a]	2.5[ab]	2.2 [b]	2.7 [a]	0.09	0.0031	0.0022
Carbohydrate Profile								
NDF (% DM)	15.7[a]	11.0[b]	11.3[b]	10.3[b]	13.8[a]	0.37	<0.0001	<0.0001
ADF (% DM)	5.7[a]	2.6[b]	2.5[b]	2.3[b]	2.8[b]	0.24	<0.0001	<0.0001
ADL (% DM)	1.3[a]	0.6[b]	0.6[b]	0.6[b]	0.5[b]	0.08	<0.0001	<0.0001
CHO (% DM)	84.0[a]	78.8[d]	83.0[ab]	81.0[c]	81.6[bc]	0.30	<0.0001	<0.0001
NSC (% CHO)	83.3[c]	88.2[ab]	89.9[a]	88.4[ab]	86.2[b]	0.58	<0.0001	<0.0001
Protein Profile								
CP (% DM)	11.3[d]	16.2[a]	14.4[b]	12.8[c]	13.6[bc]	0.30	<0.0001	<0.0001
SCP (% DM)	5.9[c]	7.8[a]	7.0[b]	6.6[bc]	7.0[b]	0.15	0.0002	0.0001
SCP (% CP)	52.5[a]	48.5[c]	48.7[bc]	51.6[a]	51.3[ab]	0.66	0.0016	0.0024
NPN (% DM)	0.7	1.0	0.8	0.8	0.8	0.14	0.3014	0.2461
NPN (% CP)	6.6	6.1	5.4	6.5	6.3	1.14	0.6789	0.5040
NPN (% SCP)	12.5	12.5	10.9	12.6	12.2	2.16	0.8412	0.7509
NDICP (% CP)	1.62	1.72	1.9	1.92	2.31	0.267	0.0836	0.0684
ADICP (% CP)	0.15	0.16	0.15	0.11	0.25	0.061	0.1777	0.6085

Note: ST: starch; DM: dry matter; CP: crude protein; NDF: neutral detergent fibre; ADF: acid detergent fibre; ADL: acid detergent lignin; ADICP: acid detergent insoluble crude protein; NDICP: neutral detergent insoluble crude protein; NPN: non-protein nitrogen; SCP: soluble crude protein; EE: ether extracts (crude fat); CHO: carbohydrate; NSC: non-structural carbohydrate; NPN: non-protein nitrogen.

SEM= Standard error of mean; Means with different letters within the same row differ (P<0.05).

Multi-treatment comparison: Tukey-Kramer method

Table 3.3 Effect of altered carbohydrate traits on protein and carbohydrate (CHO) fractions of hulless barleys in comparison with hulled barley

Item	Hulled Copeland (n=3)	Fibar (n=3)	Rattan (n=3)	McGwire (n=3)	HB08302 (n=2)	SEM	P value	Contrast Hulled vs. Hulless P value
		Hulless						
Amylose Level (% of ST)	27.0^b	2.5^d	7.7^c	25.8^b	36.9^a	0.56	<0.0001	<0.0001
Amylopectin Level (% of ST)	73.0^c	97.5^a	92.3^b	74.2^c	63.1^d	0.56	<0.0001	<0.0001
β-Glucan Level (% DM)	3.8^c	10.0^a	7.4^b	4.7^c	7.5^b	0.40	<0.0001	<0.0001
Protein fractions								
PA (% CP)	6.6	6.1	6.5	5.4	6.3	1.14	0.6789	0.5040
PB1 (% CP)	45.9^a	42.4^b	45.1^{ab}	43.3^{ab}	45.0^{ab}	0.88	0.0128	0.0142
PB2 (% CP)	33.2^{ab}	40.9^a	33.3^{ab}	38.0^{ab}	30.5^b	2.52	0.0160	0.1976
PB3 (% CP)	13.0	9.7	14.2	12.2	16.4	1.78	0.0839	0.9374
PC (% CP)	1.3	1.0	1.0	1.1	1.8	0.44	0.1277	0.5808
Carbohydrate fractions								
CA (% CHO)	23.6^c	36.3^a	28.4^b	32.4^{ab}	32.1^{ab}	1.56	0.0002	<0.0001
CB1 (% CHO)	59.7^a	51.9^c	61.5^a	56.0^b	54.1^{bc}	1.19	0.0002	<0.0001
CB2 (% CHO)	13.1^a	9.9^c	8.5^c	10.0^{bc}	12.5^{ab}	0.53	0.0008	<0.0001
CC (% CHO)	3.6^a	1.9^b	1.7^b	1.7^b	1.4^b	0.23	<0.0001	<0.0001

Note: ST: starch; PA: non-protein N; PB: true protein; PB1: rapidly degraded protein or soluble true protein fraction with degradation rate of 120–400% /h; PB2: intermediately degraded crude protein with an intermediate degradation rate of 3–16% /h; PB3: slowly degraded protein or insoluble true protein bound to fibre with degradation rate of 0.06–0.55% /h; PC: undegradable, unavailable protein; CA: Fast degradable sugars with the degradation rate of 300% /h; CB1: starch, intermediately degradable with degradation rate of 20–50% /h; CB2: slowly degradable available cell wall with a slow degradation rate of 2–10% /h; CC: the unavailable cell wall; NSC: non-structural carbohydrate.

SEM= Standard error of mean. Means with different letters within the same row differ (P<0.05).

Multi-treatment comparison: Tukey-Kramer method.

Table 3.4 Effect of altered carbohydrate traits on truly digestible nutrient and energy values of hulless barleys in comparison with hulled barley

| Item | Hulled | Hulless | | | | SEM | P value | Contrast |
	Copeland (n=3)	Fibar (n=3)	Rattan (n=3)	McGwire (n=3)	HB08302 (n=2)			Hulled vs. Hulless P value
Amylose Level (% of ST)	27.0[b]	2.5[d]	7.7[c]	25.8[b]	36.9[a]	0.56	<0.0001	<0.0001
Amylopectin Level (% of ST)	73.0[c]	97.5[a]	92.3[b]	74.2[c]	63.1[d]	0.56	<0.0001	<0.0001
β-Glucan Level (% DM)	3.8[c]	10.0[a]	7.4[b]	4.7[c]	7.5[b]	0.40	<0.0001	<0.0001
Truly digestible nutrient (% DM)								
tdNFC	71.3[b]	70.8[b]	73.0[b]	76.1[a]	71.7[b]	0.72	0.0013	0.0305
tdCP	11.2[d]	16.1[a]	14.3[b]	12.7[c]	13.6[bc]	0.31	<0.0001	<0.0001
tdNDF	7.7[a]	5.4[b]	5.6[b]	4.9[b]	7.1[a]	0.29	0.0004	0.0002
tdFA	1.1[b]	1.7[a]	1.5[ab]	1.2[b]	1.6[a]	0.09	0.0031	0.0022
Total digestible nutrient (% DM)								
TDN_{1x}	85.67[b]	89.16[a]	89.24[a]	89.43[a]	89.07[a]	0.353	<0.0001	<0.0001
Energy values								
DE_{1x} (Mcal/kg, dairy)	3.75[c]	3.96[a]	3.94[ab]	3.92[ab]	3.92[b]	0.012	<0.0001	<0.0001
DE_{p3x} (Mcal/kg, dairy)	3.08[d]	3.20[a]	3.18[b]	3.16[c]	3.16[c]	0.004	<0.0001	<0.0001
ME_{p3x} (Mcal/kg, dairy)	2.66[c]	2.78[a]	2.76[b]	2.76[b]	2.74[b]	0.005	<0.0001	<0.0001
NE_{Lp3x} (Mcal/kg, dairy)	1.68[d]	1.76[a]	1.75[ab]	1.74[c]	1.74[bc]	0.004	<0.0001	<0.0001
ME (Mcal/kg, beef)	3.07[b]	3.25[a]	3.23[a]	3.22[a]	3.22[a]	0.011	<0.0001	<0.0001
NE_m (Mcal/kg, beef)	2.09[c]	2.24[a]	2.22[ab]	2.21[b]	2.21[b]	0.008	<0.0001	<0.0001
NE_g (Mcal/kg, beef)	1.43[c]	1.55[a]	1.53[ab]	1.52[b]	1.52[ab]	0.007	<0.0001	<0.0001

Note: ST: starch; tdNFC: truly digestible non-fibre carbohydrates; tdCP: total digestible crude protein; tdNDF: total digestible neutral detergent fibre; tdFA: total digestible fatty acid; TDN_{1x}: total digestible nutrients; DE_{1x} (Mcal/kg): digestible energy; DE_{p3x} (Mcal/kg): digestible energy at a production level (3x maintenance); ME_{p3x}: metabolizable energy at a production level (3x maintenance); NE_{Lp3x}: Net energy at a production level (3x maintenance); ME: metabolizable energy; NE_m: net energy for maintainance; NE_g: net energy for gain.

SEM= Standard error of mean; Means with different letters within the same row differ (P<0.05).

Multi-treatment comparison: Tukey-Kramer method.

Table 3.5 Correlation analyses between altered carbohydrate traits (amylose level, amylopectin level, ratio of amylose to amylopectin, and β-glucan level) and chemical profiles, protein and carbohydrate fractions and energy values in hulless barley varieties

Items	Altered Starch Traits			
	Amylose Level (% DM)	Amylopectin Level (% DM)	Ratio of Amylose to Amylopectin	β-Glucan Level (% DM)
	------------------------Spearman Correlation R value --------------------------			
Basic chemical composition				
DM (%)	-0.05	0.29	-0.03	0.19
Ash (% DM)	-0.64	0.38	-0.63*	0.67*
EE (% DM)	-0.25	0.07	-0.24	0.89***
Carbohydrate profile				
NDF (% DM)	0.17	-0.09	0.18	0.20
ADF (% DM)	-0.06	0.21	-0.03	0.04
ADL (% DM)	-0.43	0.53$^+$	-0.41	0.07
CHO (% DM)	0.77**	-0.60$^+$	0.77**	-0.82**
NSC (% CHO)	-0.05	0.07	-0.07	-0.31
Starch (% DM)	0.56$^+$	-0.29	0.57	-0.93***
Protein Profile				
CP (% DM)	-0.80**	0.64*	-0.80**	0.78**
SCP (% DM)	-0.73*	0.63*	-0.72*	0.75**
SCP (% CP)	0.82**	-0.68*	0.83**	-0.62*
NPN (% DM)	-0.14	0.06	-0.16	0.34
NPN (% CP)	0.10	-0.12	0.09	0.07
NPN (% SCP)	0.00	-0.02	-0.02	0.14
NDICP (% DM)	0.55$^+$	-0.59$^+$	0.52$^+$	-0.14
ADICP (% DM)	0.21	-0.37	0.18	0.14
NDICP (% CP)	0.68*	-0.68*	0.66*	-0.35
ADICP (% CP)	0.35	-0.45	0.33	0.33
Protein subfractions				
PA (% CP)	0.10	-0.12	0.09	0.07
PB1 (% CP)	0.57$^+$	-0.50	0.60$^+$	-0.56$^+$
PB2 (% CP)	-0.85***	0.75**	-0.84**	0.51
PB3 (% CP)	0.84**	-0.80**	0.82**	-0.49
PC (% CP)	0.35	-0.45	0.33	0.05
Carbohydrate subfractions				
CA (% CHO)	-0.60$^+$	0.32	-0.61*	0.84**
CB1 (% CHO)	0.56$^+$	-0.29	0.57$^+$	-0.93***
CB2 (% CHO)	0.08	-0.09	0.09	0.28
CC (% DM)	-0.53$^+$	0.60$^+$	-0.50	0.15

$^+$ for P<0.10, * for P<0.05, ** for P<0.01, *** for P<0.001

Table 3.5 Cont'd

Items	Altered Starch Traits			
	Amylose Level (% DM)	Amylopectin Level (% DM)	Ratio of Amylose to Amylopectin	β-Glucan Level (% DM)
	------------------------Spearman Correlation R value --------------------------			
Truly digestible nutrient				
tdNFC (% DM)	0.47	-0.22	0.47	-0.79**
tdCP (% DM)	-0.79**	0.66*	-0.78**	0.83**
tdNDF (% DM)	0.16	-0.12	0.17	0.21
tdFA (% DM)	-0.25	0.07	-0.24	0.89***
TDN_{1x} (% DM)	0.16	-0.38	0.13	0.07
Energy values				
DE_{1x} (Mcal/kg, dairy)	-0.62*	0.43	-0.64*	0.52[+]
DE_{p3x} (Mcal/kg, dairy)	-0.83**	0.65*	-0.85***	0.76**
ME_{p3x} (Mcal/kg, dairy)	-0.83**	0.63*	-0.84**	0.74**
NE_{Lp3x} (Mcal/kg, dairy)	-0.79**	0.60[+]	-0.80**	0.77**
ME (Mcal/kg, beef)	-0.57[+]	0.42	-0.59[+]	0.62*
NE_m (Mcal/kg, beef)	-0.68*	0.53[+]	-0.69*	0.65*
NE_g (Mcal/kg, beef)	-0.60[+]	0.38	-0.61*	0.73*

[+] for $P<0.10$, * for $P<0.05$, ** for $P<0.01$, *** for $P<0.001$

3.3.2. Effect of Altered CHO Traits on Ruminal Degradation Kinetics of Various Nutrients

3.3.2.1. *In Situ* Rumen Degradation Kinetics of DM

Rumen degradation kinetics of nutrients varied with cultivars (Ramsey et al., 2001; Kaiser et al., 2004). In Table 3.6, dry matter (DM) degradation rates (K_d) and soluble fractions (S) of hulless barley cultivars were similar except that CDC McGwire was found higher (P<0.05) than HB08302 (15.5% vs. 9.2 %/h; 6.4 vs. 3.1%).   No significant difference was found in degradable (D) and undegradable (U) fractions of DM among hulless barleys (P>0.05), although the degradable fraction of DM in hulless barley cultivars was higher than in hulled barley (P<0.05). HB08302 was observed to have the highest bypass DM (BDM: 465 g/kg, P<0.05) but the lowest effective degradable DM (EDDM: 535 g/kg, P<0.05) among barley cultivars. On the contrary, among hulless cultivars, CDC McGwire was found lowest in BDM (335 g/kg, P<0.05), but the highest in EDDM (665 g/kg, P<0.05), although there was no significant difference when compared to hulled barley with these two parameters (P>0.05).

3.3.2.2. *In Situ* Rumen Degradation Kinetics of CP

There was no significant difference found either among hulless barley cultivars or between hulless barley and hulled barley in degradation rate (K_d), lag time (T_0), soluble fraction (S), degradable fraction (D) or undegradable fraction (U) (P>0.05) (Table 3.7), indicating that the barley varieties were similar in crude protein degradation kinetics in terms of degradation rate, retention time and crude protein fractions on a percentage basis. No significant difference was found in bypass crude protein percentage between HB08302 and CDC Rattan (BCP: 52.1% vs. 46.0 % CP, P>0.05) although HB08302 was higher than other hulless barley cultivars as well as hulled barley (P<0.05). CDC McGwire was similar (P>0.05) in rumen undegradable protein (RUP: 55 vs. 49 g/kg DM) and bypass protein (BCP: 61 vs. 55 g/kg DM) as hulled barley; these varieties were the lowest among barley varieties (P<0.05). Hulled barley CDC Copeland contained less effective degradable crude protein (EDCP: 64 g/kg DM, P<0.05) than low and normal amylose hulless barley cultivars but was similar to the high-amylose hulless cultivar (P>0.05) in this respect. Therefore, hulless barley cultivars with a lower amylose level in starch had higher effective degradabilities of CP and greater bypass CP for intestinal digestion than these in hulled barley.

Table 3.6 Effect of altered carbohydrate traits on *in situ* rumen degradation kinetics of dry matter in hulless barleys in comparison with hulled barley

Item	Hulled Copeland (n=3)	Hulless Fibar (n=3)	Rattan (n=3)	McGwire (n=3)	HB08302 (n=2)	SEM	P value	Contrast Hulled vs. Hulless P value
Amylose Level (% of ST)	27.0[b]	2.5[d]	7.7[c]	25.8[b]	36.9[a]	0.56	<0.0001	<0.0001
Amylopectin Level (% of ST)	73.0[c]	97.5[a]	92.3[b]	74.2[c]	63.1[d]	0.56	<0.0001	<0.0001
β-Glucan Level (% DM)	3.8[c]	10.0[a]	7.4[b]	4.7[c]	7.5[b]	0.40	<0.0001	<0.0001
In situ rumen DM degradation								
K_d (%/h)	16.5[a]	12.8[abc]	12.1[bc]	15.5[ab]	9.2[c]	1.29	<0.0001	0.0013
T_0 (h)	0.19	0.23	0.26	0.00	0.27	0.130	0.5144	0.3401
S (%)	8.0[a]	4.3[bc]	4.0[bc]	6.4[ab]	3.1[c]	1.60	0.0005	0.0001
D (%)	77.4[b]	84.8[a]	83.9[a]	83.7[a]	83.4[a]	1.32	0.0023	0.0001
U (%)	14.6	10.9	12.0	9. 9	13.4	1.45	0.0858	0.0368
BDM (% DM)	35.5[bc]	39.1[b]	40.1[b]	33.5[c]	46.5[a]	2.76	<0.0001	0.0054
BDM (g/kg)	355[bc]	391[b]	401[b]	335[c]	465[a]	27.6	<0.0001	0.0054
EDDM (% DM)	64.5[ab]	60.9[b]	59.9[b]	66.6[a]	53.5[c]	2.76	<0.0001	0.0054
EDDM (g/kg)	645[ab]	609[b]	599[b]	665[a]	535[c]	27.6	<0.0001	0.0054

Note: ST: starch; K_d: the rate of degradation of D fraction (%/h); U: undegradable degradable fraction; D: potentially degradable fraction; T_0: lag time in h; S: soluble fraction in the in situ incubation; BDM: rumen bypass dry matter; EDDM: effective degradability of dry matter; K_p: passage rate of 6% /h was adopted (Tamminga et al., 1994).
SEM= Standard error of mean; Means with different letters within the same row differ (P<0.05).
Multi-treatment comparison: Tukey-Kramer method.

Table 3.7 Effect of altered carbohydrate traits on *in situ* rumen degradation kinetics of crude protein in hulless barleys in comparison with hulled barley

Item	Hulled Copeland (n=3)	Hulless Fibar (n=3)	Rattan (n=3)	McGwire (n=3)	HB08302 (n=2)	SEM	P value	Contrast Hulled vs. Hulless P value
Amylose Level (% of ST)	27.0[b]	2.5[d]	7.7[c]	25.8[b]	36.9[a]	0.56	<0.0001	<0.0001
Amylopectin Level (% of ST)	73.0[c]	97.5[a]	92.3[b]	74.2[c]	63.1[d]	0.56	<0.0001	<0.0001
β-Glucan Level (% DM)	3.8[c]	10.0[a]	7.4[b]	4.7[c]	7.5[b]	0.40	<0.0001	<0.0001
In situ rumen CP degradation								
K_d (%/h)	9.3	8.0	8.4	7.9	6.0	1.39	0.0970	0.0358
T_0 (h)	0.31	0.38	0.22	0.48	0.29	0.305	0.9670	0.9082
S (%)	5.1	7.8	4.2	8.7	4.5	3.83	0.1050	0.5315
D (%)	85.7	85.6	87.2	86.3	87.7	4.43	0.9128	0.6069
U (%)	9.2	6.7	8.7	5.0	7.8	1.52	0.2936	0.1960
BCP (% CP)	43.4[b]	44.5[b]	46.0[ab]	42.8[b]	52.1[a]	4.63	0.0022	0.0494
RUP[NRC] (g/kg DM)	49[b]	72[a]	66[a]	55[b]	70[a]	6.1	<0.0001	<0.0001
BCP[DVE] (g/kg DM)	55[b]	80[a]	74[a]	61[b]	78[a]	6.8	<0.0001	<0.0001
EDCP (% CP)	56.6[a]	55.5[a]	54.0[ab]	57.1[a]	47.9[b]	4.63	<0.0001	0.0494
EDCP (g/kg DM)	64[d]	90[a]	78[b]	73[bc]	65[cd]	6.6	<0.0001	<0.0001

Note: ST: starch; K_d: the rate of degradation of D fraction (%/h); U: undegradable degradable fraction; D: potentially degradable fraction; T_0: lag time in h; S: soluble fraction in the in situ incubation; K_p: passage rate of 6% /h was adopted (Tamminga et al., 1994); BCP: rumen bypassed crude protein (DVE/OEB system); DVE: truly absorbed rumen bypass protein in the small intestine; RUP: rumen undegraded crude protein (NRC Dairy 2001 model); NRC: National Research Council; EDCP: effective degradability of crude protein.

SEM= Standard error of mean; Means with different letters within the same row differ (P<0.05).

Multi-treatment comparison: Tukey-Kramer method

3.3.2.3. *In Situ* Rumen Degradation Kinetics of NDF

No significant differences were found in S, D and U fractions of NDF among barley cultivars (P>0.05) except that the degradation rate (K_d) was higher in CDC Fibar, zero-amylose cultivar compared to hulled barley (14.8 vs. 6.2 %/h, P<0.05) (Table 3.8). Hulled barley contained a similar percentage of bypass NDF (BNDF) in total NDF (62.5%) to CDC Fibar (55.4%), but was significantly higher than the other hulless barley varieties (P<0.05) in this respect. There were no significant differences in the percentage of effective degradable NDF (%EDNDF: P>0.05) among hulless barley varieties, but a relatively higher EDNDF content (P<0.05) was found in HB08302 (74 g/kg DM) and CDC Rattan (58 g/kg DM).

3.3.2.4. *In Situ* Rumen Degradation Kinetics of Starch

High amylose hulless barley HB08302 was lower (P<0.05) in starch degradation rate compared to CDC Fibar, CDC McGwire (K_d: 8.4 vs. 15.8, 17.2 %/h) and hulled barley (17.4 %/h) (Table 3.9). CDC Rattan and HB08302 were higher in bypass starch (BST) than other barley cultivars (P<0.05); both were over 185 g/kg DM. CDC McGwire and CDC Copeland were similar in the amount of effective degradable starch (EDST) and both were higher than the other barley cultivars (P<0.05), whereas HB08302 was found lowest in the amount of EDST among hulless cultivars (P<0.05), which may due to high amylose level in starch and resulted in lower solubility of starch in rumen.

3.3.2.5. *In Situ* Rumen Degradation Kinetics of CHO

Total CHO in CDC McGwire, similar to hulled barley, was relatively rapidly degraded compared to CDC Rattan and HB08302 (P<0.05) with rumen degradation rate over 18%/h of CHO degraded in the rumen (Table 3.10). There was no difference in soluble CHO among the barley cultivars (P>0.05) but CDC Rattan contained higher potentially degradable CHO than the other hulless barleys and hulled barley (88.5% vs. 75.5% CHO, P<0.05), while hulled barley had higher undegrdable CHO (13.7% CHO, P<0.05) than hulless cultivars except CDC Fibar (10.8% CHO, P>0.05). High amylose hulless barley had the highest percentage (26.4% CHO) but

relatively higher amount of bypass CHO (179 g/kg CHO) among barley cultivars in this study (P<0.05). CDC McGwire contained the highest level of effective degradable CHO among hulless barley varieties (P<0.05) and was similar to hulled barley in the amount of EDCHO (581 vs. 584 g/kg CHO, P>0.05).

3.3.2.6. Correlation Analysis between Ruminal Degradation Kinetics and Altered Carbohydrate Traits in Hulless Barley

Results of correlation analysis (Table 3.11) indicate that degradation rate of DM (r=-0.56, P<0.05), percent and amount of EDDM (r=-0.50, P<0.05) had negative correlations with the β-glucan level in hulless barley, while percentage and amount of BDM were positively correlated with the β-glucan level (r=0.50, P<0.05). However, about 25-30% of the variation can be explained by β-glucan level, when the the significance of the relationship is expressed as the correlation of determination (r^2). Effective degradable CP (EDCP: r=0.64, P<0.001) was positively correlated to amylopectin level, but negatively correlated to amylose level (r=-0.71, P<0.001) in hulless barley. Rumen undegradable protein (RUP) and bypass crude protein (BCP) were positively correlated with β-glucan level (r=0.63, P<0.001) with about 40%-50% of the variation accounted for. Percentage of bypass NDF was negatively correlated to amylose and Ay:Ap ratio in starch (r=-0.54, P<0.01), while both percentage and amount of effective degradable NDF were positively correlated to these two starch parameters (r=0.54, P<0.01). The soluble fraction of starch was negatively correlated with amylose (r=-0.50, P<0.05) and Ay:Ap ratio (r=-0.49, P<0.05), while the potentially degradable fraction and rumen bypass fraction of starch were opposite (P<0.05). The effective degradable fraction of starch was negatively correlated with β-glucan (r=-0.60, P<0.01). CHO degradation was affected more significantly by β-glucan level, as more than 60% of the variation in the amount of EDCHO can be explained by β-glucan level due to the negative correlation between EDCHO and β-glucan level (r=-0.80, P<0.001). However, BCHO was positively correlated to β-glucan (r=0.45, P<0.05), which explained less than 20% of the variation in BCHO. Bhatty (1999) reported that a higher level of β-glucan in barley tended to protect the barley kernel from mechanical processing and resulted in a larger particle size in final processed products. A higher β-glucan in barley will result in a thicker cell wall (Oscarsson et al., 1997; Zheng et al., 2000). Therefore, a higher β-glucan level

may also keep barley from rumen degradation, resulting in less CHO, starch and protein degraded in the rumen, but more bypass protein and CHO available for digestion in the small intestine. The results of rumen degradation kinetics revealed that a higher β-glucan level in hulless barley may reduce the DM degradation rate (r=-0.56, P<0.05).

Correlation results showed that the degradable fraction and bypass content of starch were positively affected by amylose and Ay:Ap ratio in starch (P<0.05), while the effective degradable starch (EDST) was negatively responding to the β-glucan level in hulless barleys (P<0.05). However, previous studies reported that the effective degradability of starch increased as the amylose level in starch of hulless barley increased (Foley et al., 2006; Damiran and Yu, 2010). Based on the previous studies, low amylose to amylopectin ratios of barley starch showed a higher sensitivity to α-amylase and a higher starch degradation rate (MacGregor and Fincher, 1993; Hristov et al., 2002). Tang et al. (2002) and Foley et al. (2006) both mentioned that the amylopectin level in starch was negatively responding to enzyme hydrolysis, indicating a higher amylopectin level in barley starch would be more resistant to enzyme hydrolysis, due to a higher percentage of relative crystallinity in high-amylopecin barley. However, there was no relationship between altered starch traits in hulless barley and starch degradability in the current study, but a negative correlation between EDST and β-glucan level was observed (P<0.05). A possible explanation may be more of an influence on degradability of CHO or starch in rumen from differences in β-glucan level among hulless barley cultivars than for differences in altered starch traits. However, the effect of altered starch traits on starch degradation should not be neglected due to the fact that the soluble fraction was negatively correlated, while bypass starch was positively correlated, to amylose and ratio of Ay:Ap level of hulless barley starch (P<0.05). Based on Damiran and Yu (2010), starch degradation may be affected by protein to starch ratio owing to protein in the endosperm tissue of barley surrounding starch granules in a matrix form, keeping starch from digestion by rumen bacteria (Yu et al., 2004). This may explain why the altered amylose level in hulless barley was observed to have a negative correlation with the amount of EDCP in CP degradation and BNDF percentage in NDF, but a positive correlation with BST (P<0.05).

Hulless barley has a fast degradation rate and is high in intermediately degradable CHO. This may increase the possibility of cattle suffering from rumen acidosis (Damiran and Yu, 2010). In the present study, CDC Copeland contained relatively less effective degradable crude protein (EDCP: 64 g/kg DM, $P<0.05$), had a higher effective degradable starch (EDST: 75.9 %ST), a faster CHO degradation rate (19.29 %/h) and higher undegradable CHO (U: 13.8%) compared to other hulless barley cultivars ($P<0.05$), indicating the higher starch content and availability in hulless barley than in hulled barley for post-ruminal digestion and higher effective degradable crude protein for rumen degradation. In terms of nutrient availability, hulless barley lines with lower amylose and higher β-glucan level can be considered as an alternative for ruminant feeding because of its relatively higher nutrient content than in other hulless barley varieties. The high β-glucan in hulless barley protecting nutrients from rumen degradation increases the availability of bypass nutrients for post-ruminal digestion. However, concerning the incidence of rumen disorder diseases, hulless barley lines with high amylose and high β-glucan can also be considered as a grain option due to its lower starch degradation rate and effective degradable crude protein (EDCP), which reduce the risk of rumen acidosis and increase protein availability for intestinal digestion.

Table 3.8 Effect of altered carbohydrate traits on *in situ* rumen degradation kinetics of neutral detergent fibre in hulless barleys in comparison with hulled barley

Item	Hulled	Hulless				SEM	P value	Contrast
	Copeland (n=3)	Fibar (n=3)	Rattan (n=3)	McGwire (n=3)	HB08302 (n=2)			Hulled vs. Hulless P value
Amylose Level (% of ST)	27.0[b]	2.5[d]	7.7[c]	25.8[b]	36.9[a]	0.56	<0.0001	<0.0001
Amylopectin Level (% of ST)	73.0[c]	97.5[a]	92.3[b]	74.2[c]	63.1[d]	0.56	<0.0001	<0.0001
β-Glucan Level (% DM)	3.8[c]	10.0[a]	7.4[b]	4.7[c]	7.5[b]	0.40	<0.0001	<0.0001
In situ rumen NDF degradation								
K_d (%/h)	6.2[b]	14.8[a]	8.3[ab]	8.4[ab]	11.3[ab]	2.87	0.0614	0.0316
T_0 (h)	0.22	0.00	0.07	0.09	0.00	0.133	0.5940	0.1414
S (%,)	8.5	3.9	10.7	6.54	13.5	3.54	0.3314	0.9584
D (%)	40.9	56.4	53.6	57.5	50.7	4.97	0.1378	0.0201
U (%)	50.6	39.7	36.8	35.9	36.7	5.34	0.2363	0.0267
BNDF (% NDF)	62.5[a]	55.4[ab]	50.3[b]	50.1[b]	46.5[b]	3.56	0.0011	0.0001
BNDF (g/kg DM)	98[a]	61[b]	60[b]	52[b]	64[b]	5.5	<0.0001	<0.0001
EDNDF (% NDF)	37.6[b]	44.6[ab]	49.7[a]	49.9[a]	53.5[a]	3.56	0.0011	<0.0001
EDNDF (g/kg DM)	59[ab]	49[b]	58[ab]	52[b]	74[a]	4.8	0.0048	0.8403

Note: ST: starch; K_d: the rate of degradation of D fraction (%/h); U: undegradable degradable fraction; D: potentially degradable fraction; T_0: lag time in h; S: soluble fraction in the in situ incubation; K_p: passage rate of 2.5%/h was adopted (Tamminga et al., 1994); BDNDF: rumen bypass or undegraded neutral detergent fibre; EDNDF: effective degradability of neutral detergent fibre.

