NUTRITIONAL AND MICROSTRUCTURAL RESPONSES IN CEREAL GRAINS TO HEAT-RELATED PROCESSING METHODS

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ABSTRACT

Cereal grains share many common traits, but they also have different internal structures, nutrient values, degradation kinetics and digestion features. Heat treatments are commonly used in the feed industry. It is known that heat is able to change the nutrient values of the feed but the effect could be equivocal. In order to understand the effects of heat processing on internal structure and nutrient availability of cereal grains, two batches of wheat, triticale and corn were divided into three groups (control/raw (unheated), dry heating and moist heating) and processed at 121 °C for 80 min. Basic chemical analysis and *in situ*, *in vitro* assays were conducted and CNCPS, DVE/OEB and NRC-2001 models were used to determine the nutrient availability of the grains. In addition, two mid-IR molecular spectroscopy techniques (Attenuated Total Reflectance Fourier Transform Infrared (ATR-FTIR) and Synchrotron Radiation Infrared Microspectroscopy (SR-IMS)) were used to gain an insight into the heat-induced changes in the functional groups.

Significant ($P<0.05$) differences were found between the cereal grains in their nutritional availabilities, including their chemical characteristics, protein and carbohydrate fractions, energy values, the ruminal degradation kinetics, hourly effective rumen degradation ratios, potential N-to-energy synchronization, and intestinal digestion of cereal grains. Compared to dry heating, moist heating had more impact on altering the nutrient profiles and showed the potential to increase the nutrient availability of wheat and triticale for dairy cattle. Significant differences ($P<0.01$) were detected between different feeds and heat treatment groups by using the ATR-FTIR technique. Results were found in consistency with the conventional chemical and animal studies mentioned above despite when using the SR-IMS technique. Significant ($P<0.05$) correlations were detected between some structure spectral characteristics and nutrient digestion traits.

In conclusion, the moist heating had more profound impact than the dry heating in increasing nutrient supplies to ruminants in wheat and triticale. The heat-induced effects found in corn were less positive. The ATR-FTIR technique could detect the internal structural changes in cereal grains, while the sensitivity and accuracy of the SR-IMS technique were not proved in this study.

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AAs	Amino acids
AB	Autoclaving with the samples soaked in buffer
ABCP	Truly absorbed rumen bypass protein in the small intestine
AD	Autoclaving
ADF	Acid detergent fiber
ADG	Average daily gain
ADICP	Acid detergent insoluble crude protein
ADL	Acid detergent lignin
AECP	Truly absorbed rumen endogenous protein in the small intestine
AMP	Truly absorbed microbial protein in the small intestine
NDFom	Neutral detergent fiber on organic matter base
ANFs	Anti-nutritional factors
$ARUP^{NRC}$	Truly absorbed rumen undegradable protein in the small intestine (NRC Dairy 2001 model)
ATR-FTIR	Attenuated total reflectance Fourier transform infrared spectroscopy
BCHO	Rumen bypass carbohydrate
BCP	Rumen bypass crude protein (DVE/OEB system)
BDM	Rumen bypass dry matter
BNDF	Rumen bypass neutral detergent fiber
BST	Rumen bypass or undegraded starch
CA	Fast degradable CHO (CNCPS version 5)
CA1	Acetic, propionic and butyric acids with degradation rate of 0%/h
CA2	Lactic acid with degradation rate of 7%/h
CA3	Organic acids with degradation rate of 5%/h
CA4	Sugars with degradation rate of 20-40%/h
CB1	Starch with degradation rate of 3-40%/h
CB2	Soluble fiber with degradation rate of 20-40%/h

CB3	Available neutral detergent fiber with degradation rate of 3-9%/h
CC	Unavailable neutral detergent fiber
CHO	Carbohydrates
CLA or AHCA	Hierarchical cluster analysis
CNCPS	Cornell Net Crude Protein System
CP	Crude protein
D	Potentially degradable fraction
dBNDF	Digestibility of rumen by pass or undegraded NDF
dBST	Digestibility of rumen bypass or undegraded starch
DDGS	Dried distiller grains with solubles
DE_{1x}	Digestible energy at maintenance level
DE_{p3x}	Digestible energy at production level (3x maintenance)
DE_{p}	Digestible energy at productive levels of intake
DH	Dry heating
DM	Dry matter
DMI	Dry matter intake
DOM	Digested organic matter
DVE	Total truly digested absorbed protein in the small intestine
EAAs	Essential amino acids
ECP	Rumen endogenous protein
ED	Effectively degradable fractions
ED_N/ED_CHO	Effective degradability ratio of N to CHO
ED_N/ED_OM	Effective degradability ratio of N to OM
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

FA	Fatty acids
FMV	Feed Milk Values
FOM	Organic matter fermented in the rumen
FTIR	Fourier transform infrared spectroscopy
GE	Gross Energy
IADP	Intestinal absorbable feed protein
IDBCHO	Intestinal digestible rumen bypass CHO
IDBNDF	Intestinal digestible rumen bypass NDF
IDBST	Intestinal digestible rumen bypass starch
IDP	Intestinal degradable protein
IR	Infrared
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_p	Metabolizable energy at production level of intake
ME_{p3x}	Metabolizable energy at production level (3x maintenance)
MH	Moist heating
MP	Metabolizable protein
N	Nitrogen
N_MCP	Microbial protein synthesized in the rumen based on available nitrogen
NDF	Neutral detergent fiber
NDICP	Neutral detergent insoluble crude protein
NE	Net energy
NE_g	Net energy for gain
NE_{Lp3x}	Net energy for lactation at a production level (3x maintenance)
NE_m	Net energy for maintenance

NFC	Non-fiber carbohydrate
NIR	Near Infrared
NPN	Non-protein nitrogen
NSC	Non-structural carbohydrate
OEB	Degraded protein balance
PA	Ammonia (CNCPS version 6.1)
PA1	Ammonia (CNCPS version 6.5)
PA2	Soluble protein (CNCPS version 6.5)
PB	True protein (CNCPS version 6.1)
PB1	Soluble protein (CNCPS version 6.1) or moderately degradable protein (CNCPS version 6.5)
PB2	Moderately degradable protein (CNCPS version 6.1) or slowly degradable protein (CNCPS version 6.5)
PB3	Slowly degradable protein (CNCPS version 6.5)
PC	Unavailable protein
PCA	Principal component analysis
PCs	Principal components
peNDF	Physical effective NDF
QPM	Quality protein maize
RCBD	Randomized Complete Block Design
RDC	Rumen degradable carbohydrates (NRC Dairy 2001 model)
RDP	Rumen degradable protein (NRC Dairy 2001 model)
R(t)	Residue percentage at t hours of incubation in the rumen
RU	Ruminally undegradable fractions
RUC	Rumen undegradable carbohydrates (NRC Dairy 2001 model)
RUP	Rumen undegradable crude protein (NRC Dairy 2001 model)
S	Soluble fraction in the *in situ* incubation
SBW	Shrunk body weight
SC	Structural carbohydrate
SCP	Soluble crude protein
SEM	Standard error of mean

SR-IMS	Synchrotron-based Fourier Transform Infrared Microspectroscopy
ST	Starch
T_0	Lag time (h)
TCA	Trichloroacetic acid
TDCHO	Total digestible CHO
tdCPc	Total digestible crude protein for concentrates (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
VFA	Volatile Fatty Acid

1 General Introduction

Cereal grains are widely grown and used as an energy source for animals and human beings all around the world. Around 45% of the world's arable land is used to grow cereals. The harvested area for cereal grains was 703,197,068 hectares in 2012 (FAOSTAT, 2014). About 31% was wheat (*Triticum spp*.), which has the most stable growing area. The sowing area of corn (*Zea mays subsp. mays*) has increased about 50%, over the past 50 years (Guine et al., 2013). Triticale (× *Triticosecale* Wittmack) is a relatively new species of grain, being introduced in 1937. The first triticale breeding program in North America was started by the University of Manitoba in 1954 and the first commercial triticale variety was registered in 1968 (Government of Saskatchewan, 2011). Being a relatively new type of grain, the productivity of triticale is similar compared to wheat and corn. However, its sowing area also stays stable while other cereals are all decreasing in growing areas during the same period of time (Guine et al., 2013).

Despite the decrease in seeded acreage, the world's total demand for cereals will keep growing in the predictable future, as the world's population is growing. The total production of cereal grains around the world was 1,840,987,980 tonnes in 2002 and 2,305,329,750 tonnes in 2012 (FAOSTAT, 2014), and is forecast to increase by 25% in a decade. In Canada, the production of all cereal grains reached 50,066,680 tonnes, including 27,205,200 tonnes of wheat, 35,600 tonnes of triticale and 13,060,100 tonnes of corn in 2012. The total cereal production quantity increased 39% comparing to that in 2002. Among them, wheat, triticale and corn have increased by 70%, 37% and 45%, respectively, during the last decade (FAOSTAT, 2014).

Reports from the United Nations show that driven by the population growth, together with rising income levels of the world's large population, the consumption of food also increases, with increasing proportion of animal products in the diet (Gerosa et al., 2012; Nellemann et al., 2009). Approximately half of the production of cereal grains was consumed as human food, around 35-40% was used as animal feed and the rest in other industries, such as malting and bio ethanol (Batey, 2010). The increased need for cereal grains in human and animal industries requires higher yields, more growing area and more efficient use of the grains. Cereal grains play and will

continue to play a very important role in our daily life, thus it is critical to improve the nutrient availability of cereal grains.

In animal industry, cereal plants can either be grazed, used as silage, or harvested for grains (Government of Saskatchewan, 2011). Heat processing has been used as a way to improve utilization and availability of the nutrients in legume seeds (Goelema, 1999; Yu et al., 2000) and to inactivate anti-nutritional factors (ANFs) such as phytic acid and protease inhibitors in plants (Kaur et al., 2012). The heat effect alone could induce changes in protein conformation (Achouri et al., 2012) and the cross-linkages between protein and carbohydrate thus reducing the rate of rumen degradation in legume seeds (Goelema, 1999; Yu et al., 2000). With the presence of moisture, more changes such as the gelatinization and retrogradation of starch could occur. Moisture also helps the heat to penetrate into the seeds and cause more influence on the structural changes. In the animal industry, the most often used heat-related processing methods are: pelleting, extrusion, dry rolling, steam rolling, steam flaking and micronizing. Except for dry rolling and micronizing, moisture is involved in these processing methods (Owens et al., 1997).

The changes induced by dry and moist heating in different types of cereal grains are not well known. Conventional methods can detect changes in nutrient profiles, rumen degradation and intestinal digestion caused by heat processing methods, but to find out the inner structural changes in the cereal grain seeds, new techniques need to be used. Synchrotron Radiation Infrared Microspectroscopy (SR-IMS) is one of the rapid ways to directly detect heat-induced changes in functional groups without grinding and digesting the samples. Thus the information of spatial distribution and cellular dimension is able to be preserved. This cutting-edge technique has been proved effective in studying the inherent molecular structure in several feed (Yang et al., 2014; Yu, 2004; Yu, 2005b). Conventional Attenuated Total Reflectance Fourier Transform Infrared Spectroscopy (ATR-FTIR) is another rapid method to identify inherent structural information. By applying these advanced techniques, it is now possible to detect the inner structural changes caused by different heat treatments and link the alteration in microstructure to the nutritional features on a cellular level (Yu, 2004).

The objectives of this study are to understand the heat-induced changes in the nutritional availability and the inner structural of cereal grains, and it is necessary to utilize both the conventional methods and the advanced mid-IR techniques to find out 1) if these heat-induced changes are detectable using mid-IR techniques, 2) if different heat-induced changes are caused by dry and moist heating, 3) how different cereal grains response to heat effects, 4) if what is detected by the mid-IR approaches correlate with the results found by the conventional methods.

2 Literature Review

2.1 Structure of Cereal Grain Seeds (Wheat, Triticale and Corn)

Cereal grains share many things in common. In general, a cereal grain seed consists of three parts: bran, endosperm and germ. There are two parts in bran: pericarp and seed coat. The endosperm includes the aleurone layer in the outside and starch granules in the middle. The germ, or embryo, includes three parts: plumule, scutellum and radicle. As caryopses and monocots, the fruit coat in cereal grains adheres close to the seed, and there is only one cotyledon in the germ. Starch in endosperm provides nutrient for the seed (Angold, 2012; Rooney et al., 2004).

The structure of starch granules inside the endosperm is one of the crucial parts when studying cereal grains. There are two types of endosperm: the floury endosperm and the vitreous endosperm. The former one is starch granules with very little protein matrix surrounding them, while in the latter one, starch granules are embedded in a protein matrix, making the endosperm more dense and stable (Eckhoff et al., 1996). Using corn as an example, vitreous endosperm has a higher content of lysine than floury endosperm (Gibbon et al., 2003). The two kinds of endosperm granules are also different in shape, with the granules in the vitreous endosperm small, polyhedral and sometimes indented, and those in the floury endosperm large and spherical (Rooney et al., 2004). Both large and small starch granules have concentric layers of starch distribution, which can be observed in a scanning electron micrograph of the endosperm area (Wrigley, 2010).

There are also many differences in structure between different species and varieties of cereal grains, at both the macro and micro levels. Regardless of the difference in appearance such as color, size and shape, cereal grains differ in nature and numbers of aleurone layers (Wrigley, 2010). Wheat, triticale and corn have single-layered aleurone, but they differ in inner structures. Wheat and triticale have a “bi-modal” distribution of starch granules, consisting of both large (A-type: diameter > 10 μm) and small (B-type: diameter < 10 μm) granules. In triticale, higher ratio (both number and weight) of the starch granules are large, compared to wheat and corn (Naguleswaran et al., 2012). Corn is considered to have a “normal” distribution of starch granules, although the starch granules in corn range in size (5-35 μm in diameter) and have irregular shapes

(Eckhoff et al., 1996; Rooney et al., 2004; Wrigley, 2010). These differences in different cereal structures result in differences in rate and extent of ruminal fermentation. Generally, wheat and triticale are more rapidly degraded by ruminal microorganisms than corn (McAllister et al., 1993).

Starch granule features can be observed using scanning microscopy. These features include amorphous growth rings, unevenly distributed surface pores and internal channels (Naguleswaran et al., 2012). Differences were found between different cereal grains: more pores and channels were observed in corn than in wheat and triticale. Within the same variety of cereal grain (wheat, triticale or corn), surface pores were more frequently seen on large granules (Naguleswaran et al., 2012). These pores and channels were rich in protein and phospholipids (Naguleswaran et al., 2011). By making some surface areas of wheat and corn more susceptible to amylases, the pores and channels were assumed to be able to affect the pattern of amylase attack (Fannon et al., 1992, 1993). The presence of the proteins and lipids in the channels, however, also blocks binding sites of the enzyme, thus reduces the hydrolysis rate (Naguleswaran et al., 2011). Small granules have relatively larger surface area compared to larger ones, hence they were hydrolyzed faster in the initial stage. The hydrolysis process slows down in later stages due to the denser crystalline lamellae in the small granules. The hydrolysis rates after 24 h was similar between small and large granules (Naguleswaran et al., 2012).

2.2 The Nutritional Values of Cereal Grains

Being a good energy source, cereal grains are an essential part of both human food and animal feeds. Grains provide more than 56% of food energy and about 50% of the protein on earth (Cordain, 1999).

Most of the energy contained in the cereals is available in the form of carbohydrate, most of which is starch. As mono-gastric animals are not able to hydrolyze structural carbohydrate, starch is the only polysaccharide that they can utilize (Morris et al., 1996). Cereal grains are thus the primary energy supply for mono-gastric animals such as poultry and swine. About half of the

grains are consumed by human beings, while more than one-third of protein in cereal grains is consumed in pig and poultry rations (Cordain, 1999; Morris et al., 1996).

Besides carbohydrate, the endosperm, embryo and aleurone of wheat, triticale and corn also contain protein. Although the total amount only accounts for 10% on average, many kinds of proteins are involved in the grain kernels. Most storage proteins, including prolamins and a small amount of globulins/glutelins, are present in the starchy endosperm. There are also some globulins and oil bodies in the embryo and aleurone layer, and some functional proteins in cell walls, membranes, enzymes and transport systems (Shewry, 1996).

Regardless of their outstanding nutrient values, cereal grains are unsuitable to be used as complete food or feed product because of the lack of some essential amino acids, essential fatty acids, vitamins, and minerals. Low levels of lysine, methionine, tryptophan and threonine in cereals (Figure 2.1) is not a good indication of feed value for mono-gastric animals or ruminants. Cereals also have low concentration of essential fatty acids such as linoleic acid (except corn: 2.12 in corn vs. 1.2 in wheat vs. 0.95 in rye, g/100g sample) (Cordain, 1999). Vitamin A (except for yellow maize), vitamin C, vitamin B12 and β-carotene and minerals (except for potassium and phosphorus) are also insufficient for a balanced diet (Cordain, 1999; Morris et al., 1996).

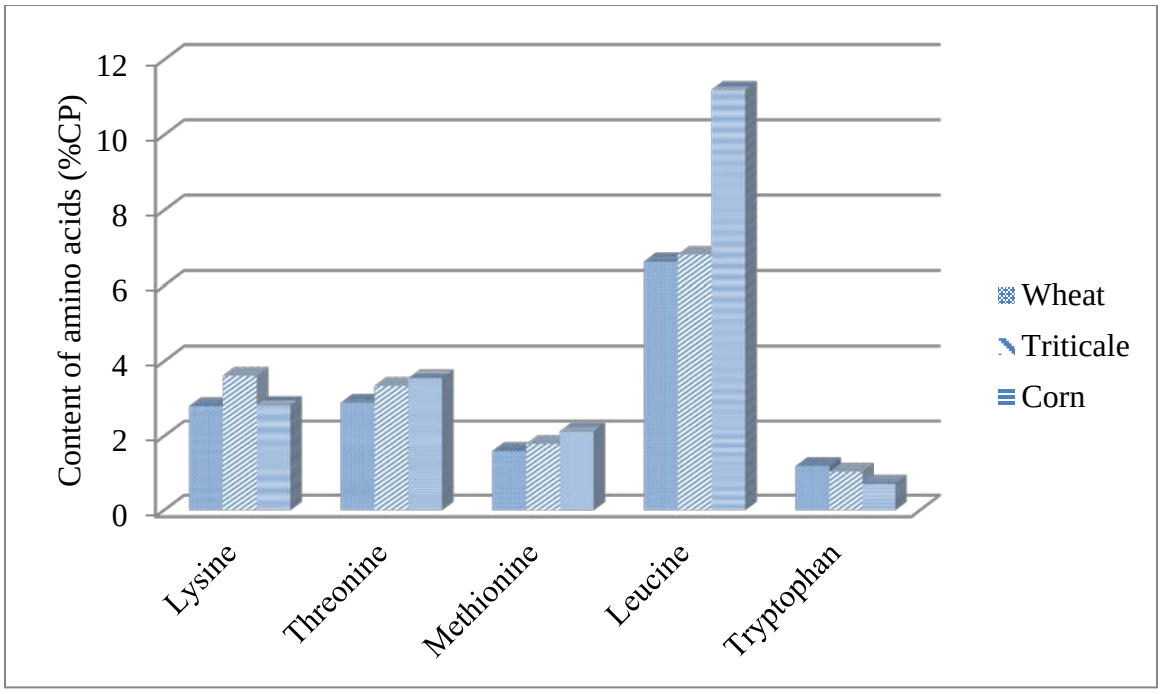

Figure 2.1 Amino acid content (g/100g crude protein) of wheat, triticale and corn (Data from National Research Council, 2001)

Although the microbes in the rumen could synthesize all the essential amino acids (EAAs), the amount is not enough for highly productive dairy cows. According to the National Research Council (2001), lysine and methionine are the first-limiting EAAs in metabolizable protein (MP). In dairy cattle, the optimal concentrations of these two AAs are 7.2% and 2.4%, respectively, in order to fulfill the needs of both maintenance and milk production (National Research Council, 2001).

Cereal grains such as wheat, corn and barley are commonly used as the energy source in the concentrate of cattle rations. The amount varies depending on the age, species and physical conditions of the cows. For instance, more than 55% (DM basis) of grains are usually included in the ration of finishing beef cattle (Owens et al., 1997).

Cereal grains are also widely used in the bioethanol industry. In Canada, wheat is used in bioethanol plants in Western Canada and corn is more commonly used in Central Canada (Wheat DDGS, 2010). By having an equivalent nutrient value, high crop yield and competitive price, triticale could be an alternative to wheat and has been processed in some ethanol plants in Western Canada (Government of Alberta, 2014). Research shows that the coproduct, triticale

DDGS, also has similar value compared to wheat DDGS and corn DDGS in the beef, dairy and lamb industry (Au et al., 2010; McKeown et al., 2010; Oba et al., 2010; Wierenga et al. 2010).

2.2.1 Wheat

Being one of the first domesticated grains, wheat is now grown and commercially consumed worldwide. Based on the different color, texture and the season planted, wheat is classified into eight different market classes in Western Canada, with different kernel sizes, shapes and nutritional values in each market class (Government of Canada, 2015). The protein content of wheat is high compared to other cereal grains, especially in the case of hard and durum wheat. Cheeke (1991) concluded that hard wheat contains about 11-14% protein, while the soft wheat has less protein content (about 8-11%).

The mature wheat grain contains about 85% carbohydrate, around 80% of which is the endosperm starch. The non-starch part includes 7% of mono-, di- and oligo-saccharides and fructans and 12% of polysaccharides in cell walls (Batey, 2010).

The use of wheat in the animal industry is affected by the market price, location and quality (Kent and Evers, 1994). Traditionally, wheat by-products after milling and wheat that is unsuitable for milling due to low quality caused by disease, insects and frost, is used as animal feed. The nutrient value, palatability and digestibility of wheat are equivalent to corn, while the amino acids profile is superior. However, often the high price of feed wheat in Canada restricts its utilization in the animal industry (Bell, 2003).

The nutritional value of wheat to the animal may vary by cultivar, as well as processing method such as steaming rolling, flaking, pelleting and dry-rolling. Coarse-grinding, dry-rolling of wheat will increase wheat feeding value compared to ground wheat when fed to cattle (Owen 1997). However, the influence of processing varies by animal species and production.

2.2.2 Triticale

As a hybrid of wheat and rye, triticale shares the name and traits of both of them. The name "triticale" is derived from *Triticum* (wheat) and *Secale* (rye) (Cheeke, 1991). Triticale has good yield on marginal lands and is tolerant to drought. Its compositional quality remains stable across environments, with nutritional characteristics between wheat and rye. Recently developed triticale varieties have lower protein content (about 13%) than the older varieties (about 17%) (Government of Saskatchewan, 2011). Nevertheless, with the vastly improved grain yield (15-20% higher yield than Canada Prairie Spring wheat), plant breeders increased the protein yield. The nutrient quality is also improved: triticale has intermediate lysine content (higher than wheat, Figure 2.1) and digestible energy compared to wheat and rye. The starch, lipid, fiber, mineral, and vitamin contents are similar or superior to those of wheat (Government of Alberta. 2014; Government of Saskatchewan, 2011).

Although triticale is not as widely grown as wheat and corn, it is potentially an equivalent feed source for livestock. The digestibility of nutrients such as starch and protein is superior to that of other Canadian cereal grains (Government of Alberta. 2014). As its extent of starch fermentation is similar to barley and oat, triticale may have higher post-ruminal digestibility. As an advantage of having higher lysine content, using triticale in the diets of mono-gastric animals often means a lower requirement of protein supplement. Reports show that triticale is a very successful alternative in swine feed, with the digestible energy (DE) equivalent to wheat and corn when fed to piglets. In the poultry industry, triticale has already been used worldwide and contradictory results were found in research on whether using triticale has a negative effect on production. Despite these differences, a consensus has arrived in economic studies that feeding triticale to mono-gastric animals is a cost-saving approach (Government of Alberta, 2014). For ruminants, there is sparse research on adding triticale in the concentrates for dairy cattle. However, since it is proved that triticale could fully substitute other grains in diets for beef cattle, similar results could be assumed for dairy cattle. Moreover, the substitutive use of triticale is already common in Australia for dairy cows when its price and supply is competitive (Government of Alberta, 2014; Government of Saskatchewan, 2011). One shortcoming of triticale is its susceptibility to ergot and

this may be one of the reasons that limited the wide application of the grain (Diana Di Mavungu et al., 2012).

2.2.3 Corn

Corn is believed to originate in Central Mexico. It is now the third most popular grain crop in the world, grown all around the world and used as food, feed, seed and in industrial products. Being the leading cereal in the U.S., about 39.2% of corn is utilized as animal feed (Győri, 2010; Serna-Saldivar et al., 2001; White, 2001).

The kernel of corn averages about 73% starch, 10% protein and 5% oil (DM basis), with about 80-90% of the starch found in the endosperm (Boyer et al., 2001; Eckhoff et al., 1996). The nutrient distribution in corn kernels is similar to other cereal grains. In the pericarp, the main components are fiber, ash and oil (Rooney et al., 2004). The endosperm mainly consists of starch, while oil and protein are concentrated in the germ (Boyer et al., 2001). Comparatively, the peripheral hard endosperm cells are high in protein content (15-50%) and low in starch content, while the center floury endosperm cells are high in starch content and low in protein content (4-5%). Proteins centralized in the germ are mainly albumins and globulins, and those in the endosperm are mostly prolamins. Zein, which was first discovered in 1821 and classified as prolamin in 1924, is the main prolamin in corn and one of the most studied proteins (Anderson and Lamsal, 2011). The cell walls are comprised of cellulose, hemicellulose, ferulic acid and some proteins high in hydroxyproline (Rooney et al., 2004).

Benefiting from being a C4 plant, corn is the cereal grain that produces the most energy per acre. It is also highly palatable, contains no intrinsic toxic or deleterious compounds and has the highest digestible energy content of cereal grains for animals (Cheeke, 1991). Another merit of corn is that it has the highest level of essential fatty acids among all the cereals (Morris et al., 1996).

The biggest limitation of corn is its low concentration of lysine and tryptophan, and the unavailability of niacin (vitamin B3). A type of nutritionally enhanced corn, called quality protein maize (QPM), with high lysine content thus has been developed (Eckhoff et al., 1996; Shewry, 1996).

In western Canada, barley-based diets are commonly fed to feedlot cattle, while corn is used as an alternative grain when barley grain is expensive (Beauchemin and Koenig, 2005). When fed to animals, the corn grain is usually processed in order to increase palatability, reduce particle size, improve digestibility, change the digestion rate, site and extent, or simply for storage purpose (Richards and Hicks, 2007).

2.3 Heat Treatments

2.3.1 Mechanism of Heat Treatments

Heat processing has been used to change the nutrient availability, sterilize samples and deactivate anti-nutritional factors (ANFs) (Goelema, 1999; Van der Poel et al., 1990; Yu et al., 2000). In the animal industry, the most often used heat processing methods are pelleting, extrusion, dry rolling, steam rolling, steam flaking and micronizing (Owens et al., 1997). Moisture is involved in many of these methods, except for dry rolling and micronizing.

The effect of heat processing is usually greater with the presence of moisture (Owens et al., 1997). Starch may swell at room temperature when wet, but gelatinization temperature varies according to starch type. With the presence of enough moisture, irreversible swelling of starch may occur when the temperature reaches 60 °C, as the granules swell too much to keep their crystalline structure (Wrigley, 2010). The intermolecular starch and protein bonds are disrupted, resulting in dissolving of the starch granules, leaching of amylose and the forming of a viscous paste or gel. On cooling, the starch retrogradation may take place, reforming amylose to an acid, heat and α-amylase resistant crystalline structure (Bornet, 1993; Cai et al. 2014; Flores-Morales et al., 2012).

Autoclaving is a commonly used method for sterilization in many fields including medicine, microbiology, mycology and plant science. By combining heat, moisture and pressure, autoclaving is able to change chemical profiles, protein subfractions, rumen degradable nutrients, potential nutrient supply to dairy cattle and the inner structure, such as amide I-to-amid II ratio and α-helix to β-sheet ratio, in flaxseed, camelina seeds, soybeans, yellow and brown canola seeds (Doiron et al., 2009; Peng et al., 2014; Samadi and Yu, 2011; Samadi et al., 2013).

Due to the branched structure, it is harder for amylopectin to undergo both the gelatinization and retrogradation processes. Hence the different amylose / amylopectin ratio in cereal grains may be the reason why they have different responses to heat treatments (Yan et al., 2014). Richards and Hicks (2007) stated that steam-rolled and steam-flaked starch granules are more susceptible to digestive enzymes after cooling, while McAllister et al. (1991) found that autoclaving could reduce the susceptibility of both the protein matrix and starch granules to microbial attack, especially when the Maillard reaction is involved.

2.3.2 Heat Processed Cereals Fed to Ruminants

Feed cost is one of the largest expenses in the ruminant industry. As cereal grains are not a cheap source of feed, it is important to optimize feed efficiency. Therefore, cereal grains are usually processed before being fed to ruminants.

The major ways of processing include providing heat, moisture and reducing the particle size. When the cows are fed with a high quantity of grain, precautions are needed to avoid acidosis. Besides coarse grinding and using buffering additives, heat processing is another measure that could help prevent acidosis (Government of Alberta, 2014).

The species of grain and the heat processing method used on grains could affect the feeding value, dry matter intake (DMI), as well as production efficiency of the cows. For instance, flame roasting cereal grains at 77-121˚C significantly decreased the ruminal degradation of dry matter (DM) and crude protein (CP) without affecting their digestibility in ruminants (McNiven et al.,

1994). Steam flaking increased the body weight-adjusted ME for corn and wheat by 15% and 13%, respectively, compared to dry rolled grain, but it was not as effective when applied to barley and oats. Steam rolling and flaking of corn, wheat and sorghum could maintain average daily gain (ADG) while reducing DMI, thus raise the feed efficiency by 10, 10 and 15%, respectively (Owens et al., 1997). Firkins et al. (2001) indicated the effectiveness of steam-flaking in increasing the starch digestibility in corn, comparing to dry rolling and steam rolling. Richards and Hicks (2007) reported that steam flaking could reduce the degradation rate of wheat and barley but not corn, compared to dry rolling, shift the digestion site of corn from rumen to intestine, compared to high moisture corn (but not dry rolling) and increase the starch digestibility, and increase the feed efficiency of cattle fed corn and sorghum, compared to dry rolling and high moisture. Goelema (1999) summarized that pressure toasting decreased *in situ* rumen degradability of protein and starch while pelleting and expander under mild conditions increased this parameter.

