

**STRUCTURAL, CHEMICAL AND NUTRIENT CHARACTERIZATION OF
ORIGINAL FEED STOCK AND CO-PRODUCTS FROM A NEWLY BUILT
BIOFUEL PLANT IN SASKATCHEWAN**

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Submitted

by

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ABSTRACT

The objective of this study was to determine the structural, chemical and nutrient variation associated with different batches of original feedstock grain (wheat) and co-products (wheat distillers dried grain with solubles, wDDGS) from bioethanol processing and to study possibility of using molecular spectroscopy FT/IR-ATR technique as a rapid tool of identifying the structure spectral differences among the batches. The samples were collected from different batches of wheat and wDDGS produced from the same and newly build bioethanol plant in western Canada with updated bioethanol processing technology.. The results of chemical composition analysis showed significant differences ($P < 0.05$) among the batches of wheat and among the batches of wDDGS. The variation in term of range among the batches of wheat for crude protein (CP), neutral detergent fibre (NDF) and acid detergent fibre (ADF) were 2.1, 2.5 and 1.0 %DM, respectively. Among the batches of wDDGS, the variation in CP, NDF and ADF were 3.9, 5.2 and 3.8 %DM, respectively. The soluble crude protein (SCP) content ranged by 7.2% of CP among the batches of wheat and by 4.2% of CP among the batches of wDDGS. In mineral profiles, variation among the batches of wheat and wDDGS in sulfur were 0.03 and 0.34 % of DM, respectively. In mineral profiles, higher phosphorus and sulfur content associated with wDDGS could be a concern. The sulfur content ranged by 0.3 % of DM among the batches of wDDGS with a mean of 1.1 %DM. For the estimated subfractions with Cornell Net Carbohydrate and Protein System (CNCPS), the immediately rumen available PA fraction (NPN) ranged by 14.8 % of CP among the batches of wheat and by 2.7 % of CP among batches of wDDGS. The particle size analysis revealed the small particle size in all three batches of wDDGS with the geometric mean of ranging from 650-690 μm . In situ rumen degradation kinetic results showed a significant differences

($P < 0.05$) among both wheat and wDDGS batches. Multivariate molecular spectral analyses showed a potential of using FT/IR molecular vibrational spectroscopy as a rapid method to identify structure difference associated with different batches. In conclusion, the structural, chemical and nutrient availability vary among the batches of wheat and among the batches of wDDGS. The molecular spectroscopy shows a potential as a rapid tool to identify batch difference in chemical and nutrient profiles and to detect the response of functional group to bioethanol processing. Further study is needed to analyse molecular spectral characteristics in details for development of a successful identification tool to identify batch differences. The variation among the batches of wDDGS should be considered in ration formulation especially at high inclusion rates (40-50% wDDGS in diet DM) even the co-products are produced from the same bioethanol plant.

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ABBREVIATIONS

ADF	Acid detergent fiber
ADICP	Acid detergent insoluble crude protein
ADIN	Acid detergent insoluble nitrogen
ADL	Acid detergent lignin
AECP	Truly absorbable endogenous protein
AMCP	Truly absorbable microbial protein synthesized in the rumen
AOAC	Association of Official Analytical Chemists
ARUP	Truly absorbed bypass feed protein in small intestine
AHCA	Agglomerative hierarchical cluster analysis
ATR	Attenuated total reflectance
CA	Rapidly fermented carbohydrate sub-fraction
CB1	Intermediately degraded carbohydrate sub-fraction
CB2	Slowly degraded carbohydrate sub-fraction
CC	Unavailable carbohydrate fraction (cell wall as per CNCPS)
CCAC	Canadian Council of Animal Care
CHO	Carbohydrate
Cfat	Crude fat
CNCPS	Cornell Net Carbohydrate and Protein System
CP	Crude Protein
Cu	Copper
D (B)	Potentially degradable fraction of in situ rumen incubation
DDGS	Distillers dried grains with solubles
DE	Digestible energy
DE _{1x}	Digestible energy at a maintenance level
DM	Dry matter
DMCP	Intestinally digestible microbial protein
DMFP	Endogenous protein losses in the faeces
DOM	Digestible organic matter
DPB	Degraded protein balance