SEM= Standard error of mean; Means with different letters within the same row differ (P<0.05).

Multi-treatment comparison: Tukey method.

Table 3.9 Effect of altered carbohydrate traits on *in situ* rumen degradation kinetics of starch in hulless barleys in comparison with hulled barley

Item	Hulled	Hulless				SEM	P value	Contrast
	Copeland (n=3)	Fibar (n=3)	Rattan (n=3)	McGwire (n=3)	HB08302 (n=2)			Hulled vs. Hulless P value
Amylose Level (% of ST)	27.0[b]	2.5[d]	7.7[c]	25.8[b]	36.9[a]	0.56	<0.0001	<0.0001
Amylopectin Level (% of ST)	73.0[c]	97.5[a]	92.3[b]	74.2[c]	63.1[d]	0.56	<0.0001	<0.0001
β-Glucan Level (% DM)	3.8[c]	10.0[a]	7.4[b]	4.7[c]	7.5[b]	0.40	<0.0001	<0.0001
In situ rumen starch (ST) degradation								
K_d (%/h)	17.4[a]	15.8[ab]	11.4[bc]	17.2[ab]	8.4[c]	1.97	0.0015	0.0132
S (%)	11.4	10.3	3.6	6.5	0.4	3.33	0.1595	0.0874
D (%)	88.6	89.7	96.4	93.5	99.6	3.33	0.1595	0.0874
BST (% ST)	24.1[c]	28.3[bc]	33.9[ab]	25.2[c]	40.3[a]	2.37	<0.0001	<0.0001
BST (g/kg DM)	144[b]	146[b]	189[a]	155[b]	218[a]	11.2	<0.0001	0.0007
EDST (% ST)	75.9[a]	71.7[ab]	66.1[bc]	74.8[a]	59.8[c]	2.37	<0.0001	<0.0001
EDST (g/kg DM)	453[a]	373[b]	371[b]	460[a]	320[c]	19.1	<0.0001	<0.0001

Note: ST: starch; K_d: the rate of degradation of D fraction (%/h); D: potentially degradable fraction; T_0: lag time in h; S: soluble fraction in the in situ incubation; K_p: passage rate of 6%/h was adopted (Tamminga et al., 1994); BST: rumen bypass or undegraded starch; EDST: effective degradability of starch.

SEM= Standard error of mean; Means with different letters within the same row differ (P<0.05). Multi-treatment comparison: Tukey-Kramer method.

Table 3.10 Effect of altered carbohydrate traits on *in situ* rumen degradation kinetics of carbohydrate in hulless barleys in comparison with hulled barley

Item	Hulled Copeland (n=3)	Hulless Fibar (n=3)	Rattan (n=3)	McGwire (n=3)	HB08302 (n=2)	SEM	P value	Contrast Hulled vs. Hulless P value
Amylose Level (% of ST)	27.0[b]	2.5[d]	7.7[c]	25.8[b]	36.9[a]	0.56	<0.0001	<0.0001
Amylopectin Level (% of ST)	73.0[c]	97.5[a]	92.3[b]	74.2[c]	63.1[d]	0.56	<0.0001	<0.0001
β-Glucan Level (% DM)	3.8[c]	10.0[a]	7.4[b]	4.7[c]	7.5[b]	0.40	<0.0001	<0.0001
In situ rumen total CHO degradation								
K_d (%/h)	19.29[a]	17.03[ab]	11.83[bc]	18.05[a]	9.05[c]	2.086	0.0003	0.0023
T_0 (h)	0.65	0.12	0.22	0.09	0.28	0.188	0.1984	0.0278
S (%)	10.7	8.9	4.6	6.3	2.4	2.65	0.2170	0.0716
D (%)	75.5[b]	80.3[ab]	88.5[a]	85.3[ab]	89.9[ab]	3.16	0.0210	0.0051
U (%)	13.7[a]	10.8[ab]	7.0[b]	8.4[b]	7.6[b]	1.19	0.0021	0.0004
BCHO (% CHO)	22.6[b]	22.2[b]	22.7[b]	19.1[c]	26.4[a]	1.73	0.0001	0.9625
BCHO (g/kg DM)	170[ab]	139[c]	152[bc]	137[c]	179[a]	10.1	<0.0001	0.0027
EDCHO (% CHO)	77.5[b]	77.8[b]	77.3[b]	80.9[a]	73.6[c]	1.73	0.0001	0.9625
EDCHO (g/kg DM)	584[a]	490[b]	521[b]	581[a]	498[b]	19.4	<0.0001	<0.0001

Note: ST: starch; K_d: the rate of degradation of D fraction (%/h); U: undegradable degradable fraction; D: potentially degradable fraction; T_0: lag time in h; S: soluble fraction in the in situ incubation; BCHO: rumen bypass or undegraded CHO; EDCHO: effective degradability of CHO；K_p: passage rate of 6%/h was adopted (Tamminga et al., 1994).

SEM= Standard error of mean; Means with different letters within the same row differ (P<0.05).

Multi-treatment comparison: Tukey-Kramer method.

Table 3.11 Correlation analysis between altered carbohydrate traits (amylose level, amylopectin level, ratio of amylose to amylopectin, and β-glucan level) and *in situ* rumen degradation of dry matter, crude protein, neutral detergent fibre, starch and carbohydrate in hulless barley varieties

Items	Altered Starch Traits			β-Glucan Level (% DM)
	Amylose Level (% DM)	Amylopectin Level (% DM)	Ratio of Amylose to Amylopectin	
	----------------Spearman Correlation R value ----------------			
In situ rumen DM degradation				
K_d (%/h)	-0.11	0.25	-0.08	-0.56*
T_0 (h)	-0.15	0.38+	-0.13	0.21
S (%)	0.04	0.00	0.07	-0.40+
D (%)	-0.05	0.05	-0.06	0.11
U (%)	0.14	-0.06	0.13	0.11
BDM (% DM)	0.12	-0.20	0.08	0.50*
BDM(g/kg)	0.12	-0.20	0.08	0.50*
EDDM (% DM)	-0.12	0.20	-0.08	-0.50*
EDDM (g/kg)	-0.12	0.20	-0.08	-0.50*
In situ rumen CP degradation				
K_d (%/h)	-0.23	0.45**	-0.19	-0.17
T_0 (h)	-0.09	0.02	-0.09	0.23
S (%)	-0.06	-0.08	-0.04	-0.07
D (%)	0.13	-0.10	0.12	-0.02
U (%)	0.00	0.18	0.00	0.15
BCP (% CP)	0.24	-0.29	0.20	0.20
RUP (g/kg DM)	-0.25	0.08	-0.28	0.63***
BCP (g/kg DM)	-0.25	0.08	-0.28	0.63***
EDCP(% CP)	-0.24	0.29	-0.20	-0.20
EDCP(g/kg DM)	-0.71***	0.64***	-0.70***	0.37+
In situ rumen NDF degradation				
K_d (%/h)	-0.07	0.06	-0.07	0.23
T_0 (h)	0.01	-0.01	0.01	-0.26
S (%)	0.33	-0.32	0.34	-0.22
D (%)	0.00	-0.03	-0.01	-0.07
U (%)	-0.33	0.38[+]	-0.32	0.26
BNDF (% NDF)	-0.54**	0.40[+]	-0.54**	0.41[+]

[+] for P<0.10, * for P<0.05, ** for P<0.01, *** for P<0.001.

Table 3.11 Cont'd

Items	Altered Starch Traits			
	Amylose Level (% DM)	Amylopectin Level (% DM)	Ratio of Amylose to Amylopectin	β-Glucan Level (% DM)
	-----------------Spearman Correlation R value -----------------			
In situ rumen NDF degradation				
BNDF (g/kg DM)	-0.25	0.19	-0.25	0.44*
EDNDF (% NDF)	0.54**	-0.40$^+$	0.54**	-0.41$^+$
EDNDF (g/kg DM)	0.54**	-0.28	0.54**	-0.20
In situ rumen starch degradation				
K_d (%/h)	-0.31	0.37$^+$	-0.28	-0.35
S (%)	-0.50*	0.34	-0.49*	0.06
D (%)	0.50*	-0.34	0.49*	-0.06
BST (% ST)	0.32	-0.31	0.29	0.35
BST (g/kg DM)	0.53*	-0.41$^+$	0.51*	0.11
EDST (% ST)	-0.32	0.31	-0.29	-0.35
EDST (g/kg DM)	-0.07	0.18	-0.04	-0.60**
In situ rumen CHO degradation				
K_d (%/h)	-0.26	0.29	-0.24	-0.26
T_0 (h)	0.16	0.02	0.16	0.08
S (%)	-0.36$^+$	0.24	-0.35	-0.08
D (%)	0.32	-0.20	0.30	0.04
U (%)	-0.26	0.13	-0.24	0.11
BCHO (% CHO)	0.08	-0.23	0.05	0.45*
BCHO (g/kg DM)	0.37$^+$	-0.41$^+$	0.35	0.13
EDCHO (% CHO)	-0.08	0.23	-0.05	-0.45*
EDCHO (g/kg DM)	0.32	-0.07	0.34	-0.80***

$^+$ for P<0.10, * for P<0.05, ** for P<0.01, *** for P<0.001.

3.3.3. Effect of Altered CHO Traits on Estimated Intestinal Digestion of CP, Starch and CHO

Intestinal digestibility of crude protein and starch in rumen degradation residues showed no significant differences among the barley cultivars (P>0.05) but intestinal digestibility of CHO was lowest in hulled barley (dCHO: 48.8% BCHO, P<0.05) (Table 3.12). Intestinal digestible protein was higher in hulless barley cultivars except that normal-amylose CDC McGwire (IDP: 43 g/kg DM) showed no significant difference from hulled barley CDC Copeland (38 g/kg DM, P>0.05). Zero-amylose hulless barley CDC Fibar was highest in total digestible protein while hulled barley was the lowest (TDP: 147 vs. 102 g/kg DM, P<0.05). Both CDC McGwire and CDC Copeland contained higher amount of total digestible starch (TDST) and CHO (TDCHO) than other hulless barley cultivars (P<0.05). HB08302, high-amylose hulless barley line was relatively higher in intestinal digestible bypass starch (IDBST) and CHO (IDBCHO) on dry matter basis than the other barley cultivars but similar to CDC Rattan in IDBST (P>0.05). Therefore, compared to hulled barley, hulless barley cultivars with altered starch traits contained higher or equivalent intestinal digestible nutrients as CDC Copeland but was relatively lower in total digestible starch and CHO (P<0.05).

From the correlation analysis results (Table 3.13), total digestible protein was negatively correlated to amylose (r=-0.85, P<0.001) and Ay:Ap ratio (r=-0.84, P<0.001) but positively correlated to amylopectin (r=0.77, P<0.001) and β-glucan level (r=0.74, P<0.001) of hulless barley cultivars. Intestinal digestible bypass starch and total digestible CHO were positively correlated with amylose and Ay:Ap ratio (P<0.05). Total digestible CHO (r=-0.89, P<0.001) and starch (r=-0.79, P<0.001) were both negatively correlated to β-glucan level in hulless barley. These indicate that hulless barley with lower amylose and higher β-glucan level would improve total digestible protein but reduce total digestible starch and CHO in ruminants. In the mean time, hulless barley with high amylose and high β-glucan contains higher or equivalent available protein and starch for intestinal digestion compared to low amylose hulless barley cultivar.

Table 3.12 Effect of altered carbohydrate traits on estimated intestinal digestion and availability of crude protein, starch and carbohydrate in hulless barleys in comparison with hulled barley

Item	Hulled Copeland (n=3)	Hulless Fibar (n=3)	Rattan (n=3)	McGwire (n=3)	HB08302 (n=2)	SEM	P value	Contrast Hulled vs. Hulless P value
Amylose Level (% of ST)	27.0[b]	2.5[d]	7.7[c]	25.8[b]	36.9[a]	0.56	<0.0001	<0.0001
Amylopectin Level (% of ST)	73.0[c]	97.5[a]	92.3[b]	74.2[c]	63.1[d]	0.56	<0.0001	<0.0001
β-Glucan Level (% DM)	3.8[c]	10.0[a]	7.4[b]	4.7[c]	7.5[b]	0.40	<0.0001	<0.0001
Intestinal CP digestion								
dIDP (% RUP)	78.0	80.0	83.1	79.2	78.5	2.72	0.2763	0.2675
IDP (% RUP)	33.8[b]	35.4[ab]	38.3[ab]	33.8[b]	40.6[a]	2.93	0.0176	0.0510
IDP (g/kg DM)	38[b]	57[a]	55[a]	43[b]	55[a]	3.9	<0.0001	<0.0001
TDP (% CP)	90.5	90.9	92.2	90.9	88.8	2.01	0.1752	0.7946
TDP (g/kg DM)	102[d]	147[a]	133[b]	116[c]	120[c]	3.4	<0.0001	<0.0001
Intestinal starch digestion								
dBST (% BST)	81.9	82.8	81.1	84.1	82.6	3.87	0.9580	0.8288
IDBST (% BST)	19.8[c]	23.6[bc]	27.4[ab]	21.2[c]	33.2[a]	2.52	<0.0001	0.0004
IDBST (g/kg DM)	118[c]	122[bc]	153[ab]	130[bc]	180[a]	11.7	0.0002	0.0028
TDST (% ST)	95.7	95.3	93.5	96.0	93.2	1.24	0.1842	0.2662
TDST (g/kg DM)	571[a]	495[b]	524[b]	590[a]	502[b]	13.8	<0.0001	<0.0001
Intestinal CHO digestion								
dBCHO (% BCHO)	48.8[b]	61.1[a]	66.4[a]	69.2[a]	67.7[a]	2.43	<0.0001	<0.0001
IDBCHO (% BCHO)	10.8[c]	13.6[b]	15.1[ab]	13.3[bc]	17.8[a]	1.12	<0.0001	<0.0001
IDBCHO (g/kg DM)	185[b]	19[b]	23[b]	18[b]	31[a]	3.0	<0.0001	0.0073
TDCHO (% CHO)	88.3[b]	91.4[a]	92.4[a]	94.2[a]	91.7[a]	0.87	0.0001	<0.0001
TDCHO (g/kg DM)	603[a]	510[c]	544[b]	600[a]	530[bc]	16.6	<0.0001	<0.0001

Note: ST: starch; CP: crude protein; IDP: intestinal digestible protein; RUP: rumen undegraded protein; TDP: total digestible protein; ST: starch; dBST: digestibility of rumen by pass or undegraded starch; IDBST: intestinal digestible rumen bypass starch; BST: rumen bypass starch; TDST: total digestible starch; CHO: carbohydrates; dBCHO: digestibility of rumen by pass or undegraded CHO; IDBCHO: intestinal digestible rumen bypass CHO; BCHO: rumen bypass CHO: TDCHO: total digestible CHO. SEM= Standard error of mean; Means with different letters within the same row differ (P<0.05); Multi-treatment comparison: Tukey-Kramer method.

Table 3.13 Correlation analyses between altered carbohydrate traits (amylose level, amylopectin level, ratio of amylose to amylopectin, and β-glucan level) and intestinal digestion of crude protein, starch and carbohydrate in hulless barley varieties

| Items | Altered Starch Traits | | | β-Glucan Level (% DM) |
	Amylose Level (% DM)	Amylopectin Level (% DM)	Ratio of Amylose to Amylopectin	
	--------------------Spearman Correlation R value ------------------			
Intestinal CP digestion				
IDP (% RUP)	0.20	-0.21	0.17	0.32
IDP (g/kg DM)	-0.39^{+}	0.27	-0.41^{+}	0.76***
TDP (% CP)	-0.24	0.34	-0.21	0.01
TDP (g/kg DM)	-0.85***	0.77***	-0.84***	0.74***
Intestinal starch digestion				
IDBST (% BST)	0.36^{+}	-0.33	0.34	0.28
IDBST (g/kg DM)	0.53*	-0.37^{+}	0.51*	0.08
TDST (% ST)	-0.14	0.24	-0.12	-0.28
TDST (g/kg DM)	0.38^{+}	-0.13	0.40^{+}	-0.79***
Intestinal CHO digestion				
IDBCHO (% BCHO)	0.30	-0.36^{+}	0.27	0.17
IDBCHO (g/kg DM)	0.39^{+}	-0.45*	0.36^{+}	0.11
TDCHO (% CHO)	0.24	-0.12	0.25	-0.62**
TDCHO (g/kg DM)	0.54**	-0.29	0.56**	-0.89***

$^{+}$ for $P<0.10$, * for $P<0.05$, ** for $P<0.01$, *** for $P<0.001$

3.3.4. Effect of Altered CHO Traits on Hourly Effective Rumen Degradation Ratios /Potential N-To-Energy Synchronization in Hulless Barley

The effective rumen degradation ratios of hulless barley lines ranged from 23 to 34 g N/kg CHO, and were greater (P<0.01) than hulled barley (19.66 g N/kg CHO) (Table 3.14). CDC Fibar and CDC Rattan had the greatest (P<0.05) ratios (34 g N/kg CHO) while McGwire (23 g N/kg CHO) had the lowest ratio (P<0.05). HB08302 was the intermediate with the ratio of 26 g N/kg CHO. According to Tamminga et al. (1990) and Sinclair et al. (1993), the optimum ratio between the effective extent of degradability of N and CHO (energy) is 25 to 32 g N per kg CHO truly digested in the rumen in order to achieve maximum microbial synthesis with minimize N loss. Therefore, hulless barley cultivars were superior to hulled barley in balancing N utilization and energy cost. Among hulless barley varieties, zero and low amylose hulless barley CDC Fibar and CDC Rattan had a more synchronized N and energy for microbial synthesis.

The hourly effective degradation pattern among the barley varieties is shown in Figure 3.1. At the beginning, a dramatic decrease of N/CHO ratio from 0h to 2 h was observed and represented the difference of total CHO and rumen available CHO after a short time incubation. From 2 h to 24 h incubation, N/CHO ratios of all barley cultivars kept increasing. Different varieties exhibited different magnitude of increase in the ratio. CDC Fibar showed the greatest increase in ratio from 25 g/kg at 2 h to 82 g/kg at incubation time of 24 h (Table 3.14). Rattan and HB08302 were increased from around 20 g/kg at incubation time of 2 h to 40 g/kg at incubation time of 24 h. Yu et al. (2008) reported the difference of hourly ED ratio of N/CHO was mainly caused by the difference in hourly effective degradation of N. In the present study, hulled barley showed lower increase in the magnitude of N/CHO ratios than hulless barley except CDC Rattan. This may be due to the hull coating which limits the rumen bacteria to access to protein and CHO. Also, the alteration of carbohydrate composition in hulless barley may also contribute to the difference in increase of N/CHO during the 24 h incubation. Hulless barley with lower amylose and higher β-glucan level contained higher crude protein and effective degradable protein in the rumen, which may explain why CDC Fibar and CDC Rattan were higher in ED ratios (Table 3.14).

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Hence, the hourly effective rumen degradation ratio differed among the hulless barley varieties but higher in ED ratios compared to hulled barley. Carbohydrate conformation of hulless barley affected hourly effective rumen degradation ratio thus potentially affecting rumen nitrogen to energy synchronization. Hulless barley with lower amylose and higher β-glucan level tended to improve nitrogen to energy synchronization, providing more efficient N and energy utilization.

3.3.5. Effect of Altered CHO Traits on Predicted Nutrient Supply to Dairy Cattle from Hulless Barley

3.3.5.1. Predicted Protein Supply of Barley Cultivars by DVE/OEB System

Hulled barley (a reference control) was lower ($P<0.05$) in true protein supplied to the small intestine (TPSI: 127 g/kg DM) and truly digested protein in the small intestine (DVE: 95 g/kg DM) than hulless barley lines but greater ($P<0.05$) in undigested inorganic matter (UASH: 9 g/kg DM) (Table 3.15). Among the hulless cultivars, CDC McGwire was greater ($P<0.05$) in rumen fermented organic matter (FOM: 683 vs. 545 g/kg DM), digested organic matter (DOM: 921 vs. 871 g/kg DM) and truly absorbed microbial protein in the small intestine (AMCP^{DVE}: 65 vs. 52 g/kg DM) but lower ($P<0.05$) in endogenous protein in the small intestine (ENDP: 5 vs. 9 g/kg DM), truly absorbed bypass protein in the small intestine (ABCP^{DVE}: 48 vs. 61 g/kg DM) and degraded protein balance (OEB: -35 vs. -24 g/kg DM) compared with HB08302. CDC Fibar had equal or higher truly digested protein in the small intestine (DVE: 117 g/kg DM) and greater degraded protein balance (OEB: -14 g/kg DM) compared to other barley varieties ($P<0.05$).

3.3.5.2. Predicted Protein Supply of Barley Cultivars by NRC Dairy 2001 Model

From NRC Dairy 2001 model, CDC Fibar was greater ($P<0.05$) in microbial protein synthesized in the rumen (MCP: 76 g/kg DM), AMCP^{NRC} (49 g/kg DM) and OEB^{NRC} (-30 g/kg DM) than the other hulless barley lines, and higher in metabolizable protein (MP: 118 g/kg DM) than the other barley cultivars. Compared with hulless barley, hulled barley was relatively lower ($P<0.01$) in truly absorbed rumen undegraded protein in the small intestine (ARUP^{NRC}: 44 g/kg DM) and total metabolizable protein (MP^{NRC}: 83 g/kg DM) (Table 3.16).

Table 3.14 Effect of altered carbohydrate traits in hulless barleys on degradability ratios between N and CHO and hourly effective degradability ratios at individual times in comparison with hulled barley

| Item | Hulled | Hulless | | | | SEM | P value | Contrast |
	Copeland (n=3)	Fibar (n=3)	Rattan (n=3)	McGwire (n=3)	HB08302 (n=2)			Hulled vs. Hulless P value
Amylose Level (% of ST)	27.0^b	2.5^d	7.7^c	25.8^b	36.9^a	0.56	<0.0001	<0.0001
Amylopectin Level (% of ST)	73.0^c	97.5^a	92.3^b	74.2^c	63.1^d	0.56	<0.0001	<0.0001
β-Glucan Level (% DM)	3.8^c	10.0^a	7.4^b	4.7^c	7.5^b	0.40	<0.0001	<0.0001
N to CHO ratio (g/kg)								
N/CHO	23.97^e	41.13^a	34.25^b	28.43^d	31.83^c	0.512	<0.0001	<0.0001
ED_N/ED_CHO	19.66^d	33.65^a	33.65^a	22.61^c	26.38^b	1.490	<0.0001	<0.0001
Hourly effective degradability ratios of N to CHO at individual time								
h0 (%)	15.32	69.03	24.55	35.16	44.78	34.229	0.4916	0.2706
h2 (%)	17.27^b	25.64^a	26.04^a	15.25^b	21.46^{ab}	1.639	0.0119	0.0001
h4 (%)	19.7^c	29.93^a	27.17^{ab}	18.05^c	22.77^{bc}	1.270	<0.0001	0.0020
h8 (%)	25.53^b	40.19^a	29.64^b	25.13^b	25.26^b	1.560	<0.0001	0.0033
h12 (%)	32.38^b	52.39^a	32.36^b	34.37^b	27.93^b	3.557	0.0002	0.2135
h24 (%)	47.01^b	81.56^a	40.51^b	66.25^{ab}	39.50^b	8.451	0.0018	0.2331

Note: ST: starch; ED: effective degradability; CHO: carbohydrates;
    SEM= Standard error of mean; Means with different letters within the same row differ (P<0.05).
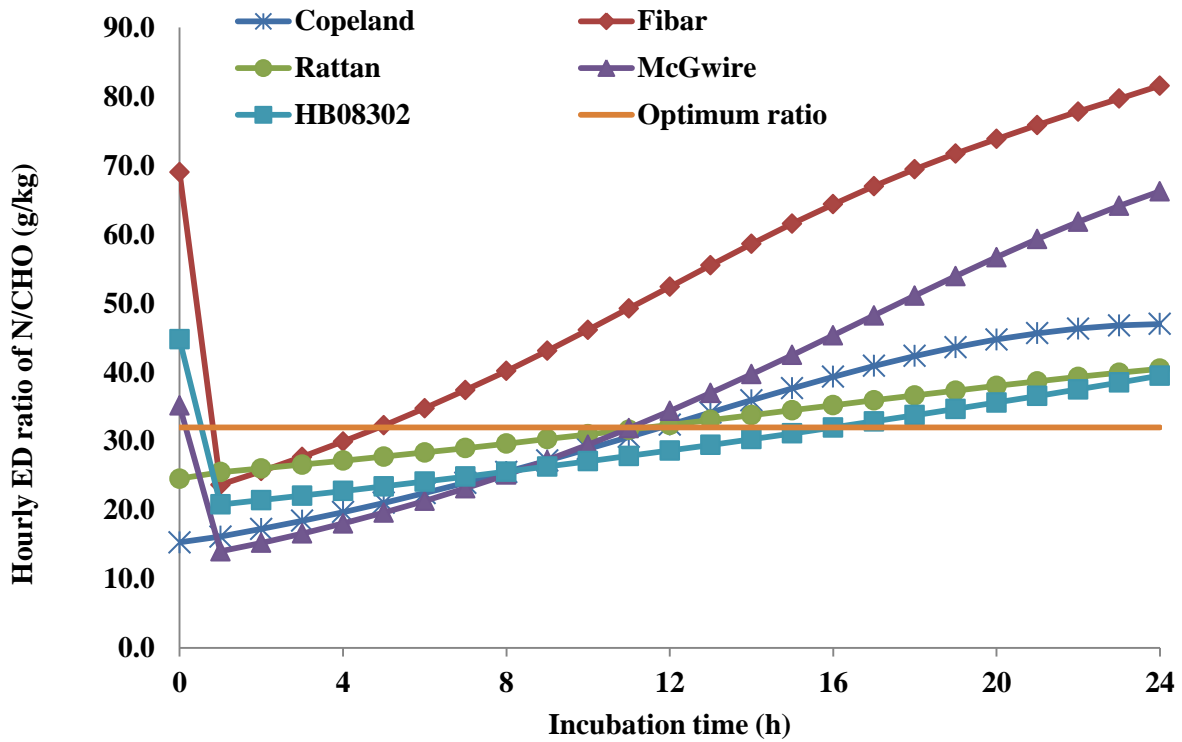   Multi-treatment comparison: Tukey-Kramer method.

Figure 3.1 Comparison of hulled barley (CDC Copeland) and four hulless barley (CDC Fibar, CDC Rattan, CDC McGwire and HB08302) with alteration in carbohydrate composition in terms of hourly effective degradability ratios between N and CHO during 24 h incubation.Optimum ratio= 32 N/CHO g/kg.

Table 3.15 Effect of altered carbohydrate traits of hulless barley varieties on potential protein supply to dairy cows predicted by DVE/OEB system in comparison with hulled barley

Item (g/kg DM)	Hulled	Hulless				SEM	P value	Contrast
	Copeland (n=3)	Fibar (n=3)	Rattan (n=3)	McGwire (n=3)	HB08302 (n=2)			Hulled vs. Hulless P value
Amylose Level (% of ST)	27.0[b]	2.5[d]	7.7[c]	25.8[b]	36.9[a]	0.56	<0.0001	<0.0001
Amylopectin Level (% of ST)	73.0[c]	97.5[a]	92.3[b]	74.2[c]	63.1[d]	0.56	<0.0001	<0.0001
β-Glucan Level (% DM)	3.8[c]	10.0[a]	7.4[b]	4.7[c]	7.5[b]	0.40	<0.0001	<0.0001
FOM	645[ab]	637[ab]	602[bc]	683[a]	545[c]	26.0	<0.0001	0.0634
DOM	866[b]	890[ab]	890[ab]	921[a]	871[b]	11.4	0.0019	0.0087
UOM	109[a]	87[ab]	88[ab]	59[b]	108[a]	11.9	0.0042	0.0249
UASH	9[a]	8[b]	7[c]	7[d]	7[c]	0.3	<0.0001	<0.0001
UDM	118[a]	95[ab]	96[ab]	66[b]	116[a]	11.8	0.0032	0.0173
TPSI	127[c]	151[a]	141[b]	138[b]	139[b]	4.6	<0.0001	<0.0001
ENDP	9[a]	7[ab]	7[ab]	5[b]	9[a]	0.9	0.0032	0.0172
Truly absorbed rumen-synthesised microbial protein in small intestine								
N_MCP	58[c]	82[a]	71[b]	67[b]	57[c]	7.2	<0.0001	<0.0001
AMCP[DVE]	62[ab]	61[ab]	58[bc]	65[a]	52[c]	2. 5	<0.0001	0.0635
Truly absorbed rumen-undegraded feed protein in small intestine								
BCP	49[b]	72[a]	66[a]	55[b]	70[a]	6.2	<0.0001	<0.0001
ABCP[DVE]	43[b]	63[a]	61[a]	48[b]	61[a]	4.2	<0.0001	<0.0001
Truly digested protein in the small intestine								
DVE	95[c]	117[a]	112[ab]	108[b]	105[b]	2.3	<0.0001	<0.0001
Degraded protein balance								
OEB[DVE]	-39[c]	-14[a]	-20[ab]	-35[c]	-24[b]	4.2	<0.0001	<0.0001

Note: ST: starch; FOM: organic matter fermented in the rumen; DOM: digested organic matter; UOM: undigested organic matter; UASH: undigested inorganic matter; UDM: undigested dry matter; TPSI: total protein supplied to the small intestine; ENDP: endogenous protein in the small intestine; N_MCP: microbial protein synthesized in the rumen based on available nitrogen; AMCP: truly absorbed microbial protein in the small intestine; BCP: rumen bypass feed crude protein; ABCP: truly absorbed bypass protein in the small intestine; DVE: truly digested protein in the small intestine; OEB: degraded protein balance.