2.4 Conventional Feed Evaluation

2.4.1 Cornell Net Carbohydrate and Protein System (CNCPS, version 6)

The Cornell Net Carbohydrate and Protein System (CNCPS) was developed by scientists in Cornell University, University of Pennsylvania and Miner Institute, released in 1991, and first published by Russell et al. (1992), Sniffen et al. (1992) and Fox et al. (1992). Based on the principals of feed digestion, rumen function and the physiological state of the animals, the model can evaluate diets and performance of all classes of cattle in their certain living situations, thus to help optimize the feed and management of cattle (Tylutki et al., 2008; Van Amburgh et al., 2013).

The latest updated model, version 6.5, has been available since Mar 20, 2015, on the CNCPS website (http://www.cncps.cornell.edu). The software has a feed library, containing almost all the nutrient values (protein, fiber, volatile fatty acids (VFA), minerals, vitamins, amino acids (AA) profiles, fatty acids (FA) profiles), intestinal digestibility and digestion rates of different protein and carbohydrate fractions of common feeds. These component values and digestion rates are used to compute the available protein, as well as the amount of structural carbohydrate (SC) and

non-structural carbohydrate (NSC) in a given feed (Tylutki et al., 2008). Many updates have been made in the version 6.1 of the model, compared to the original one, which separates protein into five fractions and carbohydrates into four fractions. Feed composition in the CNCPS version 6.1 is described by five protein fractions and eight carbohydrate fractions.

The number of protein pools doesn't change between versions, but the pool size of PA fractions is different. In CNCPS version 6.1, the PA fraction was redefined as ammonia because some small peptides and free amino acids (AA) could escape rumen degradation (Higgs et al., 2012). Therefore, old method (Licitra et al., 1996) of analyzing non-protein nitrogen (NPN) has been abandoned due to the large pore size of Whatman #54 filter paper (20μm) (Tylutki, 2010; Van Amburgh et al., 2010). In some research, a colorimetric method (AOAC 967.07) was used to measure NPN in animal feed (Haig et al., 2002). The PB part is known as "true protein", among which, the PB1 fraction is soluble in borate phosphate buffer but can be precipitated by trichloroacetic acid (TCA) and the PB3 fraction, which represents "fiber bound protein", is insoluble in neutral detergent but soluble in acid detergent solution. The PC fraction is insoluble even in acid detergent and the PB2 fraction is computed by difference (Van Amburgh et al., 2013). Further updates (version 6.5) rename PA as PA1 and PB1 as PA2 since they are both soluble, whilst PB2 is renamed as PB1 and PB3 as PB2 (Van Amburgh et al., 2013; Higgs et al., 2012).

The number of carbohydrate (CHO) pools is increased from four to eight. The former CA fraction (fast degraded: sugar) was further divided into four sub-fractions: CA1 (acetic, propionic and butyric acids), CA2 (lactic acid), CA3 (organic acids) and CA4 (sugars). The CB fraction was partitioned into three sub-fractions instead of two. The new fraction is CB2 (soluble fiber), which was previously partitioned together with starch in CB1 (version 5). The CB1 fraction remains as starch, while the former CB2 fraction (slow degraded: available cell wall) is now CB3 fraction (available NDF). Meanwhile, The CC fraction is still unavailable NDF (Sniffen et al., 1992; Lanzas et al., 2007a; Tylutki et al., 2008).

Besides the expanded scheme, the passage rate equations were also updated according to Seo et al. (2006). The soluble pools (CA, PA and PB1) are re-assigned to the liquid passage rate equation (6-12%/h), instead of the solid passage rate (4%/h). Degradation rate (K_d) for some protein and

CHO fractions are adjusted downward, to be more appropriate in accordance with the biology of the cattle. For example, K_d for sugar was 200-300% /h in previous versions, but now it is set as 40-60%/h in version 6.1. The degradation rate for NPN is also adjusted from 10,000%/h to 200%/h and K_d for PB1 regulated from 130-300%/h to 10-40%/h (Lanzas et al., 2007b; Van Amburgh et al., 2010). There are also other important changes: for instance, the physical effective NDF (peNDF) adjustment factor is no long used, because sodium sulfite is now routinely added when analyzing NDF in the feed in most labs. In the 6.5 version, NDF is further adapted to ash corrected NDFom.

Maintenance requirements are calculated using equation summarized by Fox et al. (2004), in which factors such as breed, physiological state and environmental effects are taken into consideration. According to information such as body weight, rate of body weight gain, chemical composition of gain and mature weight, energy and protein requirements for growth are predicted. Requirement of energy and protein for lactation is calculated based on actual milk production and composition. Pregnancy requirement and shrunk body weight (SBW) are computed from growth of gravid uterus depending on expected birth weight and day of gestation. CNCPS also has equations on amino acid requirements, in accordance with tissue and milk protein content of amino acids (Tylutki et al., 2008).

The new CNCPS was proved to be more biologically correct and more accurate on feed chemistry and rumen fermentation characteristics (Van Amburgh et al., 2010), thus it can be used to formulate the diets to effectively reduce the feed cost, as well as the impact of ruminant husbandry on the environment.

2.4.2 The Estimation of Energy Values

Energy is an expensive nutrient in animal industry. Thus precise estimation is required to guarantee that the energy supply in the feed meets the needs of the animal. Bomb calorimeters are commonly used to measure the Gross Energy (GE) by burning the sample to ash. However, to directly measure Digestible Energy (DE), Metabolizable Energy (ME) and Net Energy (NE), large

efforts are required. By contrast, using theoretically-based models to calculate DE, ME and NE is more convenient.

Weiss et al. (1992) developed a mathematical model to estimate the total digestible nutrient (TDN) of feeds, in which, the nutrients were partitioned into four fractions: CP, NDF, ether extract (EE) and non-fiber carbohydrate (NFC). By estimating the energy values in each part, we could add up the TDN and DE of the feed at maintenance using equations in National Research Council (2001).

Today's high producing dairy cattle actually consume about 3 to 4 times of the maintenance requirement. As the digestibility of feed declines with increasing level of feed intake, a discount needs to be applied to calculate DE at productive levels of intake (DE_p). The ME at actual intake (ME_p) is estimated according to DE_p and NE_L at actual intake (NE_{Lp}) is estimated based on ME_p (National Research Council, 2001; Robinson, 2007; Tyrrell et al., 1975). The net energy for maintenance (NE_m) and net energy for gain (NE_g) are determined according to National Research Council (1996).

2.4.3 The Estimation of Rumen Kinetics Using *in situ* Technique

The *in situ* technique was first introduced by Quin et al. (1938) and it has since been used to estimate the feed degradation in the rumen. Mehrez and Ørskov's (1977) report raised the interest in this technique by proving it as a simple and useful guide to determine the nutrient (such as protein and carbohydrate) disappearance in the rumen. After years of modification and development, the *in situ* technique has now become a reliable and rapid way to estimate the rate and extent of degradation of feedstuff in the functioning rumen (Ørskov et al., 1979 and 1980).

Despite the merits of the *in situ* method, there are also some limitations. As mentioned by Ørskov et al. (1980), the samples used in the technique are not influenced by the chewing and rumination. The particles in the artificial bags could not leave the rumen unless broken down, not necessary to simple chemical compounds, but to a size that is smaller than the pore size.

Therefore, the dimensions and pore size of the artificial bags, particle size and amount of feed in each bag, incubation time in the rumen are all critical factors that affect the outcome. The pore size was suggested to be between 15-40 μm to neither restrict microbial colonization and trap fermentation gases, nor lose solubles and undegradable particles (Mohamed and Chaudhry, 2008).

The degradation of feeds in the rumen is largely related to the colonization of rumen microorganisms to the plant tissues and the microbial colonization could significantly impact the estimation of ruminal protein degradation, especially for forages that are high in fiber and low in nitrogen content (Wanderley et al., 1999).

2.4.4 Prediction of Truly Protein Supply in Small Intestine Using Models

Several nutrition models were developed in the period from 1970 to 2010 and used to predict the true protein supply to the small intestine and feed milk protein production (Nuez-Ortín, 2010). The DVE/OEB Model and the NRC Dairy 2001 are two of these models. Both of them are modern models based on similar principles, but there are also differences due to the slight differences in conceptions and the use of different factors in the formulas, including the determination of endogenous protein losses, microbial protein synthesis and rumen bypass protein (Yu et al., 2003a, b).

The DVE/OEB Model was first developed in 1991 and introduced and revised by Tamminga et al. (1994, 2007), mainly used in European countries. The most updated version is available in Van Duinkerken et al. (2011). According to the results from chemical analysis, ruminal kinetics and protein digestibility, the DVE value is calculated as: DVE = ABCP +AMP – ENDP, where ABCP is truly absorbed rumen bypass protein in small intestine; AMP is truly absorbed microbial protein in small intestine; ENDP is endogenous protein losses in the digestive tract, which is related to the amount of undigested DM extracted in the feces. The OEB value is the balance between microbial protein synthesized from available rumen degradable protein and that from the energy extracted from the anaerobic fermentation in the rumen. Detailed equations could be found in Tamminga et al. (1994, 2007) and Yu et al. (2003a, b).

In contrast, the NRC Dairy Model is developed in 1985 and has been regularly updated. The newest version is the 7th revision in 2001. The NRC Dairy model is comparatively popular in research areas in North America and Asia. Information on TDN values of the feed is required when using this model. The major concept in this model is metabolizable protein (MP), defined as "truly digested and absorbed protein in small intestine". The MP value is calculated as: MP = ARUP + AMP + ENDP, where ARUP is truly absorbed rumen undegraded protein in small intestine; AMP is truly absorbed microbial protein in the small intestine; ENDP is endogenous protein that contributes to duodenal protein. The sources of ENDP include saliva, respiratory tract, mouth, esophagus, reticulo-rumen, omasum and abomasum (National Research Council, 2001). More detailed comparison between the two models could be found in Yu et al. (2003a,b), Yu (2005a), Gamage and Yu (2013) and Theodoridou and Yu (2013).

2.5 Microprobing the Structural Architecture of Cereal Seeds Using Mid-IR Microspectroscopy

2.5.1 Molecular Spectroscopy Techniques

According to wavelengths from short to long, the electromagnetic spectrum includes Gamma rays, Hard X-rays, Soft X-rays, ultraviolet, visible light, Near Infrared, Mid Infrared, Far Infrared, microwaves and radio waves. Gamma rays have the highest frequency and strongest penetration ability. They are produced by the most energetic objects in the universe, or by nuclear explosions, lighting and radioactive decay on earth. X-rays are widely used in medical and science areas, while Soft X-rays are also used to analyze the characterization of different layers of plant tissues (Karunakaran et al., 2009; Yu, 2012). Near Infrared (NIR), as well as Mid Infrared are effective tools in feed analysis and quality assessment. The Mid-IR spectral region (ca. 4000-400 cm^{-1}) is a domain of interest to many scientific areas because many molecules have strong characteristic vibrational transitions, especially in the wavenumber range of ca. 1800-800 cm^{-1}, which is also called the "fingerprint region" (Liu, 2009; Schliesser et al., 2012; Yu, 2004).

As we know, plants are made up of molecules and the internal molecular energy consists of the electronic, translational, rotational and vibrational energies. Under normal conditions, the functional groups in organic molecules vibrate independently and only interact weakly with each other. However, the interference from outside, such as electromagnetic radiation, could trigger the non-equilibrium phase and cause the energy transitions between the rotational and vibrational energies, which induce the net change in the electric dipole moment and the absorption of the IR. As the ratio of absorption and transmission IR differs between molecules, nearly every molecular species gives a unique IR absorption spectrum. Hence, IR spectrometry could be used to identify molecular functional groups (Yu, 2004).

2.5.2 ATR-FTIR Molecular Spectroscopy Techniques

2.5.2.1 Working Principles

Attenuated Total Reflectance Fourier Transform Infrared (ATR-FTIR) is a globar-sourced FTIR spectroscopy, which could be used to identify the molecular constituents in a wide range of samples in areas like physics, chemistry and biology. ATR-FTIR is mainly the combination of the globar light source and a microscope and based on the attenuation effect of light (Kazarian and Chan, 2013; Yu, 2004).

The core part of a FTIR spectrometer is the Michelson interferometer (Fig. 2.2, Adapted from McCluskey, 2000). The collimated light from the broadband source travels through the beamsplitter and gets split into two beams, one travels through the splitter and reflects off a movable mirror, the other travels to a fixed mirror and reflects back. A portion of the light finally reaches the sample that may be placed in a liquid-helium cryostat with IR-transparent windows made of ZnSe, KBr or polypropylene. In an ATR-FTIR machine, a crystal made of material such as zinc selenide (ZnSe), germanium (Ge) and thallium-iodide is placed under the samples and the incident beam entering at an angle larger than the critical angle, the total reflection could be achieved and only the part of energy that is absorbed by the sample is lost during the process (Ochiai, 2015; Stuart, 2004). The part that passes through the sample is sensed by the detector, which could be a photoconducting detector such as Ge:Cu placed right behind the sample or a

mercury-cadmium-telluride (MCT) mounted in the outside (McCluskey, 2000). In this way, all spectral elements are measured simultaneously on the detector and the time consumption (Fellget advantage or multiplex advantage) depends primarily on the movement of the movable mirror, which could be very short (Herres and Gronholz, 1984; Stuart, 2004).

This technology has a high spectral resolution, a broad measure range and short measure time (McCluskey, 2000). At the same time, with no slits to attenuate the infrared light, FTIR has a higher throughput of radiation compared to conventional IR methods (Jacquinot advantage) (Herres and Gronholz, 1984). Another advantage of ATR-FTIR is it only requires simple sample preparation by finely grinding them and depositing a thin layer on the infrared transparent windows (Kazarian and Chan, 2013).

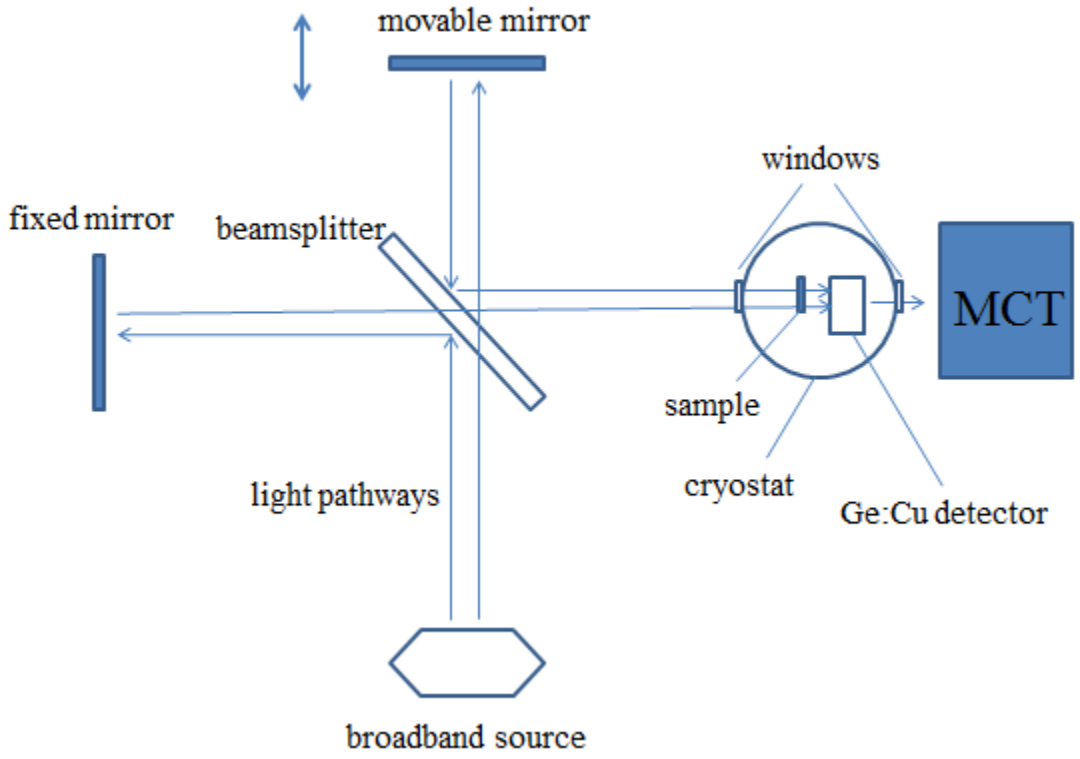

Figure 2.2 Schematic diagram of a FTIR spectrometer (Adapted from McCluskey, 2000)

Nevertheless, the technique also has its shortcomings. Due to the limited brightness, when ATR-FTIR is used to analyze a small region of interest, the decreased aperture would result in the diffraction effects and reduce the signal-to-noise ratio (Yu, 2004). It is reported that as the plant

cell size is normally between 5-30 μm, the globar sourced FTIR is not able to obtain a good signal-to-noise ratio within this dimension (Yu, 2004).

2.5.2.2 Application of ATR-FTIR Techniques in Feed Research

The ATR-FTIR technique has been proven to be effective in mineral samples in oil shale (Palayangoda et al., 2012) and biological samples such as cytology and tissue sections, live cells or biofluids (Baker et al., 2014), and plant samples including transgenic alfalfa (Jonker, 2011) and hulless barley (Yang et al., 2014) .

2.5.3 Synchrotron-based Molecular Spectroscopy Techniques (SR-IMS)

2.5.3.1 Working Principles and Advantages

The biggest advantage of SR-IMS is it could preserve the information about the spatial distribution of the objects when detecting the inner structures. This is achieved by using the synchrotron infrared light source, which is 100-1000 times brighter than the globar source (Yu, 2004). The non-divergent, intense and extremely fine beamline is created by a giant particle accelerator that turns electrons into light (Yu, 2004). Therefore, high spatial resolution and signal to noise spectra can be collected at a faster speed (Yu, 2004).

2.5.3.2 Novel Application of SR-IMS Techniques in Feed Research

The SR-IMS technology was first applied to animal feed research in 1999 (Kondo et al., 1999). Since then it has been utilized on several feeds, including transgenic alfalfa (Yu et al., 2009; Yu, 2010), hulless barley (Yu, 2004; Yang, 2013), canola seeds (Yu, 2004, 2005b), corn (Yu, 2010), flaxseeds (Yu, 2005b, 2010), sorghum seeds (Yu, 2011), wheat (Yu et al., 2007; 2010), wheat DDGS (Yu, 2010) and corn DDGS (Yu, 2010). In spite of all these applications, this research is still in its infancy.

2.5.4 Spectra Analysis

Functional groups such as amide I and amide II bonds have certain percentages of C=O, C-N and N-H stretching vibrations, the wavenumbers (per cm) at which they are absorbed are generally fixed, but they also slightly shift depending on the samples (Yu, 2004). Some typical IR absorption bands include: amide I (centered at about 1650 per cm, includes about 80% C=O stretching, 10% C-N stretching and 10% N-H bending), amide II (centered at about 1550 per cm, includes about 60% N-H bending and 40% C-N stretching), lipid carbonyl C=O (peaks at about 1738 per cm) and cellulose (at about 1100 per cm) (Jackson and Mantsch, 1996). Among them, Amide I and II are the most dominant vibrational bands of the protein backbone and amide I, due to its high C=O stretching composition, is the most sensitive and highly related to secondary structural elements of proteins (Kong and Yu, 2007).

2.5.4.1 Univariate Analysis

Using Univariate Analysis, it is possible to discover quantitative differences in the spectra information, such the component areas, peak heights and ratios between different components. Univariate analysis gives very straightforward results in terms of what changes occurred on the mathematical parameters characterizing the spectrum, such as the band intensities, integrated intensities, band frequencies and the band intensity ratios. In addition, this method makes it possible to connect the spectra information to the biological meaning on a mathematical basis (Liu, 2011).

2.5.4.2 Multivariate Analysis

Multivariate analysis is capable of analyzing multiple variables at same time. Principal component analysis (PCA) and hierarchical cluster analysis (CLA or HCHA) are two of the commonly used methods.

The PCA transforms the original set of variables based on the correlations among them, into a set of independent linear combinations called principal components (PCs) which contains most of the information in the original variables and empirically summarizes their correlations (Tabachnick and Fidell, 2007; Yu et al., 2007).The first few PCs usually account for more than 95% of the total variation among the variables (Yu, 2010).

The CLA is another data reduction method that calculates a distance matrix, searches for the two most similar objects and displays the results as dendrograms (Jobson, 1992; Yu et al., 2007). In the hierarchical approach, the object or objects are gathered as a group step by step, being nested to the previous groups. Thus, the number of clusters reduces sequentially as the clusters' sizes grow and end up with only one (Jobson, 1992).

2.6 Summary

Cereal grains are rich in energy, which mainly exists in the form of starch. Besides that, they are also comprised of protein, NDF, lipid and limited amount of vitamins. Wheat, triticale and corn share many similarities in kernel structure and nutrient composition. However, differences remain in the distribution of starch granules, levels of nutrient values, energy content, rumen degradation rate, and probably the response to heat treatment.

To optimize feeding efficiency, grains are sometimes heat-processed before feeding to animals. Heat treatment can reduce the rumen degradation rate of some plant-based feed, shifting the digestion of protein from the rumen to the small intestine, especially when moisture is included. With the participation of moisture, starch gelatinization and retrogradation could occur. As it is hard to control the heating condition to every seed, they are easy to either over- or under-heat and there are also possibilities that Maillard reaction can take place which could make the condition more complicated.

In order to better understand how different heat processing methods would affect cereal grains, conventional experiments such as the *in situ* trial and *in vitro* procedure need to be done to find out the changes on degradability and digestibility of nutritional components. Models like CNCPS, DVE/OEB and NRC-2001 could also be used to estimate the heat-induced effects on nutrient

supply for ruminants. Furthermore, advanced mid-IR approaches (e.g. ATR-FTIR and SR-IMS) can help to detect the changes that are associated with animal nutrition on inner structure and functional groups in the grain tissues caused by the heat treatments.

2.6.1 Hypotheses

1) In cereal grains, the sensitivity and responses of functional groups to heat processing differ and can be detected by SR-IMS as well as ATR-FTIR.
2) Different functional groups such as amide bonds in one type of cereal grain respond differently to different heating methods.
3) Different types of cereal grains respond differently to different heating methods. Their nutritional values, digestion sites and nutrient availability are altered by the heat treatments in different degrees.
4) Heat-induced structural changes in spectral areas of amide, CHO and cellulosic compounds detected by SR-IMS and ATR-FTIR are highly related to nutrient availability of the cereal grains in dairy cattle.

2.6.2 Overall Objectives

The objectives of the research are:

1) To use advanced Synchrotron based technique (SR-IMS) and Fourier transform infrared spectroscopy (ATR-FTIR) to directly detect the sensitivity and responses of various chemical functional groups in different types of cereal grain (wheat, triticale and corn) tissues altered by two different types of heat processing methods (moist heating vs. dry heating) in relation to nutrient utilization and availability in ruminants.
2) To increase our basic knowledge of heat effect on feed molecular structure.

3 Heat-Induced Changes in Chemical and Nutrient Profiles, Rumen Degradation and Intestinal Digestion of Different Types of Cereal Grains: Comparison of Control vs. Dry Heating vs. Moist Heating

3.1 Introduction

Cereal grains are considered as essential energy sources for modern dairy cattle. However, due to their highly fermentable characteristics, cereal grains, especially wheat and triticale, are prone to cause metabolic problems in ruminants such as acute or sub-acute rumen acidosis (Faldet et al. 1989; Nikkhah, 2014). To avoid these conditions, it is necessary to choose less degradable grain types， or take measures such as applying heat treatments on raw grain kernels, to increase the intermolecular starch-protein, starch-fat and protein-fat bounds which could reduce the exposure time of grain kernels in the rumen and prevent the instant accumulation of organic acids (Nikkhah, 2014).

As to the three types of grains, wheat is highly degradable and expected to be sensitive to the heat treatments, triticale has many similar traits compared to wheat, corn is less degradable due to its physiochemical structure of the vitreous endosperm (Nikkhah, 2014). In this Chapter, conventional methods were used to determine the nutrient values, energy levels, nutrient degradability and digestibility in the three types of cereal grains and models including CNCPS, DVE/OEB and NRC-2001 were used to detect the protein and carbohydrate availability of the grains in the digestion tract. Hence, it is possible to find out the different effects caused by the two different heat treatments.

The hypotheses were: 1) Detectable changes in nutrient values, rumen kinetics and digestion kinetics would be caused by heating at 121°C for 80 minutes; 2) The heat treatments could reduce the rumen degradation rates, shifting the digestion site of protein from rumen to the small intestine; 3) Different grains have different sensitivity to different heating methods and may react differently.

3.2 Material and Methods

3.2.1 Cereal Grains and Heat Treatment

"CDC Go", the wheat variety which was used in the study, was hard red spring wheat developed by Crop Development Centre (CDC), University of Saskatchewan, registered on 2003 and obtained in 2011. This variety of wheat is mainly grown in areas of Western Canada. The triticale variety used in the study was "AC Ultima", a type of hexaploid spring triticale that registered on 1999 by Agriculture Canada (McLeod et al., 2000). The triticale was harvested in 2011. The corn seeds were Dekalb products from Monsanto. They were also harvested in 2011, purchased in Minnesota, USA. All the grain samples before processing were stored in sealed plastic bags at room temperature.

Each grain source was divided into two batches (about 1 kg in each batch) and each batch was processed at different time. The six piles of grains were then split into three groups, one as control group without any processing (raw), and the other two were processed using dry roasting (dry heating, DH) and autoclaving (moist heating, MH), separately, without being ground or cracked. Both heat treatments lasted 80 minutes at 121°C. The dry roasting was conducted using a Forced Air Oven (Isotemp 750 F, Fisher Scientific). According to the settings of the autoclaving machine (Eagle 3031-S Scientific Gravity Sterilizer, Amsco, Steris), the autoclaving included three periods. The first period was 30 minutes of sterilization, during which time the chamber pressure was 20 psi and jacket pressure was 25 psi (atmosphere pressure = 14.7 psi). Followed was fast exhaust, which lasted for only seconds. After the exhaust, the chamber pressure went down to 0 psi but jacket pressure remained at 25 psi. The last period was dry cycle for 50 minutes. Moisture was applied only during sterilization. According to American National Standard (ANSI/AAMI ST79, 2012), less than 3% of moisture (by weight) was contained in the steam infused into the autoclave machine. Considering the size of the autoclave machine (24×36×36 inches (610×914×914 mm)) and the high pressure during sterilization, 3% was still a lot of moisture.

3.2.2 Animals and Diets

Three non-lactating Holstein Friesian cows with rumen cannula were used in an *in situ* trial to study the rumen degradation characteristics of cereal grains. Internal diameter of the cannula was 10 cm (Bar Diamond, Parma, ID). The cows were housed with *ad libitum* access to water and fed twice a day at 0800 and 1600 with 15 kg (on as fed basis) of a total mixed ration. The ration was a mixture of 50% barley silage and 50% concentrate, which consisted of wheat, barley, oats, molasses and dairy supplement pellets (Damiran and Yu, 2012). Water was available to the animals *ad libitum*. The animals were kept in the dairy barn in the University of Saskatchewan, housed in open pens with shelter and cared in accordance with the guidelines of the Canadian Council on Animal Care (CCAC, 1993).

3.2.3 Chemical Profiles

A portion of the grains were ground [Retsch ZM-1, Brinkmann Instruments (Canada) Ltd., Mississauga, ON, Canada] through 1 mm and 0.5 mm (for total starch analysis) screen. Samples were analyzed for dry matter (DM) (AOAC 930.15), ash (AOAC 942.05), crude protein (CP) (AOAC 984.13) and crude fat (ether extract, EE) (AOAC 920.39) according to AOAC (1990). Acid detergent fiber (ADF), neutral detergent fiber (NDF), and acid detergent lignin (ADL) were analyzed using ANKOM F57 filter bags (ANKOM Technology Corp., Fairport, NY), according to the procedures reported by Van Soest et al. (1991). Neutral detergent insoluble crude protein (NDICP) and acid detergent insoluble protein (ADICP) were determined using NDF (without adding sodium sulfide) and ADF residues, following methods described by Licitra et al. (1996). Soluble crude protein (SCP) was detected by incubating the samples at 39°C with borate-phosphate buffer and filtering through Whatman #54 fulter paper (Roe et al., 1990). Starch was analyzed using Megazyme total starch assay (AOAC 996.11, AACC 76.13, ICC standard method No. 168). Carbohydrates (CHO) were calculated as 100 – (ash + CP + EE). Hemicellulose was computed as (NDF – ADF) and cellulose as (ADF – ADL) (National Research Council, 2001).

3.2.4 Protein and Carbohydrate Fractions

The Level 2 Cornell Net Carbohydrate Protein System (CNCPS) version 6.1 was used to estimate the protein and carbohydrate fractions, as well as rumen degradable protein (RDP), rumen undegradable protein (RUP), rumen degradable carbohydrates (RDC) and rumen undegradable carbohydrates (RUC) in the three types of cereal grains (Jonker et al., 2010).

Protein was divided into five fractions based on different degradation rate in rumen. PA fraction, which represents ammonia in version 6.1, was estimated to be zero for the cereal grains samples. The sub-fraction PB1 was calculated as (SCP-PA), PB3 as (NDICP-ADICP) and PB2 as the difference (100-PA-PB1-PB3-PC).

Carbohydrate was divided into eight fractions. The amount of volatile fatty acids (VFA, CA1), lactic acids (CA2) and organic acids (CA3) is close to zero in cereal grains. Sugar (CA4) content was measured using the colorimetric method (Dubois et al., 1956). Starch (CB1) was analyzed using the procedure developed from Megazyme total starch assay (AOAC 996.11, AACC 76.13, ICC standard method No. 168) while CC and CB3 were calculated as:

$$CC_j \text{ (g/kg DM)} = (NDF_j \times Lignin_j \times 2.4) / 1000;$$

where, $Lignin_j$ is lignin content (g/kg DM) of the jth feed on NDF basis.

$$CB3 \text{ (g/kg DM)} = (NDF_j - CC_j)$$

where, NDF_j is the NDF of the *j*th feed (assayed with amylase and sodium sulfide, g/kg DM).
CB2 was computed by difference:

$$CB2 \text{ (g/kg DM)} = NFC_j - CA1_j – CA2_j – CA3_j – CA4_j – CB1_j$$

where, $NFC_j = CHO_j – NDF_j$ (Lanzas et al., 2007a: Tylutki et al., 2008).