SEM = Standard error of mean; Means with various letters within the same row differed by Tukey-Kramer method (P<0.05).

Table 3.16 Effect of altered carbohydrate traits on potential protein supply to dairy cows predicted by NRC Dairy 2001 model from hulless barley varieties in comparison to hulled barley

| Item (g/kg DM) | Hulled | Hulless | | | | SEM | P value | Contrast |
	Copeland (n=3)	Fibar (n=3)	Rattan (n=3)	McGwire (n=3)	HB08302 (n=2)			Hulled vs. Hulless P value
Amylose Level (% of ST)	27.0[b]	2.5[d]	7.7[c]	25.8[b]	36.9[a]	0.56	<0.0001	<0.0001
Amylopectin Level (% of ST)	73.0[c]	97.5[a]	92.3[b]	74.2[c]	63.1[d]	0.56	<0.0001	<0.0001
β-Glucan Level (% DM)	3.8[c]	10.0[a]	7.4[b]	4.7[c]	7.5[b]	0.40	<0.0001	<0.0001
MCP_{TDN}	99[b]	101[a]	101[a]	101[a]	101[a]	0.3	<0.0001	<0.0001
Truly absorbed rumen-synthesised microbial protein in small intestine								
MCP^{NRC}	54[d]	76[a]	66[b]	62[bc]	56[cd]	5.7	<0.0001	<0.0001
$AMCP^{NRC}$	35[d]	49[a]	42[b]	40[bc]	35[cd]	3.6	<0.0001	<0.0001
Truly absorbed rumen-undegraded feed protein in small intestine								
RUP^{NRC}	49[b]	72[a]	66[a]	55[b]	70[a]	6.2	<0.0001	<0.0001
$ARUP^{NRC}$	44[b]	64[a]	60[a]	49[b]	63[a]	6.2	<0.0001	<0.0001
Truly digested rumen endogenous protein in small intestine								
ECP	11	11	11	11	11	0.0	0.1591	0.0971
$AECP^{NRC}$	4	4	4	4	4	0.0	0.1211	0.0717
Total truly absorbed protein in small intestine								
MP^{NRC}	83[d]	118[a]	107[b]	93[c]	103[b]	3.1	<0.0001	<0.0001
Degraded protein balance								
OEB^{NRC}	-53[c]	-30[a]	-41[b]	-46[bc]	-54[c]	6.7	<0.0001	0.0002

Note: ST: starch; $MCP_{TDN:}$ microbial protein synthesized in the rumen based on discounted TDN; MCP: microbial protein; AMCP: truly absorbed microbial protein in the small intestine; RUP: rumen undegrdable feed crude protein; ARUP: truly absorbed rumen undegradable protein in the small intestine; ECP: rumen endogenous protein; AECP: truly absorbed rumen endogenous protein in the small intestine; MP: metabolizable protein. OEB: degraded protein balance.

SEM = Standard error of mean; Means with different letters within the same row differ (P<0.05).

Multi-treatment comparison: Tukey-Kramer method.

3.3.5.3. Correlation Analysis between Predicted Protein Supply by Two Models and Altered CHO Traits in Hulless Barley Cultivars

In agreement with previous study (Damiran and Yu 2012), both DVE/OEB system and NRC Dairy 2001 model showed negative OEB values, indicating negative balance between N and energy. This reveals a potential shortage of N when barley grain was evaluated as a single ingredient (Damiran and Yu 2012). However, among barley cultivars, zero-amylose CDC Fibar was discovered with highest OEB values obtained from both model prediction ($OEB^{DVE}=-14$ g/kg DM; $OEB^{NRC}=-30$ g/kg DM), which means that CDC Fibar contained relatively balanced N and energy compared with other barley cultivars. A possible reason may still be the high level of β-glucan which protects nutrients from rumen degradation by reducing degradation rate of a given nutrient (McAllister et al., 1993; Surber et al. 2000; Damiran and Yu 2012).

In the DVE/OEB system, altered carbohydrate traits were correlated to degraded protein balance (OEB) and truly digested protein in the small intestine (DVE) ($P<0.01$) (Table 3.17). Microbial protein synthesized in the rumen based on available nitrogen (N_MCP), DVE and OEB^{DVE} were negatively correlated ($P<0.01$) to amylose level and Ay:Ap ratio but positively correlated to amylopectin and β-glucan level. β-glucan level was positively correlated ($P<0.05$) to total protein supplied to the small intestine (TPSI: $r=0.52$), endogenous protein in the small intestine (ENDP: $r=0.46$) and truly absorbed bypass protein in the small intestine (ABCP: $r=0.76$).

In NRC Dairy 2001 model, $AMCP^{NRC}$, OEB^{NRC} and MCP^{NRC} were positively correlated to amylopectin level but negatively correlated to amylose and Ay:Ap levels ($P<0.05$) in hulless barley. Altered carbohydrate traits were correlated to total metabolizable protein ($P<0.05$) with amylose and Ay:Ap showing negative effects while amylopectin and β-glucan levels showed positive effects (Table 3.16).

According to correlation findings, hulless barley with lower amylose and higher β-glucan level contained higher amount of metabolizable protein ($r=-0.72$; $r=0.82$; $P<0.001$), greater truly

digested protein in the small intestine (DVE: r=-0.79, P<0.001; r=0.47, P<0.05) and are closer to an optimum balance between N and energy (P<0.001). CDC Fibar was greater DVE and OEB^{DVE} from DVE/OEB system (P<0.05) and greatest (P<0.05) in OEB^{NRC} and MP from NRC-2001 model than other barley cultivars.

However, the DVE/OEB system and NRC Dairy 2001 model differed in prediction parameters for protein supply. For example, AMCP was estimated from FOM in the DVE system instead of being estimated from rumen degraded protein (RDP or EDCP) in NRC Dairy 2001 model. The results reported Damiran and Yu (2012) agreed with our results that $AMCP^{DVE}$ was around 60 g/kg DM in the DVE/OEB system but lower in NRC Dairy 2001 model. Two models were not in consistent in estimation of AMCP in various feedstuffs, (Yu et al., 2003b; Heendeniya 2008; Nuez-Ort ń and Yu, 2010; Damiran and Yu, 2012), further suggesting there is a difference between the two models in estimation of AMCP.

Our results suggest that hulless barley with lower amylose and higher β-glucan (eg. CDC Fibar and CDC Rattan) would provide greater (P<0.05) truly digested protein in the small intestine (DVE) with better protein degraded balance (OEB^{DVE}) as well as higher truly absorbed rumen undegradable protein ($ABCP^{DVE}$ or $ARUP^{NRC}$) than the hulled cultivar, while the hulled barley cultivar contained higher undegradable inorganic matter (UASH) and undigested DM than hulless cultivars.

Therefore, hulless barley varieties with low amylose carbohydrate traits significantly improved the truly absorbed protein supply to dairy cattle compared to hulled barley, although all barley cultivars were observed to have a negative protein balance when evaluated as a single ingredient. Among hulless cultivars, hulless barley with lower amylose and higher β-glucan level could provide greater truly digested protein in the small intestine, better synchronized available energy and N and increase metabolizable protein supply to ruminants.

Table 3.17 Correlation analyses between altered carbohydrate traits (amylose level, amylopectin level, ratio of amylose to amylopectin, and β-glucan level) of hulless barleys and potential nutrient supply predicted to dairy cattle

Items	Altered Starch Traits			β-Glucan Level (% DM)
	Amylose Level (% DM)	Amylopectin Level (% DM)	Ratio of Amylose to Amylopectin	
	------------------Spearman Correlation R value ------------------			
DVE/OEB (g/kg DM)				
FOM	-0.31	0.26	-0.29	-0.35
DOM	-0.04	0.04	-0.02	-0.46*
UOM	0.06	-0.06	0.04	0.45*
UASH	-0.64**	0.38$^+$	-0.63**	0.67***
UDM	0.04	-0.04	0.02	0.46*
TPSI	-0.56**	0.33	-0.57**	0.52*
ENDP	0.04	-0.04	0.02	0.46*
N_MCP	-0.64**	0.58**	-0.62**	0.31
AMCP^{DVE}	-0.31	0.26	-0.29	-0.35
BCP	-0.25	0.08	-0.28	0.63**
ABCP^{DVE}	-0.39$^+$	0.27	-0.41$^+$	0.76***
DVE	-0.79***	0.60**	-0.79***	0.47*
OEB^{DVE}	-0.68***	0.63**	-0.67***	0.73***
NRC Dairy 2001(g/kg DM)				
MCP$_{TDN}$	0.17	-0.39$^+$	0.14	0.11
MCP^{NRC}	-0.71***	0.64**	-0.70***	0.37$^+$
AMCP^{NRC}	-0.71***	0.64**	-0.70***	0.37$^+$
RUP^{NRC}	-0.25	0.08	-0.28	0.63**
ARUP^{NRC}	-0.26	0.09	-0.29	0.59**
ECP	-0.03	0.29	0.00	0.08
AECP^{NRC}	-0.07	0.26	-0.05	0.13
MP^{NRC}	-0.72***	0.53*	-0.73***	0.82***
OEB^{NRC}	-0.70***	0.64**	-0.69***	0.36$^+$

$^+$ for P<0.10, * for P<0.05, ** for P<0.01, *** for P<0.001

3.4. Conclusion

In conclusion, hulless barley cultivars contained higher soluble protein and energy, greater amount of nutrients for rumen degradation and post-ruminal digestion, providing more truly absorbed protein supply to dairy cattle compare to hulled barley. Alteration of carbohydrate traits in hulless barley improved total CHO, crude protein, energy values and potential protein supply. Hulless barley with lower amylose and higher β-glucan level, contained higher energy and crude protein content, more rumen bypass nutrients available for post-rumnial digestion, better synchronized available energy and N, greater metabolizable protein supply to ruminants, but relatively lower effective digestible starch, CHO and slower degradation rate of DM than normal amylose hulless barley and hulled barley. With respect to nutrient availability, hulless barley line with lower amylose and higher β-glucan level can be considered as an alternative for ruminant feeding. However, with equavilent or higher starch and protein digestibility but lower starch degradation rate compared to low amylose hulless barley cultivar, hulless barley line with high amylose and also high β-glucan can also be considered as a grain option, which may reduce the incidence of rumen acidosis and increase rumen undegraded starch and protein for digestion in the small intestine.

4. Molecular Structure Spectral Features of CDC Hulless Barleys Using Molecular Spectroscopy

4.1. Introduction

Research presented in Chapter 3 tested how the alteration in carbohydrate traits of hulless barley varieties affected chemical and nutrient profiles, rumen degradation characteristics, intestinal digestion and potential protein supply to dairy cattle. Research on molecular structure may aid in explaining differences in nutrient availability in newly developed hulless barley varieties. Traditional wet chemistry can only provide chemical profiles of barley cultivars by destroying the external coating and internal structures of the sample, and thus inhibits the possibility of study of inherent structures. As a non-destructive method, conventional FTIR is able to investigate molecular changes in plant tissue after genetic modification, provides rapid and precise measurement of samples and increased sensitivity with good signal-to-noise ratios (Hsu, 1997; McCluskey, 2000; Kizil et al., 2002; Zotti et al., 2008). However, the weakness of this molecular technique is the requirement of an aperture size of at least 30-50 μm (Budevska, 2002; Diem et al., 2004). In the same detecting region, for example, in the mid-infrared region (ca. 4000–800 cm^{-1}) of the electromagnetic spectrum, advanced synchrotron-based FTIR uses a superior light source and small aperture settings (Miller and Dumas, 2006; Yu, 2004). Investigating the inherent molecular structures of the barley varieties used in this project, the objectives were (1) to reveal the molecular structures of four newly developed hulless barley lines in comparison with hulled barley using both conventional FTIR and synchrotron-based FTIR techniques, and (2) to quantify molecular structural features of hulless barley cultivars determined by both spectral techniques in relation to rumen degradation kinetics, intestinal nutrient digestion and predicted protein supply to dairy cattle by the DVE/OEB system and NRC Dairy 2001 model.

4.2. Materials and Methods

4.2.1. Fourier Transform Infrared-vibration Spectroscopy (FTIR)

4.2.1.1. Molecular Spectra Collection by Fourier Transform Infrared Spectroscopy (FTIR)

Samples of four barley cultivars (CDC Copeland, CDC Fibar, CDC Rattan and CDC McGwire) from three consecutive years (2008, 2009, 2010) and HB08302 samples from two consecutive years (2009, 2010) were ground through a 0.5 mm screen using a Retsch mill (Retsch ZM–1; Brinkmann Instruments of Canada Ltd., Mississauga, ON, Canada). Fourier-transform infrared-vibration spectroscopy (FTIR) was performed using a Jasco FT/IR–4200 spectroscope (Jasco Corp., Tokyo, Japan) at the University of Saskatchewan, Saskatoon, Saskatchewan, Canada. The FTIR spectroscopy was equipped with a ceramic IR light source and a deuterated L-alanine doped triglycine sulfate detector, also comprising a MIRacle ATR accessory module and a ZnSe crystal and pressure clamp (Pike Technologies, Madison, WI, USA). The molecular structural features were determined in the mid-infrared region (ca. 4000–800 cm^{-1}) [Figure 4.1.1: (a)] of the electromagnetic spectrum with 32 co-added scans. The spatial resolution was set at 4 cm^{-1}. The spectra were collected with Jasco Spectra Manager II software for five times per sample. Omnic 7.2 software (Spectra Tech, Madison, WI, USA) was applied to identify functional group spectral bands associated with protein (Figure 4.1.1 and 4.1.2), β-glucan, cellulosic compounds (Figure 4.2) and carbohydrate (Figure 4.3.1 and 4.3.2) molecular structures by analyzing absorption peak parameters (baseline, region, relative heigh and area) according to published reports (Wetzel et al., 1998, 2003; Liu and Yu, 2010; Damiran and Yu, 2011).

4.2.1.2. Univariate Analysis on Protein Amide I and II, β-Glucan, Cellulosic Compounds and Carbohydrate Molecular Structure of Barley Varieties

The assessed items of five barley cultivars included infrared intensity of protein amide I area (ca. 1732–1578 cm^{-1}) and height (ca. 1648 cm^{-1}), amide II area (ca. 1578–1483 cm^{-1}) and height (ca. 1537 cm^{-1}), α-helix height (ca. 1653 cm^{-1}), β-sheet height (ca. 1635 cm^{-1}), β-glucan

area (ca. 1448–1390 cm^{-1}) and height (ca. 1413 cm^{-1}), cellulosic compounds area (ca. 1275–1212 cm^{-1}) and height (ca. 1238 cm^{-1}), total carbohydrates area (ca. 1189–946 cm^{-1}), and areas of three major CHO peaks: 1st peak (ca. 1189–1130 cm^{-1}), 2nd peak (ca. 1130–1063 cm^{-1}) and 3rd peak (ca. 1063–946 cm^{-1}). The heights of three CHO peaks were collected at ca. 1150 cm^{-1}, ca. 1076 cm^{-1} and ca. 1016 cm^{-1}, respectively (Figure 4.1.1 and 4.3.2). Ratios of peak heights of protein amide I to amide II as well as α-helix to β-sheet height were calculated. All absorption intensities of molecular structure features related with functional groups in chemical analysis were recorded and compared for significant differences by univariate analysis using SAS 9.2 (SAS Institute, Inc., Cary, NC, USA).

4.2.1.3.   Multivariate Molecular Spectral Analysis of FTIR Spectra

In order to compare the underlying structural differences among barley cultivars, original spectra without any parameterization were used for multivariate analysis, which generates spectral correlations by using entire spectral region information of associated functional groups. Two multivariate molecular spectral analyses, agglomerative hierarchical cluster analysis (CLA), using Ward's algorithm method without prior parameterization, and principal components analysis (PCA), were used following the detailed principles reported by Yu (2008). The regions of the functional group bands were separated into protein region (ca. 1732–1483 cm^{-1}), non-starch CHO region (ca. 1483–1189 cm^{-1}) and total CHO region (ca. 1189–946 cm^{-1}) for multivariate molecular spectral analysis. CLA results were presented as dendograms, while PCA results were plotted based on the two highest factor scores and plotted as a function of those scores. Multivariate spectral analyses were performed using Statistica software 8.0 (StatSoft Inc., Tulsa, OK, USA).

4.2.2.   Synchrotron Based Fourier Transform Infrared Microspectroscopy (SR-FTIRM)

4.2.2.1.   Sample Preparation and Molecular Spectra Collection of Samples

Five kernals of each barley variety harvested in different years [four varieties harvested in three consecutive years (2008, 2009, 2010) except HB08302 harvested in two consecutive years

(2008, 2009)], were randomly selected to be cross-sectioned for anaylsis of endosperm tissue at the Western College of Veterinary Medicine, University of Saskatchewan, Saskatoon, Canada. The thin cross-sections of tissues (6 μm) were unstained and mounted on barium fluoride (BaF_2) windows (Spectral Systems, Hopewell Junction, NY, USA) according to Yu et al. (2008).

The SR-FTIRM experiment was performed with the IR microspectroscopy instrument coupled with synchrotron radiation from the U2B beamline at the National Synchrotron Light Source, Brookhaven National Laboratory (NSLS-BNL), U.S. Department of Energy (Upton, NY, USA). Molecular spectra collection was carried out using a Thermo Nicolet Magna 860 Step-Scan FTIR (Thermo Fisher Scientific Inc.,Waltham, MA, USA) spectrometer equipped with a Spectra Tech Continuum IR Microscope (Spectra-Tech, Inc., Shelton, CT, USA) and mercury cadmium telluride (MCT) detector. Liquid nitrogen was added to cool the MCT detector every eight hours of spectra collection. The molecular structural features were determined in the mid-infrared region (ca. 4000–800 cm^{-1}) of the electromagnetic spectrum. Two hundred and fifty-six scans were co-added to each spot to produce high quality IR spectrum. The spatial resolution was set at 4 cm^{-1}. The ten spectral images from ten randomly selected spots of each tissue endosperm window were collected in the mid-infrared region (ca. 4,000–800 cm^{-1}) of the electromagnetic spectrum. The total number of spectra samples were: 10 (spectra) $\times$ 5 (windows) $\times$ [3 (harvest years replicates) $\times$ 4 [three hulless barley (CDC Fibar, CDC Rattan, CDC McGwire) and one hulled barley cultivars (CDC Copeland)] + 2 (harvest years replicates) $\times$ 1 (HB08302)] = 700.

Spectral data were analyzed by Nicolet OMNIC software 7.2 (Spectra Tech, Madison, WI, USA). After baseline correction, the absorption peak parameters (baseline, region, peak area and peak center height) of functional group spectral bands representing protein, β-glucan, cellulosic compounds and carbohydrate molecular structures were recorded for univariate analysis, in which the absorption heights of protein secondary structures (α-helix and β-sheet) were identified using the second derivative option within the protein amide I region under the same baseline of total protein area.
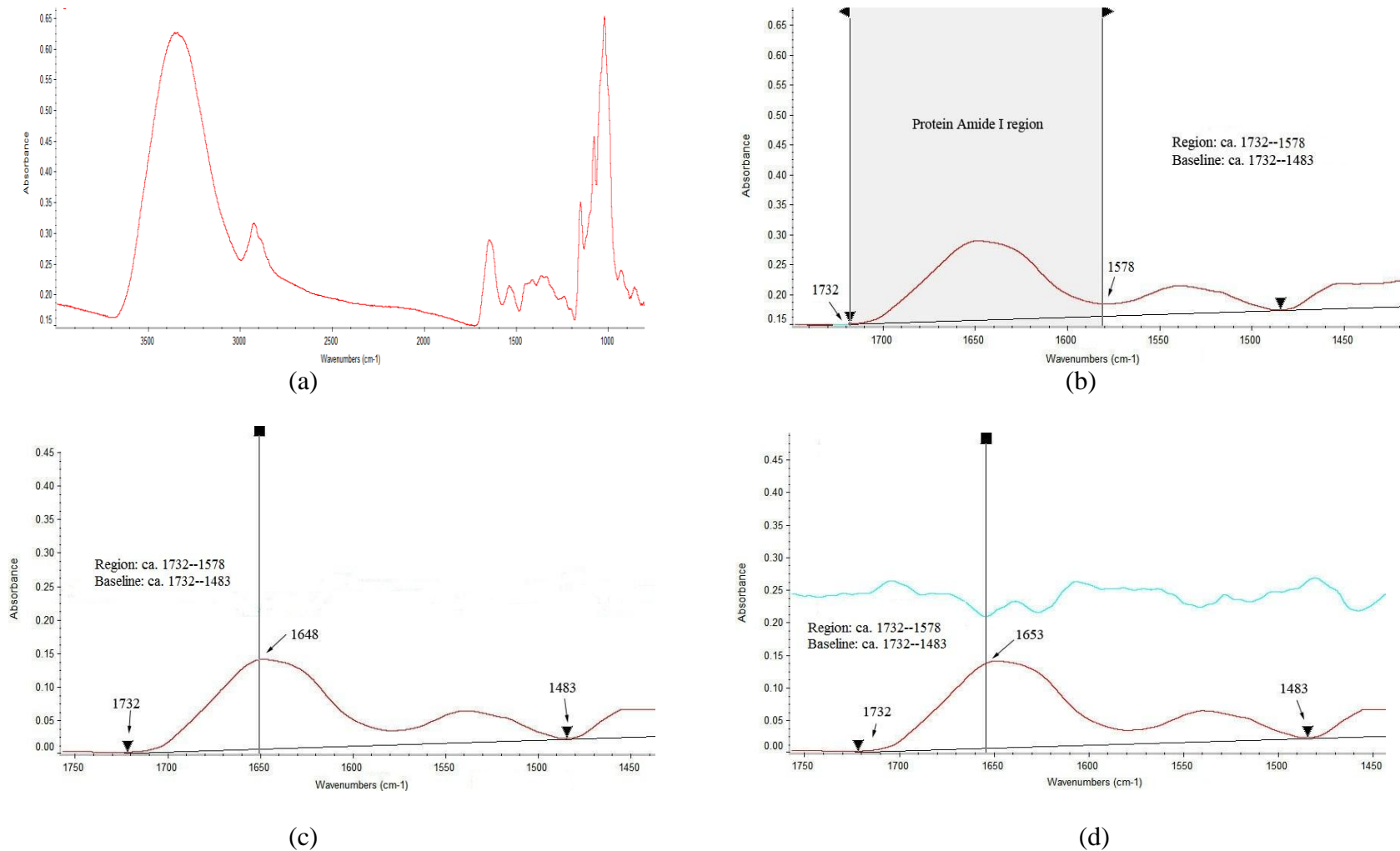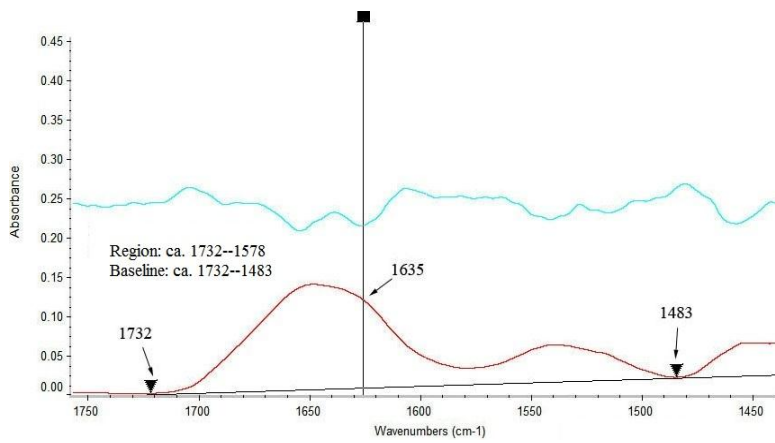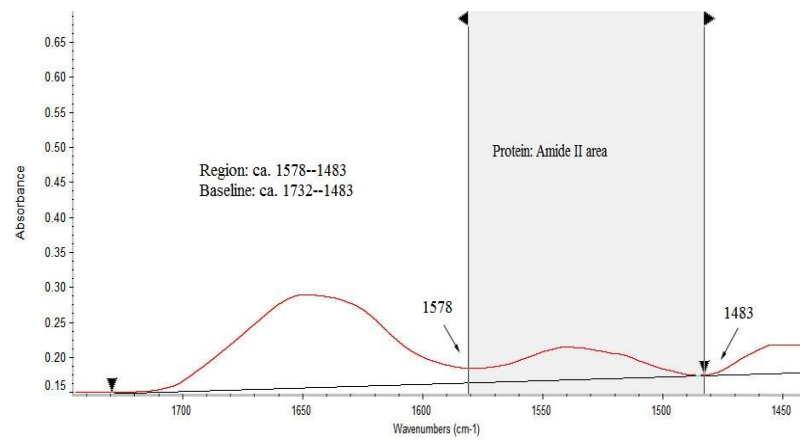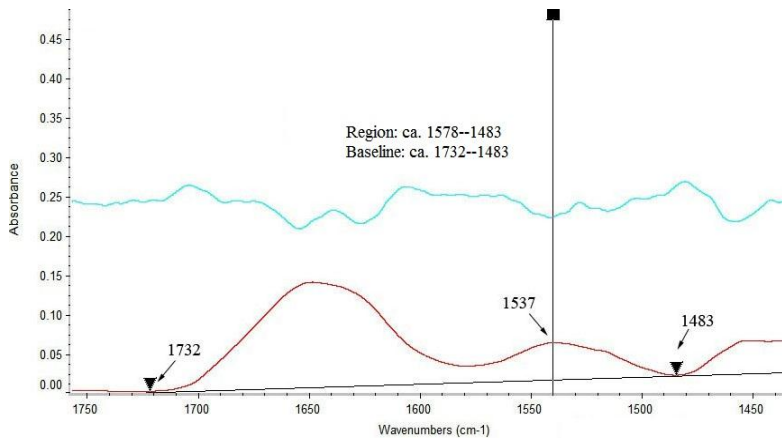
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Figure 4.1.1   Fourier transform infrared (FTIR) biomolecular spectra of (a) whole spectrum region (ca. 4,000-800 cm^{-1}); spectra parameters (peak areas and heights) of protein molecular structure (baseline region: ca. 1732-1483 cm^{-1}) of five barley cultivars used for univariate analyses including: (b) protein amide I area (ca. 1732-1578 cm^{-1}); (c) protein amide I height (ca. 1648 cm^{-1}); and (d) protein secondary structure: α-helix peak height (ca. 1653 cm^{-1}).