Modifications were also made on degradation rates (K_d) and passage rates (K_p) in version 6.5 (Van Amburgh et al., 2010). Specific K_d values for each feed were found in the feed library of CNCPS version 6.1, Table 4 of Lanzas et al. (2007a) and Tables 1 and 2 of Van Amburgh et al. (2010). Among which, K_d values for triticale were assumed to be the same as those for wheat. K_p is estimated as 6%/h for all fractions but 12%/h for CA4 and PA, as mentioned in Table 2, Van Amburgh et al. (2010).

3.2.5 Energy Values

The total digestible nutrient (TDN) fractions, including truly digestible non fiber carbohydrate (tdNFC), truly digestible CP for concentrates ($tdCP_c$), truly digestible NDF (tdNDF) and truly digestible fatty acids (tdFA) of the three types of grains were calculated based on the measured chemical profiles. The total digestible nutrient at maintenance level (TDN_{1x}) and digestible energy at maintenance level (DE_{1x}) were calculated based on the TDN fractions. The digestible energy at 3X maintenance level (DE_{p3x}) was computed as DE_{1x} multiplied by a calculated discount factor (National Research Council, 2001; Weiss et al., 1992). This DE_{p3x} was used to determine the metabolizable energy at a 3X maintenance level (ME_{p3x}); and the ME_{p3x} was used to determine the net energy at 3X maintenance level (NE_{p3x}). By multiplying DE_{1x} by 0.82, the metabolizable energy (ME) could be estimated and further used to calculate net energy for maintenance (NE_m) and net energy for gain (NE_g). All equations used for calculation were available in National Research Council (1996, 2001).

3.2.6 Rumen Incubation Procedure and Ruminal Degradation Kinetics

Three cannulated dry Holstein Friesian cows were used for the ruminal incubation. Nylon bags (10 × 20 cm) were made of Nitex nylon material (Screen Tech Inc., San Jose, CA) with a pore size of approximate 40 μm. The grain kernels were cracked using a roller mill (Sven Grain Mill, Apollo Machine and Products Ltd., Saskatoon, SK, Canada), with the roller gap set as 0.203 mm. This method guaranteed that even the smallest grain kernels (such as some wheat kernels) were cracked into at least two parts with the endosperm exposed, for the ruminal microbiota to colonize during incubation (McAllister et al., 1990b). About 7g of samples were put into each bag and the nylon bags were each tied about 2 cm below the top and put into a polyester mesh lingerie bag with a weight inside the bag and a string kept outside of the cannulae. The maximum number of bags in the rumen of each cow was 30. All 3 kinds of grains were randomly allocated to 3 cows. The bags were pushed down to the bottom of rumen to ensure sufficient contact with rumen fluid,

and incubated for 0, 2, 4, 8, 12, 24 and 48 h following a “gradual addition / all out” schedule (Yu et al., 2000).

After incubation, all the bags were hand rinsed using cold tap water without detergent in plastic tubs for 6 times, drained excess water then spread on metal trays and put into the dry oven at 55°C for 48 hours. The dry residues were pooled based on different kinds of grains, processing batches, treatments, *in situ* runs and incubation time, ground through 1 mm screen or 0.5 mm screen (for total starch assay) and stored in plastic vials for chemical analysis. Dry matter (DM, AOAC 930.15), crude protein (CP, LECO FP-528, AOAC 990.03), neutral detergent fiber (NDF, Van Soest et al, 1990), starch (AOAC 996.11, AACC 76.13, ICC standard method No. 168) and CHO were analyzed or calculated in the *in situ* residual samples. The disappearance of nutrients was calculated by the difference between the original samples and residue samples.

Rumen degradation parameters for DM, CP, NDF and CHO were determined by using the first-order rumen degradation kinetic model described by Ørskov et al. (1980) and Tamminga et al. (1994):

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

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

where, R(t) = residue percentage at t hours of incubation in the rumen (%), U = undegradable fraction (%), D = potentially degradable fraction (%), K_d = degradation rate (%/h), and T_0 = lag time (h).

The effectively degradable fractions (ED) and ruminally undegradable fractions (RU) of each nutrient were calculated using the following equations (National Research Council, 2001; Yu et al., 2003a,b):

$$ED = S + D \times K_d/(K_p + K_d)$$
$$RU = U + D \times K_p/(K_p + K_d)$$

where, S is the soluble fraction (%), K_p is the outflow of digesta from rumen, which was assumed to be equal to 6%/h (Tamminga et al., 1994; Heendeniya et al., 2012; Damiran et al., 2013).

3.2.7 Potential Nitrogen-to-Energy Synchronization

In order to utilize the nutrients efficiently, it is important to balance the ratios of rumen available protein to energy and synchronize them to optimize the microbial synthesis. Based on the results of the *in situ* trial, the total and hourly ratios of N to energy (CHO, OM) could be calculated, while the effective degradation of N, CHO and OM was computed according to Sinclair et al. (1993):

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

3.2.8 Intestinal Digestibility

The 12 hour residue from the *in situ* incubation was used in a three-step *in vitro* procedure according to Calsamiglia and Stern (1995), to estimate the intestinal digestion of crude protein (CP) in ruminants. Residue samples that contain about 15 mg of residual N were weighed to go through the three steps, including 1) adding 10 mL of a 0.1 N HCl solution containing 10 mg pepsin (Sigma P-7000, Sigma) and incubating in 38°C water bath for 1 h; 2) adding 0.5 mL of 1 N NaOH to neutralize pH and 13.5 mL of a phosphate buffer (pH 7.8) containing 37.5 mg pancreatin (Sigma P-7545, Sigma), incubating at 38°C for 24 h; 3) adding 3 mL of a 100% (wt / vol) trichloroacetic acid (TCA) solution to precipitate the undigested proteins. The samples were vortexed after adding each solution and every couple hours to ensure good mixture of sample and enzymes. After centrifuging, the supernatant was analyzed using Kjeldahl method (AOAC 984.13). Intestinal digestion of neutral detergent fiber (NDF) and starch were calculated using the parameters on rumen degradation kinetics gathered from the rumen incubation study above. The 48 h residues were considered as indigestible.

3.2.9 Prediction of Protein Supply in Small Intestine

3.2.9.1 DVE/OEB Model

Results from chemical analysis, ruminal kinetics and protein digestibility were used in the model. The equations and methods for the DVE/OEB Model could be found in Tamminga et al. (1994, 2007).

3.2.9.2 NRC Dairy 2001 Model

The detailed methods and steps to estimate the protein supply in small intestine is provided in National Research Council (2001). The concept of OEB was borrowed from the DVE/OEB Model to compare the difference of the two kinds of nutrition models.

3.2.10 Feed Milk Values

Estimating the feed milk values (FMV) is one of the objectives that Tamminga et al. (1994, 2007) established the DVE/OEB model. It is calculated based on the DVE value estimated from the model. Similarly, the FMV predicted from the NRC-2001 model means the “MP allowable milk production” (Lanzas et al., 2007b). The protein supply available for the animals to utilize should not be below the energy-allowable milk production, otherwise milk production would be affected (Lanzas et al., 2007b). The constant efficiency factor was assumed to be 0.67 (National Research Council, 2001) for both models and the milk protein level was assumed to be 3.3%.

3.2.11 Statistical Analysis

The experimental design in this research was RCBD with a 3×3 factorial arrangement. The two factors are “grain types” (wheat, triticale and corn) and “processing methods” (raw, dry heating and moist heating). The iterative nonlinear regression procedure (PROC NLIN-Gauss-Newton

method of SAS, SAS Institute Inc., Cary, NC) was used to fit the rumen degradation data to the model.

The MIXED procedure of SAS (version 9.2; SAS Institute Inc.) was used for most of the statistical analyses. The model used for the analysis was as follows:

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

in which Y_{ijkl} was an observation of the dependent variable ijkl, μ was the population mean for the variable, F_i was the effect of cereal types; H_j was the effect of heating treatments, $F_i \times H_j$ was the interaction of grain type and heat effect, B_k was the random effect of processing batches and *in situ* runs, and e_{ijkl} was the random error associated with observation ijkl.

For all statistical analyses, significance was declared at $P<0.05$. Differences among the treatments were evaluated using a multiple comparison test following the Tukey method.

3.3 Results and Discussion

3.3.1 Heat-Induced Changes in Chemical and Nutrient Profiles of Different Types of Cereal Grains: Comparison of Control vs. Dry Heating vs. Moist Heating

3.3.1.1 Heat-Induced Changes in Chemical Profiles

As shown in Table 3.1, wheat, triticale and corn showed different characteristics in chemical composition, similar to previous reports in the literature (Government of Alberta, 2014). Dry matter (DM) was increased ($P<0.001$) by both dry and moist heating in all three types of grains and dry heating had more effect than moist heating. Neutral detergent fiber (NDF), neutral detergent insoluble crude protein (NDICP) and hemicellulose were increased, while soluble crude protein (SCP) was decreased by moist heating in wheat, triticale and corn. Other changes included increased acid detergent fiber (ADF) in corn, acid detergent insoluble crude protein (ADICP) in wheat and corn, ether extract (EE) in wheat and decreased cellulose in triticale, after moist heating. These results indicated that heat induced changes in the cell wall. Samadi and Yu (2011) and Jahani-Azizabadi et al. (2010) also found similar results in soybean seeds and guar meal,

respectively. Karsli and Russell's (1999) research on alfalfa and berseem clover and Seifdavati and Taghizadeh's (2012) study on legumes also showed an increase in NDF and ADF levels after heat treatment, with the effect greater as the processing temperature increases. The amount of crude protein (CP), starch and total carbohydrate (CHO) were not changed. This does not mean that heat did not affect protein and starch, as changes may have been made at a structural level.

Table 3.1 Heat-Induced Changes in Chemical Characteristics of Cereal Grains: Raw vs. Dry Heating vs. Moist Heating

Item	Wheat			Triticale			Corn			SEM	P value		
	Raw	DH	MH	Raw	DH	MH	Raw	DH	MH		Feed	Heating	F*H
DM, %	90.3^{c}	92.1^{a}	91.1^{b}	90.3^{c}	92.4^{a}	91.4^{b}	86.5^{e}	90.0^{c}	88.4^{d}	0.08	<0.001	<0.001	<0.001
Ash, %DM	1.88	1.86	1.86	1.80	1.80	1.78	1.29	1.32	1.30	0.01	<0.001	0.357	0.301
CP, %DM	19.1	18.8	18.8	11.9	11.7	11.8	7.72	7.60	7.42	0.28	<0.001	0.026	0.393
EE, %DM	0.95^{c}	0.90^{c}	1.53^{b}	1.77^{b}	1.73^{b}	1.94^{b}	2.78^{a}	3.08^{a}	2.79^{a}	0.10	<0.001	0.018	0.003
NDF, %DM	11.4^{c}	11.2^{c}	16.2^{a}	13.0^{b}	13.2^{b}	15.8^{a}	9.1^{d}	9.1^{d}	13.0^{b}	0.47	<0.001	<0.001	<0.001
ADF, %DM	2.83^{bc}	2.94^{b}	3.06^{ab}	3.22^{a}	3.09^{ab}	3.32^{a}	2.08^{d}	1.93^{d}	2.64^{c}	0.06	<0.001	<0.001	<0.001
ADL, %DM	0.83	0.92	1.34	0.78	0.77	1.61	0.31	0.32	0.87	0.09	<0.001	<0.001	0.203
NDICP, %DM	1.20^{d}	1.08^{de}	5.16^{a}	1.08^{de}	1.03^{de}	4.21^{b}	0.62^{e}	0.74^{de}	2.53^{c}	0.14	<0.001	<0.001	<0.001
NDICP, %CP	6.91^{c}	6.25^{c}	30.1^{b}	10.1^{c}	9.48^{c}	38.9^{a}	9.32^{c}	10.75^{c}	38.6^{a}	1.15	<0.001	<0.001	0.042
ADICP, %DM	0.00^{c}	0.00^{c}	0.07^{b}	0.00^{c}	0.00^{c}	0.03^{c}	0.00^{c}	0.00^{c}	0.34^{a}	0.01	<0.001	<0.001	<0.001
ADICP, %CP	0.00^{c}	0.00^{c}	0.44^{b}	0.00^{c}	0.00^{c}	0.24^{bc}	0.00^{c}	0.00^{c}	5.16^{a}	0.08	<0.001	<0.001	<0.001
SCP, %DM	5.20^{a}	5.32^{a}	1.91^{bc}	4.89^{a}	4.88^{a}	1.77^{bc}	2.24^{b}	1.97^{b}	1.03^{c}	0.34	<0.001	<0.001	<0.001
SCP, %CP	30.0^{b}	30.7^{b}	11.1^{c}	45.7^{a}	45.0^{a}	16.4^{c}	33.5^{b}	28.7^{b}	15.7^{c}	1.83	<0.001	<0.001	<0.001
Starch, %DM	61.3	59.8	60.7	69.8	66.3	66.1	80.2	77.0	75.8	1.07	<0.001	0.004	0.469
CHO, %DM	78.0^{c}	78.5^{c}	77.8^{c}	84.6^{b}	84.7^{b}	84.5^{b}	88.2^{a}	88.0^{a}	88.5^{a}	0.31	<0.001	0.363	0.009
Hemicellulose, %DM	8.60^{cd}	8.27^{de}	13.1^{a}	9.80^{bc}	10.14^{b}	12.5^{a}	6.99^{f}	7.21^{ef}	10.4^{b}	0.50	<0.001	<0.001	0.001
Cellulose, %DM	2.00^{abc}	2.02^{abc}	1.73^{c}	2.43^{a}	2.32^{ab}	1.72^{c}	1.77^{bc}	1.61^{c}	1.76^{c}	0.12	<0.001	0.005	0.021

Note: DH = dry heating; MH = moist heating; Feed = feed effect (wheat, triticale, corn); Heating = heating effect (raw, dry heating, moist heating); F*H = interaction between feed effect and heating effect; DM = dry matter; OM = organic matter; CP = crude protein; EE = ether extract (=crude fat); NDF = neutral detergent fiber; ADF = acid detergent fiber; ADL = acid detergent lignin; NDICP = neutral detergent insoluble crude protein; ADICP = acid detergent insoluble crude protein; SCP = soluble crude protein; CHO = carbohydrates; SEM = standard error of mean; Means with different letters within the same row differ ($P<0.05$).

3.3.1.2 Heat-Induced Changes in Protein and Carbohydrate Fractions

Based on the CNCPS (version 6.1), the protein and CHO fractions of wheat, triticale and corn are shown in Table 3.2. Moist heating significantly decreased the PB1 fraction of all three types of grain, which means the protein in the grain are less soluble after autoclaving. The PB2 fraction in corn was also reduced by moist heating, indicating that the degradable protein in corn degrades slower. As a consequence, the PB3 fractions in all three types of cereals increased after being moist-heated, so do the PC fractions in wheat and corn.

Since soluble fiber (CB2) was calculated by difference, any error in the estimation of the four CA fractions, CB1 and CC fractions would lead to the over- or under-estimation of CB2 fraction. Some of the measured starch and sugar values were higher than the range reported by Dairy One (Ithaca, NY). As a result, some negative values were found in CB2 fraction and they were adjusted to zero (Higgs et al., 2012).

CNCPS model contains integration between animal and the environment and integration between physiological factors and metabolic conditions, which is the biggest advantage of the model, compared to other models. However, in the case of this study, the predicted rumen degradable and undegradable nutrients may not be very precise due to the limited size of the CNCPS feed library. Degradation rates would change after heat processing, but in the feed library, K_d values for each sub-fraction were only available for wheat and corn, without considering any heat treatment. No K_d value for triticale or any heated grain seeds were found in the CNCPS feed library or other available literature. Thus the same K_d values were used for all grains. Such limitation may cause the results of rumen degradable/undegradable nutrients to be somewhat less accurate, especially for the dry/moist heated grains.

Table 3.2 Heat-Induced Changes in the Protein and Carbohydrate Fractions of Cereal Grains: Raw vs. Dry Heating vs. Moist Heating, Using CNCPS 6.1 Version

Item	Wheat			Triticale			Corn			SEM	P value		
	Raw	DH	MH	Raw	DH	MH	Raw	DH	MH		Feed	Heating	F*H
PB1	27.1^{b}	28.3^{b}	10.1^{c}	41.2^{a}	41.6^{a}	15.0^{c}	29.0^{b}	25.8^{b}	13.9^{c}	1.65	<0.001	<0.001	<0.001
PB2	66.7^{a}	66.0^{a}	62.4^{a}	49.7^{b}	49.7^{b}	49.5^{b}	63.0^{a}	64.5^{a}	51.4^{b}	2.27	<0.001	<0.001	0.002
PB3	6.23^{c}	5.75^{c}	27.0^{b}	9.07^{c}	8.76^{c}	35.3^{a}	8.06^{c}	9.68^{c}	29.5^{b}	1.07	<0.001	<0.001	0.018
PC	0.00^{c}	0.00^{c}	0.44^{b}	0.00^{c}	0.00^{c}	0.25^{bc}	0.00^{c}	0.00^{c}	5.16^{a}	0.08	<0.001	<0.001	<0.001
CA4	4.53	4.78	4.20	5.67	6.05	5.13	4.43	4.35	4.03	0.41	0.001	0.204	0.962
CB1	78.5	76.2	78.0	82.5	78.3	78.3	90.9	87.5	85.6	1.37	<0.001	0.004	0.387
CB2	2.75	5.20	3.91	0.01	1.73	2.25	0.00	0.45	0.00	0.81	<0.001	0.073	0.550
CB3	11.6^{ab}	11.0^{bc}	9.76^{cd}	12.7^{a}	12.0^{ab}	9.78^{cd}	9.55^{cd}	9.09^{d}	10.7^{bc}	0.31	<0.001	<0.001	<0.001
CC	2.56	2.83	4.11	2.22	2.17	4.57	0.83	0.87	2.36	0.27	<0.001	<0.001	0.272
RDP	11.8	10.6	11.2	7.82	7.13	7.07	4.35	4.24	3.78	0.27	<0.001	0.004	0.201
RUP	4.53^{b}	4.19^{c}	6.14^{a}	2.80^{ef}	2.63^{f}	4.09^{c}	2.94^{e}	2.94^{e}	3.46^{d}	0.08	<0.001	<0.001	<0.001
RDC	61.6	62.0	61.5	69.0	67.6	66.0	59.9	58.1	57.1	0.69	<0.001	0.005	0.200
RUC	15.6	15.7	16.5	17.6	17.5	18.0	32.6	31.6	32.8	0.39	<0.001	0.014	0.375

Note: DH = dry heating; MH = moist heating; Feed= feed effect (wheat, triticale, corn); Heating = heating effect (raw, dry heating, moist heating); F*H = interaction between feed effect and heating effect; PB1 = true protein soluble in borate phosphate buffer and precipitated by TCA; PB2 = intermediately degradable protein; PB3 = insoluble in neutral detergent but soluble in acid detergent; PC = unavailable protein; CA4 = sugar; CB1 = starch; CB2 = soluble fiber; CB3 = available neutral detergent fiber (NDF); CC = unavailable NDF; RDP = rumen degradable protein; RUP = rumen undegradable protein; RDC = rumen degradable carbohydrates; RUC = rumen undegradable carbohydrates; SEM= Standard error of mean; Means with different letters within the same row differ (P<0.05).

3.3.1.3 Heat-Induced Changes in Energy Values

Among the three types of cereals, corn had the highest energy values (Table 3.3). Triticale is similar to wheat in total digestible nutrients (TDN), however, its energy values, such as DE, ME and NE, are low compared to wheat and corn. This is probably due to the relatively high fiber content in triticale.

The heat treatments had different effect on the energy values of different types of grains. The total digestible FA was increased in wheat (tdFA: 0.03 vs. 0.53, %DM) and total digestible NDF was reduced in triticale (tdNDF: 7.01 vs. 5.50, %DM) in response of the moist heating ($P<0.05$). Regardless of these changes, none of the energy values such as DE, ME and NE in wheat and triticale were significantly changed. While for corn, total digestible NFC (tdNFC: 78.2 vs. 76.5, %DM), DE (3.8 vs. 3.7, Mcal/kg), ME (3.13 vs. 3.06, Mcal/kg), NE_m (2.13 vs. 2.08, Mcal/kg) and NE_g (1.46 vs. 1.42, Mcal/kg) were all significantly decreased ($P<0.05$) by moist heating. As moist heating (steam flaking) usually increases the energy content (Theurer et al., 1999; Yu, 1996), the descending energy values of moist heated corn indicates that the corn kernels may be overheated during the autoclaving process.

Table 3.3 Heat-Induced Changes in Total Digestible Nutrients and Energy Values of Cereal Grains: Raw vs. Dry Heating vs. Moist Heating

Item	Wheat			Triticale			Corn			SEM	P value		
	Raw	DH	MH	Raw	DH	MH	Raw	DH	MH		Feed	Heating	F*H
TDN fractions													
tdNFC, %DM	66.45^{de}	66.98^{d}	65.43^{e}	71.17^{c}	71.09^{c}	71.41^{c}	78.18^{a}	78.01^{a}	76.45^{b}	0.632	<0.001	<0.001	<0.001
tdCPc, %DM	19.13	18.77	18.80	11.86	11.73	11.81	7.72	7.61	7.29	0.283	<0.001	0.008	0.173
tdNDF, % DM	5.73^{b}	5.51^{b}	5.51^{b}	7.01^{a}	7.23^{a}	5.50^{b}	5.45^{b}	5.38^{b}	5.84^{b}	0.276	<0.001	0.004	<0.001
tdFA, %DM	0.03^{c}	0.03^{c}	0.53^{b}	0.77^{b}	0.73^{b}	0.94^{b}	1.78^{a}	2.08^{a}	1.79^{a}	0.100	<0.001	0.032	0.008
TDN_{1x}, %DM	84.38^{c}	84.32^{c}	83.94^{c}	84.78^{c}	84.70^{c}	83.83^{c}	88.34^{a}	88.66^{a}	86.59^{b}	0.221	<0.001	<0.001	0.009
Energy values													
DE_{1x}, Mcal/kg	3.81^{a}	3.80^{a}	3.78^{a}	3.72^{bc}	3.72^{bc}	3.68^{c}	3.81^{a}	3.82^{a}	3.73^{b}	0.009	<0.001	<0.001	0.004
DE_{p3x}, Mcal/kg	3.37^{a}	3.36^{ab}	3.35^{ab}	3.29^{c}	3.28^{c}	3.26^{c}	3.33^{b}	3.33^{b}	3.28^{c}	0.006	<0.001	<0.001	0.005
ME_{p3x}, Mcal/kg	2.95^{a}	2.94^{a}	2.93^{ab}	2.87^{c}	2.86^{c}	2.84^{c}	2.91^{b}	2.92^{ab}	2.86^{c}	0.006	<0.001	<0.001	0.003
NE_{Lp3x}, Mcal/kg	1.89^{a}	1.88^{ab}	1.88^{ab}	1.83^{c}	1.83^{c}	1.81^{c}	1.86^{b}	1.86^{ab}	1.82^{c}	0.005	<0.001	<0.001	0.009
ME, Mcal/kg	3.12^{a}	3.12^{a}	3.10^{a}	3.05^{bc}	3.05^{bc}	3.02^{c}	3.13^{a}	3.13^{a}	3.06^{b}	0.008	<0.001	<0.001	0.012
NE_m, Mcal/kg	2.13^{a}	2.13^{a}	2.12^{ab}	2.07^{cd}	2.07^{cd}	2.05^{d}	2.13^{a}	2.14^{a}	2.08^{bc}	0.007	<0.001	<0.001	0.027
NE_g, Mcal/kg	1.46^{a}	1.45^{a}	1.45^{a}	1.41^{bc}	1.41^{bc}	1.39^{c}	1.46^{a}	1.47^{a}	1.42^{b}	0.005	<0.001	<0.001	0.006

Note: DH = dry heating; MH = moist heating; Feed= feed effect (wheat, triticale, corn); Heating = heating effect (raw, dry heating, moist heating); F*H = interaction between feed effect and heating effect; tdNFC = truly digestible non-fiber carbohydrates; tdCPc = truly digestible crude protein; tdNDF = trulyl digestible neutral detergent fibre; tdFA = truly digestible fatty acid; TDN_{1x} = total digestible nutrients; DE_{1x} (Mcal/kg) = digestible energy; DE_{p3x}(Mcal/kg) = digestible energy at a production level (3 x maintenance); ME_{p3x} = metabolizable energy at a production level (3 x maintenance); NE_{Lp3x} = Net eneryg at a production level (3 x maintenance); ME = metabolizable energy; NE_m = net energy for maintenance; NE_g = net energy for gain; SEM = Standard error of mean; Means with different letters within the same row differ (P<0.05).

3.3.2 Response of Different Types of Cereal Grains to Different Heating Methods in Ruminal and Intestinal Digestion in Dairy Cattle

3.3.2.1 Ruminal Digestion of Dry Matter

As Table 3.4 shows, the degradation rates (K_d) of wheat and triticale were higher compared to corn, which is consistent with what McAllister et al. (1991) observed. The moist-heated wheat and triticale showed a significant decrease in K_d ($P<0.05$), implying a slower degradation in the rumen. Autoclaving forage and grains (wheat and corn) could reduce the disappearance of dry matter (Lancaster and Patterson, 1988; McAllister et al., 1991). Similar results were found in this study: moist heating highly increased rumen bypass dry matter (BDM) for all three types of cereal grains.

3.3.2.2 Ruminal Digestion of Crude Protein

The amount of rumen undegradable protein (RUP, g/kg DM) and rumen bypass protein (BCP, g/kg DM) were increased ($P<0.05$) in wheat and triticale by moist heating, but not significantly changed ($P>0.05$) in corn (Table 3.5). This is mostly consistent with the findings of McAllister et al. (1991), who also mentioned that due to the structural difference between wheat and corn, wheat, with its loosely associated protein and starch, is more susceptible to the effects of autoclaving. Literature on legumes also showed lower solubility of protein and decreased degradation rate after autoclaving for 30 min (Goelema, 1999).

3.3.2.3 Ruminal Digestion of Neutral Detergent Fiber

As Figure 3.1 shows, moist heating increased the rumen bypass neutral detergent fiber (BNDF, g/kg DM) in corn compared to the unheated and dry heated samples. This result is consistent with Doiron's (2008) report in flaxseed that was autoclaved for 20, 40 and 60 min. Meanwhile, the effective degradable neutral detergent fiber (EDNDF, g/kg DM) also increased, when compared to those processed by dry heating ($P<0.01$). According to Table 3.6, significant

differences were found between the three types of cereal grains. The heat treatments also induced more BNDF and less EDNDF.

3.3.2.4 Ruminal Digestion of Starch

As shown in Table 3.7, none of the parameters in starch degradation was significantly changed by the heat processing, except for soluble starch (S) and degradable starch (D). Heat treatment resulted in less S and more D ($P<0.001$) starch in all three type of cereal grains. Moist heating has greater impact compared to dry heating. There was also a trend of having less effective degradability of starch (EDST) after the moist heating ($P=0.115$).

The gelatinization temperatures for the three types of grains are wheat (hard) 62.9 °C, triticale 55-62 °C, corn 62-72°C (Stone, 1996), all of which are lower than the processing temperature used in this study. During dry heating, the grains need to utilize the moisture contained inside the seeds to gelatinize, which may not be enough. For moist heating, however, we expected the occurrence of gelatinization to some degree. Wanderley et al. (1999) reported an increase in both ruminal degradation rate and potential degradability of sorghum starch after steam flaking, compared to dry roasting. Goelema (1999) shared research of similar results, but he also mentioned that prolonged autoclaving may be counterproductive.

3.3.2.5 Ruminal Digestion of Carbohydrates

As shown in Table 3.8, the degradation rates (K_d) in different cereal grains varied. The K_d of unprocessed corn (7.6 %/h) is about the half of those of unheated wheat (13.8 %/h) and triticale (15.1 %/h). The K_d values were significantly lower ($P<0.01$) in wheat and triticale after moist-heat treated. Similar trend was found in corn. The K_d descended from 7.6 %/h to 3.7 %/h after moist heating, however, this change was not statistically significant.

Rumen bypass carbohydrate (BCHO) in all three types of grain increased, while effective degradable carbohydrate (EDCHO) decreased after moist heating ($P<0.05$). These results imply

that similar to protein, the site of CHO digestion shifts from rumen to small intestine after the moist heat treatment.

Table 3.4 *In situ* Rumen Degradation Kinetics of Dry Matter in Cereal Grains: Raw vs. Dry Heating vs. Moist Heating

Item	Wheat			Triticale			Corn			SEM	P value		
	Raw	DH	MH	Raw	DH	MH	Raw	DH	MH		Feed	Heating	F*H
In situ rumen DM degradation													
K_d (%/h)	13.8^{ab}	12.1^{b}	2.84^{d}	15.1^{ab}	16.6^{a}	4.94^{cd}	7.55^{c}	7.15^{cd}	3.67^{cd}	0.93	<0.001	<0.001	0.001
T0 (h)	0.00	0.04	0.15	0.14	0.08	0.00	0.00	0.00	0.00	0.07	0.442	0.979	0.427
S (%)	7.03	6.15	3.79	8.52	9.23	5.66	15.3	15.2	13.8	0.91	<0.001	0.002	0.715
D (%)	80.8	83.1	82.1	78.6	77.4	76.4	75.1	75.4	76.9	2.05	0.003	0.961	0.824
U (%)	12.1	10.7	14.1	12.9	13.3	17.9	9.61	9.45	9.39	2.04	0.015	0.245	0.705
%BDM (=%RUDM)	37.1^{ef}	39.0^{d}e	70.0^{a}	35.3^{ef}	34.4^{f}	60.1^{b}	42.9^{cd}	43.9^{c}	57.5^{b}	0.92	<0.001	<0.001	<0.001
BDM (=RUDM, g/kg DM)	370.5^{ef}	389.8^{ed}	699.7^{a}	353.5^{ef}	344.1^{f}	600.8^{b}	428.9^{cd}	439.4^{c}	575.5^{b}	9.24	<0.001	<0.001	<0.001
%EDDM (=%RDDM)	63.0^{ab}	61.0^{bc}	30.0^{f}	64.7^{ab}	65.6^{a}	39.9^{e}	57.1^{cd}	56.1^{d}	42.5^{e}	0.92	<0.001	<0.001	<0.001
EDDM (=RDDM, g/kg DM)	630^{ab}	610^{bc}	300^{f}	647^{ab}	656^{a}	399^{e}	571^{cd}	561^{d}	425^{e}	9.24	<0.001	<0.001	<0.001

Note: DH = dry he DH = dry heating; MH = moist heating; ating; MH = moist heating; Feed= feed effect (wheat, triticale, corn); Heating = heating effect (raw, dry heating, moist heating); F*H = interaction between feed effect and heating effect; K_d = the rate of degradation of D fraction (%/h); T_0 = lag time in h; S = soluble fraction in the *in situ* incubation; D = degradable fractions; U = undegradable degradable fractions; K_p = passage rate of 6%/h was adopted (Tamminga et al., 1994). BDM = rumen bypass dry matter; EDDM = effective degradability of dry matter. SEM = Standard error of mean; Means with different letters within the same row differ (P<0.05).