Figure 4.1.2 Fourier transform infrared (FTIR) biomolecular spectra parameters (peak areas and heights) of protein molecular structure (baseline region: ca. 1732-1483 cm^{-1}) of five barley cultivars used for univariate analyses including: (e) protein secondary structure: β-sheet peak height (ca. 1635 cm^{-1}); (f) protein amide II area (ca. 1578-1483 cm^{-1}); and (g) protein amide II peak height (ca. 1537 cm^{-1}).
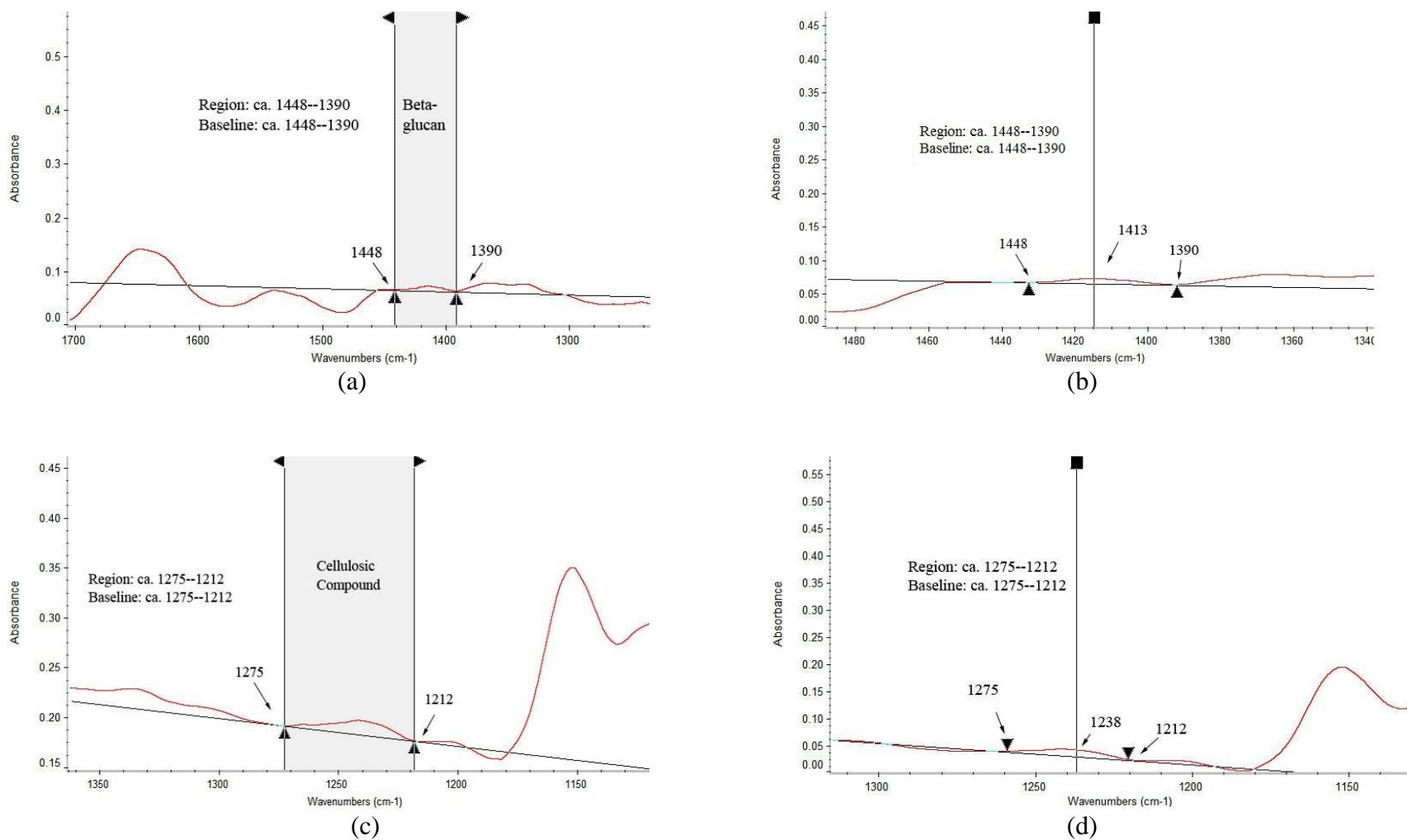
Figure 4.2   Fourier transform infrared (FTIR) biomolecular spectra of β-glucan region (baseline region: ca. 1448-1390 cm^{-1}) with its peak area [(a): ca. 1448-1390 cm^{-1}]and peak height [(b) ca. 1413 cm^{-1}] as well as spectra of cellulosic region (baseline: ca. 1275-1212 cm^{-1}) with its peak area [(c): ca. 1275-1212 cm^{-1}] and height [(d): ca. 1238 cm^{-1}].
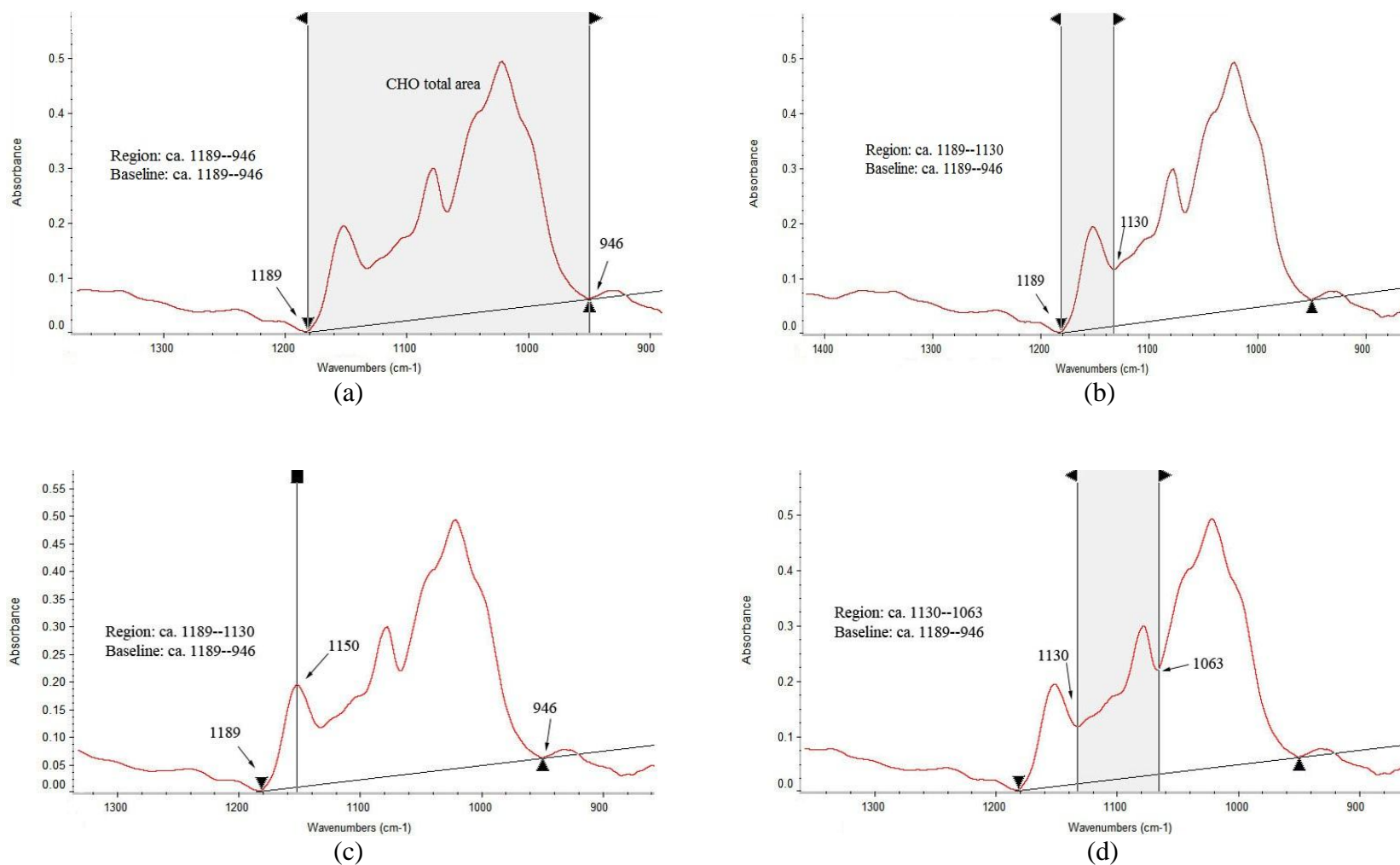
Figure 4.3.1   Fourier transform infrared (FTIR) biomolecular spectra information of CHO region (baseline: ca. 1189-946 cm^{-1}) with its (a) total area (ca. 1189-946 cm^{-1}); (b) 1st peak area (ca. 1189-1130 cm^{-1}); (c) 1st peak height (ca. 1150 cm^{-1}); (d) 2nd peak area (ca. 1130-1063 cm^{-1}).
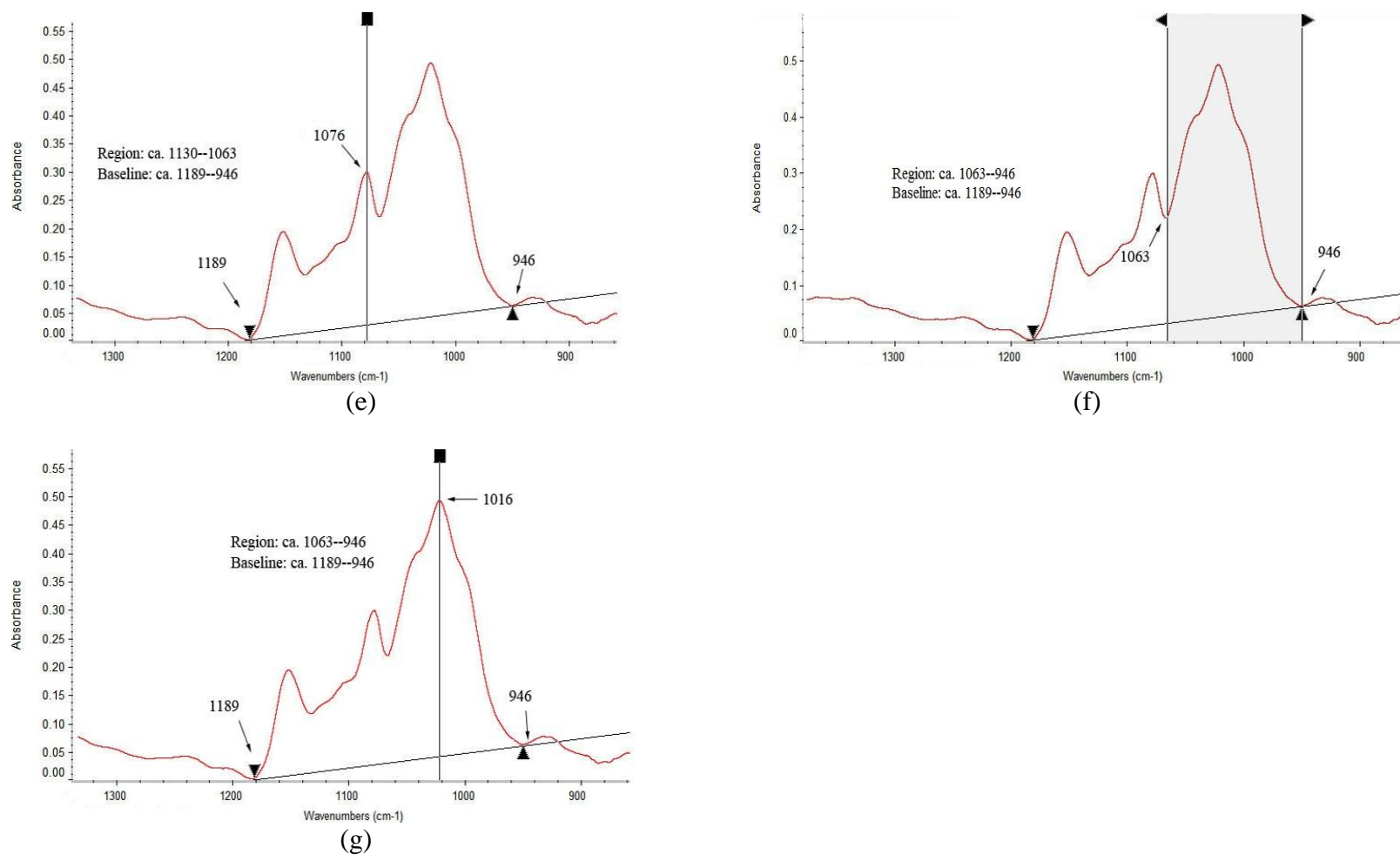
Figure 4.3.2   Fourier transform infrared (FTIR) biomolecular spectra information of CHO region (baseline: ca. 1189-946 cm^{-1}) with its: (e) 2nd peak height (ca. 1076 cm^{-1}); (f) 3rd peak area (ca. 1063-946 cm^{-1}) and (g) 3rd peak height (ca. 1016 cm^{-1}) for the use of univariate analyses.

4.2.2.2. Univariate Analysis on Protein, β-Glucan, Cellulosic Compounds and Carbohydrate Molecular Structure of Barley Varieties

The absorption peak intensities (peak area and height) of functional groups (protein, β-glucan, cellulosic compounds and carbohydrate) were recorded and compared for differences using SAS 9.2 (SAS Institute, Inc., Cary, NC, USA). The absorbance bands of specific nutrients of these barley cultivars were: protein amide I area (ca. 1768–1558 cm^{-1}) and height (ca. 1647 cm^{-1}), amide II area (ca. 1558–1475 cm^{-1}) and height (ca. 1542 cm^{-1}), α-helix height (ca. 1655 cm^{-1}), β-sheet height (ca. 1628 cm^{-1}), β-glucan area (ca. 1450–1390 cm^{-1}) and height (ca. 1415 cm^{-1}), cellulosic compounds peak area (ca.1278–1205 cm^{-1}) and height (ca. 1238 cm^{-1}) , total carbohydrates area (CHO: ca. 1195–945 cm^{-1}) and their three major CHO peaks areas: 1st peak (ca. 1195–1128 cm^{-1}), 2nd peak (ca. 1128–1049 cm^{-1}) and 3rd peak (ca. 1049–945 cm^{-1}). Heights of these CHO peaks were collected, respectively, at ca. 1152 cm^{-1}, ca. 1079 cm^{-1} and ca. 1024 cm^{-1}. Ratios of peak heights of protein amide I to amide II as well as ratios of α-helix to β-sheet height were also calculated.

4.2.2.3. Multivariate Molecular Spectral Analysis for SR-FTIRM Spectra

For SR-FTIRM spectral analysis, the regions of the functional group bands were separated as protein region (ca. 1768–1475 cm^{-1}), non-starch CHO region (ca. 1475–1195 cm^{-1}) and total CHO region (ca. 1195–945 cm^{-1}) for multivariate molecular spectral analysis using Statistica software 8.0 (StatSoft Inc., Tulsa, OK, USA). CLA results were presented as dendograms, while PCA results were plotted based on the two highest factor scores and plotted as a function of those scores.

4.2.3. Statistical Analysis

Due to uneven samples of barley cultivars, the experimental design for this study was a Randomized Incomplete Block Design. The statistical analyses were performed using the MIXED procedure of SAS 9.2 (SAS Institute, Inc., Cary, NC, USA). The model used for the analysis was $Y_{ij} = \mu + \rho_i + \alpha_j + e_{ij}$, where Y_{ij} is an observation of the dependent variable ij, μ is the

population mean for the variable, ρ_i is the random effect of harvest year, α_j is the effect of the barley cultivars, as a fixed effect, and e_{ij} is the random error associated with observation ij. Contrast statements were used to compare the difference between hulled barley and hulless barley cultivars. Means were compared using the Tukey–Kramer method and the significance was declared at P<0.05.

Because the data for the correlation study were not normally distributed, rank correlations were performed using the PROC CORR of SAS with an option of SPEARMAN to quantify molecular structural features identified using FTIR and SR-FTIRM techniques in relation to 1) rumen degradation kinetics, 2) intestinal nutrient digestion, and 3) prediction of protein supply to dairy cattle using the DVE/OEB system and the NRC Dairy 2001 model.

4.3. Results and Discussion

4.3.1. Using Conventional FTIR to Identify Molecular Structure Spectral Features of Hulless Barleys in Comparison with Hulled barley

4.3.1.1. Quantifying the Molecular Structures of Protein in Hulless Barleys in Comparison with Hulled Barley

Table 4.1 shows the absorption intensities of protein molecular structural characteristics in hulled and hulless barleys. In the protein structure region (ca. 1732−1483 cm^{-1}), CDC Fibar and CDC Rattan were significantly higher in absorption intensity than hulled barley while CDC McGwire and HB08302were more similar to hulled barley (P<0.05). Ratios of amide I to amide II area andα-helix to β-sheet height were similar among the barley cultivars (P>0.05). Among the hulless barley varieties, CDC Fibar showed relatively higher in amide I area (2.87 vs. 2.38) and height (0.042 vs. 0.035), amide II area (0.79 vs. 0.67) and height (0.015 vs. 0.012) as well as α-helix height (0.041 vs. 0.034) than CDC McGwire (P<0.05) but no significant difference with CDC Rattan and HB08302 (P>0.05). These results concur with results reported by Damiran and Yu (2011), although the protein structure characteristics of hulless barley were measured using diffuse reflectance infrared Fourier transform (DRIFT) in their study. These observations are also supported by results from PCA and CLA in the same region (Figure 4.4). CDC Copeland (C) and CDC Fibar (F) formed two separate groups with two distinct clusters below a linkage

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distance of 0.3 in CLA. PCA plots obtained from CDC Copeland and CDC Fibar show separated ellipses suggesting two distinct protein structures. The first two principal components (PC) explained 85.95% and 9.60% of the variation in the protein structures of CDC Copeland and CDC Fibar [Figure 4.4: (1)-(2)]. Similar to CDC Fibar, CDC Rattan (R) also had distinct clusters below an aggregation distance of 0.3 [Figure 4.4 (3)-(4)], while 86.06% and 9.8% of variation between CDC Rattan and CDC Copeland can be explained by PCA results. With an increase in the amylose level of hulless barley, less distinguished cluster classes and ellipses were found in comparison to the protein structures between hulled barley and hulless barley [Figure 4.4: (1)-(8)]. Both CDC McGwire (M) and HB08302 (H) showed mixed cluster classes and overlapped ellipses in CLA and PCA analysis. However, 89.38% and 6.03% of the variation was explained by first and second order principal components of protein structure in comparison to CDC Copeland and CDC McGwire, in this case 92.36% and 4.80% of the variation was explained in comparison between hulled barley and HB08302.

Among hulless barleys, the normal-hulless barley cultivar CDC McGwire (M) was found to have almost distinct cluster classes below a linkage distance of 0.2 and separated ellipses in comparison with CDC Fibar (F), indicating there were some difference in protein structure between CDC McGwire and CDC Fibar [Figure 4.4: (9)-(10)]. PCA analysis revealed that the first principal component explained 89.16% while the second principal component accounted for 7.65% of the variation between CDC McGwire and CDC Fibar in terms of protein molecular structure. However, less distinct clusters and more overlapped ellipses were found from CLA and PAC, respectively, in respect of comparison of the protein molecular structure between CDC McGwire (M) and HB08302 (H) [Figure 4.4 (13)-(14)]. This indicates there is similarity in protein molecular structural-chemical make-up between CDC McGwire and HB08302.

As detected from the previous study, hulless barley cultivars with altered carbohydrate traits differed in rumen degradation rate and intestinal digestibility, as well as in predicted protein supply to dairy cattle. The differences in protein molecular structure may be one of the factors contributing to these differences. Table 4.2 presents correlation results between structural characteristics of protein and nutrient utilization and availability in the rumen and intestine. Rumen undegrdable protein and bypass protein were positively correlated to protein amide II peak height (r=0.69, P<0.05) and protein secondary structure (r=0.61, P<0.05). Effective degradable crude protein was positively correlated to protein amide I area (r=0.79, P<0.01),

height (r=0.76, P<0.01), amide II area (r=0.64, P<0.05) and protein secondary (r=0.66 and r=0.68) structure (P<0.05). This indicates that differences in protein molecular structure affected rumen degradation of protein. The rumen degradation rate of NDF was also found to be positively correlated with protein amide I peak height (r=0.64, P<0.05) and amide II absorption intensity (r=0.63 and r=0.73, P<0.05) and to protein secondary structure α-helix (r=0.61, P<0.05). Percentage of bypass NDF and effective degradable NDF in total NDF were found to be positively and negatively correlated to protein amide I area, height and amide II area (P<0.05). Ratio of amide I to amide II area was negatively correlated with rumen bypass CHO (BCHO: r=-0.75, P<0.05) while ratio of protein secondary structure was positively correlated to effective degradable CHO (EDCHO: r=0.64, P<0.05) and effective degradable NDF (EDNDF: r=0.61, P<0.05). Protein molecular structure was also positively correlated to total digestible protein (P<0.05) while ratio of amide I to amide II area was negatively correlated intestinal digestible bypass CHO (IDCHO: r=-0.62, P<0.05). The ratio of protein secondary structure showed a positive correlation to total digestible CHO (TDCHO: r=0.75, P<0.01) (Table 4.2). As expected, prediction parameters in protein supply by both models were significantly correlated to protein structure spectral characteristics (Table 4.3). However, in the DVE/OEB system, degraded protein balance was positively correlated with structural characteristics of protein in barley (P<0.05). Ratio of amide I to amide II area was observed positively correlated with truly digested protein in the small intestine (DVE: r=0.71, P<0.05). Metabolizable protein predicted by the NRC Dairy 2001 model was positively correlated to protein structure parameters (P<0.01), indicating that differences in protein structural features among hulless barley varieties had a significant effect on metabolizable protein supply to the ruminant. Therefore, the structural characteristics of protein varied among the barley cultivars. Normal-amylose hulless barley showed a similar protein structure as hulled barley, which differed from hulless barley with altered starch traits. Among the hulless barleys, CDC Fibar showed more significant spectral features in protein than did CDC McGwire. The protein spectral characteristics positively correlated to rumen degradation and potential protein supply with the higher absorption intensity of protein structure, the greater effective degradable protein in the rumen, total digestible protein for post-ruminal digestion, greater metabolizable protein supply to ruminants, and better N to energy balance.

Table 4.1 Effect of altered carbohydrate traits on structure spectral characteristics of protein amide I and II, protein secondary structure α-helix, β-sheet in whole seeds of hulless barley varieties in comparison with hulled barley using FTIR molecular spectroscopy

Item	Peak region and center (cm^{-1})	Hulled Copeland (n=3)	Hulless Fibar (n=3)	Rattan (n=3)	McGwire (n=3)	HB08302 (n=2)	SEM	P value	Contrast Hulled vs. Hulless P value
Amylose Level (% of ST)		27.0^b	2.5^d	7.7^c	25.8^b	36.9^a	0.56	<0.0001	<0.0001
Amylopectin Level (% of ST)		73.0^c	97.5^a	92.3^b	74.2^c	63.1^d	0.56	<0.0001	<0.0001
β-Glucan Level (% DM)		3.8^c	10.0^a	7.4^b	4.7^c	7.5^b	0.40	<0.0001	<0.0001
Baseline	1732—1483								
Amide I area	1732—1578	2.20^c	2.87^a	2.60^{ab}	2.38^{bc}	2.45^{bc}	0.084	<0.0001	<0.0001
Amide II area	1578—1483	0.61^c	0.79^a	0.74^{ab}	0.67^{bc}	0.72^{ab}	0.024	<0.0001	<0.0001
Amide I peak height	~1648	0.031^c	0.042^a	0.038^b	0.035^{bc}	0.036^b	0.0011	<0.0001	<0.0001
Amide II peak height	~1537	0.011^c	0.015^a	0.013^{ab}	0.012^{bc}	0.013^{ab}	0.0004	<0.0001	<0.0001
α-helix height	~1653	0.031^c	0.041^a	0.037^{ab}	0.034^{bc}	0.036^{ab}	0.0010	<0.0001	<0.0001
β-sheet height	~1635	0.029	0.059	0.035	0.032	0.034	0.0097	0.1419	0.2770
Ratio of Amide I to Amide II area		3.60	3.67	3.52	3.56	3.41	0.063	0.0940	0.3488
Ratio of α-helix to β-sheet height		1.09	0.97	1.06	1.08	1.08	0.032	0.0506	0.2483

Note: ST: starch; SEM= Standard error of mean; Means with different letters within the same row differ (P<0.05);
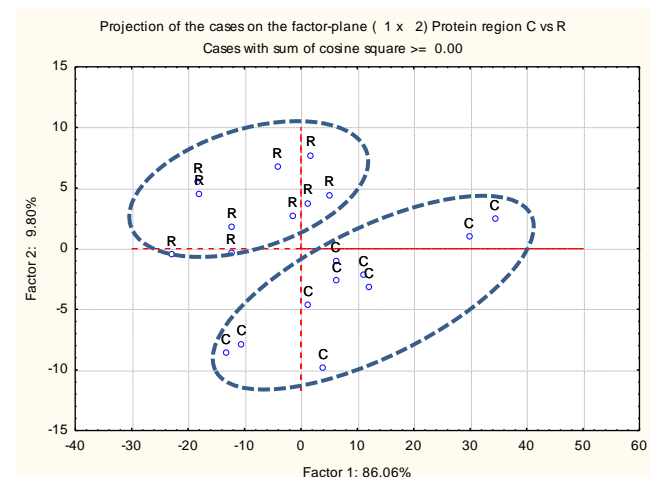Multi-treatment comparison: Tukey-Kramer method.
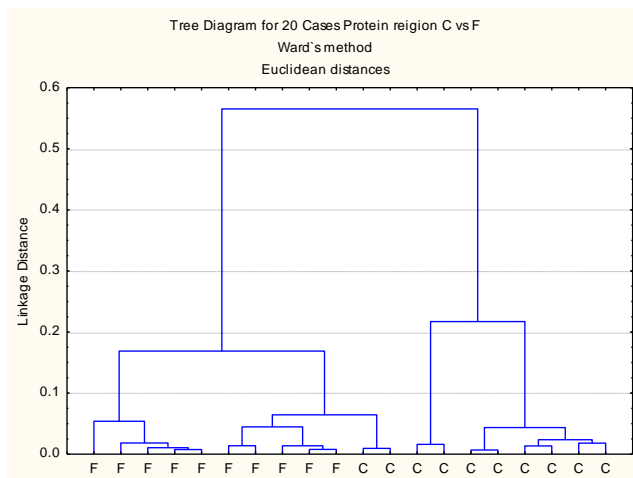
(1) CLA Comparison: CDC Copeland (C) and CDC Fibar (F)
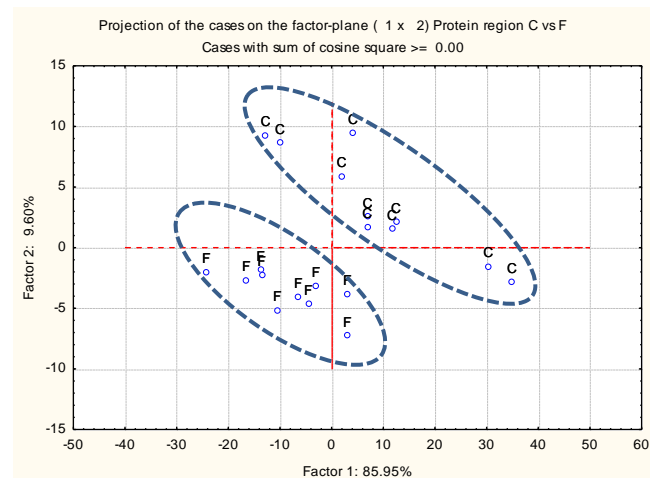
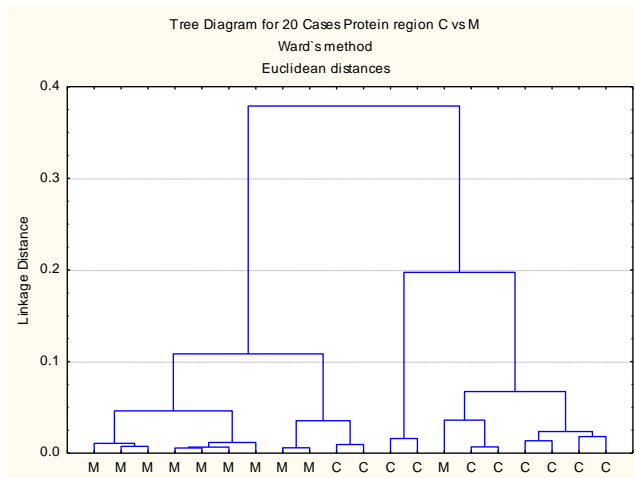(2) PCA Comparison: CDC Copeland (C) and CDC Fibar (F)
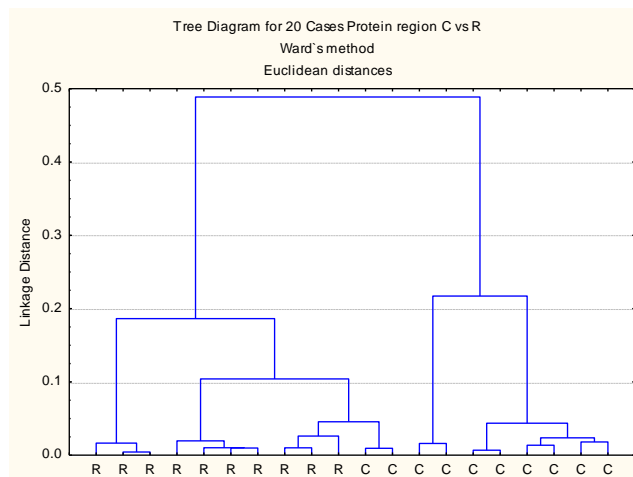
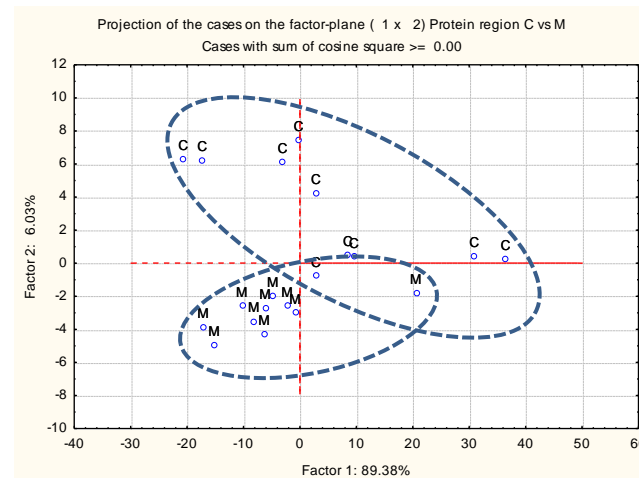(3) CLA Comparison: CDC Copeland (C) and CDC Rattan (R)

(4) PCA Comparison: CDC Copeland (C) and CDC Rattan (R)

Figure 4.4 Multivariate molecular spectral analyses of hulless barley varieties [CDC Fibar (F), CDC Rattan (R), CDC McGwire (M) and HB08302 (H)] in comparison with hulled barley [CDC Copeland: (C)] at FTIR protein fingerprint region: ca. 1732-1483 cm^{-1}. CLA (cluster analysis): (1) Cluster method: Ward's algorithm, (2) Distance method: Euclidean; PCA (principal component analysis): Scatter plots of the 1st principal components (PC1) vs. the 2nd principal components (PC2).

(5) CLA Comparison: CDC Copeland (C) and CDC McGwire (M)

(6) PCA Comparison: CDC Copeland (C) and CDC McGwire (M)
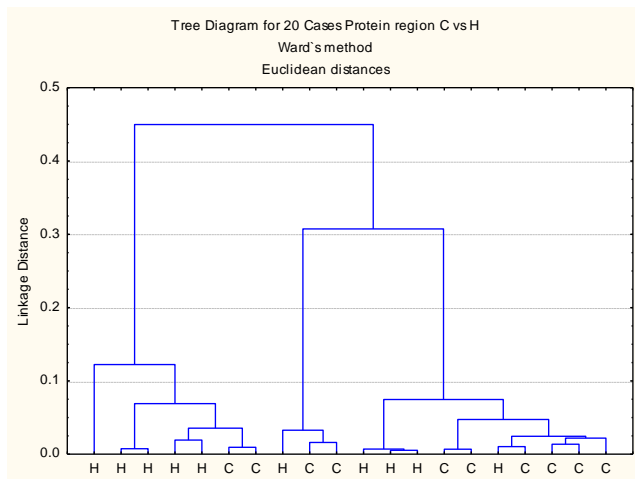
(7) CLA Comparison: CDC Copeland (C) and HB08302 (H)
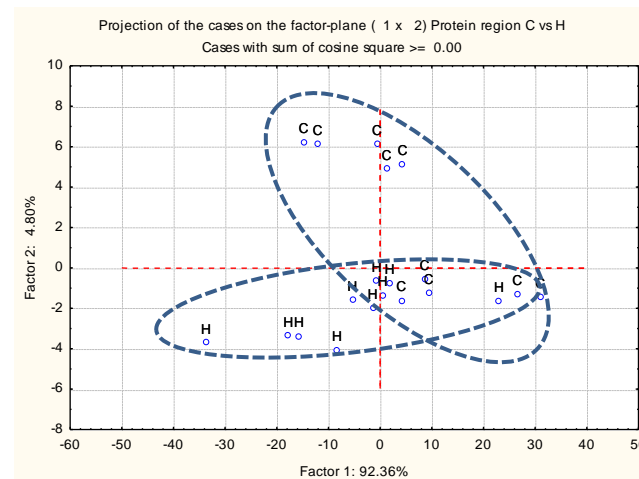
(8) PCA Comparison: CDC Copeland (C) and HB08302 (H)

Figure 4.4 Cont'd

(9) CLA Comparison: CDC McGwire (M) and CDC Fibar (F)
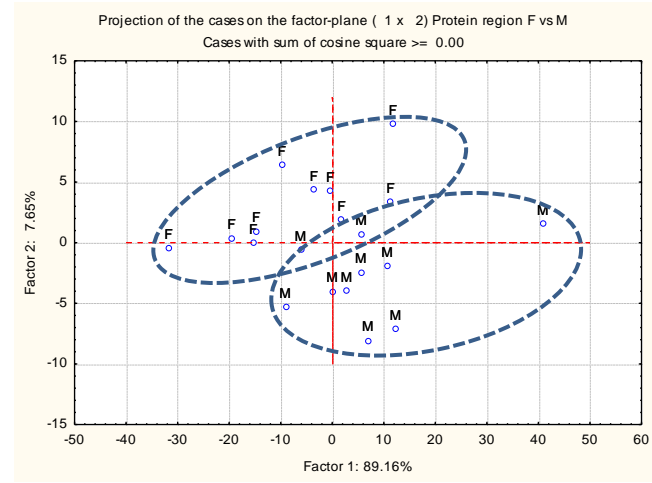
(10) PCA Comparison: CDC McGwire (M) and CDC Fibar (F)

(11) CLA Comparison: CDC McGwire (M) and CDC Rattan (R)

(12) PCA Comparison: CDC McGwire (M) and CDC Rattan (R)

Figure 4.4 Cont'd

Tree Diagram for 20 Cases Protein region M vs H
Ward`s method
Euclidean distances

(13) CLA Comparison: CDC McGwire (M) and HB08302 (H)

Projection of the cases on the factor-plane (1 x 2) Protein region M vs H
Cases with sum of cosine square >= 0.00
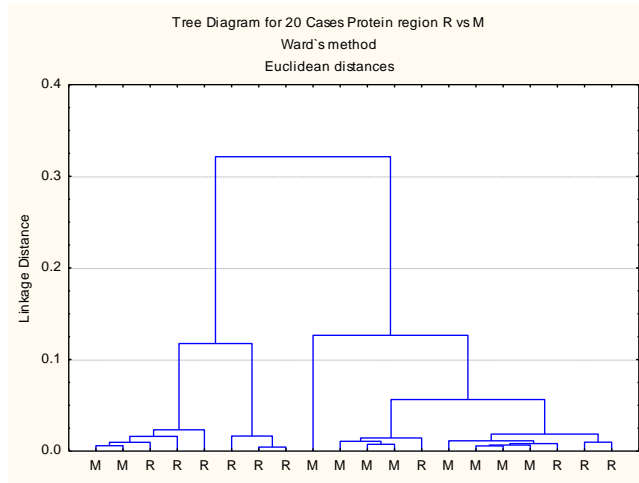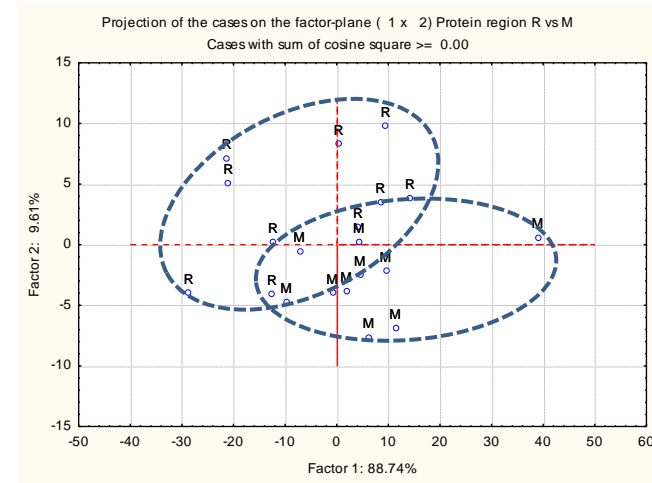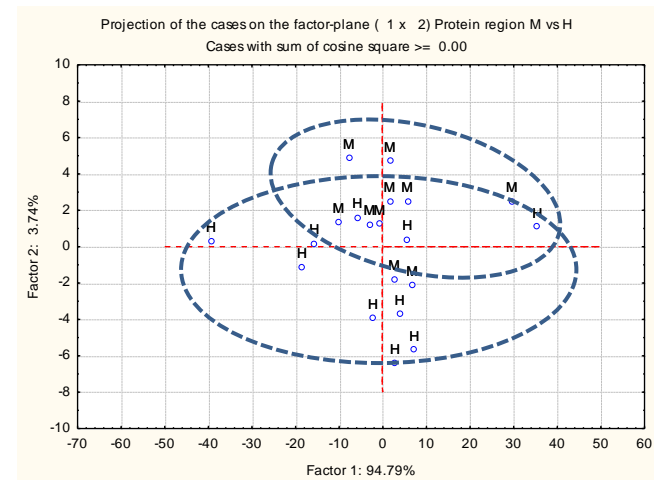
(14) PCA Comparison: CDC McGwire (M) and HB08302 (H)

Figure 4.4 Cont'd

Table 4.2 Correlation analysis between structure characteristics of protein amide I and II, protein secondary structure α-helix, β-sheet of hulless barley with altered carbohydrate traits and nutrient utilization and availability

Items	Amide I area	Amide I peak height	Amide II area	Amide II peak height	α-helix height	β-sheet height	Ratio of Amide I to Amide II area	Ratio of α-helix to β-sheet height
			--------Spearman Correlation R values--------					
In situ rumen CHO degradation								
BCHO (g/kg DM)	-0.19	-0.21	0.06	-0.04	-0.11	-0.20	-0.75**	0.24
EDCHO (g/kg DM)	-0.33	-0.30	-0.29	-0.29	-0.33	-0.41	-0.03	0.64*
In situ rumen CP degradation								
RUP (g/kg DM)	0.55^{+}	0.58^{+}	0.56^{+}	0.69*	0.61*	0.61*	-0.11	-0.34
BCP (g/kg DM)	0.55^{+}	0.58^{+}	0.56^{+}	0.69*	0.61*	0.61*	-0.11	-0.34
EDCP (g/kg DM)	0.79**	0.76**	0.64*	0.60^{+}	0.66*	0.68*	0.37	-0.40
In situ rumen NDF degradation								
K_d (%/h)	0.57^{+}	0.64*	0.63*	0.73*	0.61*	0.57^{+}	-0.20	-0.05
BNDF (% NDF)	0.65*	0.67*	0.66*	0.52	0.59^{+}	0.52	-0.06	-0.29
BNDF (g/kg DM)	0.55^{+}	0.57^{+}	0.75**	0.52^{+}	0.54^{+}	0.46	-0.53^{+}	0.11
EDNDF (% NDF)	-0.65*	-0.67*	-0.66*	-0.52	-0.59^{+}	-0.52	0.06	0.29
EDNDF (g/kg DM)	-0.28	-0.30	-0.08	-0.19	-0.27	-0.32	-0.55^{+}	0.61*
Intestinal CP digestion								
TDP (% CP)	0.02	-0.01	-0.26	-0.20	-0.04	0.07	0.75**	-0.31
TDP (g/kg DM)	0.76**	0.73*	0.63*	0.61*	0.67*	0.73*	0.34	-0.57^{+}
Intestinal CHO digestion								
IDBCHO (g/kg DM)	-0.23	-0.24	-0.04	-0.09	-0.13	-0.20	-0.62*	0.15
TDCHO (g/kg DM)	-0.44	-0.41	-0.35	-0.40	-0.42	-0.53^{+}	-0.18	0.75**

Note: K_d: the rate of degradation of degradable fraction (%/h); BCHO: rumen bypass or undegraded CHO; EDCHO: effective degradability of CHO; BCP or RUP: rumen bypass or undegraded crude protein; EDCP or RDP: effective degradability of crude protein; BDNDF: rumen bypass or undegraded neutral detergent fibre; EDNDF: effective degradability of neutral detergent fibre; TDP: total digestible protein; IDBCHO: intestinal digestible bypass CHO; TDCHO: total digestible CHO. + for P<0.10, * for P<0.05, ** for P<0.01, *** for P<0.001

Table 4.3 Correlation analysis between structure spectral characteristics of protein amide I and II, protein secondary structure α-helix, β-sheet of hulless barley with altered carbohydrate traits and predicted nutrients by DVE/OEB system and NRC Dairy 2001 model

Items (g/kg DM)	Amide I area	Amide I peak height	Amide II area	Amide II peak height	α-helix height	β-sheet height	Ratio of Amide I to Amide II area	Ratio of α-helix to β-sheet height
	--Spearman Correlation R values--							
DVE/OEB system								
DVE	0.55^{+}	0.52	0.25	0.36	0.46	0.49	0.71*	-0.59^{+}
OEB^{DVE}	0.74**	0.72*	0.69*	0.66*	0.70*	0.76**	0.24	-0.52
NRC Dairy 2001 model								
$AMCP^{NRC}$	0.79**	0.76**	0.64*	0.60^{+}	0.66*	0.68*	0.37	-0.40
$ARUP^{NRC}$	0.55^{+}	0.57^{+}	0.56^{+}	0.69^{+}	0.61*	0.61*	-0.11	-0.34
MP^{NRC}	0.90***	0.90***	0.84**	0.88***	0.87***	0.89***	0.11	-0.55^{+}
OEB^{NRC}	0.79**	0.76**	0.64*	0.60^{+}	0.66*	0.68*	0.37	-0.40

Note: DVE: truly digested protein in the small intestine; OEB: degraded protein balance; AMCP: truly absorbed microbial protein in the small intestine; ARUP: truly absorbed rumen undegraded protein in the small intestine; MP: metabolizable protein
$^{+}$ for P<0.10, * for P<0.05, ** for P<0.01, *** for P<0.001

4.3.1.2.   Quantifying the Molecular Structures of Non-Starch CHO (β-Glucan and Cellulosic Compounds) in Hulless Barleys in Comparison with Hulled barley

The non-starch CHO region in the spectra was separated as β-glucan and cellulosic compounds regions for univariate analysis. Both β-glucan and cellulosic compounds were associated with cell wall structures in which β-glucan can be mainly found in the internal aleurone and endosperm cell walls of cereals, while cellulosic compounds include phenolic-carbohydrate complexes, hemicellulose encrustation and cellulose crystallinity (Garleb et al., 1988, 1991; Gordon et al., 1977; Liu and Yu, 2011). According to previous studies (Wetzel et al., 1998, 2003; Liu and Yu, 2010; Damiran and Yu, 2011), β-glucan was determined in the region of ca. 1448-1390 cm^{-1} while cellulosic compounds were detected at ca. 1275-1212 cm^{-1} (Table 4.4). Hulless barley cultivars, except CDC McGwire, were higher in β-glucan content than hulled barley (P<0.05). Hulless barley lines showed no significant difference on β-glucan absorption peak intensity (P>0.05). Hulless barley varieties were similar in absorption peak intensity in area and height at cellulosic compounds regions (P<0.05), while CDC Fibar and CDC Rattan were both significantly higher in cellulosic compounds peak area and height compared to hulled barley (P<0.05). These results further confirm that the absorption intensity of certain functional groups cannot be regarded as representative of actual carbohydrate content (Liu and Yu, 2011), but molecular structure makeup.

Figure 4.5 shows the CLA and PCA analysis of structural differences between hulled barley and hulless barley in the region of ca. 1483−1189 cm^{-1}, which mainly consists of functional groups of β-glucan and cellulosic compounds. The clusters were formed below the linkage distance of 0.3. Although there was an overlap area between CDC Fibar (F) and CDC Copeland (C), the two ellipses were still distinguishable [Figure 4.5: (1)-(2)]. 97.55% of the variation can be explained by the first principal component, while 2.15% of variation was associated with the second principal component. CDC Fibar and CDC Rattan (R) were not fully distinguished from CDC Copeland (C) in the PCA plot with 97.78% of the variation associated with first principal component while second principal component only accounted for about 2% of the variation [Figure 4.5 (3)-(4)]. CDC McGwire (M) and HB08302 (H) both had overlapped

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ellipses with CDC Copeland and cluster classes were mixed instead of grouped separately [Figure 4.5: (5)−(8)]. Both variations were mainly associated with first principal components around 98% of their variation. Among hulless barley, there was no fully distinguished cluster or ellipse found, although the variations between altered starch hulless barley cultivars compared to normal-amylose hulless barley were more than 95% explained by first principal component [Figure 4.5: (9)−(14)]. The results of multivariate analysis further supported the univariate analysis that there was no distinguishable difference among hulless barleys in terms of spectral characteristics of β-glucan and cellulosic compounds.

Previous studies reported that high β-glucan in cereal grains will increase the cell wall thickness (Oscarsson et al., 1997; Zheng et al., 2000) and particle size after mechanical processing, while the structures of cellulosic compounds will reduce the susceptibility to digestive enzymes in animals (Van Soest 1975; Garleb et al., 1988, 1991; Gordon et al., 1977; Bhatty 1999; Damiran and Yu, 2010; Liu and Yu, 2011). Although the spectral characteristics of related functional group bands cannot be interpreted as a representation of accurate biological compound content (Liu and Yu, 2011), the spectral structure characteristics of β-glucan and cellulosic compounds could still affect nutrient degradation in the rumen, intestinal digestion and potential protein supply. Table 4.5 showed only effective degradable crude protein (EDCP) was correlated with cellulosic compounds area (r=0.67, P<0.05) and height (r=0.86, P<0.05). β-glucan height was negatively correlated with intestinal digestible bypass starch (IDBST: r=-0.68, P<0.05) while cellulosic compounds was found to be positively correlated with total digestible protein (r=0.68, P<0.05) (Table 4.5). Compared with β-glucan, spectral charactersitics of cellulosic compounds showed a more positive correlation with OEB (r=0.70, 0.79; P<0.05) and metabolizable protein (r=0.63, 0.71; P<0.05). This implies the complex structures of cellulosic compounds in hulless barley lines played more important role in nutrient availability for ruminant than β-glucan in powdered seed.

Therefore, hulless barley varieties were similar in non-starch CHO although a difference was observed between hulled and hulless cultivars. Spectral features of cellulosic compounds showed a positive effect on effective degradable protein in the rumen, protein degraded balance, and metabolizable protein supply to the dairy cow.

Table 4.4 Effect of altered carbohydrate traits on structure spectral characteristics of β-glucan and cellulosic compounds in whole seeds of hulless barley varieties in comparison with hulled barley using FTIR molecular spectroscopy

Item	Peak region and center (cm^{-1})	Hulled Copeland (n=3)	Hulless Fibar (n=3)	Rattan (n=3)	McGwire (n=3)	HB08302 (n=2)	SEM	P value	Contrast Hulled vs. Hulless P value
Amylose Level (% of ST)		27.0[b]	2.5[d]	7.7[c]	25.8[b]	36.9[a]	0.56	<0.0001	<0.0001
Amylopectin Level (% of ST)		73.0[c]	97.5[a]	92.3[b]	74.2[c]	63.1[d]	0.56	<0.0001	<0.0001
β-Glucan Level (% DM)		3.8[c]	10.0[a]	7.4[b]	4.7[c]	7.5[b]	0.40	<0.0001	<0.0001
β-glucan									
Peak area	1448−1390	0.05[a]	0.04[b]	0.03[b]	0.04[b]	0.04[b]	0.002	<0.0001	<0.0001
Peak height	~1413	0.0021[a]	0.0019[ab]	0.0018[ab]	0.0019[ab]	0.0016[b]	0.00013	0.0642	0.0211
Cellulosic compounds									
Peak area	1275−1212	0.12[b]	0.15[a]	0.14[a]	0.13[ab]	0.13[ab]	0.004	0.0011	0.0010
Peak height	~1238	0.0043[b]	0.0053[a]	0.0050[a]	0.0047[ab]	0.0047[ab]	0.00018	0.0021	0.0019

Note: ST: starch; SEM= Standard error of mean; Means with different letters within the same row differ (P<0.05);
   Multi-treatment comparison: Tukey-Kramer method.
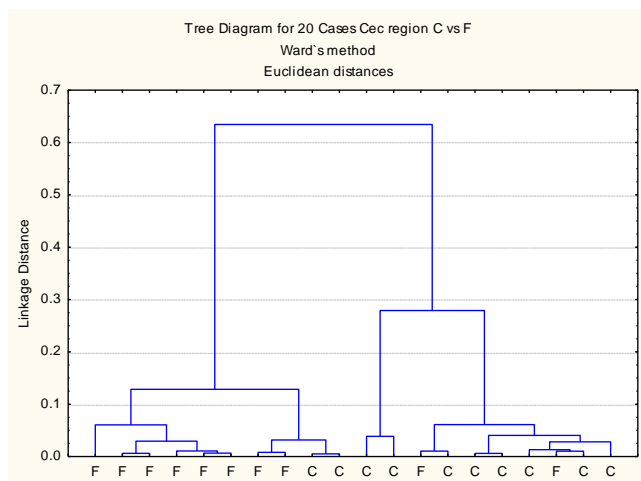
(1) CLA Comparison: CDC Copeland (C) and CDC Fibar (F)
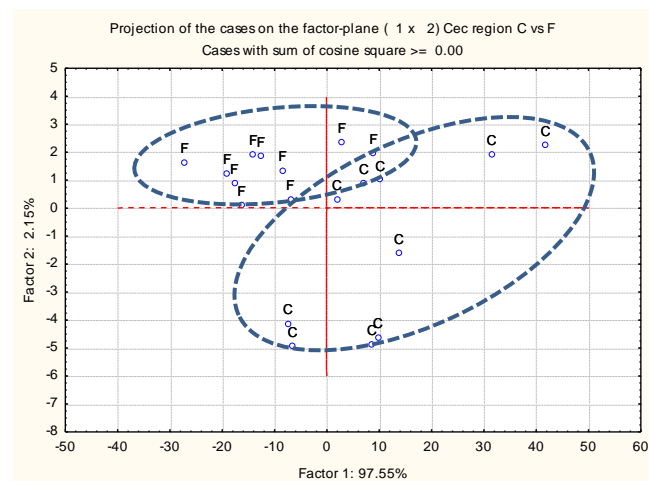
(2) PCA Comparison: CDC Copeland (C) and CDC Fibar (F)
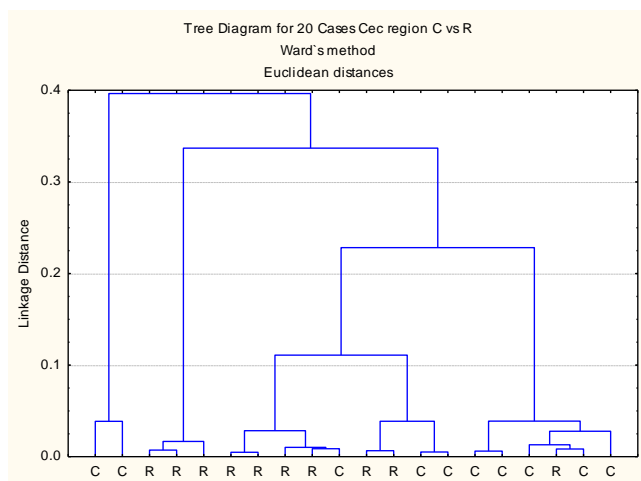
(3) CLA Comparison: CDC Copeland (C) and CDC Rattan (R)

(4) PCA Comparison: CDC Copeland (C) and CDC Rattan (R)

Figure 4.5 Multivariate molecular spectral analyses of hulless barley varieties [CDC Fibar (F), CDC Rattan (R), CDC McGwire (M) and HB08302 (H)] in comparison with hulled barley [CDC Copeland: (C)] at FTIR non-starch carbohydrate fingerprint region: ca. 1483-1189 cm^{-1}. CLA (cluster analysis): (1) Cluster method: Ward's algorithm, (2) Distance method: Euclidean; PCA (principal component analysis): Scatter plots of the 1st principal components (PC1) vs. the 2nd principal components (PC2).
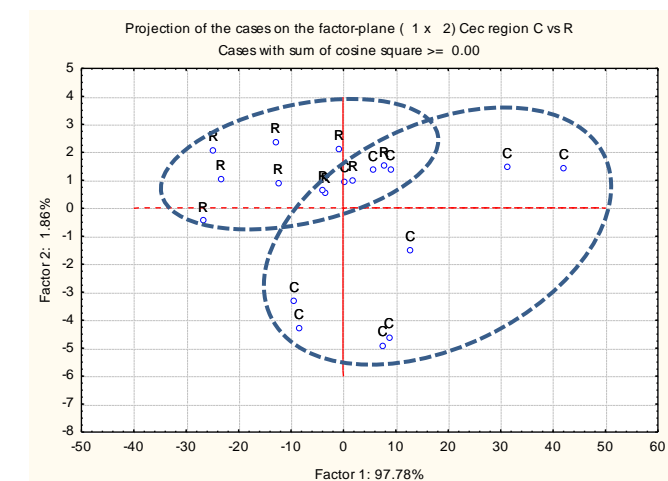
(5) CLA Comparison: CDC Copeland (C) and CDC McGwire (M)

(6) PCA Comparison: CDC Copeland (C) and CDC McGwire (M)

(7) CLA Comparison: CDC Copeland (C) and HB08302 (H)

(8) PCA Comparison: CDC Copeland (C) and HB08302 (H)

Figure 4.5 Cont'd

(9) CLA Comparison: CDC McGwire (M) and CDC Fibar (F)

(10) PCA Comparison: CDC McGwire (M) and CDC Fibar (F)

(11) CLA Comparison: CDC McGwire (M) and CDC Rattan (R)

(12) PCA Comparison: CDC McGwire (M) and CDC Rattan (R)

Figure 4.5 Cont'd

Tree Diagram for 20 Cases Cec region M vs H
Ward`s method
Euclidean distances

(13) CLA Comparison: CDC McGwire (M) and HB08302 (H)

Projection of the cases on the factor-plane (1 x 2) Cec region M vs H
Cases with sum of cosine square >= 0.00
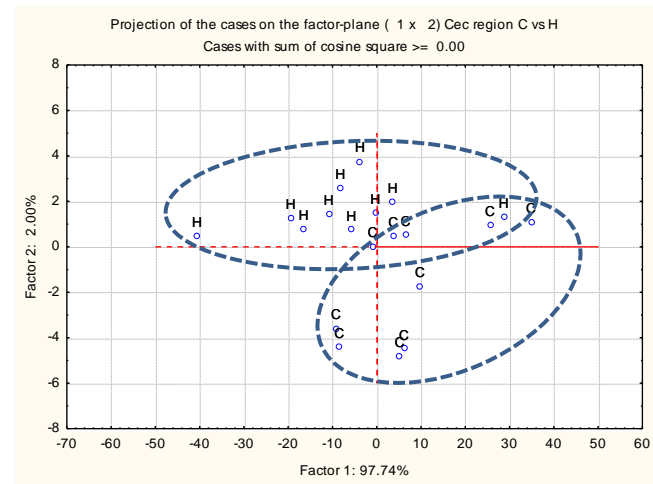
(14) PCA Comparison: CDC McGwire (M) and HB08302 (H)

Figure 4.5 Cont'd

Table 4.5 Correlation analysis between structure spectral characteristics of β-glucan, cellulosic compounds of hulless barley with altered carbohydrate traits and nutrients availability and utilization in the rumen and intestine

Items (g/kg DM)	β-glucan		Cellulosic compounds	
	Area	Height	Area	Height
	--------------------Spearman Correlation R values-----------------			
In situ rumen CP degradation				
EDCP	-0.28	0.39	0.67*	0.76*
Intestinal CP digestion				
TDP	-0.36	0.34	0.60^+	0.68*
Intestinal starch digestion				
IDBST	-0.13	-0.68*	-0.20	-0.23
DVE/OEB system				
OEB^{DVE}	-0.46	0.21	0.70*	0.79**
NRC Dairy 2001 model				
$AMCP^{NRC}$	-0.28	0.39	0.67*	0.76**
MP^{NRC}	-0.12	0.28	0.63*	0.71*
OEB^{NRC}	-0.28	0.39	0.67*	0.76**

Note: EDCP: effective degradability of crude protein; TDP: total digestible protein; IDBST: intestinal digestible bypass starch; OEB: degraded protein balance; AMCP: truly absorbed microbial protein; MP: metabolizable protein.

  + for $P<0.10$, * for $P<0.05$, ** for $P<0.01$, *** for $P<0.001$

4.3.1.3.   Quantifying the Molecular Structures of CHO in Hulless Barleys in Comparison with Hulled Barley

The spectral features of carbohydrate were detected by FTIR at the region of ca. 1189-946 cm^{-1}. The intensity of three CHO absorption peaks were recorded in the regions of ca. 1189-1130 cm^{-1}, 1130-1063 cm^{-1} and 1063-946 cm^{-1} (Table 4.6). Hulless barley varieties were similar in CHO peak 1 and peak 3 areas, although CDC Fibar was higher (P<0.05) in CHO peak 2 area (4.10 vs. 3.67) and height (0.096 vs. 0.088) compared to CDC McGwire. CDC Copeland was lower in absorption intensity for all these peaks of CHO than CDC Fibar (P<0.05).

In Figure 4.6, the molecular spectra of barley cultivars were compared using CLA and PCA at the region of ca. 1189-945 cm^{-1}. Hulless barley cultivars were not fully distinguished from hulled barley [Figure 4.6: (1)-(8)] although approximately 80% of the variation could be explained by the first principal components, large overlap areas between the two ellipses were found. A similar situation was found in the comparison among hulless barley cultivars. Altered starch hulless barley was not fully separated into cluster groups and ellipses overlapped on CLA and PCA plots [Figure 4.6: (9)-(14)]. The first principal component explained approximately 70% of the variation between hulless barley cultivars with altered amylose level and the normal-amylose hulless cultivar CDC McGwire. These results indicate that there was limited variation among the barley cultivars in molecular spectral characteristics for the CHO region.

Table 4.7 shows the correlation analysis between the related parameters of nutrient availability and structure spectral characteristics of CHO of hulless barley. Unexpectedly, spectral features of CHO showed significant correlation with protein availability instead of CHO availability for ruminants. Some spectral characteristics of CHO exhibited a positive correlation to rumen undegradable or bypass protein (P<0.05) but no relationship with starch or CHO degradation in the rumen was found (P>0.05). CHO peak 2 area (r=0.70, P<0.05) and height (r=0.62, P<0.05) were positively correlated to total digestible protein. On potential protein supply predicted by the two models, CHO spectral features only showed positive correlation to truly absorbed rumen undegrdable protein (ARUP^{NRC}) and metabolizable protein (MP^{NRC}) estimated by the NRC Dairy 2001 model (P<0.05). There was no correlation between CHO spectral features and CHO degradation or digestion, which was in agreement with Liu and Yu

(2010) who also found a weak correlation between spectral characteristics of barley cultivars and *in situ* degradation kinetics (rate and extent). A possible reason may be that the differences among hulless barley are not large enough to identify the actual relationship between spectral features of carbohydrates and their effect on nutrient availability. However, FTIR still can be used to detect the structural features of barley varieties in the CHO region. The structural differences of functional group bands of hulless barley with altered carbohydrate traits may be one of the factors for different metabolizable protein supply to ruminants and may also influence the rumen degradation of protein.