Table 3.5 *In situ* Rumen Degradation Kinetics of Crude Protein in Cereal Grains: Raw vs. Dry Heating vs. Moist Heating

Item	Wheat			Triticale			Corn			SEM	P value		
	Raw	DH	MH	Raw	DH	MH	Raw	DH	MH		Feed	Heating	F*H
In situ rumen CP degradation													
K_d (%/h)	9.98	9.02	1.06	10.6	10.8	4.75	3.59	3.45	0.81	1.25	<0.001	<0.001	0.154
T0 (h)	0.61^{b}	0.91^{b}	1.64^{b}	0.20^{b}	0.04^{b}	5.78^{ab}	1.12^{b}	1.72^{b}	9.80^{a}	1.26	0.0142	<0.001	0.033
S (%)	8.40	6.44	7.02	14.5	16.0	13.5	30.8	27.9	27.4	1.65	<0.001	0.267	0.534
D (%)	84.3	87.9	93.0	76.9	75.7	86.5	68.0	70.2	72.6	1.76	<0.001	<0.001	0.094
U (%)	7.27^{a}	5.67^{ab}	0.00^{c}	8.59^{a}	8.27^{a}	0.00^{c}	1.17^{bc}	1.91^{bc}	0.00^{c}	1.12	<0.001	<0.001	0.017
%BCP	39.2	41.1	79.1	36.4	35.7	59.0	43.8	46.8	64.1	4.04	0.016	<0.001	0.071
RUP (g/kg DM)	75.7^{b}	79.7^{b}	153.9^{a}	47.4^{c}	46.7^{c}	77.6^{b}	42.0^{c}	43.8^{c}	60.2^{bc}	5.12	<0.001	<0.001	<0.001
BCP (g/kg DM)	84.0^{b}	88.5^{b}	170.8^{a}	52.6^{c}	51.9^{c}	86.1^{b}	46.6^{c}	48.6^{c}	66.8^{bc}	5.69	<0.001	<0.001	<0.001
%EDCP (=%RDP)	60.8	58.9	20.9	63.6	64.3	41.0	56.2	53.2	35.9	4.04	0.016	<0.001	0.071
EDCP (=RDP, g/kg DM)	117.4^{a}	114.5^{a}	40.7^{c}	82.7^{b}	84.3^{b}	54.3^{c}	53.9^{c}	49.7^{c}	33.7^{c}	5.54	<0.001	<0.001	<0.001

Note: DH = dry heating; MH = moist heating; Feed= feed effect (wheat, triticale, corn); Heating = heating effect (raw, dry heating, moist heating); F*H = interaction between feed effect and heating effect; K_d = the rate of degradation of D fraction (%/h); T_0 = lag time in h; S = soluble fraction in the *in situ* incubation; D = degradable fractions; U = undegradable degradable fractions; K_p = passage rate of 6%/h was adopted (Tamminga et al., 1994). BCP or RUP = rumen bypass or undegraded feed crude protein; EDCP = effective degradability of feed crude protein; SEM = Standard error of mean; Means with different letters within the same row differ (P<0.05).

Table 3.6 *In situ* Rumen Degradation Kinetics of Neutral Detergent Fiber in Cereal Grains: Raw vs. Dry Heating vs. Moist Heating

Item	Feed			SEM	P value	Heating			SEM	P value
	Wheat	Triticale	Corn			Raw	DH	MH		
In situ rumen NDF degradation										
K_d (%/h)	7.56^{b}	12.4^{a}	5.92^{b}	0.83	<0.001	8.95	8.09	8.87	0.83	0.720
T0 (h)	0.00	0.00	0.40	0.13	0.051	0.00	0.23	0.17	0.13	0.428
S (%, Washable)	1.71^{b}	0.46^{b}	13.1^{a}	1.47	<0.001	7.19	3.48	4.55	1.47	0.203
D (%)	36.2	39.9	35.5	2.99	0.448	39.0	39.0	32.6	2.99	0.115
U (%)	62.0	59.6	51.5	3.26	0.074	53.8	56.5	62.8	3.26	0.154
%BNDF (=%RUNDF)	72.0^{a}	66.6^{b}	65.7^{b}	1.01	<0.001	65.5^{b}	68.3^{ab}	70.5^{a}	1.01	0.006
BNDF (=RUNDF, g/kg DM)	78.4^{a}	81.4^{a}	64.4^{b}	0.96	<0.001	71.7^{b}	71.8^{b}	80.8^{a}	1.67	<0.001
%EDNDF (=%RDNDF)	28.0^{b}	33.4^{a}	34.3^{a}	1.01	<0.001	34.5^{a}	31.7^{ab}	29.5^{b}	1.76	0.006
EDNDF (=RDNDF, g/kg DM)	30.6^{b}	40.9^{a}	33.7^{b}	1.10	<0.001	37.7^{a}	33.5^{b}	34.0^{ab}	1.10	0.022

Note: DH = dry heating; MH = moist heating; Feed= feed effect (wheat, triticale, corn); Heating = heating effect (raw, dry heating, moist heating); F*H = interaction between feed effect and heating effect; K_d = the rate of degradation of D fraction (%/h); T_0 = lag time in h; S = soluble fraction in the *in situ* incubation; D = degradable fractions; U = undegradable degradable fractions; K_p = passage rate of 6%/h was adopted (Tamminga et al., 1994). BNDF = rumen bypass feed 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).

Table 3.7 *In situ* Rumen Degradation Kinetics of Starch in Cereal Grains: Raw vs. Dry Heating vs. Moist Heating

Item	Feed			SEM	P value	Heating			SEM	P value
	Wheat	Triticale	Corn			Raw	DH	MH		
In situ rumen starch (ST) degradation										
K_d (%/h)	9.77	10.1	6.83	1.79	0.231	9.62	9.43	7.68	1.79	0.591
S (%)	11.0^{c}	16.1^{b}	21.1^{a}	1.04	<0.001	19.6	17.1	11.5	1.04	<0.001
D (%)	89.0^{a}	83.9^{b}	78.9^{c}	1.04	<0.001	80.4	82.9	88.5	1.04	<0.001
%BST (=%RUST)	38.6	38.0	41.2	3.98	0.814	36.3	36.7	44.7	3.98	0.231
BST (=RUST, g/kg DM)	234.3	255.7	318.7	25.85	0.073	255.5	249.8	303.4	25.85	0.290
%EDST (=%RDST)	61.4	62.0	58.8	3.98	0.814	63.7	63.3	55.3	3.98	0.231
EDST (=RDST, g/kg DM)	371.6	418.4	457.7	25.91	0.080	448.6	427.3	371.8	25.91	0.115

Note: DH = dry heating; MH = moist heating; Feed= feed effect (wheat, triticale, corn); Heating = heating effect (raw, dry heating, moist heating); F*H = interaction between feed effect and heating effect; K_d = the rate of degradation of D fraction (%/h); T_0 = lag time in h; S = soluble fraction in the *in situ* incubation; D = degradable fractions; U = undegradable degradable fractions; K_p = passage rate of 6%/h was adopted (Tamminga et al., 1994). BST = rumen bypass starch; EDST = effective degradability of starch; SEM = Standard error of mean; Means with different letters within the same row differ (P<0.05).

Table 3.8 *In situ* Rumen Degradation Kinetics of Carbohydrate in Cereal Grains: Raw vs. Dry Heating vs. Moist Heating

Item	Wheat			Triticale			Corn			SEM	P value		
	Raw	DH	MH	Raw	DH	MH	Raw	DH	MH		Feed	Heating	F*H
In situ rumen CHO degradation													
K_d (%/h)	16.4^{a}	13.5^{a}	3.07^{b}	15.9^{a}	16.6^{a}	4.77^{b}	7.96 ^{b}	6.93^{b}	3.51^{b}	1.11	<0.001	<0.001	0.001
T0 (h)	0.00	0.19	0.42	0.26	0.00	0.00	0.09	0.00	0.13	0.17	0.595	0.689	0.410
S (%)	12.5	9.17	6.55	17.3	14.7	7.95	23.3	21.2	16.2	1.57	<0.001	<0.001	0.726
D (%)	74.5	78.6	84.5	70.2	72.1	79.2	67.6	70.7	77.7	2.64	0.007	<0.001	0.996
U (%)	13.0	12.3	8.93	12.5	13.2	12.8	9.09	8.09	6.11	2.25	0.029	0.436	0.889
BCHO (%CHO)	23.2^{de}	25.0^{cde}	47.9^{a}	22.1^{e}	22.9^{de}	40.5^{b}	25.3^{cd}	26.9^{c}	38.8^{b}	0.65	<0.001	<0.001	<0.001
BCHO (g/kg DM)	168^{d}	176^{d}	342^{a}	182^{d}	179^{d}	316^{b}	225^{c}	231^{c}	338^{a}	3.93	<0.001	<0.001	<0.001
EDCHO (%CHO)	76.8^{ab}	75.1^{abc}	52.1^{e}	77.9^{a}	77.1^{ab}	59.5^{d}	74.7^{bc}	73.1^{c}	61.3^{d}	0.65	<0.001	<0.001	<0.001
EDCHO (g/kg DM)	556^{cd}	530^{d}	373^{f}	642^{ab}	604^{bc}	466^{e}	668^{a}	627^{ab}	535^{d}	10.55	<0.001	<0.001	0.041

Note: DH = dry heating; MH = moist heating; Feed= feed effect (wheat, triticale, corn); Heating = heating effect (raw, dry heating, moist heating); F*H = interaction between feed effect and heating effect; K_d = the rate of degradation of D fraction (%/h); T_0 = lag time in h; S = soluble fraction in the *in situ* incubation; D = degradable fractions; U = undegradable degradable fractions; K_p = passage rate of 6%/h was adopted (Tamminga et al., 1994). BCHO = rumen bypass CHO; EDCHO = effective degradability of CHO; SEM = Standard error of mean; Means with different letters within the same row differ (P<0.05).

3.3.2.6 Hourly Effective Degradation Ratios

As shown in Table 3.9, significant differences were found between the three types of cereal grains, while the heat effect was only significant on the N to CHO ratio. According to Figure 3.2, the ratio of effective degradable nitrogen to effective degradable carbohydrate (ED_N/ED_CHO, g available N/kg available CHO) is decreased ($P<0.05$) in wheat by moist heating and approaches the optimal ratio (OP) of 32 (Tamminga et al., 1990; Sinclair et al., 1993). On the other hand, the N to OM ratio wasn't significantly changed ($P>0.05$) by the heat effect. The optimal ratio of effective degradable nitrogen to effective degradable organic matter (ED_N/ED_OM) is 25 (Tamminga et al., 1990; Sinclair et al., 1993).

Nevertheless, as shown in Figure 3.3 and 3.4, the hourly effective degradable ratio of N/OM in moist-heated wheat and triticale shows a similar declining trend and approaches the optimal ratio, while the dry-heated wheat and triticale fail to show much difference compared to the raw groups. Likewise, the hourly effective degradable ratio of N/OM in raw corn kernels and dry-heated corn are similar (Figure 3.5). The N/OM ratio for moist-heated corn also descended, only to be further away from the optimal ratio. This implies that the moist heating restrained the amount of protein participating in rumen fermentation. In the case of corn, there is already excessive OM and not enough protein to synchronize with, so the moist heating applied in this study may not be suitable for corn. However, for feed that contains high level of protein, the moist heating would be helpful to better synchronize the N and OM degradation and increase microbial protein production.

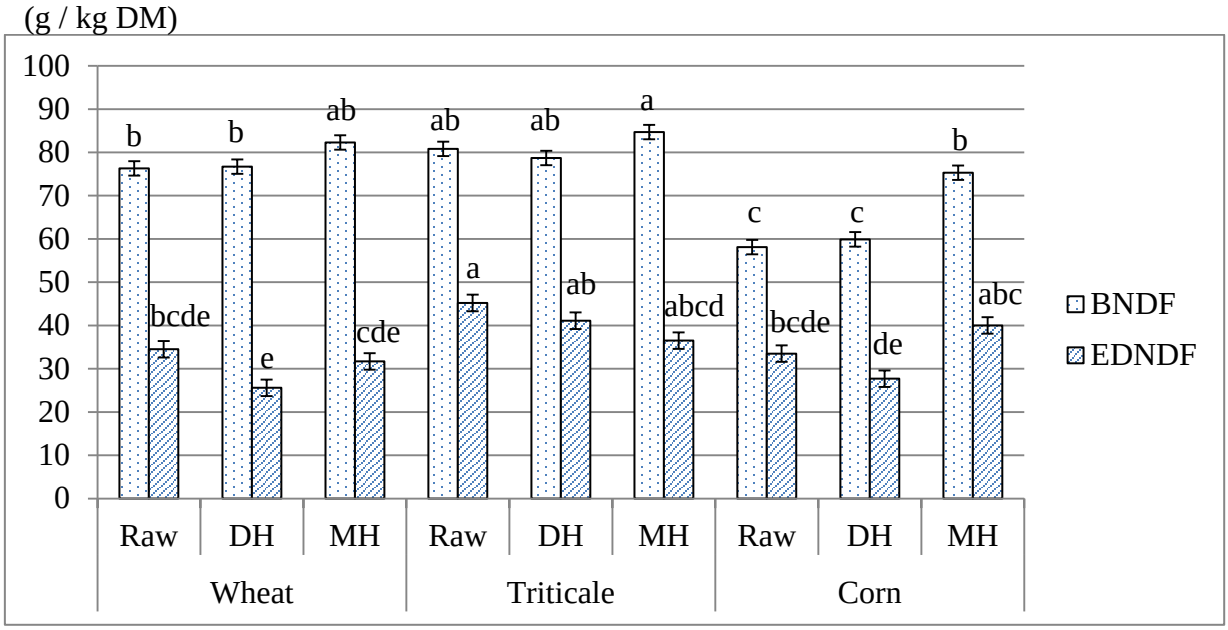

Figure 3.1 *In situ* Rumen Degradation Kinetics of NDF in Cereal Grains

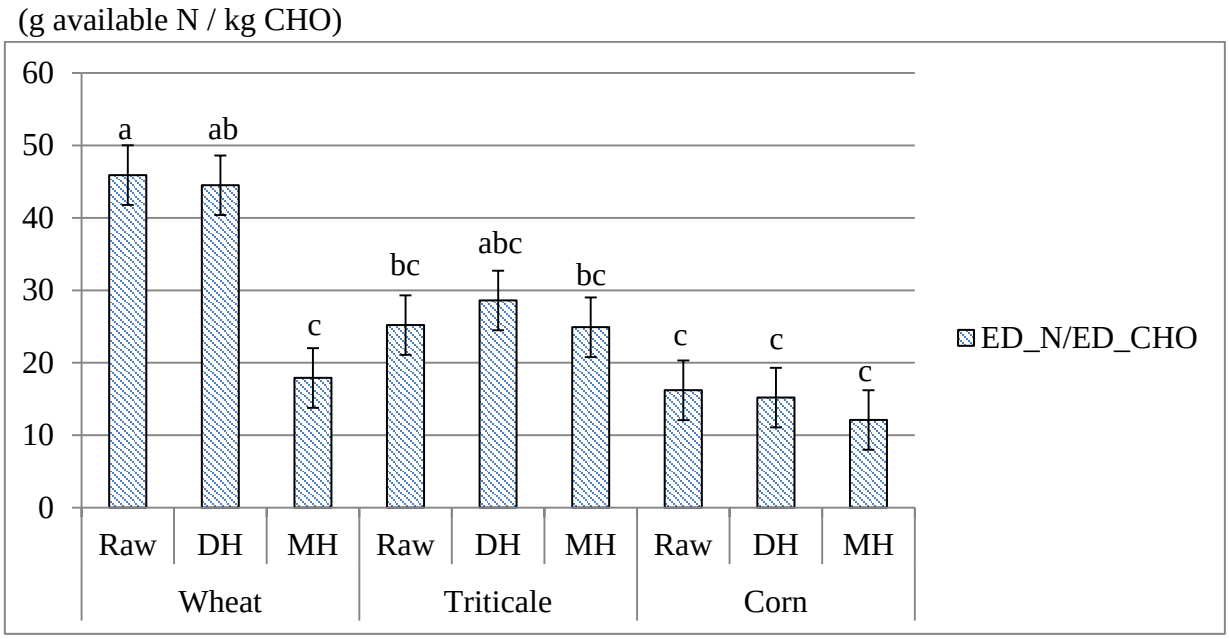

Figure 3.2 Hourly Effective Degradation Nitrogen to Hourly Effective Degradation CHO Ratio in Cereal Grains

Table 3.9 Hourly Effective Degradation ratios in Cereal Grains: Raw vs. Dry Heating vs. Moist Heating

Item	Feed			SEM	P value	Heating			SEM	P value
	Wheat	Triticale	Corn			Raw	DH	MH		
N to OM ratio										
N/OM	34.4[a]	21.3[b]	15.3[c]	0.22	<0.001	23.6	23.6	23.7	0.22	0.758
ED_N/ED_OM	27.8[a]	21.2[b]	13.7[c]	1.52	<0.001	21.8	22.2	18.7	1.52	0.102
N to CHO ratio										
N/CHO	47.2[a]	26.3[b]	17.3[c]	0.48	<0.001	29.6[b]	30.7[a]	30.5[ab]	0.48	0.026
ED_N/ED_CHO	36.1[a]	26.2[b]	14.5[c]	2.47	<0.001	29.1[a]	29.5[a]	18.3[b]	2.47	0.015

Note: Feed= feed effect (wheat, triticale, corn); Heating = heating effect (raw, dry heating, moist heating); F*H = interaction between feed effect and heating effect; K_d = the rate of degradation of D fraction; N = nitrogen; OM = organic matter; ED = effective degradability; CHO = carbohydrates; SEM = Standard error of mean; Means with different letters within the same row differ (P<0.05).

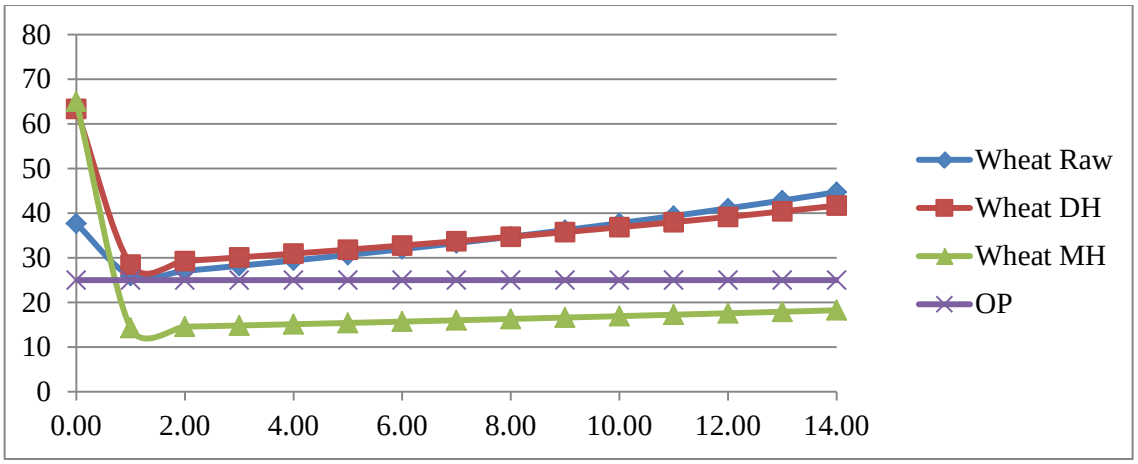

Figure 3.3 Hourly Effective Degradable Ratio of N/OM in Wheat

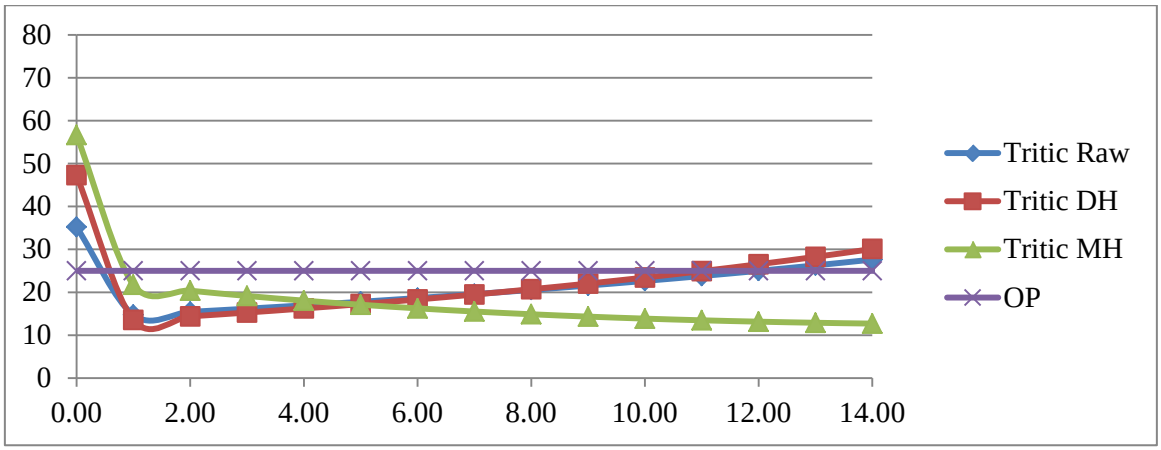

Figure 3.4 Hourly Effective Degradable Ratio of N/OM in Triticale

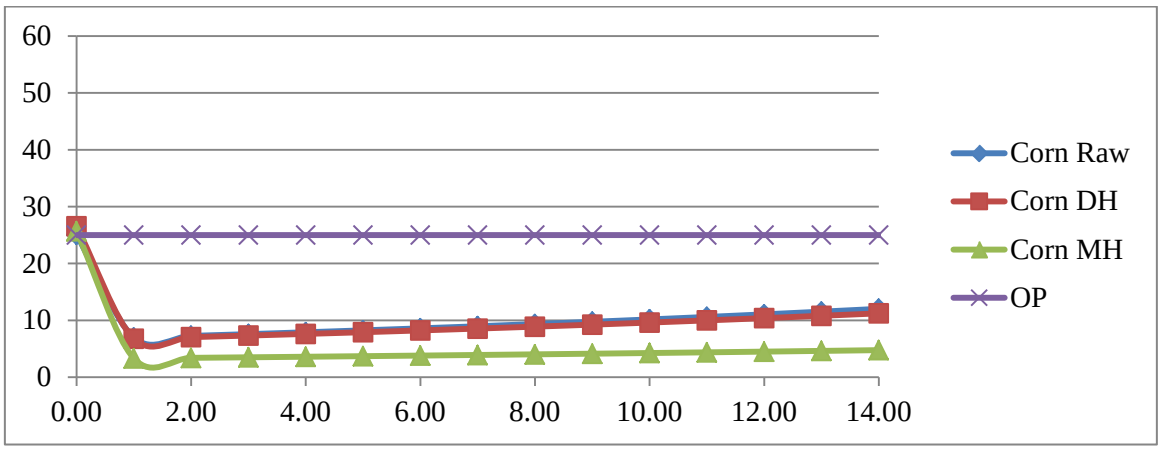

Figure 3.5 Hourly Effective Degradable Ratio of N/OM in Corn

3.3.2.7 Intestinal Digestion of Crude Protein

As showed in Figure 3.6 and 3.7, the intestinal digestible rumen undegradable protein (IDP, g/kg DM) and intestinal absorbable feed protein (IADP, %CP) were both increased ($P<0.05$) by moist heating in wheat and triticale. Changes in moist-heated corn were limited ($P>0.05$). Total digestible protein (TDP) for all three types of grains are not significantly changed ($P>0.05$), which is consistent with Goelema's (1999) finding on peas, faba beans and lupins. However, there were significant heat effect on IDP (g/kg DM), IADP (%CP), TDP (%CP) and TDP (g/kg DM), and significant differences were also found between the three types of cereal grains (Table 3.10).

3.3.2.8 Intestinal Digestion of Neutral Detergent Fiber

The three types of cereal grains had different levels of intestinal digestible rumen bypass neutral detergent fiber (INBDNF, g/kg DM, $P<0.001$) and there are signs of lower IDBNDF (g/kg DM) and total digestible neutral detergent fiber (TDNDF, %NDF) after heating ($P\approx0.05$). The values of TDNDF (%NDF) are similar to what Ferraretto et al. (2013) found in wheat and corn. The TDNDF (g/kg DM) is decreased by moist heating in triticale, however, in corn, it is increased by moist heating, when compared to the dry-heated corn (Table 3.11, $P<0.01$). Seifdavati and Taghizadeh's (2012) experiment, in which the legume seeds were autoclaved at 127 °C for 20 min, also didn't find significant change in NDF digestibility.

3.3.2.9 Intestinal Digestion of Starch

Table 3.12 indicates that the digestibility of rumen bypass starch (dBST) decreases in both wheat and corn, and triticale as well, if compared to the dry-heated group ($P<0.01$). At the same time, the total digestible starch (TDST, %ST) declines in all three types of grains by moist heating ($P<0.01$).

We expected to find a higher degree of starch digestion as discussed by Pagan (2009) and Goelema (1999), however, the result shows the opposite. Significant decrease of TDST (%ST)

was found after moist heating in all three types of cereal grains. According to McAllister et al. (1991) and Seifdavati and Taghizadeh (2012), this may be caused by the gelatinization and retrogradation and had there been a presence of larger amount of moisture (autoclaving with the kernels cracked and soaked in buffer, for instance), the gelatinization may be more complete and the starch digestion improved. Another reason may be that all the grain kernels were heated as whole seeds. The seed coat may have prevented the moisture from penetrating into the seed and gelatinizing the starch granules to some degree.

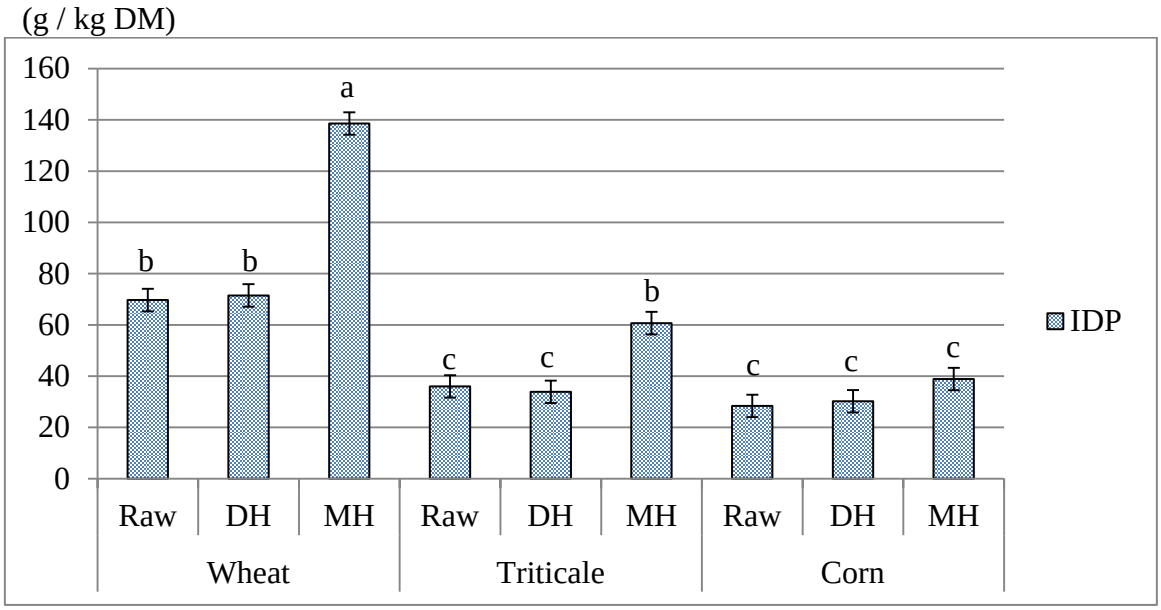

Figure 3.6 Intestinal Digestible Rumen Undegradable Protein in Cereal Grains

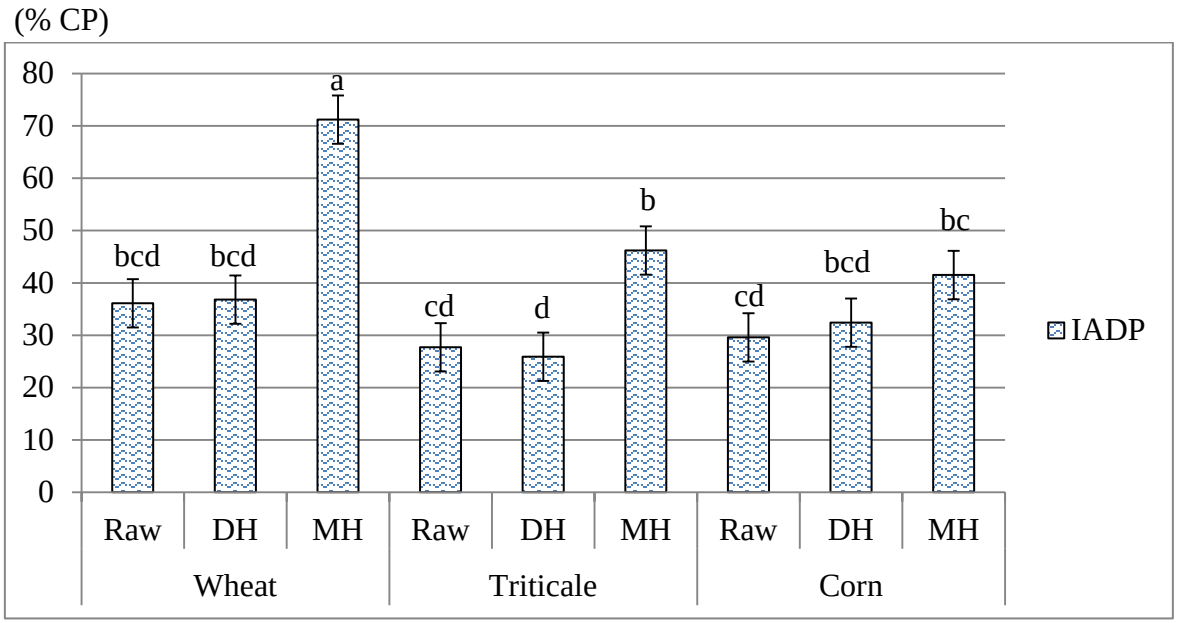

Figure 3.7 Intestinal Absorbable Feed Protein in Cereal Grains

Table 3.10 Intestinal and Total Digestion of Crude Protein in Cereal Grains: Raw vs. Dry Heating vs. Moist Heating

Item	Feed			SEM	P value	Heating			SEM	P value
	Wheat	Triticale	Corn			Raw	DH	MH		
Intestinal crude protein (CP) digestion										
IDP (% RUP)	90.7[a]	75.6[b]	67.1[c]	2.43	<0.001	78.7	77.1	77.6	2.43	0.464
IDP (g/kg DM)	93.3[a]	43.5[b]	32.5[c]	2.76	<0.001	44.7[b]	45.2[b]	79.4[a]	2.76	<0.001
IADP (% CP)	48.1[a]	33.2[b]	34.5[b]	2.44	<0.001	31.1[b]	31.7[b]	53.0[a]	2.44	<0.001
TDP (% CP)	95.0[a]	89.6[b]	82.9[c]	1.24	<0.001	91.3[a]	90.5[a]	85.6[b]	1.24	<0.001
TDP (g/kg DM)	184.1[a]	117.3[b]	78.3[c]	1.59	<0.001	129.4[a]	128.0[a]	122.3[b]	1.59	<0.001

Note: DH = dry heating; MH = moist heating; Feed= feed effect (wheat, triticale, corn); Heating = heating effect (raw, dry heating, moist heating); F*H = interaction between feed effect and heating effect; CP = crude protein; IDP = intestinal digestible rumen undegradable protein; IADP = intestinally absorbable feed protein; TDP = total digestible protein; SEM = Standard error of mean; Means with different letters within the same row differ (P<0.05).

Table 3.11 Intestinal and Total Digestion of Neutral Detergent Fiber in Cereal Grains: Raw vs. Dry Heating vs. Moist Heating

Item	Wheat			Triticale			Corn			SEM	P value		
	Raw	DH	MH	Raw	DH	MH	Raw	DH	MH		Feed	Heating	F*H
Intestinal neutral detergent fiber (NDF) digestion													
dBNDF (% BNDF)	13.1	14.3	9.31	15.7	13.1	10.4	10.4	10.5	11.7	1.68	0.272	0.141	0.202
IDBNDF (% NDF)	8.99	10.1	7.06	10.1	8.60	7.20	6.71	7.14	7.63	1.13	0.174	0.267	0.334
IDBNDF (g/kg DM)	9.95	11.0	7.62	12.7	10.3	8.80	6.19	6.25	8.83	1.28	0.005	0.481	0.052
TDNDF (% NDF)	40.1	39.4	30.8	46.0	42.9	37.2	43.3	38.7	42.3	2.00	0.006	0.003	0.053
TDNDF (g/kg DM)	44.5^{bcde}	42.7^{bcde}	33.2^{e}	58.0^{a}	51.4^{ab}	45.3^{bcd}	39.7^{cde}	34.0^{de}	48.8^{abc}	2.49	<0.001	0.031	<0.001

Note: DH = dry heating; MH = moist heating; Feed = feed effect (wheat, triticale, corn); Heating = heating effect (raw, dry heating, moist heating); F*H = interaction between feed effect and heating effect; dBNDF = digestibility of rumen by pass or undegraded neutral detergent fiber; IDBNDF = intestinal digestible rumen bypass neutral detergent fiber; TDNDF = total digestible neutral detergent fiber; SEM = Standard error of mean; Means with different letters within the same row differ ($P < 0.05$).

Table 3.12 Intestinal and Total Digestion of Starch in Cereal Grains: Raw vs. Dry Heating vs. Moist Heating

Item	Wheat			Triticale			Corn			SEM	P value		
	Raw	DH	MH	Raw	DH	MH	Raw	DH	MH		Feed	Heating	F*H
Intestinal starch (ST) digestion													
dBST (% BST)	94.4^{a}	92.4^{ab}	39.1^{d}	90.4^{abc}	92.8^{ab}	67.0^{c}	94.0^{a}	90.3^{abc}	69.1^{bc}	5.02	0.068	<0.001	0.009
IDBST (% ST)	37.1	32.3	19.2	28.5	35.6	32.1	36.5	33.9	33.1	6.64	0.657	0.479	0.561
IDBST (g/kg DM)	227	194	117	200	237	212	291	260	251	44.84	0.071	0.427	0.704
TDST (% ST)	98.0^{a}	97.6^{a}	77.2^{c}	97.5^{a}	97.4^{a}	87.4^{b}	97.6^{a}	96.5^{a}	85.7^{b}	0.75	<0.001	<0.001	<0.001
TDST (g/kg DM)	600	584	468	681	646	578	783	743	649	11.38	<0.001	<0.001	0.232

Note: DH = dry heating; MH = moist heating; Feed= feed effect (wheat, triticale, corn); Heating = heating effect (raw, dry heating, moist heating); F*H = interaction between feed effect and heating effect; dBST = digestibility of rumen by pass or undegraded starch; IDBST = intestinal digestible rumen bypass starch; TDST = total digestible starch; SEM = Standard error of mean; Means with different letters within the same row differ ($P<0.05$).

3.3.3 Alteration of Modeling Nutrient Supply of Different Types of Cereal Grains to Dairy Cattle through Different Heating Methods

3.3.3.1 Potential Protein Supply to Dairy Cows Predicted by DVE/OEB System

As Table 3.13 shows, the total protein supplied to the small intestine (TPSI) is increased ($P<0.01$) by moist heating in wheat. The microbial protein synthesized in the rumen based on available nitrogen (N_MCP) was decreased ($P<0.01$) by moist heating in wheat and triticale, but not changed ($P>0.01$) in corn. Rumen bypass feed crude protein (BCP) and truly absorbed bypass protein in the small intestine (ABCP) were both increased ($P<0.01$) by moist heating in wheat and triticale. Truly digested protein in the small intestine (DVE) was increased ($P<0.01$) while the degraded protein balance (OEB) decreased ($P<0.05$) in wheat by moist heating. Such results are consistent with other studies on pressure toasted legumes (Yu et al., 2004). The unheated wheat (raw) and dry-heated wheat had positive OEB values, which turned negative after being moist-heated. It indicates that the raw and dry-heated wheat have potential N loss in the rumen while moist-heated wheat has insufficient N supply compared to available energy in the feed samples (Damiran and Yu, 2012; Yu et al., 2004).

3.3.3.2 Potential Protein Supply to Dairy Cows Predicted by NRC Dairy 2001 Model

The results predicted by NRC Dairy is slightly different compared to those estimated using DVE/OEB System. As Table 3.14 shows, the microbial protein (MCP) and truly absorbed microbial protein in the small intestine (AMP) were both decreased, while the rumen undegradable protein (RUP) and truly absorbed rumen undegradable protein in the small intestine (ARUP) were increased by moist heating in wheat and triticale ($P<0.01$). As a result, the metabolizable protein was increased and the degraded protein balance (OEB^{NRC}) was decreased by moist heating in both wheat and triticale ($P<0.01$).

Comparing to what was found using the DVE/OEB system, the results estimated from the NRC-2001 were similar. The major difference between the two models is that in the DVE/OEB system, the metabolic losses of each individual feed in the digestive tract is taken into

consideration, hence the maintenance requirement of the animal was not counted for metabolic losses as NRC-1985 does (Tamminga et al., 1994). Both of them are modern protein evaluation systems developed to fit the circumstances in certain country or areas.

Table 3.13 Potential Protein Supply to Dairy Cows Predicted by DVE/OEB System from Cereal Grains: Raw vs. Dry Heating vs. Moist Heating

Item (g/kg DM)	Wheat			Triticale			Corn			SEM	P value		
	Raw	DH	MH	Raw	DH	MH	Raw	DH	MH		Feed	Heating	F*H
FOM	572	599	454	620	583	500	530	545	452	43.5	0.275	0.007	0.909
DOM	905	905	895	908	906	901	914	912	906	1.0	<0.001	<0.001	0.356
UOM	76.4	76.7	86.1	74.4	75.9	81.6	73.4	75.0	80.9	1.0	0.002	<0.001	0.418
UASH	6.56	6.50	6.49	6.31	6.29	6.21	4.51	4.62	4.55	0.0	<0.001	0.177	0.152
UDM	83.0	83.2	92.5	80.8	82.2	87.8	77.9	79.6	85.4	1.0	<0.001	<0.001	0.397
TPSI	148^{bc}	156^{b}	222^{a}	122^{bcd}	117^{cd}	142^{bcd}	106^{d}	110^{d}	118^{bcd}	8.1	<0.001	<0.001	0.003
ENDP	6.22	6.24	6.94	6.06	6.16	6.59	5.85	5.97	6.41	0.1	<0.001	<0.001	0.397
Truly absorbed rumen-synthesised microbial protein in small intestine													
N_MCP	109^{a}	106^{ab}	23.8^{d}	77.5^{c}	79.1^{bc}	45.7^{d}	49.3^{d}	44.9^{d}	27.1^{d}	6.1	<0.001	<0.001	<0.001
AMP^{DVE}	54.7	57.3	43.4	59.2	55.7	47.8	50.6	52.2	43.2	4.2	0.275	0.007	0.909
Truly absorbed rumen-undegraded feed protein in small intestine													
BCP	84.0^{b}	88.5^{b}	171^{a}	52.6^{c}	51.9^{c}	86.1^{b}	46.6^{c}	48.6^{c}	66.8^{bc}	5.7	<0.001	<0.001	<0.001
$ABCP^{DVE}$	77.4^{b}	79.4^{b}	154^{a}	40.0^{c}	37.6^{c}	67.4^{b}	31.5^{c}	33.6^{c}	43.2^{c}	4.9	<0.001	<0.001	<0.001
Truly digested protein in the small intestine													
DVE	126^{b}	130^{b}	190^{a}	93.2^{cd}	87.2^{cd}	109^{bc}	76.3^{d}	79.8^{cd}	80.0^{cd}	6.8	<0.001	<0.001	<0.001
Degraded protein balance													
OEB^{DVE}	23.2^{a}	15.9^{a}	-44.3^{b}	-15.4^{ab}	-8.31^{ab}	-29.2^{b}	-30.2^{b}	-36.9^{b}	-40.7^{b}	9.2	<0.001	<0.001	0.014

Note: DH = dry heating; MH = moist heating; Feed= feed effect (wheat, triticale, corn); Heating = heating effect (raw, dry heating, moist heating); F*H = interaction between feed effect and heating effect; 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; AMP = 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 3.14 Potential Protein Supply to Dairy Cows Predicted by NRC Dairy 2001 Model in Cereal Grains: Raw vs. Dry Heating vs. Moist Heating

Item (g/kg DM)	Wheat			Triticale			Corn			SEM	P value		
	Raw	DH	MH	Raw	DH	MH	Raw	DH	MH		Feed	Heating	F*H
MCP_{TDN}	97.0^{cd}	96.9^{cd}	96.6^{d}	97.3^{c}	97.3^{c}	96.5^{d}	100^{a}	101^{a}	98.8^{b}	0.1	<0.001	<0.001	<0.001
Truly absorbed rumen-synthesised microbial protein in small intestine													
MCP^{NRC}	97.0^{a}	95.4^{a}	34.6^{c}	70.3^{b}	71.6^{b}	46.1^{c}	45.8^{c}	42.3^{c}	28.6^{c}	4.6	<0.001	<0.001	<0.001
AMP^{NRC}	62.1^{a}	61.1^{a}	22.1^{c}	45.0^{b}	45.8^{b}	29.5^{c}	29.3^{c}	27.1^{c}	18.3^{c}	3.0	<0.001	<0.001	<0.001
Truly absorbed rumen-undegraded feed protein in small intestine													
RUP^{NRC}	75.7^{b}	79.7^{b}	154^{a}	47.4^{c}	46.7^{c}	77.6^{b}	42.0^{c}	43.8^{c}	60.2^{bc}	5.1	<0.001	<0.001	<0.001
$ARUP^{NRC}$	69.7^{b}	71.5^{b}	139^{a}	36.0^{c}	33.9^{c}	60.7^{b}	28.4^{c}	30.2^{c}	38.9^{c}	4.4	<0.001	<0.001	<0.001
Truly digested rumen endogenous protein in small intestine													
ECP	10.7^{c}	10.9^{a}	10.8^{b}	10.7^{c}	11.0^{a}	10.9^{b}	10.3^{e}	10.7^{c}	10.5^{d}	0.0	<0.001	<0.001	<0.001
$AECP^{NRC}$	4.29^{c}	4.38^{a}	4.33^{b}	4.29^{c}	4.39^{a}	4.34^{b}	4.11^{e}	4.28^{c}	4.20^{d}	0.0	<0.001	<0.001	<0.001
Total truly absorbed protein in small intestine													
MP^{NRC}	136^{b}	137^{b}	165^{a}	85.3^{d}	84.1^{d}	94.5^{c}	61.8^{e}	61.6^{e}	61.4^{e}	2.0	<0.001	<0.001	<0.001
Degraded protein balance													
OEB^{NRC}	2.93^{a}	0.10^{a}	-73.3^{c}	-32.1^{b}	-30.5^{b}	-59.6^{c}	-64.5^{c}	-68.9^{c}	-82.9^{c}	5.5	<0.001	<0.001	<0.001

Note: DH = dry heating; MH = moist heating; Feed= feed effect (wheat, triticale, corn); Heating = heating effect (raw, dry heating, moist heating); F*H = interaction between feed effect and heating effect; MCP_{TDN} = microbial protein synthesized in the rumen based on discounted TDN; MCP = microbial protein; AMP = 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 ($P<0.05$).

3.3.3.3 Estimating Milk Values of Cereal Grains Based on Metabolizable Protein

Dairy cows are capable of converting feed nutrients into milk. Based on the DVE and MP values estimated using the DVE/OEB and the NRC models, the nitrogen available for milk production was calculated as FMV^{DVE} and FMV^{MP}, respectively. The two values are slightly different, but similar in their trend (Table 3.15). FMV estimated from both models increased ($P<0.05$) in wheat after moist heating, but in triticale, only FMV estimated from the NRC model was increased ($P<0.05$) by moist heating. Neither of the FMV values predicted from the two models was significantly changed in corn after the two types of heat processing ($P>0.05$). These results confirm that the heat conditions in this study are probably not suitable for corn and do no benefit in increasing its nutritional value. Although cereal grains are not a typical protein source, high FMV in the cereal grains is a bonus feature, as the market prices for protein feeds are usually higher than the grains.

Table 3.15 Estimating Milk Values of Cereal Grains Based on Metabolizable Protein

Item (kg milk per kg DM)	Wheat			Triticale			Corn			SEM	P value		
	Raw	DH	MH	Raw	DH	MH	Raw	DH	MH		Feed	Heating	F*H
Estimated from Metabolizable Protein													
FMV^{DVE}	2.56^{b}	2.65^{b}	3.86^{a}	1.89^{cd}	1.77^{cd}	2.20^{bc}	1.55^{d}	1.62^{cd}	1.62^{cd}	0.13	<0.001	<0.001	<0.001
FMV^{MP}	2.76^{b}	2.78^{b}	3.35^{a}	1.73^{d}	1.71^{d}	1.92^{c}	1.25^{e}	1.25^{e}	1.25^{e}	0.04	<0.001	<0.001	<0.001

Note: DH = dry heating; MH = moist heating; Feed= feed effect (wheat, triticale, corn); Heating = heating effect (raw, dry heating, moist heating); F*H = interaction between feed effect and heating effect; FMV^{DVE} = estimated feed milk value based on truly digested protein in the small intestine using DVE/OEB system; FMV^{MP} = estimated feed milk value based on metabolizable protein according to NRC Dairy 2001; SEM = Standard error of mean; Means with different letters within the same row differ ($P<0.05$).

3.4 Conclusion

All three hypotheses are accepted. The heat processing induced changes in the nutrient profile and availability of cereal grains. Between the two types of heat treatment, moist heating had greater impact compared to dry heating on nutrient availability of cereal grains. The presence of heat made it easier for moisture to penetrate into the grain kernels and induce a series of changes in nutritional level. The detailed mechanism of heat-induced changes is still unclear. Nevertheless, based on the results gathered using the conventional methods and models, there is a high possibility that Maillard Reaction occurred in the moist-heated grains. The dry heating didn't show much influence on the grains possibly due to two reasons: 1) the processing temperature was not high enough to make a change; 2) the particle sizes of the grain seeds were too big, as they were heated as whole seeds (Khan and Yu, 2013).

Meanwhile, differences were found between different types of grains in response to heat treatment. For instance, protein degradability of all three types of grain was reduced by moist heating, which implies a shift of protein digestion from rumen to intestine. Moist heating was found to increase nutrient utilization without affecting their digestibility in wheat and triticale. However, the heat condition applied in this study was not suitable for corn. With the occurrence of reduced PB1, PB2 fractions and increased PC fraction, decreased energy levels, lower total digestible starch and worse synchrotron of energy and nitrogen in rumen, corn seemed to be over-heated after the moist heat treatment.

For a better understanding of the heat-induced inner structural changes in the cereal grains, as well as their relationship and consistency with the nutritional studies conducted in this chapter, the advanced mid infrared microspectroscopy is introduced as a suitable approach in the next chapter.

4 Structural Responses of Chemical Functional Groups in Cereal Grains to Heat Processing Methods

4.1 Introduction

After applying the two types of heat treatment (moist heating and dry heating) to the three types of grain kernels (wheat, triticale and corn), different degrees of changes were discovered on nutrient values, degradation rates, digestion and potential nutrient supply, which implies that there may also be different changes in the inner structure of the grain seeds as a result of heating.

Infrared spectroscopy is a well-established technique for analyzing chemical functional groups (Kong and Yu, 2007). Amongst the infrared, the Mid-IR is especially useful in the medical and biology areas. With wavelength range from 2.5 to 10 μm, Mid-IR could trigger fundamental rotational-to-vibrational molecular energy transitions and be utilized to analyze some important bands that are associated with the C-H, N-H and O-H stretches.

In this chapter, two Mid-IR techniques, namely ATR-FTIR (Attenuated total reflectance Fourier transform infrared spectroscopy) and SR-IMS (Synchrotron Radiation Infrared Microspectroscopy), were used to probe the inherent functional groups in grain seeds that associate with nutritent digestion and supply. The major difference between these two methods is the light source: the synchrotron IR light is formed by accelerated electrons and usually 100-1000 times brighter than the globar sourced IR light (Yu, 2004). The brightness of synchrotron light makes it possible for SR-IMS to explore the molecular chemical make-up in the plant tissues and reserve the inner structure at the same time (Yu, 2006). Comparatively, the advantages of ATR-FTIR technique include the simple sample preparation, easy manual operation, relatively low cost and fast data collection at a high spectral resolution.

The objectives of this study were to directly detect the sensitivity and responses of various chemical functional groups to the two different heating methods in tissues of the three types of cereal grains and their correlation to the nutrient utilization and availability in ruminants. The hypotheses were: 1) the sensitivity and responses of functional groups can be detected by ATR-

FTIR as well as SR-IMS; 2) different functional groups in cereal grain tissues respond differently to the heating methods; 3) heat-induced structural changes are highly related to the nutrient availability of cereal grains in ruminants.

4.2 Material and Methods

4.2.1 Attenuated Total Reflectance Fourier Transform Infrared Spectroscopy (ATR-FTIR)

4.2.1.1 Sample Preparation and Spectra Collection

Three types of cereal grains (wheat, triticale, corn) were divided into three groups (raw, dry-heated and moist-heated) and processed in two batches. Random grain seeds were sampled and finely ground through the 0.5 mm screen using a Retsch mill (Retsch ZM–1; Brinkmann Instruments of Canada Ltd., Mississauga, ON, Canada). The molecular structural features were determined in the mid-infrared region (ca. 4000–800 cm^{-1}) of the electromagnetic spectrum using a JASCO FT/IR–4200 spectroscope (JASCO Corp., Tokyo, Japan) at the University of Saskatchewan. Thirty-two scans were accumulated for each spectrum and five spectra were collected for each sample using JASCO Spectra Manager II software.

4.2.1.2 Univariate Analysis

The Omnic 7.2 software (Spectra Tech, Madison, WI) was used to identify functional spectral bands associated with protein, lipid, cellulosic compound and carbohydrate molecular structures according to published reports (Miller, et al., 2000). The assessed items (Figure 4.1) included infrared intensity of protein amide I (ca. 1725–1578 cm^{-1}), amide II (ca. 1578–1482 cm^{-1}), amide I peak height (ca. ~1647 cm^{-1}), amide II peak height (ca. ~1537 cm^{-1}), α-helix height (ca. ~1653 cm^{-1}), β-sheet (ca. ~1632 cm^{-1}), lipid (ca. 1798-1709 cm^{-1}) and its peak height (ca. ~1744 cm^{-1}), cellulosic compounds (ca. 1291–1184 cm^{-1}) and its peak height (ca. ~1238 cm^{-1}), total carbohydrates (CHO; ca. 1191–944 cm^{-1}) , three major CHO peaks: 1st peak (ca. 1191–1132 cm^{-1}) and its peak height (ca. ~1150 cm^{-1}), 2nd peak (ca. 1132–1066 cm^{-1}) and its peak height (ca.

~1078 cm^{-1}), 3rd peak (ca. 1066–944 cm^{-1}) and its peak height (ca. ~1012 cm^{-1}). As Figure 4.2 and 4.3 show, the 2nd derivative process was used to enhance the information of α-helix and β-sheet (Griffiths and Pariente, 1986).The detailed method to locate each particular band in the spectrum could be found in Yang (2013).

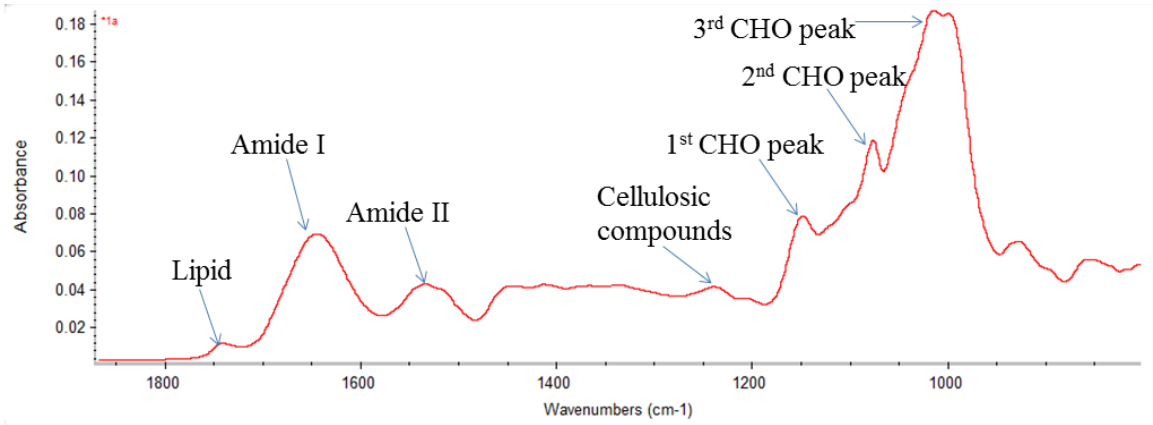

Figure 4.1 ATR-FTIR detected spectrum information in a raw wheat sample

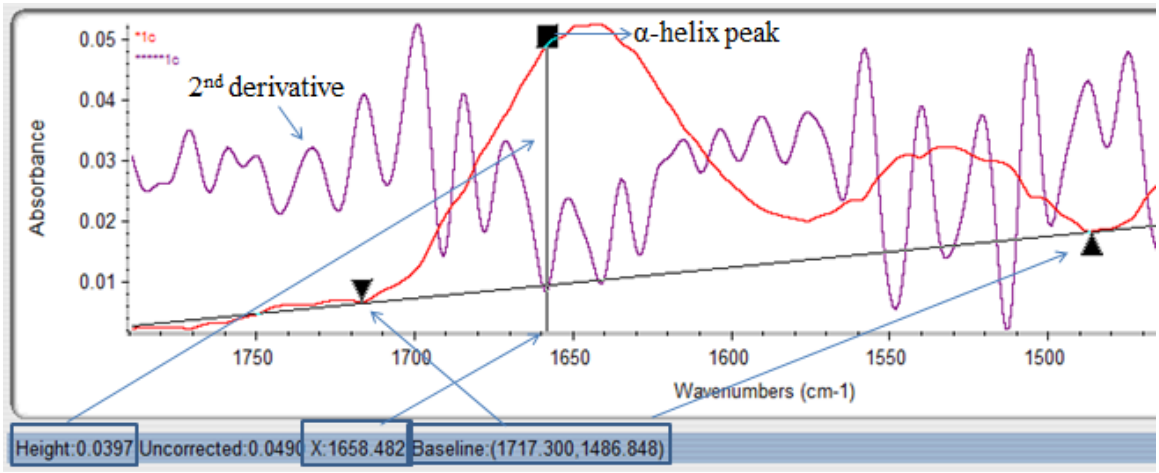

Figure 4.2 ATR-FTIR detected α-helix peak in a raw wheat sample

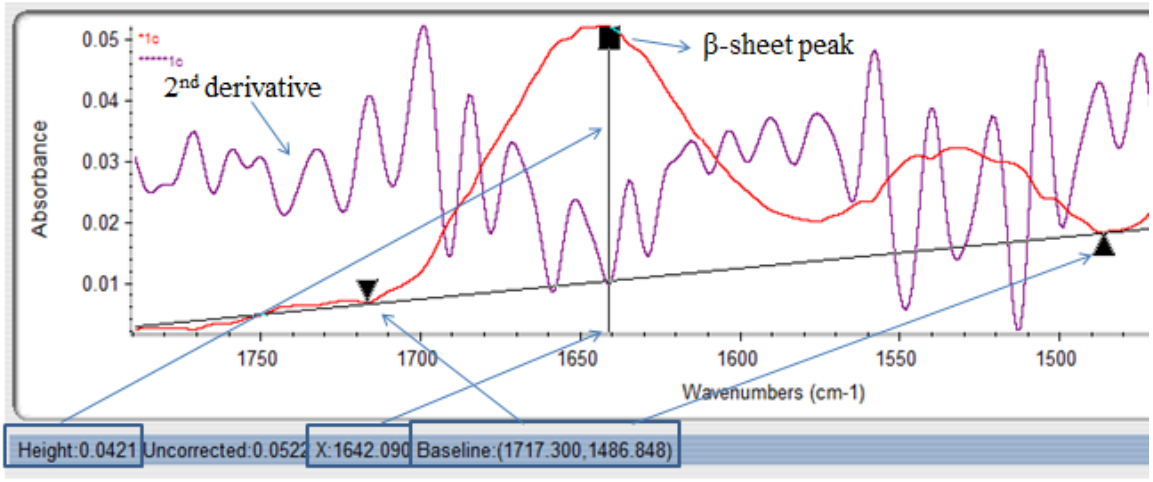

Figure 4.3 ATR-FTIR detected β-sheet peak in a raw wheat sample

4.2.1.3 Multivariate Analysis

Spectra in [.JWS] form were converted into [.CSV] form and used for hierarchical cluster analysis (CLA or HCHA) and principal components analysis (PCA). Detailed principles and analysis process followed the report of Yu (2008). Ward's algorithm method was used for clustering in CLA and clusters were displayed as dendrograms (Miller et al., 2000). PCA results were plotted based on the two highest factor scores and plotted as a function of the scores. The first two factors usually accounted for over 85% of the variability in the data. Multivariate spectral analyses were performed using Statistica software 8.0 (StatSoft Inc., Tulsa, OK, USA).

4.2.2 Synchrotron Radiation Infrared Microspectroscopy (SR-IMS)

4.2.2.1 Sample Preparation and Spectra Collection

Nine grain seed samples (three grains types (wheat, triticale and corn) × three processing methods (raw, dry heating and moist heating)) were randomly selected to be cross-sectioned at Western College of Veterinary Medicine, University of Saskatchewan, Saskatoon, Canada. The thin cross-sections of endosperm tissues (6 μm) were unstained and mounted on barium fluoride (BaF2) window (Spectral Systems, Hopewell Junction, NY, USA) according to the report by Yu

et al. (2008).

The SR-IMS experiment was performed with the IR microspectroscopy instrument coupled with synchrotron radiation from U2B beamline at National Synchrotron Light Source, Brookhaven National Laboratory, U.S. Department of Energy (NSLS-BNL, Upton, NY). Molecular spectra collection was carried out using a Thermo Nicolet Magna 860 Step-Scan FTIR (Thermo Fisher Scientific Inc.,Waltham,MA) spectrometer equipped with a Spectra Tech Continuum IR Microscope (Spectra-Tech, Inc., Shelton, CT) and mercury cadmium telluride (MCT) detector. Atlus software (Thermo Nicolet, Madison, WI, USA) was used to produce the visual image of the sample. Twenty spots were randomly chosen and their spectra images (128 scans per spectrum) were collected for each window at the tissue endosperm region in the mid-infrared region (ca. 4,000–800 cm^{-1}) of the electromagnetic spectrum, with the resolution set at 4 per cm and the aperture size 10 μm × 10 μm.

Spectral data were processed by OMNIC software 7.2 (Spectra Tech, Madison, WI, USA). After baseline correction, the absorption peak parameters (baseline, region, relative height, and area) of functional spectral bands associated with protein, cellulosic compound and carbohydrate molecular structures were recorded for univariate analysis.