Table 4.6 Effect of altered carbohydrate traits on structure spectral characteristics of CHO in whole seeds of hulless barley varieties in comparison with hulled barley using FTIR molecular spectroscopy

Item	Peak region and center (cm^{-1})	Hulled Copeland (n=3)	Hulless				SEM	P value	Contrast Hulled vs. Hulless P value
			Fibar (n=3)	Rattan (n=3)	McGwire (n=3)	HB08302 (n=2)			
Amylose Level (% of ST)		27.0^b	2.5^d	7.7^c	25.8^b	36.9^a	0.56	<0.0001	<0.0001
Amylopectin Level (% of ST)		73.0^c	97.5^a	92.3^b	74.2^c	63.1^d	0.56	<0.0001	<0.0001
β-Glucan level (% DM)		3.8^c	10.0^a	7.4^b	4.7^c	7.5^b	0.40	<0.0001	<0.0001
Total area	1189—946	16.26^b	18.61^a	17.67^{ab}	17.39^{ab}	18.02^{ab}	0.431	0.0033	0.0007
CHO Peak 1 area	1189—1130	1.38^b	1.52^a	1.49^{ab}	1.46^{ab}	1.52^a	0.039	0.0473	0.0045
CHO Peak 2 area	1130—1063	3.49^b	4.10^a	3.80^{ab}	3.67^b	3.83^{ab}	0.093	0.0002	0.0007
CHO Peak 3 area	1063—946	11.39^b	12.99^a	12.38^{ab}	12.27^{ab}	12.66^{ab}	0.306	0.0051	0.0006
CHO Peak 1 height	~1150	0.07	0.05	0.05	0.05	0.10	0.022	0.4449	0.6104
CHO Peak 2 height	~1076	0.083^c	0.096^a	0.091^{ab}	0.088^{bc}	0.092^{ab}	0.0021	0.0003	0.0002
CHO Peak 3 height	~1016	0.16^b	0.18^a	0.17^{ab}	0.17^{ab}	0.18^a	0.004	0.0025	0.0002

Note: ST: starch; CHO: carbohydrates;
SEM= Standard error of mean; Means with different letters within the same row differ (P<0.05);
Multi-treatment comparison: Tukey-Kramer method.
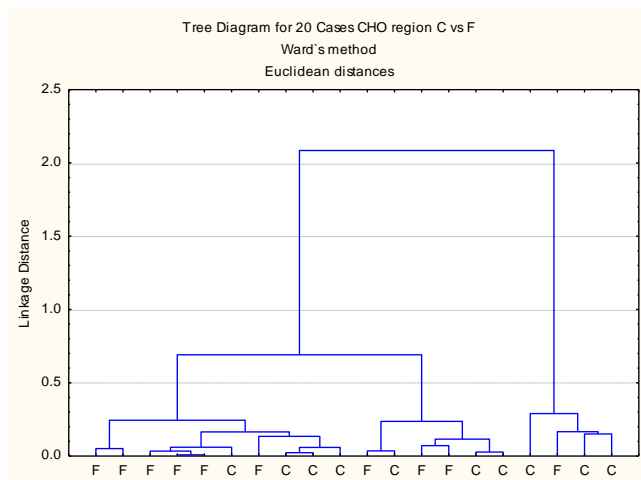
(1) CLA Comparison: CDC Copeland (C) and CDC Fibar (F)
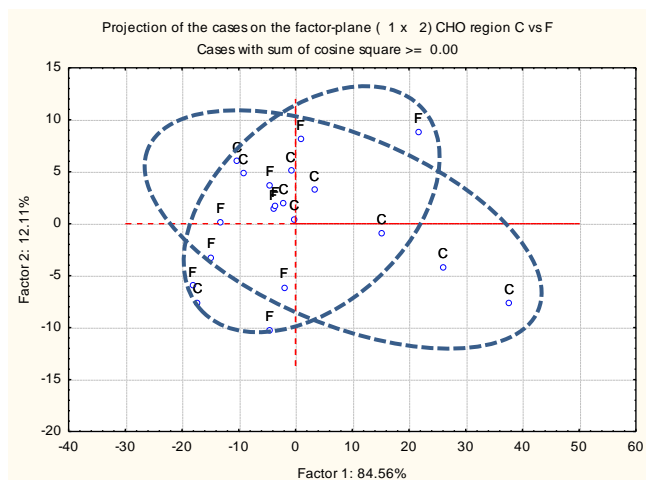
(2) PCA Comparison: CDC Copeland (C) and CDC Fibar (F)
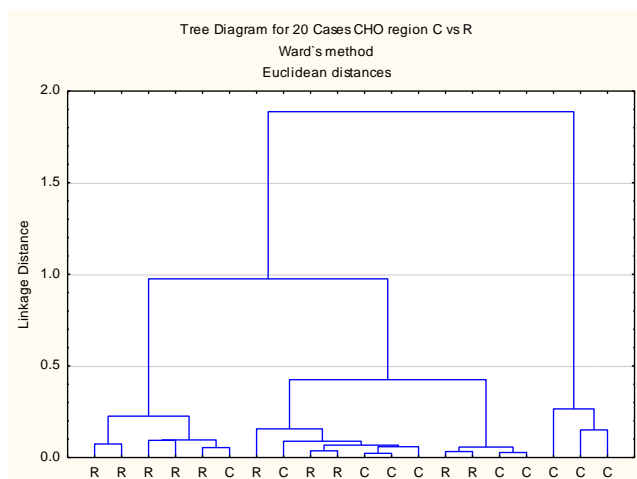
(3) CLA Comparison: CDC Copeland (C) and CDC Rattan (R)
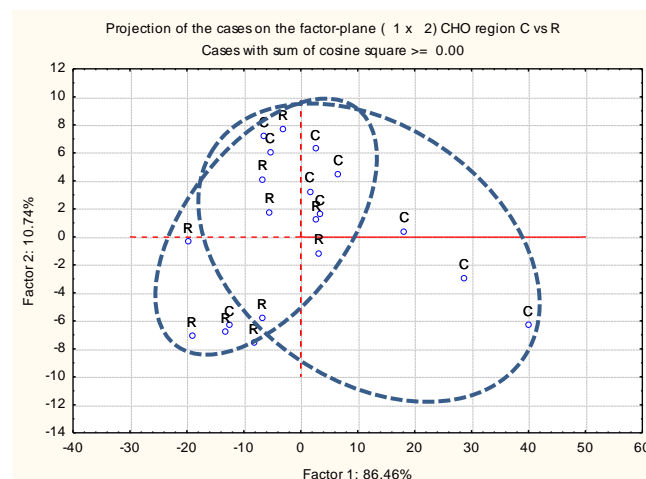
(4) PCA Comparison: CDC Copeland (C) and CDC Rattan (R)

Figure 4.6 Multivariate molecular spectral analyses of hulless barley varieties [CDC Fibar (F), CDC Rattan (R), CDC McGwire (M) and HB08302 (H)] in comparison with hulled barley [CDC Copeland: (C)] at FTIR carbohydrate fingerprint region: ca. 1189-945 cm^{-1}. CLA (cluster analysis): (1) Cluster method: Ward's algorithm, (2) Distance method: Euclidean; PCA (principal component analysis): Scatter plots of the 1st principal components (PC1) vs. the 2nd principal components (PC2).

(5) CLA Comparison: CDC Copeland (C) and CDC McGwire (M)

(6) PCA Comparison: CDC Copeland (C) and CDC McGwire (M)

(7) CLA Comparison: CDC Copeland (C) and HB08302 (H)

(8) PCA Comparison: CDC Copeland (C) and HB08302 (H)

Figure 4.6 Cont'd
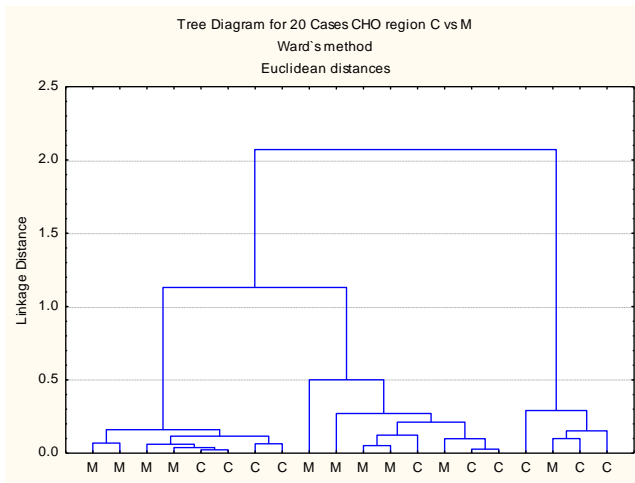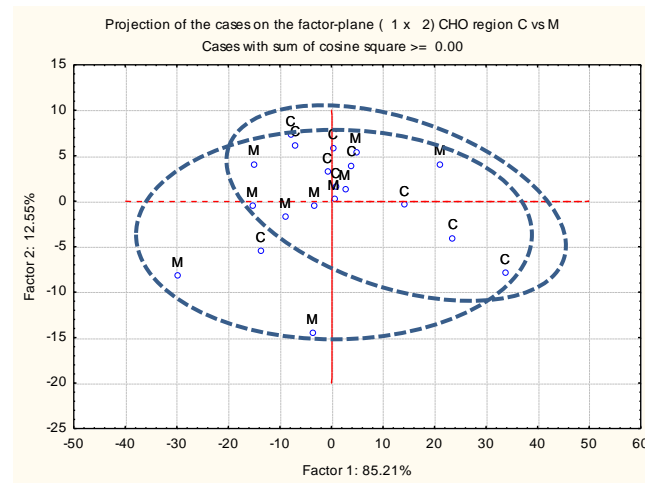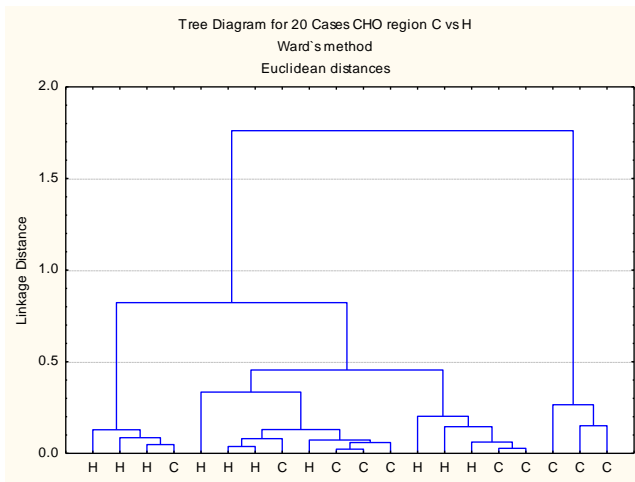
(9) CLA Comparison: CDC McGwire (M) and CDC Fibar (F)

(10) PCA Comparison: CDC McGwire (M) and CDC Fibar (F)

(11) CLA Comparison: CDC McGwire (M) and CDC Rattan (R)
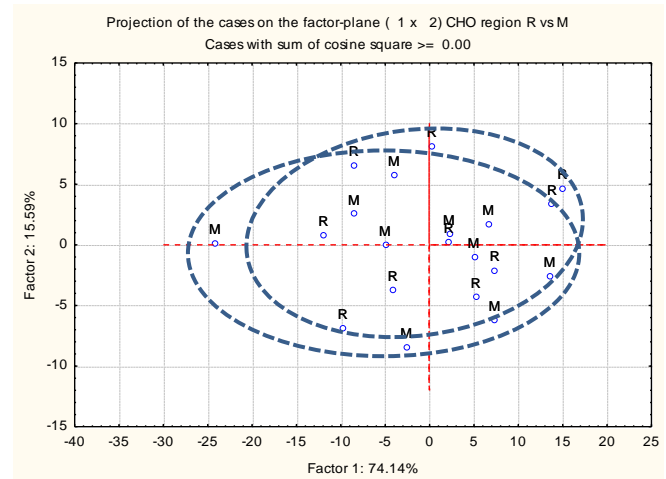
(12) PCA Comparison: CDC McGwire (M) and CDC Rattan (R)

Figure 4.6 Cont'd

(13) CLA Comparison: CDC McGwire (M) and HB08302 (H)
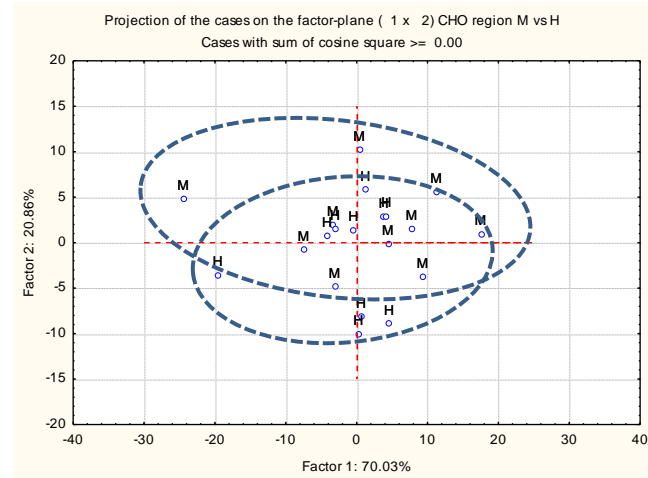
(14) PCA Comparison: CDC McGwire (M) and HB08302 (H)

Figure 4.6 Cont'd

Table 4.7 Correlation analysis between structure spectral characteristics of carbohydrates of hulless barley with altered carbohydrate traits detected by FTIR and nutrient utilization and availability in the rumen and intestine

Items	Total area	CHO Peak 1 area	CHO Peak 2 area	CHO Peak 3 area	CHO Peak 1 height	CHO Peak 2 height	CHO Peak 3 height
	---Spearman Correlation R values---						
In situ rumen CP degradation (g/kg DM)							
RUP	0.65*	0.55[+]	0.65*	0.65*	0.52	0.63*	0.64*
BCP	0.65*	0.55[+]	0.65*	0.65*	0.52	0.63*	0.64*
Intestinal CP digestion (g/kg DM)							
TDP	0.41	0.15	0.70*	0.41	0.00	0.62*	0.28
NRC Dairy 2001 model (g/kg DM)							
ARUP^{NRC}	0.65*	0.55[+]	0.65*	0.65*	0.52	0.63*	0.64*
MP^{NRC}	0.70*	0.45	0.86***	0.70*	0.37	0.82**	0.61*

Note: BCP or RUP: rumen bypass or undegraded crude protein; TDP: total digestible protein; ARUP: truly absorbed rumen undegraded protein in the small intestine; MP: metabolizable protein

+ for P<0.10, * for P<0.05, ** for P<0.01, *** for P<0.001

4.3.2. **Using Synchrotron-Based Fourier Transformed Vibrational Infrared Microspectroscopy (SR-FTIRM) to Identify Molecular Structure Spectral Features of Hulless Barleys in Comparison with Hulled Barley**

Synchrotron based Fourier transform vibrational infrared Microspectroscopy (SR-FTIRM) was used to identify molecular structure spectral features of newly developed CDC hulless barleys in terms of protein, non-starch CHO (β-glucan, cellulosic compounds) and total CHO. Sample preparation differed from conventional FTIR, as only endosperm tissues of barley cultivars were sectioned and placed in the BaF_2 window for SR-FTIRM work under transmission mode. The absorption characteristics associated with chemical functional groups (protein, β-glucan, cellulosic compounds and CHO) of barley grains were collected and recorded for univariate, multivariate and correlation analyses.

4.3.2.1. **Quantifying Spectral Features of Protein in the Endosperm Tissue of Hulless Barleys In Comparison With Hulled Barley**

Table 4.8 shows the absorbance peak area and height intensities of protein in the endosperm tissue of hulled and hulless barley varieties in region of ca. 1768−1475 cm^{-1}. Compared to hulled barley, hulless barley CDC Fibar exhibited similar absorbance peak intensity in terms of protein amide I and II area, height as well as protein secondary structure height as CDC Copeland (P>0.05). However, they were both lower in amide I area, amide II peak height and α-helix height than other hulless barley varieties (P<0.05). CDC Rattan, CDC McGwire and HB08302 were similar at most of the protein spectral features in the endosperm tissue although CDC Rattan was higher in amide II area (2.11) than other barley cultivars (P<0.05). Ratios of α-helix to β-sheet height among the barley varieties ranged from 1.12 to 1.17 instead of 1.4 to 2.0 for hulled barley (Yu, 2007) and hulless barley (Damiran and Yu, 2011), and there were no significant differences (P>0.05) among barley varieties in terms of ratios of amide I to amide II area and ratios of α-helix to β-sheet height.

The CLA and PCA analysis were conducted to identify the protein structural differences among the barley cultivars in the endosperm tissue (Figure 4.7). Hulless barley and hulled barley

varieties were not fully distinguished from each other at the protein region ca. 1768−1475 cm^{-1}. When comparing CDC Copeland (C) to CDC Fibar (F), no clear separate cluster classes were grouped and 94.29% of the variation in protein structure between the two cultivars was explained by first principal components [Figure 4.7: (1)-(2)]. There were overlapped areas of the two ellipses found in PCA plot indicating there was similarities of protein spectral features between the two varieties. Similar results were found for hulless barley varieties when compared to hulless barley. Altered amylose level to normal-amylose hulless barley the clusters were not separated [(Figure 4.7: (9)-(14)). It seems as the level of amylose increases in starch of hulless barley, larger overlapped ellipses areas were found in PCA plot when compared to normal amylose CDC McGwire (M). Multivariate results suggested that there were no fully distinguished differences in protein structures existing among hulless barley and hulled barley.

Yu (2007) reported that protein secondary structures will have an influence on protein value and protein availability by affecting access to digestive enzymes. Table 4.9 shows the correlation between protein structural features in endosperm tissue of hulless barley and correlated parameters estimated by rumen degradation, intestinal digestion as well as predicted protein supply from two models. With respect to rumen degradation of nutrients, effective degradable crude protein (EDCP) was negatively correlated to ratio of amide I to amide II area (r=−0.66, P<0.05) while effective degradable NDF was positively correlated to amide I area (r=0.74, P<0.05) and protein secondary structure (r=0.65, r=0.82; P<0.05). There was no correlation found between intestinal nutrient digestion and most of the protein spectral features except negative correlation between ratio of amide I to amide II area and percentage of total digestible protein in total crude protein (r=−0.75, P<0.01), and between the ratio of protein secondary structure on absorption peak intensity and intestinal digestible bypass CHO (r=−0.61, P<0.05). There was a positive correlation between total digestible CHO and amide II peak height (r=0.63, P<0.05). Ratio of amide I to amide II area was negatively correlated with OEB, MCP and AMCP (r=−0.64, P<0.05) while MCP estimated from TDN was negatively correlated to protein spectral structure (P<0.05).

The results suggested that protein structure differences among hulless barley cultivars may affect availability and utilization of protein and CHO to the dairy cow, which was partially

supported by Damiran and Yu (2011) who reported that protein utilization was affected by protein secondary structure. The results also indicated there was similarity in protein molecular structure make-up in endosperm tissue between CDC Fibar and hulled barley, although a significant difference between these two cultivars on protein profiles were found in chemical analysis (Table 3.2.2). These results differed from results of FTIR, CDC Fibar exhibited similarity as hulled barley in terms of protein spectral characteristics. A possible reason may be sampling area in which whole seeds were ground to be detected by FTIR, while only endosperm tissue was used in SR−FTIRM. Although SR−FTIRM was superior in intensity of light source and capable to detect the sample molecular spectral features with thin layer of tissue, there was less even distribution of nutrient in tissue sample for SR−FTIRM than the powdered seed prepared for FTIR.

Table 4.8 Effect of altered carbohydrate traits on spectral characteristics of protein amide I and II, protein secondary structure α-helix and β-sheet in the endosperm region of hulless barley varieties in comparison with hulled barley using synchrotron−based FTIR microspectroscopy

Item	Peak region and center (cm^{-1})	Hulled Copeland (n=3)	Hulless Fibar (n=3)	Rattan (n=3)	McGwire (n=3)	HB08302 (n=2)	SEM	P value	Contrast Hulled vs. Hulless P value
Amylose Level (% of ST)		27.0^b	2.5^d	7.7^c	25.8^b	36.9^a	0.56	<0.0001	<0.0001
Amylopectin Level (% of ST)		73.0^c	97.5^a	92.3^b	74.2^c	63.1^d	0.56	<0.0001	<0.0001
β-Glucan Level (% DM)		3.8^c	10.0^a	7.4^b	4.7^c	7.5^b	0.40	<0.0001	<0.0001
Baseline	1768−1475								
Amide I area	1768−1558	6.70^b	6.97^b	9.28^a	8.71^a	9.07^a	0.381	<0.0001	<0.0001
Amide II area	1558−1475	1.33^c	1.55^{bc}	2.11^a	1.75^b	1.66^{bc}	0.154	<0.0001	<0.0001
Amide I peak height	~1647	0.10^b	0.10^b	0.13^a	0.13^a	0.13^a	0.006	<0.0001	<0.0001
Amide II peak height	~1542	0.02^b	0.03^{ab}	0.03^a	0.03^a	0.03^{ab}	0.003	0.0006	0.0011
α-helix height	~1655	0.09^b	0.10^b	0.12^a	0.12^a	0.12^a	0.005	<0.0001	<0.0001
β-sheet height	~1628	0.08^c	0.09^{bc}	0.11^a	0.10^{ab}	0.11^{ab}	0.005	<0.0001	<0.0001
Ratio of Amide I to Amide II area		8.27	6.06	5.62	8.61	7.24	1.449	0.3579	0.3320
Ratio of α-helix to β-sheet height		1.17	1.16	1.14	1.13	1.12	1.058	0.1281	0.0474

Note: ST: starch;

SEM= Standard error of mean; Means with different letters within the same row differ (P<0.05);

Multi-treatment comparison: Tukey-Kramer method.
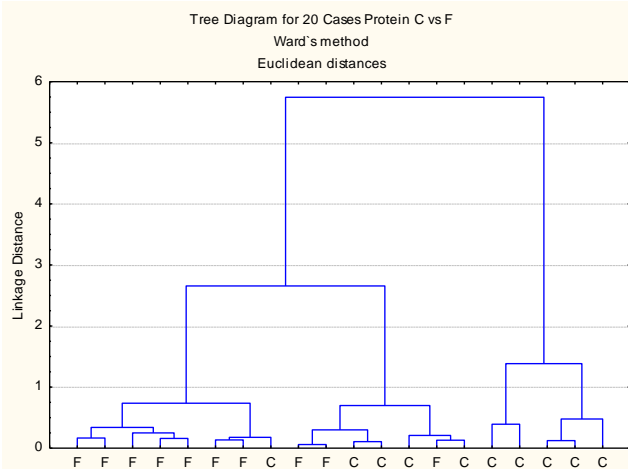
(1) CLA Comparison: CDC Copeland (C) and CDC Fibar (F)
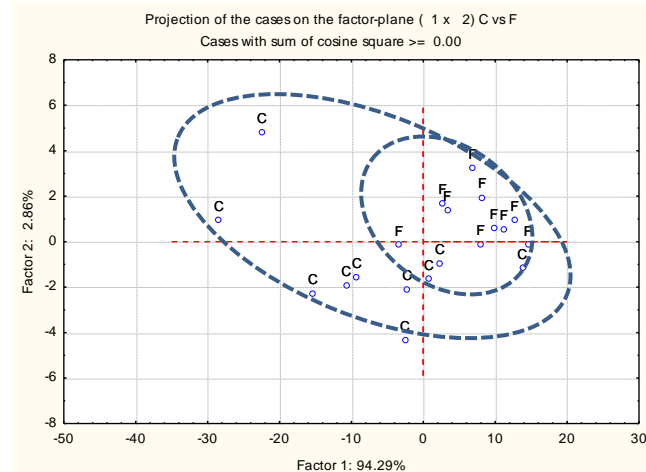
(2) PCA Comparison: CDC Copeland (C) and CDC Fibar (F)
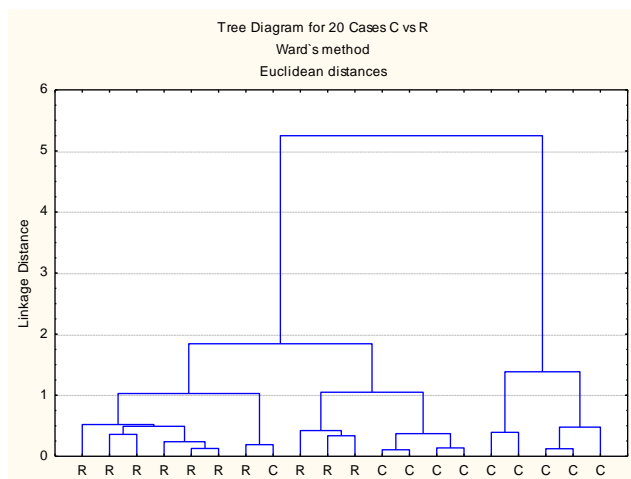
(3) CLA Comparison: CDC Copeland (C) and CDC Rattan (R)
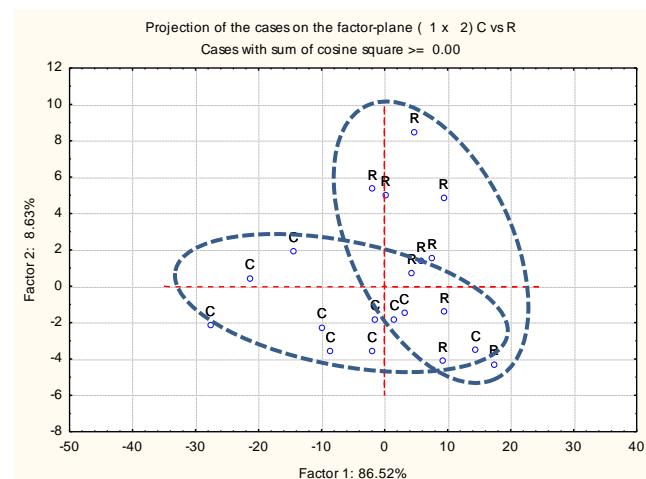
(4) PCA Comparison: CDC Copeland (C) and CDC Rattan (R)

Figure 4.7 Multivariate molecular spectral analyses of hulless barley varieties [CDC Fibar (F), CDC Rattan (R), CDC McGwire (M) and HB08302 (H)] in comparison with hulled barley [CDC Copeland: (C)] at SR-FTIRM protein fingerprint region: ca. 1768-1475 cm^{-1}. CLA (cluster analysis): (1) Cluster method: Ward's algorithm, (2) Distance method: Euclidean; PCA (principal component analysis): Scatter plots of the 1st principal components (PC1) vs. the 2nd principal components (PC2).
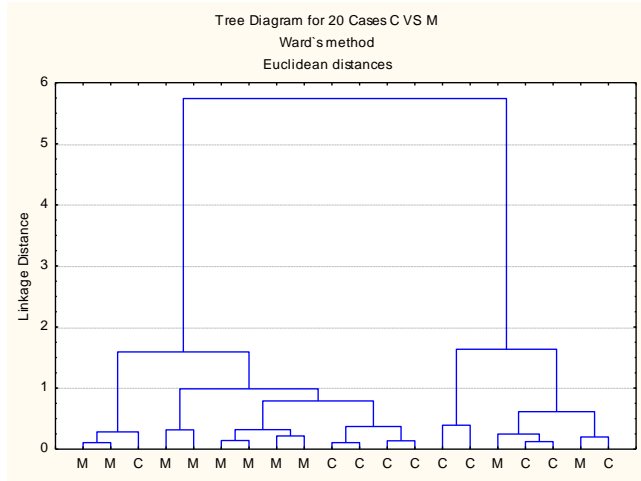
(5) CLA Comparison: CDC Copeland (C) and CDC McGwire (M)
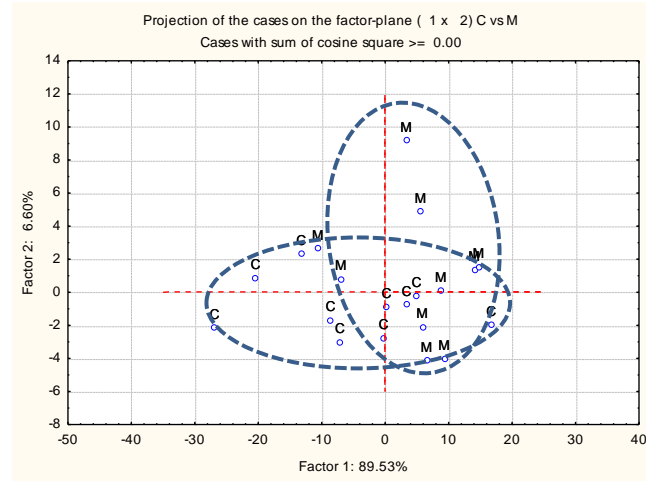
(6) PCA Comparison: CDC Copeland (C) and CDC McGwire (M)
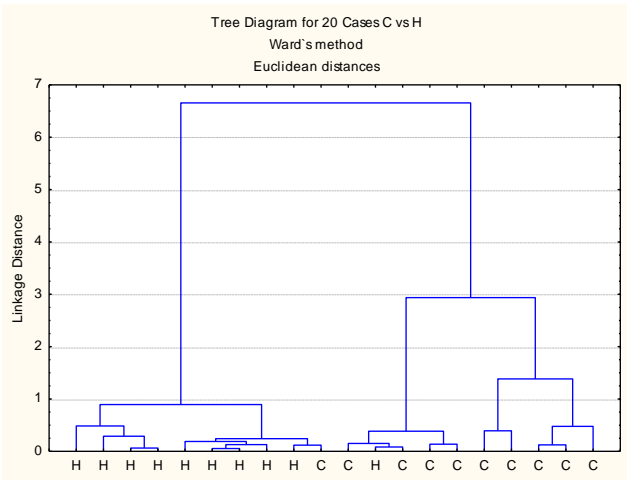
(7) CLA Comparison: CDC Copeland (C) and HB08302 (H)
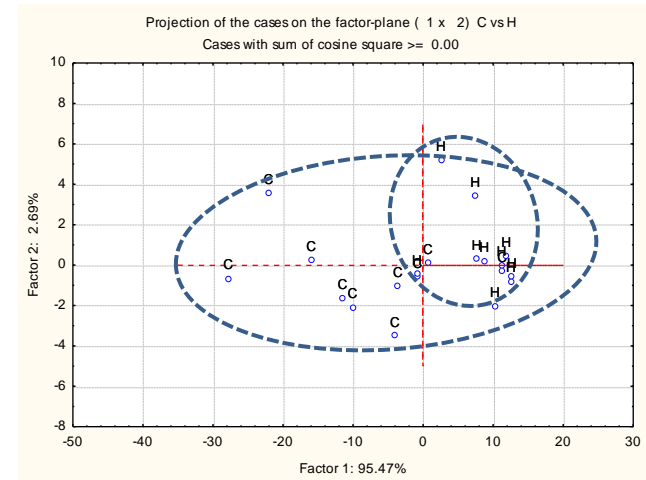
(8) PCA Comparison: CDC Copeland (C) and HB08302 (H)