4.2.2.2 Univariate Analysis

The absorbance bands (Figure 4.4) of associated with specific nutrients of grain samples were: protein amide I (ca. 1804–1574 cm^{-1}), amide II (ca. 1574–1471 cm^{-1}), amide I peak height (ca. ~1649 cm^{-1}), amide II peak height (ca. ~1543 cm^{-1}), α-helix height (ca. ~1656 cm^{-1}), β-sheet (ca. ~1627 cm^{-1}), cellulosic compound (ca. 1291–1172 cm^{-1}) and its peak height (ca. ~1236 cm^{-1}), total carbohydrates (CHO; ca. 1194–933 cm^{-1}) , three major CHO peaks: 1st peak (ca. 1194–1117 cm^{-1}) and its peak height (ca. ~1151 cm^{-1}), 2nd peak (ca. 1117–1067 cm^{-1}) and its peak height (ca. ~1079 cm^{-1}), 3rd peak (ca. 1067–933 cm^{-1}) and its peak height (ca. ~1022 cm^{-1}). As all the spots were sampled from the endosperm area of grains, no visible lipid peaks were found

in the spectra. The spectra analysis methods were similar to that of the ATR-FTIR spectra, despite for the requirement of an automatic baseline correction before attempting to locate any functional group.

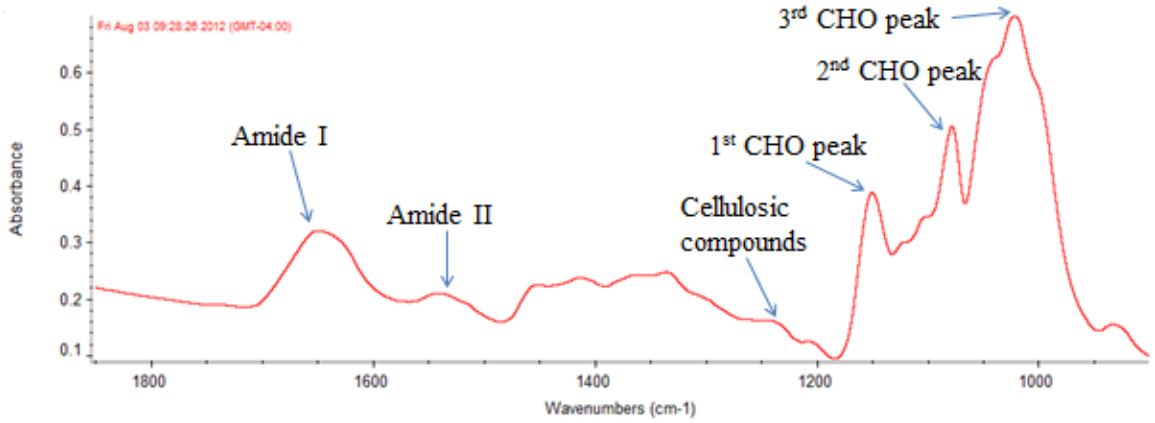

Figure 4.4 SR-IMS detected spectrum information in a raw wheat sample

4.2.2.3 Multivariate Analysis

In order to identify the effect of the grain types and heat treatment on associated nutrient region, the grain samples were randomly selected and analyzed using PCA and CLA. CLA results were presented as dendograms while PCA results were plotted based on the two highest factor scores. Same as ATR-FTIR, multivariate spectral analyses for SR-IMS spectra were also performed using Statistica software 8.0 (StatSoft Inc., Tulsa, OK, USA).

4.2.3 Statistical Analysis

The experimental design for this study is RCBD with treatment design as 3 × 3 Factorial arrangement, with the two factors “grain types” (wheat, triticale and corn) and “processing methods” (raw, dry heating and moist heating). The Statistical analyses were performed using the MIXED procedure of SAS 9.2 (SAS Institute, Inc., Cary, NC).

The model used for the analysis was as follows:

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

in which Y_{ijkl} was an observation of the dependent variable ijkl, μ was the population mean for the variable, F_i was the effect of cereal types; H_j was the effect of heating treatments, $F_i \times H_j$ was the interaction of grain type and heat effect, B_k was the random effect of processing batches and *in situ* runs, and e_{ijkl} was the random error associated with observation ijkl.

RCBD model assumptions were tested. Normality tests were performed using the UNIVARIATE procedure of SAS with Normal and PLOT options. Means were compared using the Tukey–Kramer method and the significance was declared at $P<0.05$.

Correlations were performed using the PROC CORR of SAS with an option of SPEARMAN to quantify molecular structural features identified using ATR-FTIR and SR-IMS techniques in relation to 1) chemical profiles, 2) rumen degradation kinetics, 3) intestinal nutrient digestion, 4) prediction of protein supply to dairy cattle using the DVE/OEB system and the NRC Dairy 2001 model and 5) Feed milk values predicted from DVE/OEB and NRC Dairy 2001 models.

4.3 Results and Discussion

4.3.1 Detecting the Sensitivity and Responses of Functional Groups in Cereal Grains Using ATR-FTIR Techniques

4.3.1.1 Univariate and Correlation Analysis of Protein Structure

Significant effects of heat processing on protein spectral characteristics of different grains were found (Table 4.1). The absorbance peak area and height of protein, including the amide I, amide II areas and peak height and α-helix, β-sheet peak height, all decreased after the moist heating treatments in wheat and triticale ($P<0.05$), indicating protein structural alterations in the two types of cereal grains. In wheat, the ratio of α-helix to β-sheet is also significantly increased (1.06 vs. 1.23, $P<0.05$).

Among the three types of grains, corn was found to be the lowest in protein absorbance intensity to spectra, compared to wheat and triticale ($P<0.05$). As amide I, which consists of about 80% of C=O stretching vibration in amide C=O group, is particularly sensitive and could be used to predict the relation to protein values (Yu et al., 2009). The result is consistent with the fact that corn has lower protein levels among the three cereal grains. Heat treatment made no impact on peak area and height of protein structure of corn ($P>0.05$), except that unprocessed corn was found higher in β-sheet height than that in moist-heated corn (0.03 vs. 0.02 AU, $P<0.05$). As a result, the ratio of α-helix to β-sheet is increased in corn (0.99 vs. 1.28 AU, $P<0.05$). This indicates that heat treatments had limited impact on protein structure in corn, which is probably caused by its relatively big kernel size and vitreous endosperm. The result also corresponds to those of the conventional studies.

Positive correlations were found between absorbance peak areas and heights of protein in three cereal grains and their protein profiles, nitrogen to energy ratios, as well as protein digestion kinetics ($P<0.01$) (Table 4.2). Relatively weak correlation between protein absorbance intensity and DVE values was detected ($P<0.05$), while ratios of amide I and amide II absorbance areas showed strong negative correlation to the protein degradation and digestion profiles, especially for CP and TDP ($R=-0.87$, $P<0.001$). It is unexpected that the ratio of α-helix to β-sheet, which was reported to be very sensitive to the heat treatment, showed almost no relation to the protein values (Yu, 2005b).

The correlation between the protein absorbance peak areas and heights and other nutrient parameters analyzed in the conventional studies are listed in Appendix (Table 7.1).

Table 4.1 Heat-Induced Changes in Spectral Characteristics of Protein Amide I and II, Protein Secondary Structure α-Helix, β-Sheet in Cereal Grains Using ATR-FTIR Molecular Spectroscopy

Item	Peak region and center (cm^{-1})	Wheat			Triticale			Corn			SEM	P value		
		Raw	DH	MH	Raw	DH	MH	Raw	DH	MH		Feed	Heating	F*H
Baseline	1725-1428													
Amide I area	1725-1578	3.14^{a}	2.99^{ab}	2.56^{bc}	2.42^{c}	2.43^{c}	1.93^{d}	1.78^{d}	1.54^{d}	1.67^{d}	0.100	<0.001	<0.001	0.017
Amide II area	1578-1482	1.01^{a}	0.92^{ab}	0.87^{b}	0.70^{c}	0.71^{c}	0.52^{d}	0.44^{d}	0.41^{d}	0.41^{d}	0.028	<0.001	<0.001	0.008
Amide I peak height	~1647	0.045^{a}	0.040^{ab}	0.035^{b}	0.035^{b}	0.036^{b}	0.027^{c}	0.027^{c}	0.022^{c}	0.024^{c}	0.001	<0.001	<0.001	0.003
Amide II peak height	~1537	0.017^{a}	0.016^{ab}	0.014^{bc}	0.013^{c}	0.013^{c}	0.009^{d}	0.009^{d}	0.008^{d}	0.007^{d}	0.001	<0.001	<0.001	0.016
α-helix height	~1653	0.043^{a}	0.040^{ab}	0.034^{b}	0.035^{b}	0.035^{b}	0.026^{c}	0.026^{c}	0.022^{c}	0.023^{c}	0.001	<0.001	<0.001	0.003
β-sheet height	~1632	0.041^{a}	0.036^{ab}	0.028^{cd}	0.033^{b}	0.032^{bc}	0.023^{de}	0.026^{d}	0.021^{e}	0.018^{e}	0.001	<0.001	<0.001	0.025
Ratio of Amide I to Amide II area		3.10^{ef}	3.25^{def}	2.96^{f}	3.49^{cd}	3.44^{cde}	3.73^{bc}	4.03^{ab}	3.76^{abc}	4.09^{a}	0.076	<0.001	0.221	<0.001
Ratio of α-helix to β-sheet height		1.06^{bc}	1.10^{b}	1.23^{a}	1.06^{bc}	1.08^{bc}	1.12^{b}	0.99^{c}	1.06^{bc}	1.28^{a}	0.031	0.008	<0.001	<0.001

Note: DH = dry heating; MH = moist heating; SEM = Standard error of mean; Means with different letters within the same row differ (P<0.05).

Table 4.2 Correlation Analysis between Spectral Characteristics of Protein Amide I and II, Protein Secondary Structure α-Helix and β-Sheet Detected by ATR-FTIR Technique and Protein Profiles and Digestion Kinetics of Cereal Grains

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	Ratio of α-helix to β-sheet height
	----------Spearman Correlation R value----------							
Protein profiles								
Crude protein (% DM)	0.89***	0.85***	0.95***	0.90***	0.84***	0.73***	-0.87***	0.07
Soluble crude protein (%DM)	0.74***	0.81***	0.69**	0.75***	0.80***	0.90***	-0.51*	-0.49*
Protein degradation in rumen								
Ratio of N to OM	0.88***	0.83***	0.94***	0.89***	0.83***	0.70**	-0.86***	0.13
Ratio of ED_N to EN_OM	0.88***	0.85***	0.88***	0.86***	0.85***	0.78***	-0.73***	-0.04
Ratio of N to CHO	0.88***	0.82***	0.94***	0.88***	0.82***	0.68**	-0.85***	0.15
Ratio of ED_N to ED_CHO	0.83***	0.84***	0.79***	0.80***	0.83***	0.84***	-0.58*	-0.22
Protein digestion in small intestine								
IDP (%CP)	0.84***	0.79***	0.90***	0.84***	0.77***	0.69**	-0.82***	-0.00
TDP (%CP)	0.87***	0.88***	0.90***	0.90***	0.86***	0.88***	-0.82***	-0.35
TDP (g/kg DM)	0.91***	0.87***	0.96***	0.93***	0.86***	0.76***	-0.87***	0.06
Predicted protein supply in small intestine using DVE/OEB								
DVE (g/kg DM)	0.58*	0.50*	0.69**	0.61**	0.49*	0.32	-0.75***	0.33
OEB (g/kg DM)	0.77***	0.80***	0.70**	0.74***	0.80***	0.84***	-0.45+	-0.32
Modeling protein supply in small intestine using NRC Dairy 2001								
MP (g/kg DM)	0.79***	0.73***	0.87***	0.81***	0.72***	0.57*	-0.85***	0.21
OEB (g/kg DM)	0.83***	0.85***	0.77***	0.81***	0.85***	0.90***	-0.55*	-0.35
Estimated Feed Milk Values								
FMV^{DVE} (kg milk / kg feed)	0.58*	0.50*	0.69**	0.61**	0.49*	0.32	-0.75**	0.33
FMV^{MP} (kg milk / kg feed)	0.79***	0.73***	0.87***	0.81***	0.72***	0.57*	-0.85***	0.21

Note: R = Correlation coefficient calculated using spearman method; DM = dry matter; CP = crude protein; ED_N = effective degradable nitrogen; ED_OM = effective degradable organic matter; ED _CHO = effective degradable carbohydrate; IDP = intestinal digestible protein; TDP = total digestible protein; DVE = truly digested protein in the small intestine; OEB = degraded protein balance; MP = metabolizable protein; FMV^{DVE} =

estimated feed milk value based on truly digested protein in the small intestine using DVE/OEB system; FMV^{MP} = estimated feed milk value based on metabolizable protein according to NRC Dairy 2001; $^{+}$for $P<0.10$, * for $P<0.05$, **for $P<0.01$, *** for $P<0.001$.

4.3.1.2 Univariate and Correlation Analysis of Carbohydrate Structure

Table 4.3 shows structure spectral characteristics detected by ATR-FTIR spectroscopy in CHO spectra region (ca. 1191-944 cm^{-1}). Unlike triticale and corn, both dry- and moist-heated wheat showed no impact on absorbance intensity of CHO peaks and heights compared to the raw wheat ($P>0.05$). Raw and dry-heated triticale showed decreased the area of the third CHO peak (11.20 vs. 9.44 AU/cm), descended heights for the 2nd (0.078 vs. 0.066 AU) and 3rd CHO peak (0.15 vs. 0.13 AU) after being moist-heated ($P<0.05$). Nevertheless, for corn, dry heating appears to have more impact than moist heating. All the absorbance intensity of CHO peaks are reduced by dry heating while moist heating only decreased height of the 3rd CHO peak, with less extent compared to the dry heating (0.16 vs. 0.12 vs. 0.13 AU, $P<0.05$). The 3rd CHO peak, with the peak region 1066-944 cm^{-1} and the peak center 1012 cm^{-1} (close to 1025 cm^{-1}), could be the peak represents non-structural CHO such as starch in the endosperm (Yu, 2004; Liu and Yu, 2010).

Table 4.4 shows correlation between all CHO absorbance intensities to spectra and CHO profiles. The area and height of the 1st CHO peak are found negatively correlated to the CHO degradation parameters (except for EDCHO) and feed milk value, and positively correlated to the CHO profiles and CHO digestion parameters. Some strong correlation are shown, for instance, correlation between the area of the 1st CHO peak and ratio of nitrogen to CHO ($R=-0.80$, $P<0.001$) and that between the area of the 1st CHO peak and FMV^{MP} ($R=-0.81$, $P<0.001$).

In Table 4.5, the correlation between CHO absorbance intensity and some of the protein digestion parameters are shown. Except for the weak relationship with EDCP, negative relationship is found between all of them. Similar to Table 4.4, most strong correlations are found between the area and heights of 1st and 3rd CHO peaks and nutrient values detected by conventional methods. The correlation between the CHO absorbance peak areas and heights and other nutrient parameters analyzed in the conventional studies are listed in Appendix (Table 7.2).

Table 4.3 Effect of Heat Treatments on Spectral Characteristics of CHO in Cereal Grains Using ATR-FTIR Molecular Spectroscopy

Item	Peak region and center (cm^{-1})	Wheat			Triticale			Corn			SEM	P value		
		Raw	DH	MH	Raw	DH	MH	Raw	DH	MH		Feed	Heating	F*H
Total area	1191-944	12.07^{cd}	11.51^{cd}	10.70^{d}	15.81^{ab}	16.05^{a}	13.59^{bc}	15.50^{ab}	12.22^{cd}	13.50^{bc}	0.568	<0.001	<0.001	0.003
Peak 1 area	1191-1132	1.01^{de}	0.97^{e}	0.95^{e}	1.34^{abc}	1.40^{ab}	1.22^{bcd}	1.51^{a}	1.16^{cde}	1.36^{abc}	0.055	<0.001	0.016	<0.001
Peak 2 area	1132-1066	2.69^{bc}	2.55^{c}	2.44^{c}	3.27^{a}	3.27^{a}	2.79^{abc}	3.04^{ab}	2.46^{c}	2.71^{bc}	0.118	<0.001	<0.001	0.015
Peak 3 area	1066-944	8.37^{cd}	7.99^{cd}	7.31^{d}	11.20^{a}	11.38^{a}	9.44^{bc}	10.95^{ab}	8.60^{cd}	9.42^{bc}	0.387	<0.001	<0.001	0.002
Peak 1 height	~1150	0.034^{de}	0.033^{e}	0.032^{e}	0.046^{ab}	0.047^{ab}	0.041^{bcd}	0.051^{a}	0.039^{cde}	0.045^{abc}	0.002	<0.001	0.002	<0.001
Peak 2 height	~1078	0.062^{c}	0.058^{c}	0.055^{c}	0.078^{a}	0.079^{a}	0.066^{bc}	0.075^{ab}	0.059^{c}	0.066^{bc}	0.003	<0.001	<0.001	0.003
Peak 3 height	~1012	0.11^{cd}	0.11^{d}	0.10^{d}	0.15^{ab}	0.16^{ab}	0.13^{c}	0.16^{a}	0.12^{cd}	0.13^{bc}	0.006	<0.001	<0.001	<0.001

Note: DH = dry heating; MH = moist heating; SEM = Standard error of mean; Means with different letters within the same row differ (P<0.05).

Table 4.4 Correlation Analysis between Spectral Characteristics of Carbohydrates Detected by ATR-FTIR Technique and Carbohydrate Profiles and Digestion Kinetics of Cereal Grains

Items	Total CHO area	CHO peak 1 area	CHO peak 1 height	CHO peak 2 area	CHO peak 2 height	CHO peak 3 area	CHO peak 3 height
	----------Spearman Correlation R value----------						
CHO profiles							
Starch (% DM)	0.43^{+}	0.67**	0.65**	0.18	0.35	0.44^{+}	0.53*
CHO (%DM)	0.58*	0.80***	0.77***	0.34	0.49*	0.59**	0.66**
Cellulose (% DM)	0.52*	0.18	0.26	0.67**	0.58*	0.52*	0.43^{+}
CHO degradation in rumen							
BCHO (%CHO)	-0.43^{+}	-0.24	-0.30	-0.45^{+}	-0.45^{+}	-0.46^{+}	-0.42^{+}
EDCHO (g/kg DM)	0.60**	0.57*	0.60**	0.49*	0.58*	0.63**	0.65**
Ratio of N to OM	-0.58*	-0.79***	-0.77***	-0.35	-0.50*	-0.60**	-0.67**
Ratio of ED_N to EN_OM	-0.29	-0.58*	-0.54*	-0.07	-0.22	-0.31	-0.39
Ratio of N to CHO	-0.60**	-0.80***	-0.77***	-0.36	-0.51*	-0.61**	-0.68**
Ratio of ED_N to ED_CHO	-0.15	-0.44^{+}	-0.39	0.04	-0.08	-0.16	-0.24
CHO digestion in small intestine							
IDBST (g/kg DM)	0.48*	0.59*	0.58*	0.35	0.44^{+}	0.49*	0.54*
TDST (g/kg DM)	0.55*	0.65**	0.65**	0.36	0.49*	0.57*	0.63**
TDNDF (g/kg DM)	0.46^{+}	0.29	0.34	0.50*	0.49*	0.47*	0.43^{+}
TDCHO (g/kg DM)	0.61**	0.64**	0.66**	0.47^{+}	0.57*	0.64**	0.67**
Estimated Feed Milk Values							
FMV^{DVE} (kg milk / kg feed)	-0.69**	-0.77***	-0.77***	-0.51*	-0.63**	-0.70**	-0.74***
FMV^{MP} (kg milk / kg feed)	-0.64**	-0.81***	-0.79***	-0.41^{+}	-0.56*	-0.65**	-0.71***

Note: R = Correlation coefficient calculated using spearman method; CHO = carbohydrates; BCHO = rumen bypass or undegraded CHO; EDCHO = effective degradability of CHO; ED_N = effective degradable nitrogen; ED_OM = effective degradable organic matter; ED _CHO = effective degradable carbohydrate; IDBST = intestinal digestible rumen bypass starch; TDST = total digestible starch; TDNDF = total digestible neutral detergent fiber; TDCHO = total digestible carbohydrates; FMV^{DVE} = estimated feed milk value based on truly digested protein in the small intestine using DVE/OEB system; FMV^{MP} = estimated feed milk value based on metabolizable protein according to NRC Dairy 2001; $^{+}$for P<0.10, * for P<0.05, **for P<0.01, *** for P<0.001.

Table 4.5 Correlation Analysis between Spectral Characteristics of Carbohydrates Detected by ATR-FTIR Technique and Protein Digestion Kinetics of Cereal Grains Affected by Heat-Related Processing

Items	Total CHO area	CHO peak 1 area	CHO peak 1 height	CHO peak 2 area	CHO peak 2 height	CHO peak 3 area	CHO peak 3 height
		----------Spearman Correlation R value----------					
Protein degradation in rumen							
RUP (g/kg DM)	-0.68**	-0.67**	-0.69**	-0.53*	-0.63**	-0.70**	-0.71***
EDCP (%CP)	0.50*	0.28	0.33	0.53*	0.52*	0.52*	0.47*
Protein digestion in small intestine							
IDP (g/kg DM)	-0.69**	-0.73***	-0.74***	-0.53*	-0.64**	-0.71***	-0.74***
TDP (g/kg DM)	-0.53*	-0.77***	-0.74***	-0.29	-0.44+	-0.54*	-0.62**
Predicted protein supply in small intestine using DVE/OEB							
ABCP (g/kg DM)	-0.69**	-0.73***	-0.74***	-0.53*	-0.64**	-0.71***	-0.73***
DVE (g/kg DM)	-0.69**	-0.77***	-0.77***	-0.51*	-0.63**	-0.70**	-0.74***
Predicted protein supply in small intestine using NRC Dairy 2001							
ARUP (g/kg DM)	-0.69**	-0.73***	-0.74***	-0.53*	-0.64**	-0.71***	-0.74***
MP (g/kg DM)	-0.64**	-0.81***	-0.79***	-0.41+	-0.56*	-0.65**	-0.71***

Note: R = Correlation coefficient calculated using spearman method; CHO = carbohydrates; RUP = rumen undegraded feed crude protein; EDCP = effective degradability of feed crude protein; IDP = intestinal digestible rumen undegradable protein; TDP = total digestible protein; ABCP or ARUP = truly absorbed rumen bypass protein or truly absorbed rumen undegradable protein in the small intestine; DVE = truly digested protein in the small intestine; MP = metabolizable protein; +for P<0.10, * for P<0.05, **for P<0.01, *** for P<0.001.

4.3.1.3 Univariate and Correlation Analysis of Lipid and Cellulose Structure

Heat treatment effect on spectral characteristics of lipid and cellulosic compounds were analyzed by ATR-FTIR in the region of (ca. 1798-1709 cm^{-1}) and (ca. 1291-1184 cm^{-1}) respectively (Table 4.6). Moist heating showed increased influence on both the area and height of lipid absorbance peak in wheat (0.045 vs. 0.137 AU/cm and 0.002 vs. 0.007 AU, $P<0.05$), while dry heating depressed both in corn (0.245 vs. 0.178 AU/ cm and 0.013 vs. 0.009 AU, $P<0.05$). No significant change is detected in absorbance intensity of lipid compounds in triticale after either heat treatment. There is also no change found in the absorbance intensity of cellulosic compounds in any of the cereal grains after both dry and moist heating ($P>0.05$).

Results of the correlation study are listed in Table 4.7. The area of cellulosic compounds peak has positive correlation to cellulose ($R=0.62$, $P<0.01$), lipid ($R=-0.50$, $P<0.05$), effective degradability of feed crude protein (EDCP, $R=0.48$, $P<0.05$), as well as nitrogen and energy balance value (OEB^{DEV}, $R=0.52$, $P<0.05$; OEB^{NRC}, $R=0.49$, $P<0.05$). Comparatively, absorbance peak area and height of lipid has much stronger relationship to nutrient profiles, rumen degradation kinetics and protein availabilities, such as lipid ($R=0.83$ and 0.81, $P<0.001$) and OEB^{NRC} ($R=-0.80$ and -0.78, $P<0.001$). The correlation between the lipid and cellulosic absorbance peak areas and heights and other nutrient parameters analyzed in the conventional studies are listed in Appendix (Table 7.3).

Table 4.6 Heat-Induced Changes in Spectral Characteristics of Lipid and Cellulosic compounds in Cereal Grains Using ATR-FTIR Molecular Spectroscopy

Item	Peak region and center (cm^{-1})	Wheat			Triticale			Corn			SEM	P value		
		Raw	DH	MH	Raw	DH	MH	Raw	DH	MH		Feed	Heating	F*H
Lipid														
Peak area	1798-1709	0.045^{e}	0.059^{e}	0.137^{bc}	0.071^{de}	0.078^{cde}	0.126^{bcd}	0.245^{a}	0.178^{b}	0.261^{a}	0.016	<0.001	<0.001	0.022
Peak height	~1744	0.002^{e}	0.003^{de}	0.007^{bc}	0.004^{de}	0.004^{cde}	0.006^{bcd}	0.013^{a}	0.009^{b}	0.013^{a}	0.001	<0.001	<0.001	<0.001
Cellulosic Compounds														
Peak area	1291-1184	0.225	0.217	0.209	0.230	0.224	0.212	0.211	0.192	0.211	0.009	0.055	0.211	0.454
Peak height	~1238	0.006	0.005	0.005	0.006	0.006	0.005	0.006	0.005	0.006	0.000	0.432	0.003	0.057

Note: DH = dry heating; MH = moist heating; SEM = Standard error of mean; Means with different letters within the same row differ (P<0.05);

Table 4.7 Correlation Analysis between Spectral Characteristics of Cellulosic and Lipid Compounds Detected by ATR-FTIR Technique and Nutrient Availability of Cereal Grains Affected by Heat-Related Processing

Items	Cellulosic Compounds		Lipid Compounds	
	Area	Peak	Area	Peak
	----------Spearman Correlation R value----------			
Nutrient profiles				
Cellulose (%DM)	0.62**	0.45+	-0.59**	-0.57*
Lipid (%DM)	-0.50*	0.00	0.83***	0.81***
Rumen degradation				
EDCP (g/kg DM)	0.48*	0.24	-0.78***	-0.75***
BST (g/kg DM)	-0.13	0.11	0.72***	0.71***
BCHO (g/kg DM)	-0.34	-0.34	0.59**	0.56*
Ratio of N to OM	0.24	-0.21	-0.67**	-0.65**
Ratio of ED_N to EN_OM	0.44+	-0.01	-0.77***	-0.75***
Ratio of N to CHO	0.23	-0.22	-0.65**	-0.64**
Ratio of ED_N to ED_CHO	0.47*	0.12	-0.75***	-0.73***
Intestinal digestion				
IDP (g/kg DM)	-0.06	-0.45+	-0.26	-0.26
TDP (g/kg DM)	0.28	-0.18	-0.73***	-0.71**
IDBST (g/kg DM)	0.07	0.38	0.39	0.41+
TDST (g/kg DM)	-0.13	0.38	0.40	0.41+
IDBNDF (g/kg DM)	0.35	0.12	-0.58*	-0.58*
TDNDF (g/kg DM)	0.25	0.24	-0.35	-0.35

Table 4.7 Cont'd

Items	Cellulosic Compounds		Lipid Compounds	
	Area	Peak	Area	Peak
	----------Spearman Correlation R value----------			
Predicted protein supply in small intestine using DVE/OEB				
AMP (g/kg DM)	0.21	0.19	-0.59*	-0.58*
OEB (g/kg DM)	0.52*	0.28	-0.68**	-0.66**
Predicted protein supply in small intestine using NRC Dairy 2001				
AMP (g/kg DM)	0.48*	0.23	-0.78***	-0.76***
MP (g/kg DM)	0.16	-0.31	-0.58*	-0.57*
OEB (g/kg DM)	0.49*	0.22	-0.80***	-0.78***
Estimated Feed Milk Values				
FMV^{DVE} (kg milk / kg feed)	-0.02	-0.43+	-0.38	-0.38
FMV^{MP} (kg milk / kg feed)	0.16	-0.31	-0.58*	-0.57*

Note: R = Correlation coefficient calculated using spearman method; EDCP = effective degradability of feed crude proten; BST = rumen bypass or undegraded starch; BCHO = rumen bypass or undegraded carbohydrates; ED_N = effective degradable nitrogen; ED_OM = effective degradable organic matter; ED _CHO = effective degradable carbohydrate; IDP = intestinal digestible protein; TDP = total digestible protein; IDBST = intestinal digestible rumen bypass starch; TDST = total digestible starch; IDBNDF = intestinal digestible rumen bypass neutral detergent fiber; TDNDF = total digestible neutral detergent fiber; AMP = truly absorbed microbial protein in the small intestine; OEB = degraded protein balance; MP = metabolizable protein; FMV^{DVE} = estimated feed milk value based on truly digested protein in the small intestine using DVE/OEB system; FMV^{MP} = estimated feed milk value based on metabolizable protein according to NRC Dairy 2001; +for P<0.10, * for P<0.05, **for P<0.01, *** for P<0.001.

4.3.1.4 Multivariate Analysis

Both CLA and PCA based on original spectra without any parameterization failed to fully distinguish the treatments from one another in all three types of grains. Differences were only detected between the different types of grains. Those comparisons that can be distinguished from each other include: lipid and protein in raw wheat and raw corn; lipid and protein in dry-heated wheat and dry-heated corn; protein in dry-heated triticale and dry-heated corn; CHO in dry-heated wheat and dry-heated triticale. Detailed figures are attached in the Appendix (Figure 7.2, 7.3 and 7.4).

4.3.2 Detecting the Sensitivity and Responses of Functional Groups in Cereal Grains Using SR-IMS Techniques

4.3.2.1 Univariate Analysis of Protein Structure

The absorbance intensity of protein peak area and height in the endosperm of cereal grains were analyzed in the region of ca. 1804-1471 cm^{-1} (Table 4.8). Unlike the results found in the conventional study, dry heating, instead of moist heating, induced significantly positive effect on absorption intensity of protein in wheat and triticale ($P<0.05$). This is probably due to the different sample area in the SR-IMS study and other studies. The grain kernels were all ground and mixed well before all the other experiments, however, in the SR-IMS study, all the spots were sampled only in the endosperm area (100-600 μm from the seed coats), and the results may not be very representative. Meanwhile, the massive changes of amide I area in dry-heated wheat and triticale imply the great alterations of protein secondary structure in their endosperm (Yu et al., 2009).