Figure 4.7 Cont'd

(9) CLA Comparison: CDC McGwire (M) and CDC Fibar (F)
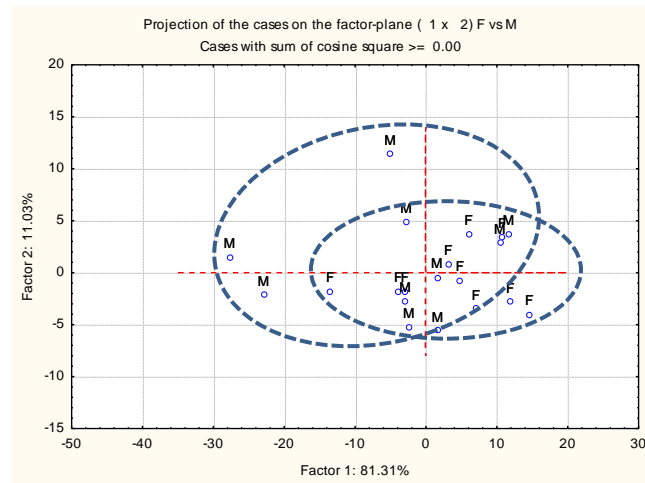
(10) PCA Comparison: CDC McGwire (M) and CDC Fibar (F)
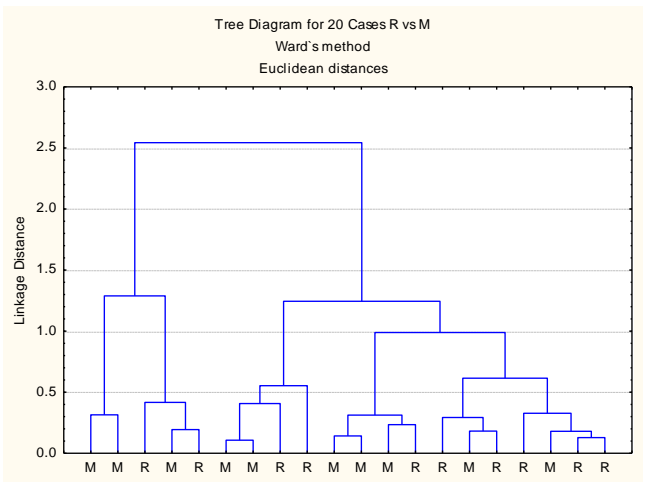
(11) CLA Comparison: CDC McGwire (M) and CDC Rattan (R)
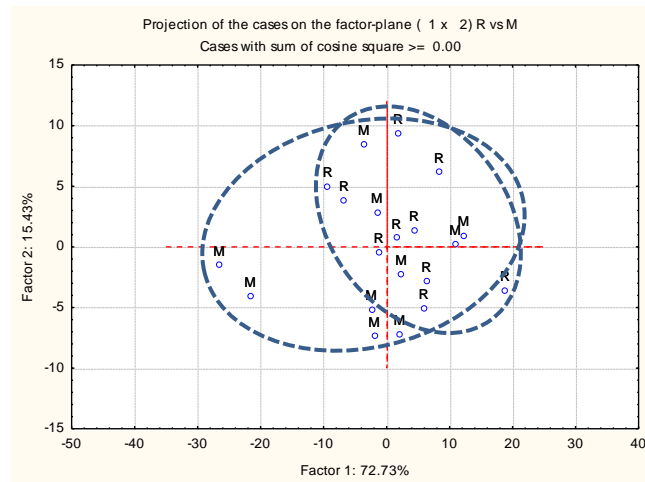
(12) PCA Comparison: CDC McGwire (M) and CDC Rattan (R)
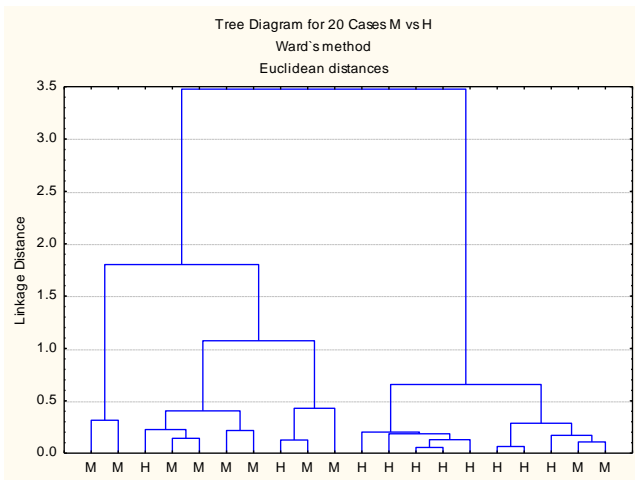
Figure 4.7 Cont'd

(13) CLA Comparison: CDC McGwire (M) and HB08302 (H)
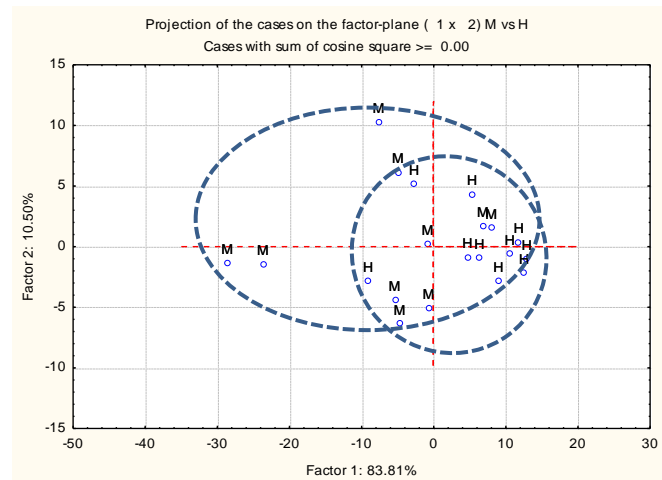
(14) PCA Comparison: CDC McGwire (M) and HB08302 (H)

Figure 4.7 Cont'd

Table 4.9 Correlation analysis between structure spectral characteristics of protein amide I and II, protein secondary structure α-helix and β-sheet in endosperm region (SR-FTIRM) of hulless barley with altered carbohydrate traits and nutrients availability and utilization in the rumen and intestine

Items	Amide I area	Amide I peak height	Amide II area	Amide II peak height	α-helix height	β-sheet height	Ratio of Amide I to Amide II area	Ratio of α-helix to β-sheet height
	---------------------------------------Spearman Correlation R values-------------------------------------							
In situ rumen CP degradation								
BCP (% CP)	0.10	-0.21	-0.16	-0.34	0.05	0.03	0.66*	-0.44
EDCP(% CP)	-0.10	0.21	0.16	0.34	-0.05	-0.03	-0.66*	0.44
EDCP(g/kg DM)	-0.22	0.03	0.30	0.06	-0.23	-0.16	-0.66*	0.50
In situ rumen NDF degradation								
EDNDF (g/kg DM)	0.74**	0.49	0.52	0.12	0.65*	0.82**	-0.05	-0.36
Intestinal CP digestion								
TDP (% CP)	-0.15	-0.17	0.14	-0.09	-0.27	-0.08	-0.75**	0.15
Intestinal CHO digestion								
IDBCHO (% BCHO)	0.30	-0.06	-0.05	-0.16	0.24	0.18	0.56$^+$	-0.61*
IDBCHO (g/kg DM)	0.37	0.04	0.13	-0.08	0.30	0.26	0.43	-0.61*
TDCHO (g/kg DM)	0.47	0.51	0.08	0.63*	0.55$^+$	0.49	0.04	0.08
NRC Dairy 2001 model								
MCP_{TDN}	-0.64*	-0.83**	-0.79**	-0.53$^+$	-0.66*	-0.71*	0.54$^+$	-0.29
$AMCP^{NRC}$	-0.22	0.03	0.30	0.06	-0.23	-0.16	-0.64*	0.50
OEB^{NRC}	-0.22	0.03	0.30	0.06	-0.23	-0.16	-0.64*	0.50

Note: BCP: rumen bypass crude protein; EDCP: effective degradability of crude protein; EDNDF: effective degradability of neutral detergent fibre; TDP: total digestible protein; IDBCHO: intestinal digestible bypass CHO; BCHO: rumen bypass CHO; MCP_{TDN}: microbial crude protein estimated from TDN; OEB: degraded protein balance; AMCP: truly absorbed microbial protein.
$^+$ for P<0.10, * for P<0.05, ** for P<0.01, *** for P<0.001.

4.3.2.2. **Quantifying Spectral Features of Non-Starch CHO (β-Glucan and Cellulosic Compounds) in the Endosperm Tissue of Hulless Barleys in Comparison with Hulled Barley**

Table 4.10 shows the absorbance intensity for non-starch CHO, including β-glucan at region ca. 1450-1390 cm^{-1} and cellulosic compounds at region ca. 1278−1205 cm^{-1} for barley cultivars. There was no notable difference found in β-glucan absorbance intensity among the hulless barley cultivars except for CDC Fibar ($P>0.05$). Hulled barley showed lower β-glucan peak area (0.35 vs. 0.42, $P<0.05$) and height (0.013 vs. 0.016, $P<0.05$) than CDC McGwire but higher than CDC Fibar (0.35 vs. 0.21; 0.013 vs. 0.009; $P<0.05$). CDC McGwire also exhibited higher peak area (0.33) of cellulosic compounds absorbance intensity compared to other barley cultivars ($P<0.05$). CDC Fibar was lowest in peak area for cellulosic compounds (0.21, $P<0.05$). There were similar peak heights (0.01) among barley cultivars except for CDC McGwire ($P>0.05$).

Although there were significant differences between barley cultivars in terms of absorbance intensity of non-starch CHO, the difference was not fully distinguishable from cluster classes and PCA plots at the whole non-starch CHO region ca. 1475−1195 cm^{-1} (Figure 4.8). When CDC Copeland (C) was used as a control, CDC Fibar (F), the lower amylose hulless barley was found to have less overlapped areas compared to other hulless barley varieties in ellipses [Figure 4.8: (1)-(8)]. A significant portion of the variation (95.5%) could be explained by the first principal component between CDC Fibar and CDC Copeland [Figure 4.8: (1)-(2)]. As the level of amylose increases in hulless barley, more similarity of spectral features in non-starch CHO region was found with more overlapped areas of the two ellipses from the PCA plots and mixed cluster classes ($P>0.05$) [Figure 4.8: (1)-(8)]. Similarly in comparison with CDC Copeland, no fully distinguished structures were found among hulless barley cultivars in terms of non-starch CHO ($P>0.05$) [Figure 4.8: (9)-(14)]. Different from univariate study results, the multivariate analysis focused on a larger non-starch CHO area compared to more specific β-glucan and cellulosic compounds spectral regions used for univariate analysis. Therefore, there might be some other non-starch CHO components in this region that may affect spectral features resulting in no notable structural difference from CLA clusters and PCA plots.

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Since the difference in spectral characteristics of β-glucan and cellulosic compounds were found among hulless barley cultivars (Table 4.10), the variation of molecular structure may affect nutritional value in terms of rumen degradation, intestinal digestion and potential protein supply (Table 4.11). Correlation results between structural features of β-glucan and cellulosic compounds from FITR and rumen digestive parameters showed significant correlations with rumen degradation kinetics and intestinal digestion of protein with cellulosic compounds in hulless barley but few correlations found with β-glucan. However, spectral features of β-glucan detected by SR−FTIRM showed more significant correlation to protein and CHO metabolic characteristics than spectral features of cellulosic compounds. Peak area and height of β-glucan showed negative correlation with protein availability in rumen and small intestine including total digestible protein (TDP: $r=−0.73$, $P<0.05$; $r=-0.84$, $P<0.01$) and degraded protein balance in DVE/OEB system (OEB^{DVE}: $r=-0.61$, $P<0.05$; $r=-0.72$, $P<0.05$). There was a positive correlation between β-glucan peak area and total digestible CHO ($r=0.71$, $P<0.05$). The peak area and height of cellulosic compounds was positively correlated with effective degradable CHO (TDCHO: $r=0.78$, $P<0.01$; $r=0.69$, $P<0.05$) in the rumen as well as total digestible CHO in small intestine (TDCHO: $r=0.85$, $P<0.001$; $r=0.69$, $P<0.05$), whereas there were a negative correlation between peak area of cellulosic compounds and truly absorbed bypass crude protein (ABCP: $r=-0.65$, $P<0.05$) as well as metabolizable protein ($r=-0.61$, $P<0.05$).

SR−FTIRM detects endosperm cell tissues which include structural CHO such as cellulosic compounds and non-structural CHO like β-glucan in barley (Garleb et al., 1988, 1991; Gordon et al., 1977). This could explain the greater absorption intensity (area and height) of both compounds observed by SR−FTIRM (Table 4.10) than those by FTIR (Table 4.4), as well as more significant correlation between metabolic characteristics of crude protein and CHO and β-glucan than cellulosic compounds (Table 4.11), due to the factor that β-glucan is mainly located in endosperm cell wall of barley.

Hence, molecular structures of β-glucan and cellulosic compounds have an effect on protein and CHO supply to ruminants. Higher spectral absorption intensity of β-glucan could be associated with lower truly absorbed protein supply to rumen but higher total digestible CHO.

Table 4.10 Effect of altered carbohydrate traits on spectral characteristics of non-starch carbohydrates (β-glucan and cellulosic compounds) in the endosperm region of hulless barley varieties in comparison with hulled barley using synchrotron−based FTIR microspectroscopy

Item	Peak region and center (cm^{-1})	Hulled Copeland (n=3)	Hulless Fibar (n=3)	Rattan (n=3)	McGwire (n=3)	HB08302 (n=2)	SEM	P value	Contrast Hulled vs. Hulless P value
Amylose Level (% of ST)		27.0[b]	2.5[d]	7.7[c]	25.8[b]	36.9[a]	0.56	<0.0001	<0.0001
Amylopectin Level (% of ST)		73.0[c]	97.5[a]	92.3[b]	74.2[c]	63.1[d]	0.56	<0.0001	<0.0001
β-Glucan Level (% DM)		3.8[c]	10.0[a]	7.4[b]	4.7[c]	7.5[b]	0.40	<0.0001	<0.0001
β-glucan									
Peak area	1450-1390	0.35[b]	0.21[c]	0.39[ab]	0.42[a]	0.41[a]	0.014	<0.0001	0.4291
Peak height	~1415	0.013[b]	0.009[c]	0.016[ab]	0.016[a]	0.016[ab]	0.0010	<0.0001	0.3486
Cellulosic compounds									
Peak area	1278-1205	0.26[b]	0.21[c]	0.28[b]	0.33[a]	0.28[b]	0.014	<0.0001	0.2903
Peak height	~1238	0.01[b]	0.01[b]	0.02[ab]	0.02[a]	0.01[ab]	0.001	<0.0001	0.1673

Note: ST: starch;

    SEM= Standard error of mean; Means with different letters within the same row differ (P<0.05);

    Multi-treatment comparison: Tukey-Kramer method.
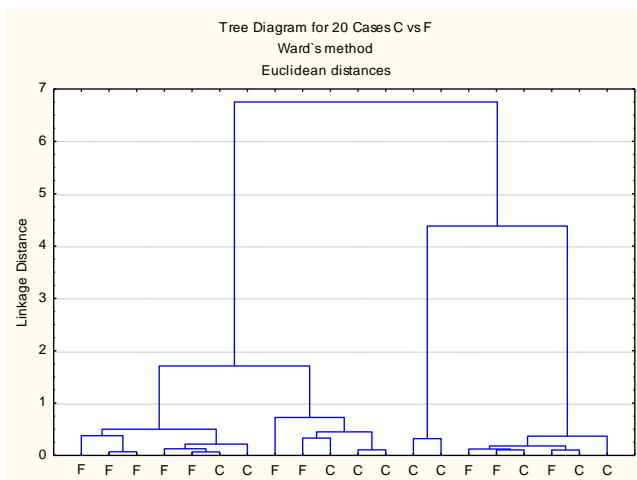
(1) CLA Comparison: CDC Copeland (C) and CDC Fibar (F)
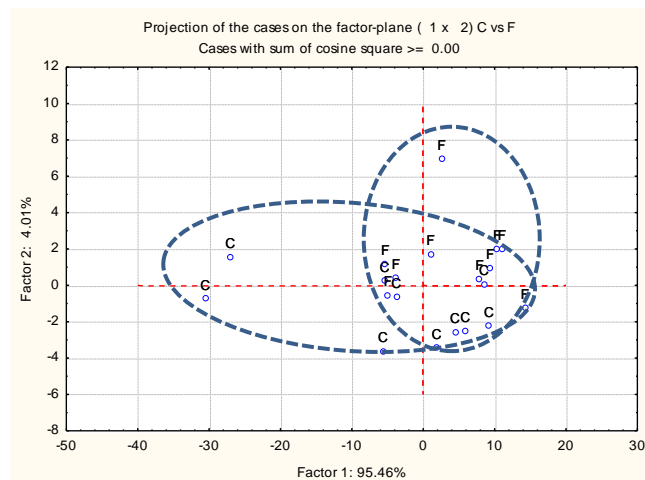
(2) PCA Comparison: CDC Copeland (C) and CDC Fibar (F)
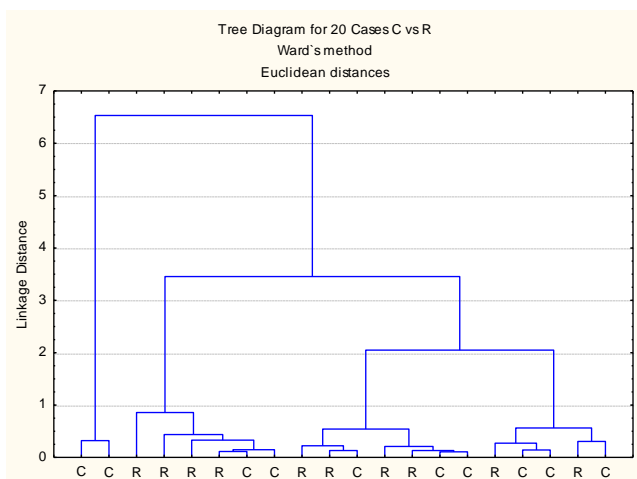
(3) CLA Comparison: CDC Copeland (C) and CDC Rattan (R)
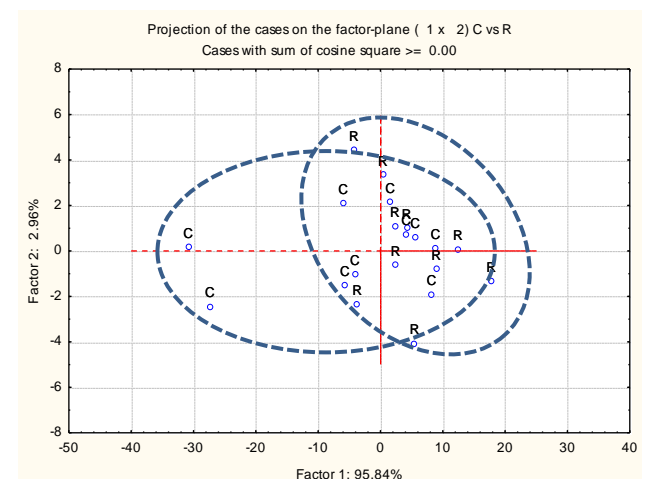
(4) PCA Comparison: CDC Copeland (C) and CDC Rattan (R)

Figure 4.8 Multivariate molecular spectral analyses of hulless barley varieties [CDC Fibar (F), CDC Rattan (R), CDC McGwire (M) and HB08302 (H)] in comparison with hulled barley [CDC Copeland: (C)] at SR-FTIR non-starch carbohydrate fingerprint region: ca. 1475-1195 cm^{-1}. CLA (cluster analysis): (1) Cluster method: Ward's algorithm, (2) Distance method: Euclidean; PCA (principal component analysis): Scatter plots of the 1st principal components (PC1) vs. the 2nd principal components (PC2).
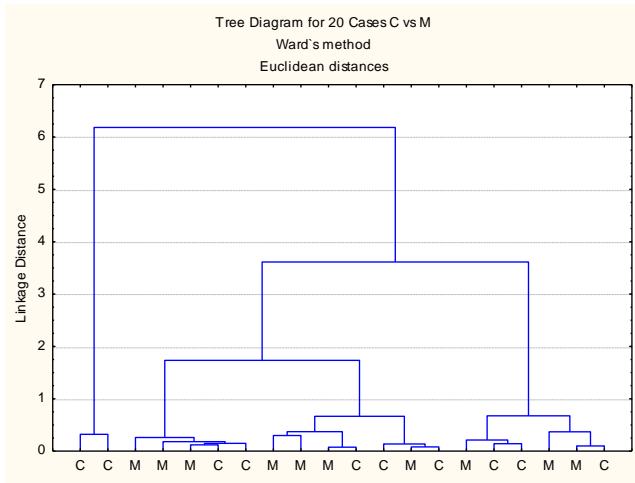
(5) CLA Comparison: CDC Copeland (C) and CDC McGwire (M)
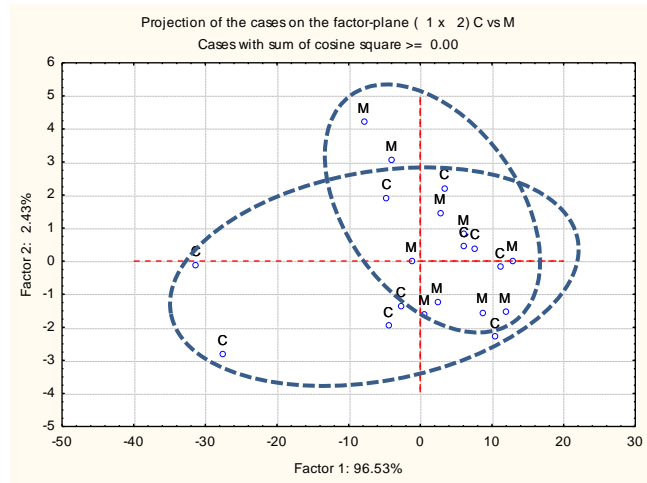
(6) PCA Comparison: CDC Copeland (C) and CDC McGwire (M)
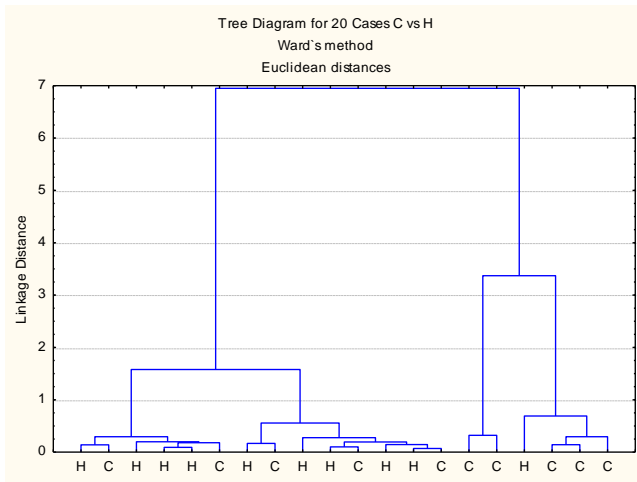
(7) CLA Comparison: CDC Copeland (C) and HB08302 (H)
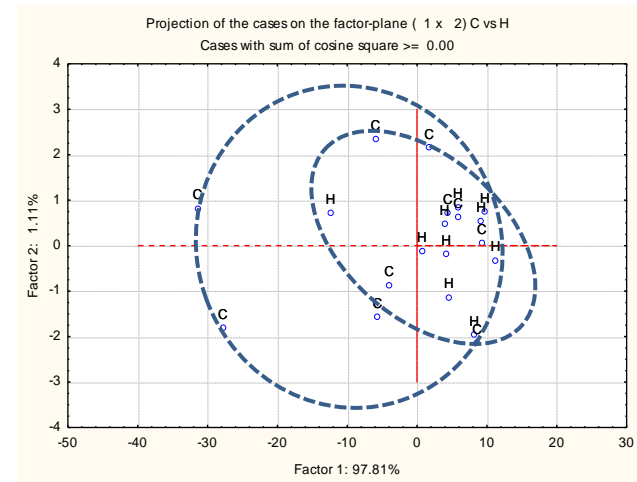
(8) PCA Comparison: CDC Copeland (C) and HB08302 (H)

Figure 4.8 Cont'd

(9) CLA Comparison: CDC McGwire (M) and CDC Fibar (F)
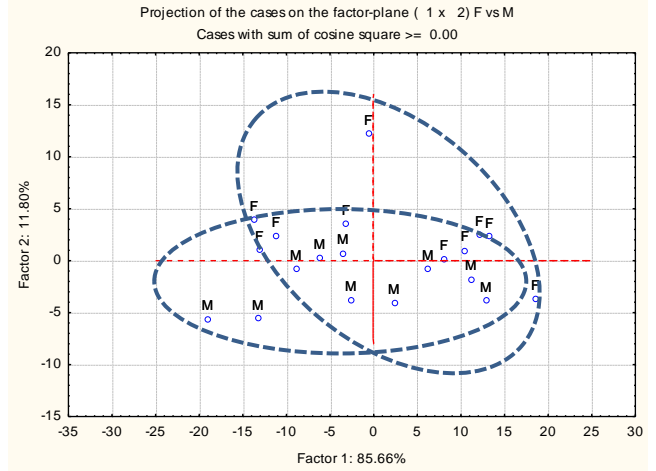
(10) PCA Comparison: CDC McGwire (M) and CDC Fibar (F)
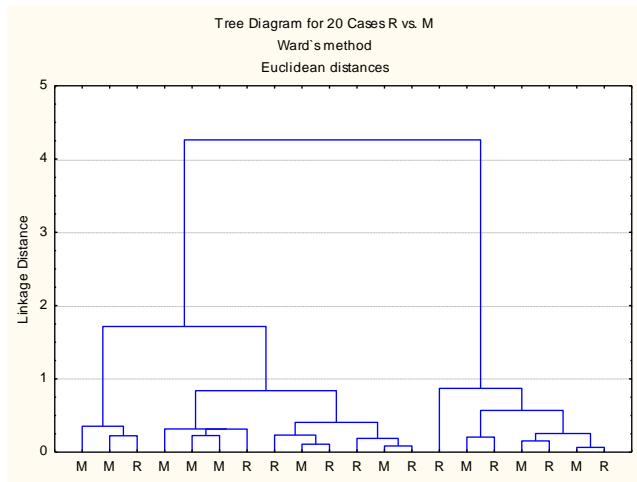
(11) CLA Comparison: CDC McGwire (M) and CDC Rattan (R)
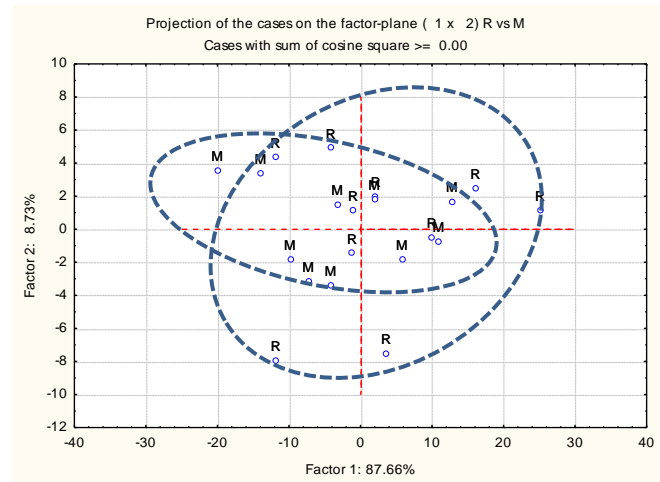
(12) PCA Comparison: CDC McGwire (M) and CDC Rattan (R)

Figure 4.8 Cont'd
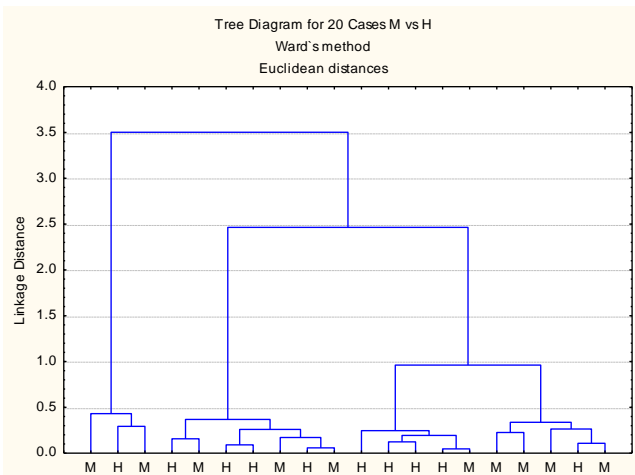
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(13) CLA Comparison: CDC McGwire (M) and HB08302 (H)
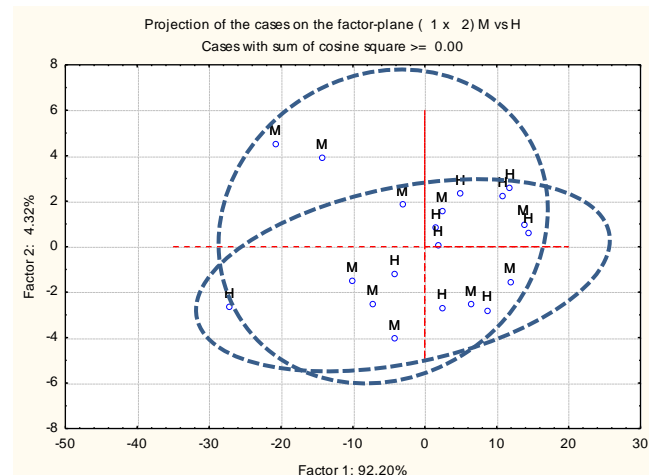
(14) PCA Comparison: CDC McGwire (M) and HB08302 (H)

Figure 4.8 Cont'd

Table 4.11 Correlation analysis between structural characteristics of β-glucan, cellulosic compounds in the endosperm region of hulless barley with altered carbohydrate traits and nutrient utilization and availability in dairy cattle

Items	β-glucan		Cellulosic compounds	
	Area	Height	Area	Height
	----------------------Spearman Correlation R values-----------------			
In situ rumen CHO degradation (g/kg DM)				
EDCHO	0.57^+	0.48	0.78**	0.69*
In situ rumen CP degradation (g/kg DM)				
EDCP	-0.54^+	-0.78**	-0.33	0.00
Intestinal CP digestion				
IDP (g/kg DM)	-0.64*	-0.60^+	-0.65	-0.40
TDP (% CP)	-0.44	-0.42	-0.24	-0.17
TDP (g/kg DM)	-0.73*	-0.84**	-0.62*	-0.29
Intestinal CHO digestion (g/kg DM)				
TDCHO	0.71*	0.60^+	0.85***	0.69*
DVE/OEB system (g/kg DM)				
ABCP^{DVE}	-0.64*	-0.60^+	-0.65*	-0.40
DVE	-0.76**	-0.84**	-0.53^+	-0.29
OEB^{DVE}	-0.61*	-0.72*	-0.58^+	0.23
NRC Dairy 2001 model (g/kg DM)				
AMCP^{NRC}	-0.54^+	-0.78**	-0.33	0.00
MP^{NRC}	-0.71*	-0.84**	-0.61*	-0.23
OEB^{NRC}	-0.54^+	-0.78**	-0.33	0.00

Note: EDCHO: effective degradability of CHO; EDCP: effective degradability of crude protein; OEB: degraded protein balance; AMCP: truly absorbed microbial protein in the small intestine; MP: metabolizable protein.
  $+$ for $P<0.10$, $*$ for $P<0.05$, $**$ for $P<0.01$, $***$ for $P<0.001$

4.3.2.3. **Quantifying Spectral Features of CHO in the Endosperm Tissue of Hulless Barleys in Comparison with Hulled Barley**

Table 4.12 shows the differences of spectral features of CHO in the endosperm tissue of barley cultivars at the region ca. 1195-945 cm^{-1}. Three CHO peak areas were separated from total region at ca. 1195-1128 cm^{-1}, ca. 1128-1049 cm^{-1} and 1049-945 cm^{-1}, respectively. Among hulless barley cultivars, McGwire and HB08302 had the greatest total absorption area (72.27 and 73.15; $P<0.05$), CHO Peak 2 area (9.47 and 9.38; $P<0.05$), CHO peak 1 height (0.34, $P<0.05$) and peak 2 height (0.47 and 0.49, $P<0.05$) than low amylose hulless barley cultivars, and also higher than those in hulled barley ($P<0.05$).