No change was found on protein structure in response to heat-treated corn ($P>0.05$) except for a higher amide I to amide II area ratio in dry-heated corn (5.51 vs. 6.78 AU/cm, $P<0.05$). Similar to the results found with ATR-FTIR, the α-helix to β-sheet ratio didn't show its sensitivity to heat treatments as former research indicated (Yu, 2005).

As shown in Table 4.9, the absorbance intensity of protein detected by SR-IMS in endosperm region of grains showed no correlation with either protein digestion kinetics, or metabolizable protein levels ($P>0.05$). The ratio of amide I to amide II is the only spectral parameter that has significant relationship with the protein values. Negative correlation ($R=-0.76$, $P<0.05$) between the ratio of amide I to amide II and the ratio of N to OM, as well as the ratio of N to CHO were detected. Total digestible protein (TDP) ($R=--0.76$) and metabolizable protein (MP) ($R=-0.69$) also have significant relationships with the ratio of amide I to amide II ($P<0.05$). The advantages of using ratios are: 1) the spectra variation due to the thickness of the tissue cross is eliminated; 2) as relative biological component contents, the ratio has its own biological meaning and could be used as an index to predict the characteristics and nutrient value of the subject (Yu et al., 2005).

Table 4.8 Heat-Induced Changes in Spectral Characteristics of Protein Amide I and II, Protein Secondary Structure α-Helix, β-Sheet in Endosperm Tissue of Cereal Grains Using Synchrotron Technique (SR-IMS): Raw vs. Dry heating vs. Moist Heating

Item	Peak region and center (cm^{-1})	Wheat			Triticale			Corn			SEM	P value		
		Raw	DH	MH	Raw	DH	MH	Raw	DH	MH		Feed	Heating	F*H
Baseline	1804-1471													
Amide I area	1804-1574	13.39^{bcd}	25.20^{a}	14.31^{bc}	11.83^{cd}	16.09^{b}	10.98^{d}	15.18^{b}	13.93^{bc}	14.25^{bc}	0.670	<0.001	<0.001	<0.001
Amide II area	1574-1471	3.05^{bcd}	6.80^{a}	3.29^{bc}	2.16^{de}	3.87^{b}	2.00^{e}	3.40^{bc}	2.62^{cde}	2.88^{cde}	0.211	<0.001	<0.001	<0.001
Amide I peak height	~1649	0.18^{bc}	0.31^{a}	0.19^{b}	0.16^{bc}	0.20^{b}	0.15^{c}	0.20^{b}	0.17^{bc}	0.18^{b}	0.008	<0.001	<0.001	<0.001
Amide II peak height	~1543	0.06^{bcd}	0.11^{a}	0.06^{bc}	0.04^{de}	0.07^{b}	0.04^{e}	0.06^{bc}	0.05^{cde}	0.06^{bcd}	0.003	<0.001	<0.001	<0.001
α-helix height	~1656	0.18^{b}	0.31^{a}	0.19^{b}	0.16^{bc}	0.19^{b}	0.14^{c}	0.19^{b}	0.16^{bc}	0.18^{bc}	0.008	<0.001	<0.001	<0.001
β-sheet height	~1627	0.13^{d}	0.27^{a}	0.15^{cd}	0.13^{d}	0.18^{b}	0.12^{d}	0.16^{bc}	0.15^{cd}	0.15^{cd}	0.007	<0.001	<0.001	<0.001
Ratio of Amide I to Amide II area		4.59^{cd}	3.97^{d}	4.49^{cd}	6.19^{ab}	4.65^{cd}	7.09^{a}	5.51^{bc}	6.78^{a}	6.28^{ab}	0.239	<0.001	0.001	<0.001
Ratio of α-helix to β-sheet height		1.40	1.15	1.31	1.24	1.04	1.32	1.14	1.10	1.31	0.053	0.029	<0.001	0.098

Note: DH = dry heating; MH = moist heating; SEM = Standard error of mean; Means with different letters

Table 4.9 Correlation Analysis between Structural Characteristics of Protein Amide I and II, Protein Secondary Structure α-Helix, β-Sheet Detected by Synchrotron Technique (SR-IMS) and Protein Digestion Kinetics of Cereal Grains Affected by Heat-Related Processing

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	Ratio of α-helix to β-sheet height
	----------Spearman Correlation R value----------							
Protein degradation in rumen								
EDCP (g/kg DM)	0.47	0.50	0.53	0.49	0.52	0.45	-0.59^{+}	-0.01
Ratio of N to OM	0.41	0.50	0.50	0.48	0.54	0.34	-0.76*	0.40
Ratio of ED_N to EN_OM	0.46	0.52	0.54	0.51	0.56	0.42	-0.66^{+}	0.26
Ratio of N to CHO	0.43	0.51	0.52	0.50	0.56	0.37	-0.76*	0.39
Ratio of ED_N to ED_CHO	0.47	0.52	0.54	0.51	0.54	0.43	-0.61^{+}	0.16
Protein digestion in small intestine								
IDP (%CP)	0.31	0.40	0.39	0.38	0.45	0.24	-0.65^{+}	0.45
TDP (g/kg DM)	0.40	0.49	0.49	0.48	0.53	0.35	-0.76*	0.37
Predicted protein supply in small intestine using DVE/OEB								
OEB (g/kg DM)	0.47	0.50	0.53	0.50	0.52	0.44	-0.59^{+}	0.05
Predicted protein supply in small intestine using NRC Dairy 2001								
MP (g/kg DM)	0.31	0.40	0.40	0.39	0.44	0.25	-0.69*	0.44
OEB (g/kg DM)	0.46	0.50	0.52	0.49	0.52	0.45	-0.60^{+}	0.01
Estimated Feed Milk Values								
FMV^{MP} (kg milk / kg feed)	0.31	0.40	0.40	0.39	0.44	0.24	-0.69*	0.50

Note: R = Correlation coefficient calculated using spearman method; EDCP = effective degradability of feed crude protein; ED_N = effective degradable nitrogen; ED_OM = effective degradable organic matter; ED_CHO = effective degradable carbohydrate; IDP = intestinal digestible rumen undegradable protein; TDP = total digestible protein; OEB = degraded protein balance; MP = metabolizable protein; FMV^{MP} = estimated feed milk value based on metabolizable protein according to NRC Dairy 2001; $^{+}$for P<0.10, * for P<0.05, **for P<0.01, *** for P<0.001.

4.3.2.2 Univariate Analysis of Carbohydrate and Cellulose Structure

Spectral structural characteristics of CHO (ca. 1194-993 cm^{-1}) and cellulosic compounds (ca. 1291-1172 cm^{-1}) in response to heat treatments were analyzed by SR-IMS via detecting endosperm tissues of cereal grains (Table 4.10). No significant effect was found in wheat regarding the absorbance intensity of CHO at endosperm region ($P>0.05$). However, in triticale, dry heating significantly lessened all the CHO peak areas and heights ($P<0.05$), leaving the dry-heated triticale to be the lowest among all the treatments in the CHO spectra parameters ($P<0.05$). In contrast to CHO spectra features, absorbance intensity of cellulose compounds in wheat are improved by dry heating (area: 0.77 vs. 1.03 AU/cm and peak height: 0.02 vs. 0.03 AU, $P<0.05$).

Weak correlations are found in the correlation analysis between structural characteristics of CHO and CHO profiles, CHO subfractions, as well as nutrient digestion kinetics (Table 4.11). The strongest ones lie between the area of 2^{nd} CHO peak and starch or EE ($R=0.67$, $P<0.05$), as well as TDP ($R=-0.67$, $P<0.05$). Besides, cellulose compound area is negatively correlated to rumen bypass starch (BST, $R=-0.71$, $P<0.05$) and positively related to truly absorbed microbial protein in the small intestine (AMP, $R=0.77$, $P<0.05$) and OEB^{NRC} ($R=0.69$, $P<0.05$).

Table 4.10 Heat-Induced Changes in Spectral Characteristics of Carbohydrate in Endosperm Tissue of Cereal Grains Using Synchrotron Technique (SR-IMS): Raw vs. Dry heating vs. Moist Heating

Item	Peak region and center (cm^{-1})	Wheat			Triticale			Corn			SEM	P value		
		Raw	DH	MH	Raw	DH	MH	Raw	DH	MH		Feed	Heating	F*H
Total area	1194-933	68^{bc}	66^{c}	65^{c}	77^{a}	55^{d}	74^{ab}	71^{abc}	79^{a}	78^{a}	1.94	<0.001	<0.001	<0.001
CHO Peak 1 area	1194-1117	12^{bcd}	13^{bcd}	12^{cd}	15^{a}	11^{d}	14^{ab}	14^{abc}	15^{a}	16^{a}	0.48	<0.001	0.029	<0.001
CHO Peak 2 area	1117-1067	14^{bc}	14^{bcd}	14^{cd}	17^{a}	12^{d}	17^{a}	16^{ab}	18^{a}	18^{a}	0.53	<0.001	<0.001	<0.001
CHO Peak 3 area	1067-933	41^{cde}	39^{e}	40^{de}	45^{ab}	32^{f}	43^{abcd}	41^{bcde}	47^{a}	44^{abc}	0.99	<0.001	<0.001	<0.001
CHO Peak 1 height	~1151	0.33^{bcd}	0.33^{bc}	0.32^{cd}	0.39^{a}	0.28^{d}	0.37^{ab}	0.36^{abc}	0.40^{a}	0.40^{a}	0.01	<0.001	0.032	<0.001
CHO Peak 2 height	~1079	0.41^{bc}	0.39^{c}	0.40^{c}	0.48^{a}	0.33^{d}	0.46^{ab}	0.43^{abc}	0.48^{a}	0.47^{a}	0.01	<0.001	<0.001	<0.001
CHO Peak 3 height	~1022	0.58^{bcd}	0.54^{d}	0.57^{cd}	0.64^{ab}	0.44^{e}	0.61^{abc}	0.54^{d}	0.64^{a}	0.58^{abcd}	0.01	0.018	<0.001	<0.001
Cellulose Compounds														
Peak area	1291-1172	0.77^{bcd}	1.03^{a}	0.69^{cd}	0.90^{ab}	0.78^{bcd}	0.73^{cd}	0.84^{bc}	0.72^{cd}	0.64^{d}	0.04	0.006	<0.001	<0.001
Peak height	~1236	0.02^{bc}	0.03^{a}	0.02^{c}	0.02^{bc}	0.02^{bc}	0.02^{c}	0.03^{ab}	0.02^{bc}	0.02^{bc}	0.00	0.109	<0.001	<0.001

Note: DH = dry heating; MH = moist heating; CHO = carbohydrates; SEM = Standard error of mean; Means with different letters within the same row differ (P<0.05);

Table 4.11 Correlation Analysis between Structural Characteristics of Carbohydrates Detected by Synchrotron Based SR-IMS Technique and Carbohydrate Profiles, Carbohydrate Subfractions and Digestion Kinetics of Cereal Grains Affected by Heat-Related Processing

Items	Total CHO area	CHO peak 1 area	CHO peak 1 height	CHO peak 2 area	CHO peak 2 height	CHO peak 3 area	CHO peak 3 height	Cellulose area	Cellulose peak
	----------Spearman Correlation R value----------								
CHO profiles									
Starch (% DM)	0.58	0.66^{+}	0.62^{+}	0.67*	0.55	0.48	0.22	-0.27	-0.01
CHO (%DM)	0.51	0.64^{+}	0.58	0.64^{+}	0.49	0.38	0.13	-0.30	-0.01
CHO fractions									
CA4	-0.42	-0.33	-0.37	-0.40	-0.37	-0.45	-0.37	0.38	0.04
CB1	0.59^{+}	0.63^{+}	0.60^{+}	0.65^{+}	0.55	0.52	0.27	-0.22	0.05
CB2	-0.56	-0.63^{+}	-0.58^{+}	-0.64^{+}	-0.55	-0.47	-0.27	0.35	0.26
CB3	-0.31	-0.25	-0.29	-0.34	-0.30	-0.33	-0.25	0.38	0.11
CC	-0.19	-0.28	-0.24	-0.22	-0.14	-0.16	0.06	-0.15	-0.32
CHO degradation in rumen									
BCHO (%CHO)	0.14	0.07	0.10	0.15	0.15	0.15	0.21	-0.61^{+}	-0.49
BST (g/kg DM)	0.44	0.48	0.46	0.58	0.41	0.33	0.11	-0.71*	-0.40
Ratio of N to OM	-0.51	-0.64^{+}	-0.58^{+}	-0.63^{+}	-0.50	-0.39	-0.15	0.32	0.14
Ratio of ED_N to EN_OM	-0.51	-0.59^{+}	-0.55	-0.60^{+}	-0.48	-0.41	-0.19	0.56	0.30
Ratio of N to CHO	-0.52	-0.64^{+}	-0.58^{+}	-0.64^{+}	-0.51	-0.40	-0.16	0.32	0.15
Protein digestion in small intestine									
TDP (% CP)	-0.54	-0.64^{+}	-0.60^{+}	-0.67*	-0.51	-0.41	-0.18	0.58	0.27
AMP (g/kg DM)	-0.22	-0.17	-0.19	-0.28	-0.21	-0.20	-0.14	0.77*	0.53
OEB^{DVE} (g/kg DM)	-0.44	-0.46	-0.45	-0.49	-0.43	-0.40	-0.28	0.63^{+}	0.42
MP (g/kg DM)	-0.48	-0.61^{+}	-0.56	-0.60^{+}	-0.46	-0.35	-0.10	0.19	0.02
OEB^{NRC} (g/kg DM)	-0.46	-0.48	-0.47	-0.53	-0.45	-0.41	-0.26	0.69*	0.43
Estimated Feed Milk Values									
FMV^{MP} (kg milk / kg feed)	-0.48	-0.61^{+}	-0.56	-0.60^{+}	-0.46	-0.34	-0.10	0.19	0.02

Note: R = Correlation coefficient calculated using spearman method; CHO = carbohydrates; CA4 = sugar; CB1 = starch; CB2 = soluble fiber; CB3 = available neutral detergent fiber (NDF); CC = unavailable NDF; BCHO = rumen bypass or undegraded CHO; BST = rumen bypass starch;

ED_N = effective degradable nitrogen; ED_OM = effective degradable organic matter; TDP = total digestible protein; AMP = truly absorbed microbial protein in the small intestine; OEB^{DVE} = estimated degraded protein balance using DVE/OEB model; MP = metabolizable protein; OEB^{NRC} = estimated degraded protein balance according to NRC Dairy 2001; FMV^{MP} = estimated feed milk value based on metabolizable protein according to NRC Dairy 2001; $^{+}$for $P<0.10$, * for $P<0.05$, **for $P<0.01$, *** for $P<0.001$.

4.3.2.3 Multivariate Analysis

No difference between grain types or heat treatments was detected by either CLA or PCA. However, the result may be different if parameterized spectra were used instead of the original ones. Further studies could be conducted.

4.4 Conclusion

According to the findings above, we conclude that the sensitivity and responses of functional groups can be detected by both ATR-FTIR and SR-IMS and different functional groups in cereal grain tissues respond differently to the heating methods. The first two hypotheses are accepted but as not all heat-induced structural changes detected by the two mid-IR technique are highly related to the nutrient availability of cereal grains in ruminants, we partly accept the third hypothesis. Although in conventional studies, moist heating was found to have greater impact compared to dry heating on nutrient availability, the results discovered by the SR-IMS technique indicated that dry heating also played a big role in changing the secondary structures and functional groups of the grains. As the peak areas and peak heights represent the combined information of nutrient amount and molecular structure, when many nutrient contents were affected by moist heating, it is possible that the molecular structures in the endosperm were also changed, however, the difference was not shown as a combination. The grains were ground and well-mixed before using the ATR-FTIR technique. It was found that FTIR Spectral structural alterations, especially the changes on protein secondary structure, were highly related with the nutrient availability in cereal grains. In comparison with results found by the ATR-FTIR technique, less and weaker correlation was discovered between the heat-induced structural changes and the nutrient availability in the endosperm area of cereal grains in ruminants by the SR-IMS technique.

5 General Discussions and Conclusion

The objectives of this study were 1) to use advanced synchrotron based technique (SR-IMS) and globar sourced Fourier transform infrared spectroscopy (ATR-FTIR) to directly detect the sensitivity and responses of various chemical functional groups in three types of cereal grains (wheat, triticale and corn) tissues to two types of heat processing methods (dry heating and moist heating); 2) to gain more knowledge about heat effect on cereal grains and their molecular structures. To achieve these goals, seven conventional ruminant feed evaluation methods were applied, including 1) chemical profiles, 2) CNCPS, 3) energy values, 4) *in situ* degradability trial, 5) *in vitro* intestinal digestibility trial, 6) models that predict nutrient supplies (DVE/OEB and NRC-2001 model), 7) feed milk values.

According to previous research (Doiron et al., 2009; Goelema, 1999; McAllister, 1991), the following is expected to happen to moist-heated cereal grains: 1) decreased SCP, 2) lower protein but higher starch degradation in rumen, 3) higher digestible protein and starch in small intestine, 4) higher or unchanged total protein and starch digestibility, 5) better synchrotron of N to energy in rumen, 6) increased potential nutrient supply to dairy cows, 7) different sensitivity and responses in internal functional groups, 8) strong correlations between the spectroscopy detected changes in functional groups and conventional methods detected nutrient availability changes. It is also anticipated that dry heating would affect the cereal grains in some degree, but not as much as the moist heating would, different cereal grains would response differently to the different heating methods, and both the ATR-FTIR and SR-IMS mid-IR techniques would detect the changes. In this study, we found 1) decreased SCP, 2) lower protein but unchanged starch degradation in rumen, 3) higher digestible protein and unchanged digestible starch in small intestine, 4) unchanged total protein and lower starch digestibility, 5) better synchrotron of N to energy in wheat in rumen, 6) increased potential nutrient supply to dairy cows in wheat, 7) different sensitivity and responses in internal functional groups, 8) strong correlations between the ATR-FTIR detected changes in functional groups and conventional methods detected nutrient availability changes.

We did find differences between the three types of cereal grains. Corn tended to be slower degraded than wheat and triticale, which is probably because the rumen microbes are more prone

to colonize the starch granules in wheat and triticale, as the corn endosperm is hornier and only large and distinctive coccoid bacteria such as *Sarcina* would colonize it. The floury part of corn endosperm, however, can be colonized by various bacteria; the vitreous part, with starch granules embedded in the protein matrix, is less susceptible to the bacteria colonization, while the germ is uncolonized even after 48 h of incubation in the rumen (McAllister et al., 1990a, b). As the ruminal degradation of the cereal grains depend largely on the efficiency and extent of microbial colonization, the different bacterial morphotypes stated above may account for part of the reason that cause the differences in degradation kinetics between the three types of grains.

Differences were detected between the heating methods. Khan and Yu (2013) used the Differential Scanning Calorimetry technique to study the thermal stability and mobility on molecular levels of the three cereal grain kernels. They found that the endothermic peak of the dry-heated cereal rose while that of the moist-heated grains decreased, suggesting the high thermal stability of grain kernels when undergoing dry heating. This may be the reason why dry heat turned out to have very limited effect on the nutritional availability of grains, implying that higher dry heating temperature could be used.

When supplied with enough water, starch granules undergo gelatinization at about 62 to 72°C (Rooney et al., 2004). However, although the processing temperature was as high as 121°C, there was still no sign of sufficient gelatinization in this study. McAllister et al. (1991) compared autoclaving (AD) and autoclaving with the samples soaked in buffer (AB) using ground wheat and corn, and found that AD decreased the CP rumen degradation of both cereals while AB increased the DM, CP and starch degradation of wheat ($P<0.001$). It is likely that the cereal starch in the AB treatment accomplished sufficient gelatinization while Maillard Reaction occurred after the AD treatment (McAllister et al., 1991; Seifdavati and Taghizadeh, 2012). Although moisture with pressure was already included in the autoclaving process, the amount of water may still not be enough to create the adequate amount of gelatinization, especially when processing with the grain kernels uncracked. Goelema (1999) also discovered that toasting broken seeds resulted in higher rumen bypass protein in peas and faba beans, but not in lupins. With the protection of the seed coat, it could be hard for the moisture to penetrate into the kernels and affect the endosperm and germ tissues (Rooney et al., 2004). The heat may end up

causing the reaction between the proteins and reduced sugars, fibers, as well as amino acids in the out-layer of grains. The non-enzymatic browning, probably a sign of Millard Reaction, was discovered in all three types of cereal grains after autoclaving (Figure 7.1).

In addition to the Maillard reaction, some other interactions between different nutrient molecules or compounds inside the kernels may also have occurred. Possible reactions during the heating include: 1) the protein-polyphenolic bonds, including the protein-tannins bond and protein-lignin bond; 2) the protein-protein bonds, including the disulfide crosslinks between proteins, as well as the compounds formed by the free carboxyl groups of dicarboxylic amino acids (such as aspartic acid and glutamic acid) and ε-amino group of lysine (Gerrard, 2002; Newkirk, 2002; Zahedifar et al., 2002). The formation of such bonds would certainly reduce the degradability and digestibility of the cereals, but it also explains some detected changes. For example, the increase of NDF, NDICP and ADICP, the digestion tract shift from rumen to small intestine, and possibly differing results found by SR-IMS technique from that detected by ATR-FTIR technique. However, the amount of compounds that could have undergone such reactions is unknown. One possible way to find out the quantity of AAs participated in the protein-protein interactions is analyzing the amount of lysine before and after the heat treatment.

The CNCPS system estimated the decrease in PB1 fraction and the increase of PB3 fraction for all three types of grains, the decrease in PB2 fraction for corn, and the increase in PC fraction for wheat and corn, indicating lower degradability in the rumen and possibly lower protein digestibility. Consistent with these findings, the *in situ* trial results also indicated that the protein digestion tract shifted from rumen to small intestine. However, the intestinal protein digestibility wasn't reduce based on the results from the *in vitro* study. It was also found that in moist-heated wheat and triticale, the synchronization of N to energy was better, which means that the available protein and energy were utilized more efficiently. This point was further confirmed by the DVE/OEB system and NRC-2001 model predicted results. Having more protein supply in the small intestine of ruminants also offered the possibility of producing more milk. In accordance with the increasing DVE and MP values estimated from the two models, the FMV predicted from them also aggrandized.

In order to gain some insights of the internal structural changes, two types of the Fourier Transformed Infrared Microspectroscopy were used in the study. Compared to the ATR-FTIR, the SR-IMS is more advanced in several ways. However, due to the special heating and processing conditions and specific sample area (endosperm) in this study, the ATR-FTIR technique tends to be a more suitable method for this study, as it requires the grinding and mixture of the sample, making it possible for the kernel parts to be evenly distributed on the windows, without holding its nature spatial information. The results from correlation study back up this point, too. Nevertheless, this doesn't mean that SR-IMS is not a good technique, although its sensitivity and accuracy is unclear according to this study. Examinations using SR-IMS technique in some other areas, such as seed coat, aluerone layer and pericarp, are suggested in the future studies in order to understand more about the specific heat-induced alteration in the inner structure of cereal grains.

As a result, we accept our 1st hypothesis that “in cereal grains, the sensitivity and responses of functional groups to heat processing differ and can be detected by SR-IMS as well as ATR-FTIR”, our 2nd hypothesis that “different functional groups such as amide bonds in one type of cereal grain respond differently to different heating methods”, and our 3rd hypothesis that “different types of cereal grains respond differently to different heating methods, their nutritional values, digestion sites and nutrient availability are altered by the heat treatments in different degrees” and partly accept our 4th hypothesis that “heat-induced structural changes in spectral areas of amide, CHO and cellulosic compounds detected by SR-IMS and ATR-FTIR are highly related to nutrient availability of the cereal grains in dairy cattle”, as what is detected by the SR-IMS technique only reflects the changes in the endosperm areas and doesn’t correlated well with the results in conventional studies.

In conclusion, moist heating is suggested when processing wheat and triticale in industry, as it can improve the protein efficiency, availability, and feed milk values in these two grains. However, more research needs be done to find out the optimal processing condition (temperature, time period, moisture level and pressure), as well as a cost-benefit analysis. Meanwhile, due to the possibility of reaction between lysine and the dicarboxylic amino acids, it is suggested to measure the amino acids contents before and after the moist heat treatment to prevent a lack of

essential amino acids in the diets, especially if the grains were heated together with some high-protein feed.

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7 Appendix

Figure 7.1 Color difference of heat-processed cereal grains (ground through 0.5 mm screen)

Table 7.1 Correlation Analysis between Spectral Characteristics of Protein Amide I and II, Protein Secondary Structure α-Helix and β-Sheet Detected by ATR-FTIR Technique and Nutrient Profiles, Protein and Carbohydrate Subfractions and Digestion Kinetics of Cereal Grains

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	Ratio of α-helix to β-sheet height
	----------Spearman Correlation R value----------							
Nutrient profiles								
EE (%DM)	-0.94***	-0.92***	-0.94***	-0.92***	-0.92***	-0.83***	0.76***	-0.01
NDF (%DM)	0.16	0.10	0.22	0.14	0.10	-0.06	-0.33	0.47*
ADF (%DM)	0.51*	0.47*	0.50*	0.47*	0.49*	0.37	-0.48*	0.25
ADL (%DM)	0.25	0.16	0.29	0.20	0.16	-0.01	-0.34	0.45+
Starch (%DM)	-0.84***	-0.78**	-0.88***	-0.84***	-0.80***	-0.64**	0.80***	-0.21
CHO (%DM)	-0.87***	-0.83***	-0.94***	-0.89***	-0.82***	-0.71***	0.87***	-0.08
Hemicellulose (% DM)	0.08	0.00	0.14	0.06	0.00	-0.15	-0.28	0.49*
Cellulose (% DM)	0.47*	0.55*	0.40	0.49*	0.57*	0.60**	-0.29	-0.24
Protein fractions								
PB1	0.22	0.34	0.13	0.25	0.33	0.52*	-0.02	-0.67**
PB2	0.32	0.30	0.38	0.38	0.30	0.27	-0.35	-0.06
PB3	-0.39	-0.48*	-0.34	-0.44+	-0.48*	-0.62**	0.21	0.61**
PC	-0.38	-0.42+	-0.40	-0.45+	-0.41+	-0.54*	0.46+	0.67**
CHO fractions								
CA4	0.22	0.26	0.17	0.25	0.28	0.32	-0.18	-0.24
CB1	-0.72***	-0.66**	-0.73***	-0.71**	-0.67**	-0.51*	0.65**	-0.28
CB2	0.64**	0.53*	0.65**	0.61**	0.54*	0.38	-0.54*	0.23
CB3	0.53*	0.59**	0.47+	0.55*	0.61**	0.61**	-0.32	-0.12
CC	0.32	0.23	0.37	0.27	0.23	0.06	-0.42+	0.44+

Table 7.1 Cont'd

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	Ratio of α-helix to β-sheet height
	----------Spearman Correlation R value----------							
Dry matter degradation in rumen								
K_d	0.52*	0.62**	0.44^{+}	0.53*	0.61**	0.77***	-0.28	-0.59**
T_0	0.22	0.24	0.30	0.33	0.26	0.18	-0.44^{+}	0.13
S	-0.73***	-0.66**	-0.77***	-0.71***	-0.66**	-0.50*	0.73***	-0.24
D	0.76***	0.67**	0.78***	0.76***	0.68**	0.50*	-0.64**	0.29
U	0.17	0.17	0.21	0.15	0.16	0.15	-0.31	0.01
BDM (%DM)	-0.29	-0.40	-0.19	-0.30	-0.39	-0.59*	0.02	0.68**
BDM (g/kg DM)	-0.29	-0.40	-0.19	-0.30	-0.39	-0.59*	0.02	0.68**
EDDM (g/kg DM)	0.29	0.40	0.19	0.30	0.39	0.59*	-0.02	-0.68**
Protein degradation in rumen								
K_d	0.61**	0.68**	0.53*	0.61**	0.68**	0.79***	-0.40	-0.48*
T_0	-0.52*	-0.60**	-0.53*	-0.61**	-0.60**	-0.70**	0.51*	0.60**
S	-0.81***	-0.78***	-0.86***	-0.83***	-0.78***	-0.67**	0.81***	-0.11
D	0.64**	0.56*	0.71**	0.64**	0.56*	0.38	-0.70**	0.34
U	0.55*	0.65**	0.50*	0.58*	0.65**	0.78***	-0.39	-0.51*
BCP (%CP)	-0.26	-0.35	-0.15	-0.26	-0.35	-0.53*	0.01	0.64**
BCP (g/kg DM)	0.38	0.29	0.50*	0.40^{+}	0.29	0.09	-0.60**	0.49*
EDCP (g/kg DM)	0.81***	0.84***	0.75***	0.79***	0.84***	0.89***	-0.53*	-0.38
Neutral detergent fiber degradation in rumen								
K_d	0.24	0.28	0.16	0.16	0.27	0.31	-0.06	-0.13
T_0	-0.56*	-0.59**	-0.51*	-0.52*	-0.59*	-0.56*	0.32	0.08
S	-0.60**	-0.58*	-0.58*	-0.57*	-0.59*	-0.47^{+}	0.49*	-0.21
D	0.17	0.22	0.13	0.21	0.20	0.32	-0.14	-0.46^{+}
U	0.40	0.34	0.40^{+}	0.34	0.36	0.19	-0.32	0.46^{+}
BNDF (%NDF)	0.38	0.30	0.43^{+}	0.35	0.29	0.13	-0.41^{+}	0.41^{+}
BNDF (g/kg DM)	0.49*	0.43^{+}	0.48*	0.44^{+}	0.45^{+}	0.28	-0.42^{+}	0.43^{+}
EDNDF (g/kg DM)	-0.38	-0.30	-0.43^{+}	-0.35	-0.29	-0.13	0.41^{+}	-0.41^{+}

Table 7.1 Cont'd

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	Ratio of α-helix to β-sheet height
Starch degradation in rumen								
K_d	0.42^{+}	0.39	0.41^{+}	0.45^{+}	0.41^{+}	0.38	-0.42^{+}	-0.13
S	-0.49*	0.12	-0.53*	-0.45^{+}	-0.38	-0.16	0.49*	-0.54*
D	0.49*	0.12	0.53*	0.45^{+}	0.38	0.16	-0.49*	0.54*
BST (%ST)	-0.30	-0.30	-0.28	-0.34	-0.31	-0.38	0.29	0.39
BST (g/kg DM)	-0.63**	-0.61**	-0.65**	-0.68**	-0.62**	-0.62**	0.65**	0.26
EDST (g/kg DM)	-0.38	-0.34	-0.41^{+}	-0.34	-0.34	-0.17	0.32	-0.47*
Carbohydrate degradation in rumen								
K_d	0.61**	0.70**	0.52*	0.60**	0.69**	0.84***	-0.33	-0.59*
T_0	0.13	0.13	0.22	0.21	0.15	0.07	-0.34	0.19
S	-0.56*	-0.46^{+}	-0.60**	-0.52*	-0.47^{+}	-0.25	0.54*	-0.50*
D	0.30	0.17	0.36	0.27	0.18	-0.08	-0.36	0.72***
U	0.62**	0.67**	0.57*	0.60**	0.66**	0.74***	-0.45^{+}	-0.42^{+}
BCHO (%CHO)	-0.23	-0.34	-0.14	-0.25	-0.33	-0.54*	-0.03	0.71**
BCHO (g/kg DM)	-0.48*	-0.58*	-0.40^{+}	-0.51*	-0.57*	-0.74***	0.24	0.68**
EDCHO (g/kg DM)	-0.27	-0.16	-0.37	-0.25	-0.16	0.08	0.44^{+}	-0.66**
Carbohydrate degradation in small intestine								
IDBST (g/kg DM)	-0.42^{+}	-0.33	-0.51*	-0.45^{+}	-0.34	-0.18	0.64**	-0.31
TDST (%ST)	0.17	0.27	0.06	0.17	0.25	0.47^{+}	0.12	-0.71***
TDST (g/kg DM)	-0.56*	-0.46^{+}	-0.63**	-0.55*	-0.47*	-0.24	0.65**	-0.55*
IDBNDF (%DNF)	0.46^{+}	0.49*	0.41^{+}	0.48*	0.51*	0.54*	-0.25	-0.19
IDBNDF (g/kg DM)	0.45^{+}	0.47*	0.40	0.46^{+}	0.50*	0.49*	-0.26	-0.08
TDNDF (%NDF)	-0.20	-0.11	-0.27	-0.16	-0.10	0.07	0.30	-0.45^{+}

Note: R = Correlation coefficient calculated using spearman method; DM = dry matter; EE = ether extract (=crude fat); NDF = neutral detergent fiber; ADF = acid detergent fiber; ADL = acid detergent lignin; CHO = carbohydrates; PB1 = true protein that soluble in borate phosphate buffer and precipitated by TCA; PB2 = intermediately degradable protein; PB3 = insoluble in neutral detergent but soluble in acid detergent; PC = unavailable protein; CA4 = sugar; CB1 = starch; CB2 = soluble fiber; CB3 = available neutral detergent fiber (NDF); CC = unavailable NDF; K_d = the rate of degradation of D fraction (%/h); T_0 = lag time in h; S = soluble fraction in the *in situ* incubation; D = degradable fractions; U = undegradable degradable fractions; BDM = rumen bypass dry matter; EDDM = effective degradability of dry matter; BCP = rumen bypass feed crude protein; EDCP = effective degradability of feed crude protein; BNDF = rumen bypass feed neutral detergent fibre; EDNDF = effective

degradability of neutral detergent fibre; BST = rumen bypass starch; EDST = effective degradability of starch; BCHO = rumen bypass CHO; EDCHO = effective degradability of CHO; IDBST = intestinal digestible rumen bypass starch; TDST = total digestible starch; IDBNDF = intestinal digestible rumen bypass neutral detergent fiber; TDNDF = total digestible neutral detergent fiber. $^{+}$ for $P < 0.10$, * for $P < 0.05$, ** for $P < 0.01$, *** for $P < 0.001$.

Table 7.2 Correlation Analysis between Spectral Characteristics of Carbohydrates Detected by ATR-FTIR Technique and Nutritional Profiles, Protein and Carbohydrate Subfractions and Digestion Kinetics of Cereal Grains

Items	Total CHO area	CHO peak 1 area	CHO peak 1 height	CHO peak 2 area	CHO peak 2 height	CHO peak 3 area	CHO peak 3 height
	----------Spearman Correlation R value----------						
Nutrient profiles							
CP (%DM)	-0.56*	-0.78***	-0.75***	-0.31	-0.46+	-0.57*	-0.64**
EE (%DM)	0.28	0.58*	0.54*	0.02	0.18	0.29	0.39
Protein fractions							
PB1	0.60**	0.35	0.42+	0.65**	0.63**	0.63**	0.57*
PB2	-0.53*	-0.48*	-0.49*	-0.50*	-0.52*	-0.53*	-0.51*
PB3	-0.21	-0.04	-0.09	-0.27	-0.25	-0.23	-0.20
PC	-0.02	0.23	0.17	-0.12	-0.05	-0.03	0.02
CHO fractions							
CA4	0.48*	0.23	0.27	0.55*	0.51*	0.49*	0.42+
CB1	0.28	0.51*	0.49*	0.07	0.21	0.29	0.39
CB2	-0.55*	-0.65**	-0.64**	-0.40+	-0.50*	-0.55*	-0.60**
CB3	0.36	0.06	0.13	0.53	0.44	0.37	0.28
CC	-0.38	-0.46+	-0.47*	-0.25	-0.35	-0.41+	-0.45+
Dry matter degradation in rumen							
K_d	0.42+	0.11	0.18	0.55*	0.48*	0.44+	0.35
T_0	0.04	-0.10	-0.08	0.15	0.08	0.03	-0.01
S	0.34	0.59**	0.57*	0.11	0.27	0.36	0.45+
D	-0.49*	-0.66**	-0.64**	-0.29	-0.42+	-0.50*	-0.56*
U	0.08	-0.09	-0.07	0.18	0.09	0.05	-0.00
BDM (%DM)	-0.44+	-0.23	-0.29	-0.48*	-0.47*	-0.47*	-0.42+
BDM (g/kg DM)	-0.44+	-0.23	-0.29	-0.48*	-0.47*	-0.47*	-0.42+
EDDM (g/kg DM)	-0.44+	-0.23	-0.29	-0.48*	-0.47*	-0.47*	-0.42+

Table 7.2 Cont'd

Items	Total CHO area	CHO peak 1 area	CHO peak 1 height	CHO peak 2 area	CHO peak 2 height	CHO peak 3 area	CHO peak 3 height
	----------Spearman Correlation R value----------						
Protein degradation in rumen							
K_d	0.36	0.01	0.07	0.51*	0.42^{+}	0.37	0.27
T_0	-0.11	0.14	0.07	-0.25	-0.17	-0.13	-0.07
S	0.37	0.65**	0.61**	0.13	0.29	0.39	0.48*
D	-0.54*	-0.70**	-0.69**	-0.33	-0.48*	-0.56*	-0.62**
U	0.31	-0.01	0.07	0.45^{+}	0.37	0.32	0.23
BCP (%CP)	-0.50*	-0.28	-0.33	-0.53*	-0.52*	-0.52*	-0.47*
BCP (g/kg DM)	-0.68**	-0.67**	-0.69**	-0.53*	-0.63**	-0.70**	-0.71**
EDCP (g/kg DM)	-0.01	-0.32	-0.26	0.17	0.06	-0.00	-0.09
Neutral detergent fiber degradation in rumen							
K_d	0.49*	0.26	0.29	0.60**	0.53*	0.48*	0.40^{+}
T_0	-0.25	-0.03	-0.06	-0.42^{+}	-0.32	-0.23	-0.17
S	-0.00	0.27	0.24	-0.22	-0.07	0.01	0.12
D	0.24	0.01	0.07	0.28	0.26	0.26	0.21
U	-0.14	0.23	-0.24	0.01	-0.10	-0.17	-0.22
BNDF (%NDF)	-0.47^{+}	-0.48*	-0.51*	-0.35	-0.44^{+}	-0.49*	-0.52*
BNDF (g/kg DM)	-0.04	-0.25	-0.23	0.14	0.02	-0.07	-0.15
EDNDF (g/kg DM)	0.52*	0.39	0.43^{+}	0.51*	0.53*	0.53*	0.50*
Starch degradation in rumen							
K_d	-0.03	-0.24	-0.20	0.08	0.01	-0.02	-0.09
S	0.51*	0.57*	0.59*	0.36	0.47*	0.54*	0.58*
D	-0.51*	-0.57*	-0.59*	-0.36	-0.47*	-0.54*	-0.58*
BST (%ST)	-0.04	0.14	0.09	-0.08	-0.06	-0.06	-0.01
BST (g/kg DM)	0.20	0.46^{+}	0.41^{+}	0.05	0.14	0.19	0.26
EDST (g/kg DM)	0.31	0.35	0.37	0.17	0.27	0.34	0.37

Table 7.2 Cont'd

Items	Total CHO area	CHO peak 1 area	CHO peak 1 height	CHO peak 2 area	CHO peak 2 height	CHO peak 3 area	CHO peak 3 height
	----------Spearman Correlation R value----------						
Carbohydrate degradation in rumen							
K_d	0.34	0.03	0.10	0.49^{*}	0.41^{+}	0.36	0.27
T_0	-0.19	-0.23	-0.22	-0.10	-0.16	-0.20	-0.20
S	0.43^{+}	0.55^{*}	0.56^{*}	0.26	0.38	0.46^{+}	0.52^{*}
D	-0.58^{*}	-0.55^{*}	-0.58^{*}	-0.48^{*}	-0.56^{*}	-0.60^{**}	-0.63^{**}
U	0.27	-0.07	-0.02	0.45^{+}	0.33	0.26	0.16
BCHO (%CHO)	-0.43^{+}	-0.24	-0.30	-0.45^{+}	-0.45^{+}	-0.46^{+}	-0.42^{+}
BCHO (g/kg DM)	-0.28	-0.02	-0.09	-0.37	-0.33	-0.31	-0.25
EDCHO (g/kg DM)	0.60^{**}	0.57^{*}	0.61^{**}	0.49^{*}	0.58^{*}	0.63^{**}	0.65^{**}

Note: R = Correlation coefficient calculated using spearman method; DM = dry matter; CP = crude protein; EE = ether extract (=crude fat); PB1 = true protein that soluble in borate phosphate buffer and precipitated by TCA; PB2 = intermediately degradable protein; PB3 = insoluble in neutral detergent but soluble in acid detergent; PC = unavailable protein; CA4 = sugar; CB1 = starch; CB2 = soluble fiber; CB3 = available neutral detergent fiber (NDF); CC = unavailable NDF; K_d = the rate of degradation of D fraction (%/h); T_0 = lag time in h; S = soluble fraction in the *in situ* incubation; D = degradable fractions; U = undegradable degradable fractions; BDM = rumen bypass dry matter; EDDM = effective degradability of dry matter; BCP = rumen bypass feed crude protein; EDCP = effective degradability of feed crude protein; BNDF = rumen bypass feed neutral detergent fibre; EDNDF = effective degradability of neutral detergent fibre; BST = rumen bypass starch; EDST = effective degradability of starch; BCHO = rumen bypass CHO; EDCHO = effective degradability of CHO. $^{+}$ for $P < 0.10$, * for $P < 0.05$, ** for $P < 0.01$, *** for $P < 0.001$.

Table 7.3 Correlation Analysis between Spectral Characteristics of Cellulosic and Lipid Compounds Detected by ATR-FTIR Technique and Nutritional Profiles, Protein and Carbohydrate Subfractions and Digestion Kinetics of Cereal Grains

Items	Cellulosic Compounds		Lipid Compounds	
	Area	Peak	Area	Peak
	----------Spearman Correlation R value----------			
Nutrient profiles				
CP (%DM)	0.27	-0.21	-0.71***	-0.70**
ADF (%DM)	0.44+	-0.04	-0.61**	-0.62**
Starch (%DM)	-0.32	0.24	0.74***	0.74***
CHO (%DM)	-0.23	0.24	0.70**	0.68**
NDICP (%CP)	-0.23	-0.32	0.41+	0.38
ADICP (%CP)	-0.07	0.13	0.59*	0.56*
Protein fractions				
PB1	0.36	0.42+	-0.44+	-0.42+
PB2	-0.15	-0.11	-0.03	-0.01
PB3	-0.24	-0.36	0.37	0.33
PC	-0.07	0.13	0.59*	0.56*
CHO fractions				
CA4	0.44+	0.16	-0.54*	-0.55*
CB1	-0.34	0.21	0.67**	0.68**
CB2	0.03	-0.34	-0.46+	-0.46+
CB3	0.55*	0.38	-0.62**	-0.61**
CC	0.07	-0.39	-0.32	-0.34

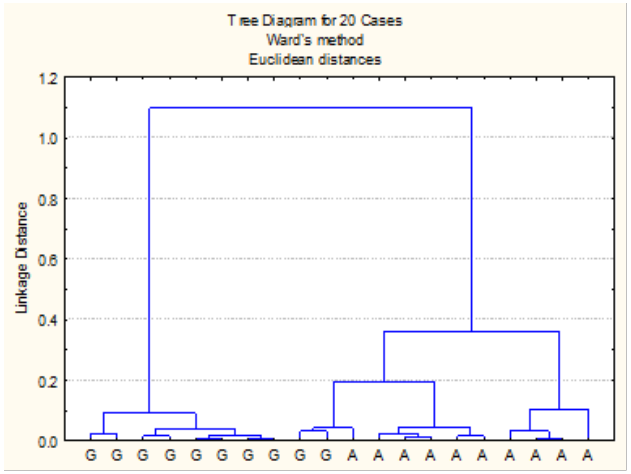
Table 7.3 Cont'd

Items	Cellulosic Compounds		Lipid Compounds	
	Area	Peak	Area	Peak
	----------Spearman Correlation R value----------			
Dry matter degradation in rumen				
K_d	0.47*	0.40	-0.65**	-0.64**
T_0	0.23	-0.06	-0.25	-0.25
S	-0.33	0.25	0.67**	0.67**
D	0.23	-0.18	-0.56*	-0.55*
U	0.20	-0.14	-0.30	-0.32
BDM (%DM)	-0.32	-0.44+	0.43+	0.41+
BDM (g/kg DM)	-0.32	-0.44+	0.43+	0.41+
EDDM (g/kg DM)	-0.32	-0.44+	0.43+	0.41+
Protein degradation in rumen				
K_d	0.57*	0.36	-0.75***	-0.73***
T_0	-0.25	-0.13	0.56*	0.53*
S	-0.36	0.22	0.77***	0.77***
D	0.19	-0.38	-0.53*	-0.53*
U	0.45+	0.34	-0.69**	-0.68**
BCP (%CP)	-0.38	-0.46+	0.41+	0.39
BCP (g/kg DM)	-0.10	0.46+	-0.17	-0.17
EDCP (g/kg DM)	0.48*	0.24	-0.78***	-0.75***

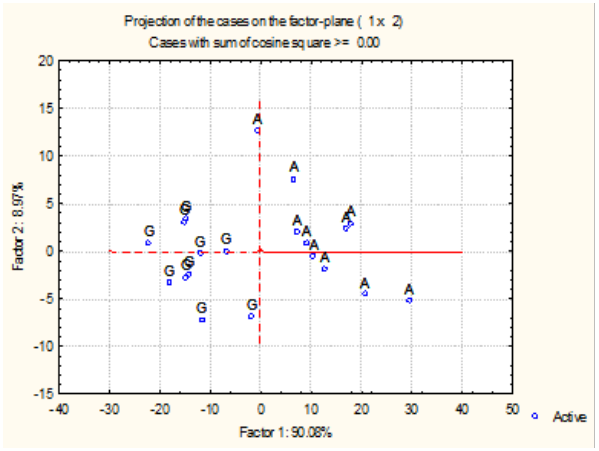
Items	Cellulosic Compounds		Lipid Compounds	
	Area	Peak	Area	Peak
	----------Spearman Correlation R value----------			
Neutral detergent fiber degradation in rumen				
K_d	0.50*	0.27	-0.39	-0.41+
T_0	-0.70**	-0.38	0.40	0.36
S	-0.57*	-0.01	0.60**	0.63**
D	0.15	0.03	-0.52*	-0.50*
U	0.37	-0.01	-0.18	-0.21
BNDF (%NDF)	0.13	-0.25	-0.09	-0.10
BNDF (g/kg DM)	0.43+	-0.06	-0.47*	-0.49*
EDNDF (g/kg DM)	0.18	0.26	-0.23	-0.23
Starch degradation in rumen				
K_d	0.16	-0.01	-0.50*	-0.50*
S	-0.14	0.36	0.31	0.33
D	0.14	-0.36	-0.31	-0.33
BST (%ST)	-0.02	-0.05	0.42+	0.42+
BST (g/kg DM)	-0.13	0.11	0.72***	0.71***
EDST (g/kg DM)	-0.25	0.17	0.19	0.19
Carbohydrate degradation in rumen				
K_d	0.49*	0.41+	-0.68**	-0.66**
T_0	0.03	-0.14	-0.03	-0.01
S	-0.24	0.32	0.42+	0.43+
D	-0.01	-0.43+	-0.10	-0.12
U	0.56*	0.20	-0.73***	-0.71***
BCHO (%CHO)	-0.27	-0.43+	0.38	0.35
BCHO (g/kg DM)	-0.34	-0.34	0.59**	0.56*
EDCHO (g/kg DM)	0.06	0.47*	0.08	0.10

Note: R: Correlation coefficient calculated using spearman method; DM = dry matter; CP = crude protein; EE = ether extract (=crude fat); ADF = acid detergent fiber; CHO = carbohydrates; NDICP = neutral detergent insoluble crude protein; ADICP = acid detergent insoluble crude protein;

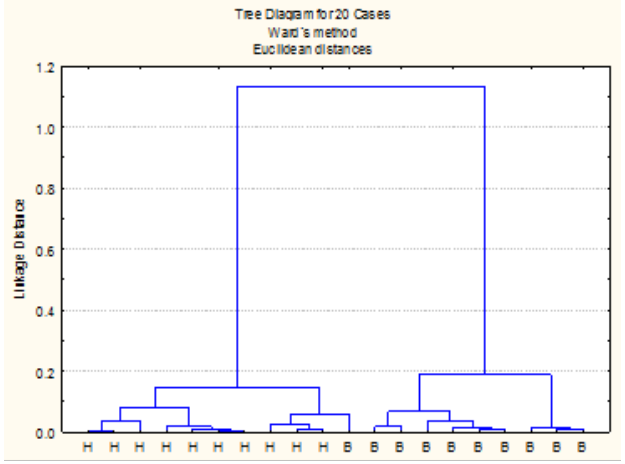
PB1 = true protein that soluble in borate phosphate buffer and precipitated by TCA; PB2 = intermediately degradable protein; PB3 = insoluble in neutral detergent but soluble in acid detergent; PC = unavailable protein; CA4 = sugar; CB1 = starch; CB2 = soluble fiber; CB3 = available neutral detergent fiber (NDF); CC = unavailable NDF; K_d = the rate of degradation of D fraction (%/h); T_0 = lag time in h; S = soluble fraction in the *in situ* incubation; D = degradable fractions; U = undegradable degradable fractions; BDM = rumen bypass dry matter; EDDM = effective degradability of dry matter; BCP = rumen bypass feed crude protein; EDCP = effective degradability of feed crude protein; BNDF = rumen bypass feed neutral detergent fibre; EDNDF = effective degradability of neutral detergent fibre; BST = rumen bypass starch; EDST = effective degradability of starch; BCHO = rumen bypass CHO; EDCHO = effective degradability of CHO. $^{+}$ for $P < 0.10$, * for $P < 0.05$, ** for $P < 0.01$, *** for $P < 0.001$.



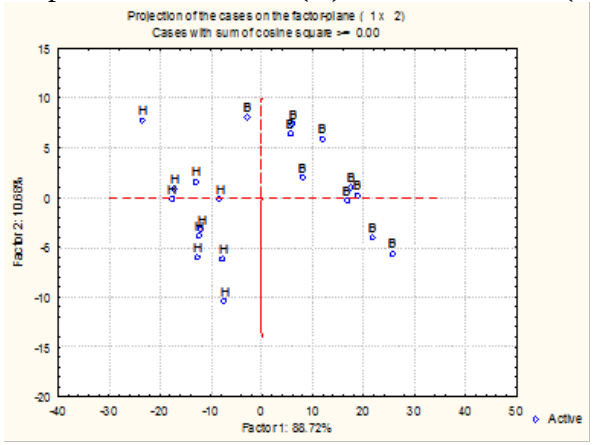
I. CLA Comparison: Raw Corn (G) and Raw Wheat (A)



II. PCA Comparison: Raw Corn (G) and Raw Wheat (A)

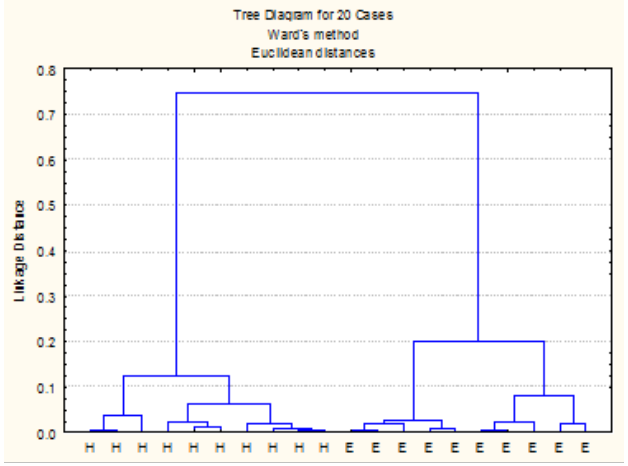


III. CLA Comparison: DH Corn (H) and MH Wheat (B)

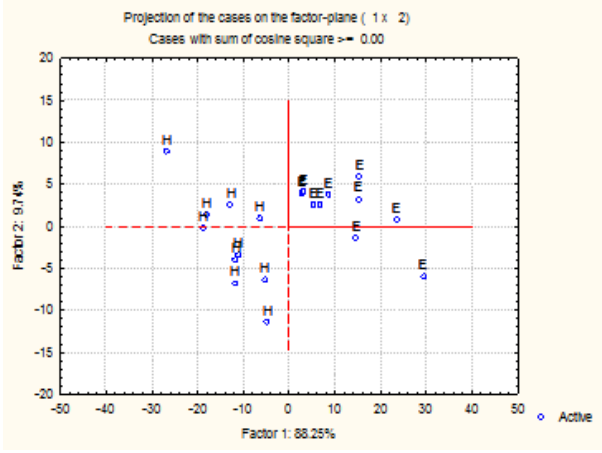


IV. PCA Comparison: DH Corn (H) and MH Wheat (B)

Figure 7.2 Multivariate molecular spectral analyses of the cereal grains at ATR-FTIR protein fingerprint region (ca. 1482-1725 cm^{-1})
I, III and V: cluster analysis (1) Cluster method: Ward's algorithm; (2) Distance method: Euclidean;
II, IV and VI: principal component analysis: Scatter plots of the 1st principal components (PC1) vs. the 2nd principal components (PC2).

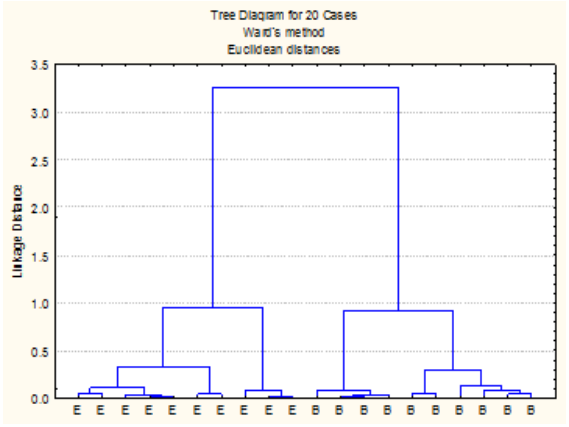


V. CLA Comparison: DH Corn (H) and DH Triticale (E)

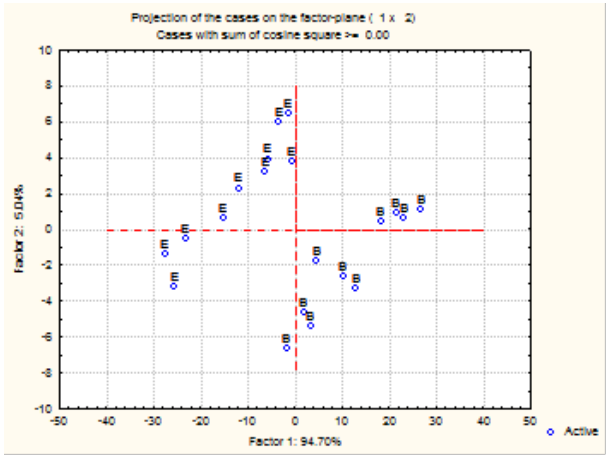


VI. PCA Comparison: DH Corn (H) and DH Triticale (E)

Figure 7.2 Cont'd

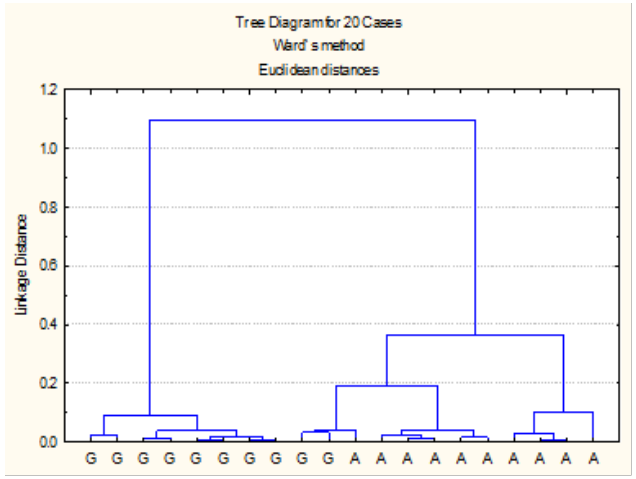


I. CLA Comparison: DH Corn (H) and MH Wheat (B)

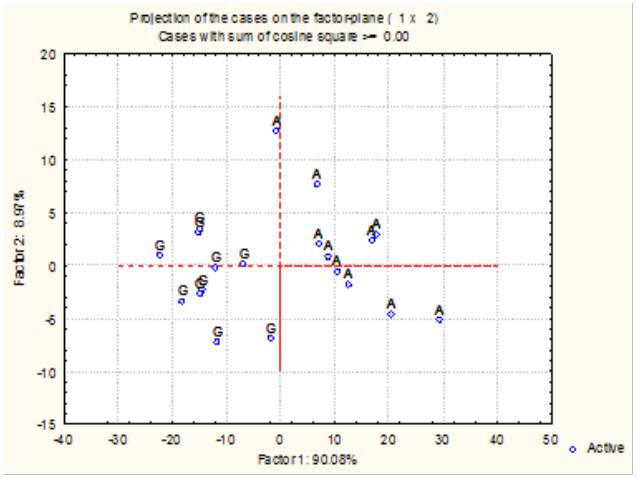


II. PCA Comparison: DH Corn (H) and MH Wheat (B)

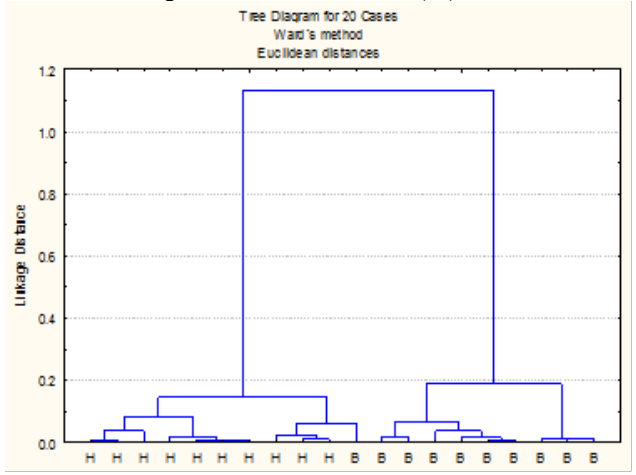
Figure 7.3 Multivariate molecular spectral analyses of the cereal grains at ATR-FTIR CHO fingerprint region (ca. 945-1191 $cm^{-1)}$
I: cluster analysis (1) Cluster method: Ward's algorithm; (2) Distance method: Euclidean;
II: principal component analysis: Scatter plots of the 1st principal components (PC1) vs. the 2nd principal components (PC2).



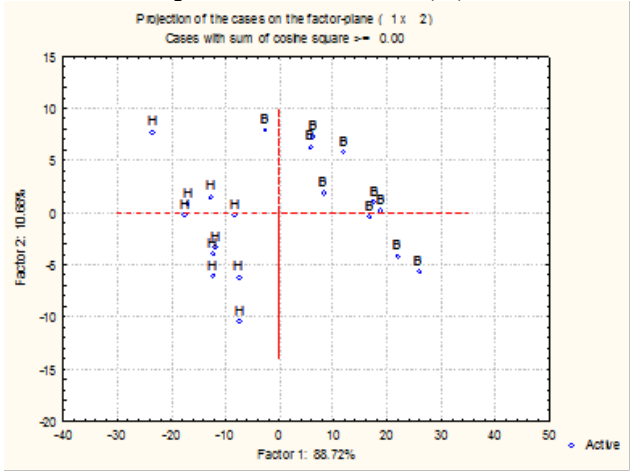
I. CLA Comparison: Raw Corn (G) and Raw Wheat (A)



II. PCA Comparison: Raw Corn (G) and Raw Wheat (A)



III. CLA Comparison: DH Corn (H) and MH Wheat (B)



IV. PCA Comparison: DH Corn (H) and MH Wheat (B)

Figure 7.4 Multivariate molecular spectral analyses of the cereal grains at ATR-FTIR lipid fingerprint region (ca. 1709-1798 cm^{-1})
I and III: cluster analysis (1) Cluster method: Ward's algorithm; (2) Distance method: Euclidean;
II and IV: principal component analysis: Scatter plots of the 1st principal components (PC1) vs. the 2nd principal components (PC2).