There was no distinguishable cluster classes or ellipses found in each comparison of barley cultivars at the region ca. 1195-945 cm^{-1}. For example, with the comparison between CDC Fibar (F) and CDC Copeland (C) (Figure 4.9), there was no clear separation between the two clusters. PCA plot had well overlapped ellipses with plots representing the two cultivars and there was only 70% of variation explained by the first principal component. There was no sufficient difference to be detected in whole CHO region among the barley cultivars in the endosperm tissue.

However, variation in CHO absorption peak intensity among the hulless barley varieties was observed with mostly negative effects on rumen degradation, total tract digestion and potential protein supply (Table 4.13). Absorption peak intensity of CHO were weakly correlated to effective degradable crude protein, protein degraded balance (both in DVE/OEB system and NRC-2001 model), truly absorbed microbial protein and metabolizable protein ($P<0.05$), intermediately strongly correlated to total digestible protein ($P<0.01$) and strongly correlated to truly digested protein in small intestine ($P<0.001$). This may explain the similar negative correlation results between altered starch traits (amylose and ratio of Ay:Ap) and the same parameters for metabolic characteristics. The only positive correlation was found between absorption intensity of non-starch CHO and total digestible CHO, which was also supported by previous observation that amylose ($r=0.54$, $P<0.01$) and ratio of Ay:Ap were positively correlated to TDCHO ($r=0.56$, $P<0.01$). Because starch and protein are the major components in endosperm

tissue, spectral features of CHO in endosperm tissue were relevant to starch level in relation to nutrient availability although there was no notable difference found by CLA and PCA among the hulless barley cultivars with altered carbohydrate composition. A possible reason may also be the insufficient difference detected by SR−FTIRM among barley varieties in terms of total CHO molecular structures, which include all CHO structural features instead of the one specific CHO structure such as starch.

Table 4.12 Effect of altered carbohydrate traits on spectral characteristics of total carbohydrates in the endosperm region of hulless barley in comparison to hulled barley using synchrotron−based FTIR microspectroscopy

Item	Peak region and center (cm^{-1})	Hulled Copeland (n=3)	Hulless				SEM	P value	Contrast Hulled vs. Hulless P value
			Fibar (n=3)	Rattan (n=3)	McGwire (n=3)	HB08302 (n=2)			
Amylose Level (% of ST)		27.0^b	2.5^d	7.7^c	25.8^b	36.9^a	0.56	<0.0001	<0.0001
Amylopectin Level (% of ST)		73.0^c	97.5^a	92.3^b	74.2^c	63.1^d	0.56	<0.0001	<0.0001
β-Glucan Level (% DM)		3.8^c	10.0^a	7.4^b	4.7^c	7.5^b	0.40	<0.0001	<0.0001
Total area	1195−945	60.92^b	52.78^c	65.82^b	72.27^a	73.15^a	2.184	<0.0001	0.0056
CHO Peak 1 area	1195−1128	8.31^b	5.76^c	8.28^b	9.47^a	9.38^a	0.343	<0.0001	0.7480
CHO Peak 2 area	1128−1049	16.69^{bc}	16.5^c	18.22^{bc}	19.77^{ab}	23.28^a	0.868	<0.0001	0.0034
CHO Peak 3 area	1049−945	35.95^c	30.29^d	39.03^{bc}	42.56^a	42.50^{ab}	1.215	<0.0001	0.0101
CHO Peak 1 height	~1152	0.29^b	0.20^c	0.29^b	0.34^a	0.34^a	0.012	<0.001	0.9015
CHO Peak 2 height	~1079	0.40^{bc}	0.36^c	0.43^b	0.47^a	0.49^a	0.016	0.0001	0.0015
CHO Peak 3 height	~1024	0.54^c	0.44^d	0.58^{bc}	0.64^a	0.63^{ab}	0.022	0.0001	0.0332

Note: ST: starch; CHO: carbohydrates;
SEM= Standard error of mean; Means with different letters within the same row differ (P<0.05).
Multi-treatment comparison: Tukey-Kramer method.
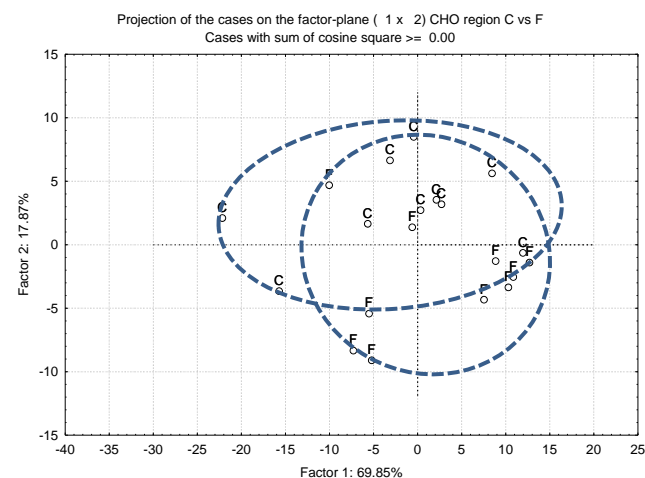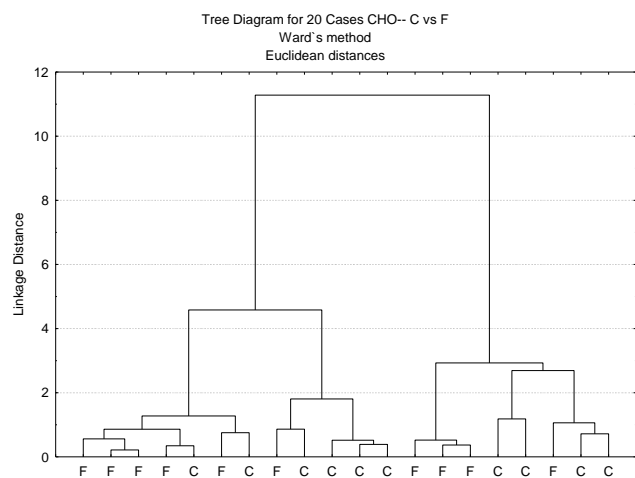
Figure 4.9 Multivariate molecular spectral analyses of CDC Copeland: (C) compared to CDC Fibar (F) at SR-FTIR carbohydrate fingerprint region: ca. 1195−945 cm^{-1}. CLA (cluster analysis): (1) Cluster method: Ward's algorithm, (2) Distance method: Euclidean; PCA (principal component analysis): Scatter plots of the 1st principal components (PC1) vs. the 2nd principal components (PC2).

Table 4.13 Correlation analysis between structure spectral characteristics of carbohydrates in the endosperm region (SR-FTIRM) of hulless barley with altered carbohydrate traits and nutrient availability and utilization in dairy cattle

Items (g/kg DM)	Total area	CHO Peak 1 area	CHO Peak 2 area	CHO Peak 3 area	CHO Peak 1 height	CHO Peak 2 height	CHO Peak 3 height
---		-Spearman Correlation R values-					--------------
In situ rumen CP degradation							
EDCP	-0.73*	-0.75**	-0.72*	-0.68*	-0.76**	-0.70*	-0.73*
Intestinal CP digestion							
IDP	-0.64*	-0.61*	-0.52	-0.60$^+$	-0.63*	-0.53$^+$	-0.60$^+$
TDP	-0.83**	-0.84**	-0.75**	-0.82**	-0.87***	-0.76**	-0.85***
Intestinal CHO digestion							
TDCHO	0.68*	0.67*	0.55$^+$	0.70*	0.70*	0.62*	0.70*
DVE/OEB system							
ABCP^{DVE}	-0.64*	-0.61*	-0.52	-0.60$^+$	-0.63*	-0.53$^+$	-0.60$^+$
DVE	-0.91***	-0.92***	-0.88***	-0.89***	-0.91***	-0.90***	-0.92***
OEB^{DVE}	-0.68*	-0.70*	-0.56$^+$	-0.71*	-0.74**	-0.59$^+$	-0.72*
NRC Dairy 2001 model							
OEB^{NRC}	-0.73*	-0.75**	-0.72*	-0.68*	-0.76**	-0.70*	-0.73*
AMCP^{NRC}	-0.73*	-0.75**	-0.72*	-0.68*	-0.76**	-0.70*	-0.73*
MP^{NRC}	-0.72*	-0.71*	-0.65*	-0.67*	-0.73*	-0.67*	-0.69*

Note: EDCP: effective degradability of feed crude protein; IDP: intestinal degradable protein; TDP: total digestible protein; TDCHO: total digestible CHO; ABCP: truly absorbed bypass protein in the small intestine; DVE: truly digested protein in the small intestine; OEB: degraded protein balance; MP: metabolizable protein.
$^+$ for $P<0.10$, * for $P<0.05$, ** for $P<0.01$, *** for $P<0.001$

4.4. Conclusion

In conclusion, inherent structural differences of five barley varieties can be detected by FTIR in powdered whole seed and SR−FTIRM on endosperm tissue. Univariate molecular spectral analysis and multivariate analysis can be applied to analyze absorption intensity of peaks associated with functional group bands including protein, non-starch CHO and total CHO. The molecular structure features of hulless barley with altered starch traits do have significant effects on protein, CHO and NDF availability to ruminants. Metabolizable protein (MP) was positively affected by protein molecular structure characteristics. Absorption intensity of functional group bands in barley cultivars detected by FTIR was relevant to chemical profiles, protein and carbohydrate supply to dairy cattle. SR−FTIRM can be considered as a new approach to identify structural molecular characteristics of cereal grain at cellular dimension due to its brilliant light source and small aperture size. More research is needed to investigate the relationship between absorption intensity of molecular structures associated with functional groups and metabolic characteristics of nutrients at other different seed layers.

5. General Discussion, Overall Conclusion and Future Research

Barley is used to meet the energy and protein needs of beef and dairy cattle, especially in Northern areas where the environmental conditions are not suitable for corn cultivation (Hunt 1996, Bleidere and Gaile, 2012). Feed barley breeding is dependent on various selection criteria (Bleidere and Gaile, 2012). The feed value of barley is influenced by physical quality (hulled or hulless) and also chemical composition (CHO, protein, non-starch polysaccharides, fibre and fat) (Bleidere and Gaile, 2012). The significance of high quality barley grain for animal feed is not only to satisfy the basic growth requirements of the animal, but also to increase the utilization efficiency of available nutrients of grain and reduce the environment impact from undigested compounds (Bleidere and Gaile, 2012).

Hulled and hulless barley can be distinguished physically by the husk cover. Hulless barley is a line with loose or no husk cover (Thomason et al. 2009), which improves nutrient content on a dry matter basis due to the lack of a fibre coating. A previous study states that the proportion of hulls positively affects fibre content of grain, resulting in a decrease of metabolizable energy (Bell et al., 1983). In first research chapter, the comparison between hulled barley and hulless barley in terms of chemical profiles revealed the absence of the hull resulting in advantage in nutrient content and availability such as less NDF, lower intermediately, slowly degradable carbohydrate, higher SCP and greater energy content than hulled barley. In the analysis of metabolic characteristics of barley cultivars, hulless barley showed higher ($P<0.05$) effective degradable crude protein (EDCP), lower CHO degradation rate and lower undegrdable (U) CHO, higher effective degradable starch (EDST), intestinal digestible nutrients and greater truly digested protein in the small intestine (DVE) with a better protein degraded balance. In agreement with previous studies (Bowman et al. 2001; Shon et al., 2007; Pieper et al., 2008; Jha et al., 2010), hulless barley cultivars improved nutrient content and availability to the animal.

Hulless barleys with altered carbohydrate traits were primarily developed for food use. However, the alteration in carbohydrate conformation may improve nutrient availability and extend the use from food to feed. The alteration of carbohydrate traits involves amylose and β-glucan. In normal barley, amylose accounts for 15-25% of total barley starch while β-glucan accounts for 2–7% of total dry matter of barley (Zhang et al., 2000; Ullrich, 2011). Hulless

barley cultivars with altered carbohydrate traits include zero-amylose waxy, CDC Fibar; 5%-amylose waxy, CDC Rattan and high-amylose, HB08302. All contained high β-glucan (>7 %DM) in which CDC Fibar contained high β-glucan that was approximately 10% of DM). Higher β-glucan level may be correlated with grain ground particle size because higher β-glucan in the endosperm region will increase cell wall thickness, especially in the barleycell wall where β-glucan accounts for 75% of the cell wall composition (Evers et al., 1999; Oscarsson et al., 1997; Zheng et al., 2000; Damiran and Yu, 2010). This may protect nutrients from rumen degradation. As to starch, the chemical structures and proportions of amylose to amylopectin ratio are the key factors (Song and Jane, 2000). Lower amylose and high amylopectin level in barley starch may result in higher starch degradation rate while high-amylose barley is less susceptible to enzymatic degradation (Pomeranz et al., 1972; Newman and Newman, 1992; MacGregor and Fincher, 1993; Hristov et al., 2002). Therefore, high-amylose or high Ay:Ap ratio as well as high β-glucan barley variety is more suitable for ruminant feeding due to its advantages of lower dry matter digestibility and lower rate of starch digestion when considering the risk of digestive disorders from fast starch digestion (Hunt, 1996; Bowman et al., 2001). However, from a plant breeding point of view, barley cultivars with high amylose and high β-glucan may not be feasible due to the negative correlation between β-glucan and amylose content (Hang et al., 2007), whereas, a positive correlation between β-glucan and protein was discovered in previous studies (Ullrich et al., 1986; Hang et al., 2007). In our study, chemical profiles and metabolic characteristics of hulless barley with altered CHO traits in the first study revealed that hulless barley with lower amylose and higher β-glucan level contained higher protein and energy contents with greater nutrient availability in the rumen and truly absorbed protein supply for post-ruminal digestion, as well as better synchronization of N and energy than other barley cultivars (P<0.05). Combining barley quality for feed and nutrient availability for ruminant, hulless barley with lower amylose and higher β-glucan level can be regarded as an alternative for ruminant feeding, although the inclusion level in the diet needs to be adjusted to protect rumen health from severe acid challenge and reduce inefficient energy utilization.

Chapter 4 discussed differences in the internal structures of the five barley cultivars and the effect of the molecular structures of barley cultivars on the metabolic characteristics of nutrients to ruminants. Two molecular spectroscopy techniques were applied in this study—Fourier Transformed Infrared Spectroscopy and Synchrotron−based Fourtier

Transformed Infrared Microspectroscopy. As non-destructive methods, both techniques were shown to detect the molecular structures among barley cultivars in powdered whole grain by FTIR and at a cellular dimension level by SR−FTIRM, respectively. As to the relationship between spectral features of functional group bands and nutrient availability and utilization, parameters of absorption peak intensity of all detected functional group bands [protein, structure CHO (β-glucan and cellulosic compounds) and total CHO] in hulless barley cultivars were observed to have significant effects on protein, CHO and NDF availability estimated from rumen degradation, intestinal digestion and model predictions. This implies there are differences in the molecular structure make-up in terms of protein, non-starch CHO and total CHO in barley varieties, which provide a possible explanation for various metabolic characteristics of hulless barley varieties from a molecular structure perspective. However, owing to the similarity of CHO among barley cultivars in response to IR source, both molecular spectroscopy techniques failed to fully distinguish the differences of spectral features in total CHO region of barley varieties, although the starch composition differed among hulless barley varieties. Distinct differences in absorption intensity of CHO molecular structures were observed in the comparison between low amylose hulless barley and hulled barley using univariate analyses. Therefore, more precise detection of specific compounds within the total CHO region may be needed to identify the possible inherent structural factors for the differences of hulless barley varieties in the total CHO region.

In conclusion, there are significant effects of alteration of carbohydrate traits on nutrient availability and utilization in hulless barley. Hulless barley cultivars with lower amylose and higher β-glucan level improved nutrient content and utilization to dairy cow with greater soluble protein and energy supply to ruminant, more synchronized N and energy, and greater truly absorbed protein in the small intestine compared to hulled barley. FTIR and SR−FTIRM are capable of detecting inherent structural differences of five barley varieties either in powdered form or in endosperm tissue. The spectral features of molecular structures of hulless barley with altered starch traits have effects on protein, CHO and NDF availability and utilization in dairy cattle. Molecular structure spectral characteristics of protein amide I, II and secondary structures detected by both techniques were positively correlated to potential protein supply although the absorption intensity of barley cultivars detected by FTIR were more relevant to chemical profiles, and protein and carbohydrate supply to dairy cattle than those by SR−FTIRM.

The findings of this study will be beneficial to the feed industry, plant breeders and animal nutrition researchers. From a financial point of view, grain quality is highly associated with the cost of the final product (Bleidere and Gaile, 2012). For the feed industry, this study suggests hulless barley with lower amylose and higher β-glucan contains higher energy and protein supply to ruminants compared with regular hulled barley or high amylose hulless barley. It is well known that barley is high in carbohydrate which is rapidly fermented in the rumen. Higher β-glucan was shown to have a protective role for starch from fast degradation, which may lower the incidence of rumen digestive disorders. Considering animal health and nutritional feedback, hulless barley with lower amylose and higher β-glucan could be used as a barley grain alternative for feeding ruminants. In addition, FTIR, similar to the near infrared spectroscopy technique as an infrared spectroscopy technique, can also be used to detect the chemical composition of feedstuffs. This study revealed that absorption peak intensity of functional group bands for hulless barley obtained by FTIR were relevant to related chemical composition, therefore, FTIR can be used to distinguish the structural difference of chemical composition in different samples from molecular structure perspective without damaging the inherent structures.

For plant breeders, SR−FTIRM, compared to FTIR, is more advanced in probing the molecular structure of functional groups at a cellular level, in other words capable of more precisely focusing on the targeted chemical compound in certain plant tissue. SR−FTIRM is useful to identify a chemical compound at a specific location with a small aperture size. In this study, non-starch CHO and total CHO observed in the endosperm tissue of hulless barley cultivars showed higher absorption intensities than those observed by FTIR with ground whole seed, resulting in significant correlations between metabolic characteristics of protein, NDF, CHO and spectral features of CHO molecular structures. Taking advantage of SR−FTIRM, it is possible to identify or separate specific chemical compounds from plant tissue, for example, analyzing chemical composition in plant tissue for genetic modification.

For animal nutrition researchers, newly developed hulless barleys with alteration in carbohydrate traits provides a new grain option for cattle with high energy and truly absorbed protein. This study revealed that hulless barley with lower amylose and higher β-glucan contain higher SCP, CHO, energy, greater CHO and protein for rumen degradation and intestinal digestion, better N and energy synchronization and greater potential true protein supply to small intestine. An animal feeding trial with different inclusion ratios of hulless barley with low

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amylose and high β-glucan level and regular hulled barley should be conducted to look for the optimum inclusion ratio of hulless barley with altered CHO traits in regular cattle diet. Molecular spectroscopy can be applied in evaluating feedstuffs or for distinguishing the chemical compounds within feedstuffs without using traditional chemical methods. In this study, although the absorption intensity of functional group bands differed when detected by FTIR and SR−FTIRM, there was a significant influence of different spectral features of functional group bands in relation to metabolic characteristics of nutrients. However, since SR−FTIRM is more expensive and requiring a large lab facility investment, FTIR could be considered as a better technique for feed science.

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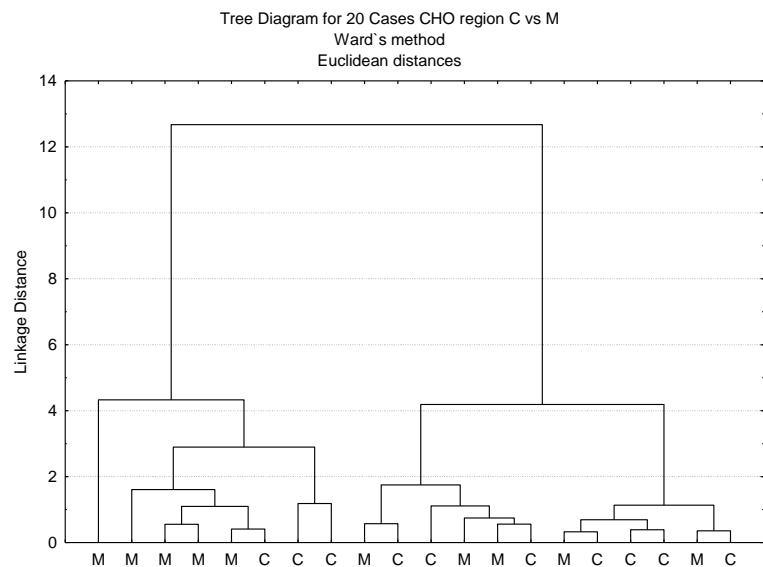
(1) CLA Comparison: CDC Copeland (C) and CDC McGwire
(M)
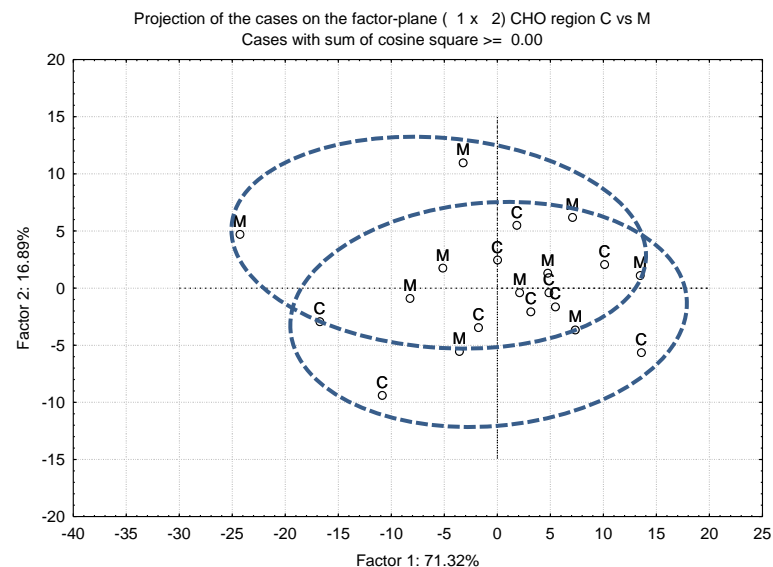
(2) PCA Comparison: CDC Copeland (C) and CDC McGwire
(M)

Figure 1 Multivariate molecular spectral analyses of fingerprint region (ca. 1195-945 cm^{-1}): hulled barley vs. hulless barley at SR−FTIR carbohydrate fingerprint region: ca. 1195-945 cm^{-1}. (1): cluster analysis; (2): principal component analysis: Scatter plots of the 1st principal components (PC1) vs. the 2nd principal components (PC2).
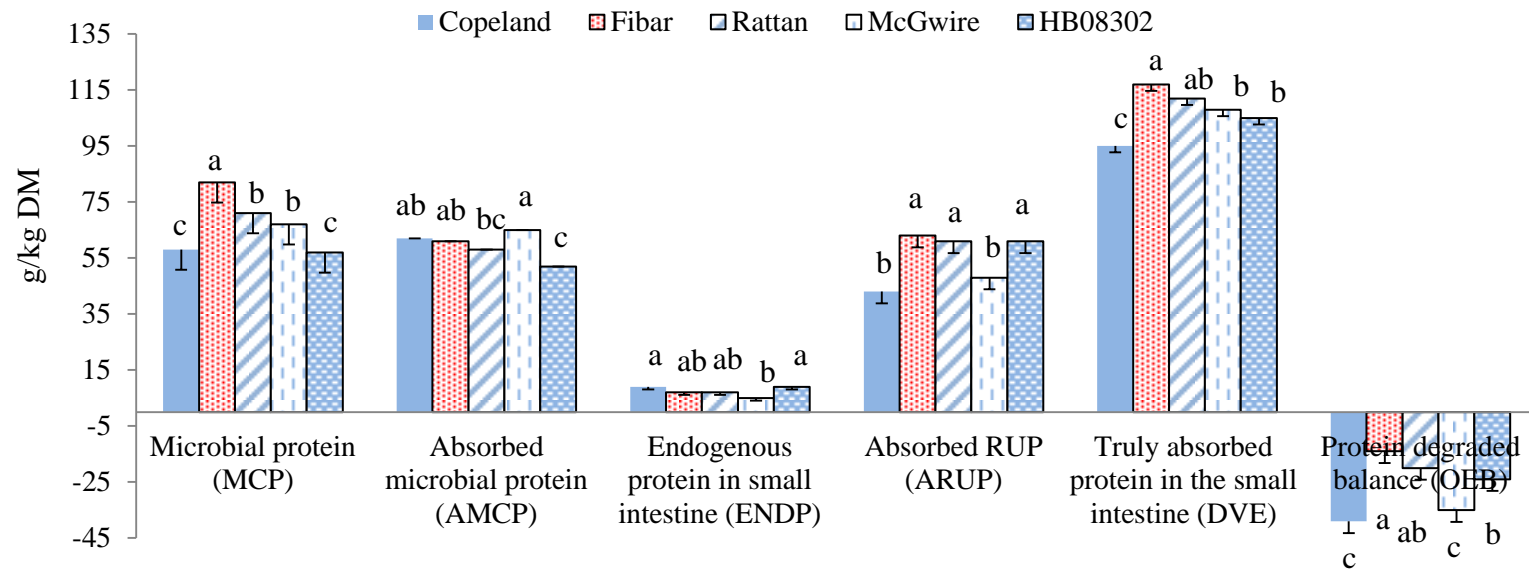
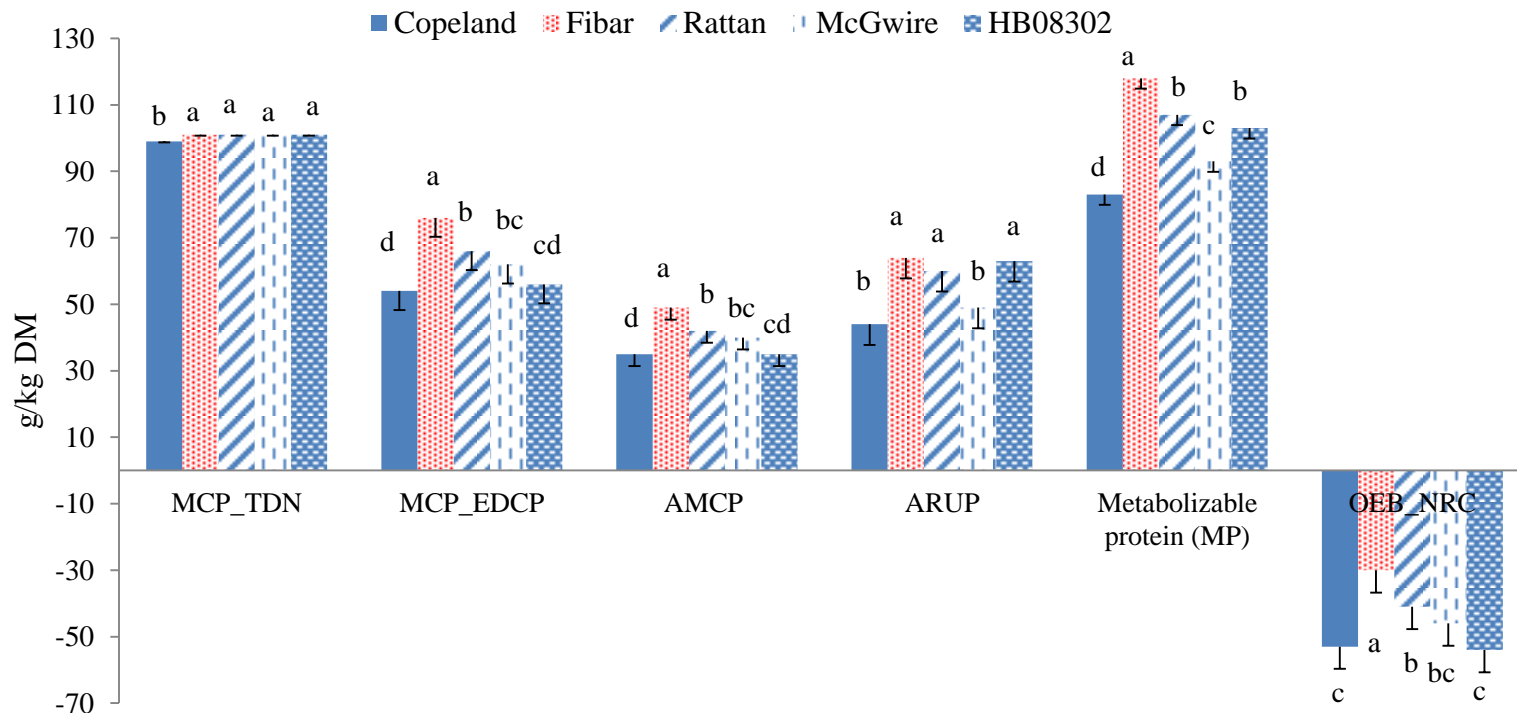Figure 2 Protein supply parameters predicted by DVE/OEB system

Figure 3 Protein supply parameters predicted by NRC Dairy 2001 model