DRUP	Intestinally digestible rumen undegradable protein
DVBE	Rumen undegraded feed protein digested and absorbed in small intestine
DVME	Microbial protein synthesized in the rumen and absorbed in the small intestine
DVMFE	Endogenous protein in the faeces associated with the digestion
DVE/OEB	Dutch protein evaluation system
ECP	Correction for endogenous protein losses associated with digestion process
ED	Effective degradability
EDCP	Effective degradability of crude protein
EE	Ether extract
ESC	Ethanol soluble carbohydrate
FA	Fatty acids
FCOMP	Effectively rumen degradable component
FCP	Fermentable crude protein
Ffat	Fermentable fat
FOM	Fermentable organic matter in the rumen
FNDF	Fermentable neutral detergent fibre
FRNSP	Fermentable rumen non-starch polysaccharides
FSTA	Fermentable starch
FP	Fermentable products
FTIR	Fourier Transform Infrared Vibration Spectroscopy
HEDN	Hourly effective degradability of nitrogen
HEDOM	Hourly effective degradability of organic matter
IADP	Estimated intestinally absorbable feed protein
IDP	Estimated intestinal digestibility of rumen bypass protein
IR	Infrared
ISWF	Insoluble washable fraction at 0 h in situ incubation
K_d	Rate constant for in situ rumen degradation of D fraction
K_p	Rate of passage
MCP_{FOM}	Microbial crude protein produced based on fermentable organic matter
MCP_{RDP}	Microbial crude protein produced based on rumen degradable protein

MCPE	Microbial protein synthesized based on rumen available energy from anaerobic fermentation
MCPN	Microbial protein synthesized based on rumen available N
ME _{3x}	Metabolizable energy at a production level when intake is 3 times maintenance intake
MP	Metabolizable protein
MREN	Microbial protein synthesized from rumen degradable feed protein
MREE	Microbial protein synthesized based on energy from anaerobic fermentation
NDF	Neutral detergent fibre
NE _L	Net energy for lactation
NPN	Non-protein nitrogen
NRC	National Research Council
NSC	Non-structural carbohydrate
N	Nitrogen
Na	Sodium
NDICP	Neutral detergent insoluble crude protein
NE	Net energy
NE _g	Net energy for gain
NE _m	Net energy for maintenance
NEL _{3x}	Net energy for lactation when intake is 3 times maintenance intake
NFC	Non-fibrous carbohydrate
nNDF	Nitrogen corrected neutral detergent fibre
OM	Organic matter
P	Phosphorus
PAF	Processing adjustment factor
PB1	Rapidly degradable true protein
PB2	Intermediately degradable true protein
PB3	Slowly degradable true protein
PC	Undegradable protein
PCA	Principal component analysis
R(t)	Residue present at a specific time t during in situ ruminal incubation

RDP	Rumen degraded protein
RNSP	Residual non-starch polysaccharides
S	Soluble fraction
SCP	Soluble crude protein
SRC	Saskatchewan Research Council
SWF	Soluble washable fraction
T ₀	Lag time
TCA	Tri-chloro acetic acid
tdCP	Total digestible crude protein
tdFA	Total digestible fatty acids
TDN	Total digestible nutrients
TDN _{1x}	Total digestible nutrient at a maintenance level
TDN _{3x}	Total digestible nutrient at three times maintenance level
tdNDF	Truly digestible NDF
tdNFC	Truly digestible non-fibrous carbohydrate
TP	True protein
TPSI	True protein supply to the small intestine
U	Undegradable fraction
WI	Washable insoluble fraction
Zn	Zinc

1.0. INTRODUCTION

Distillers dried grains with solubles (DDGS) are nutritional co-products which are residual products of the fermentation of grains into bioethanol. Bioethanol production has intensified during past few years in Canada because of the federal and provincial mandates to use 5% renewable fuel in gasoline by 2010. The increased bioethanol production resulted in the production of large quantities of DDGS (CRFA, 2010). Increased price and demand for feed grain made DDGS as an ideal substitute, in the place of grain to certain extent. Since more than half of Canada's wheat production is grown in western Canada (CGC, 2010), wheat is a main feedstock for bioethanol production in western Canada. The main drawback of using DDGS for the livestock feed industry is the inconsistency of its nutritional quality. The variation in nutritional quality is due to many factors and affects nutrient composition, digestibility and availability to the animal (Cromwell et al., 1993; Shurson et al., 2001; Kleinschmit et al., 2006; Nuez-Ortín and Yu, 2009, 2010; Belyea et al., 1998, 2004, 2010). Variation among ethanol plants is due to the type of grain feedstock (Boila and Ingalls, 1994 a,b; Spiels et al., 2002; Belyea et al., 2010; Nuez-Ortín and Yu, 2010), processing methods (Martinez-Amezcuca et al., 2007), complete, partial or no adding back of solubles (Ham et al., 1994; Harty et al., 1998; Martinez-Amezcuca et al., 2007) and drying temperatures and conditions (Shurson and Noll, 2005; Saunders and Rosentrater, 2009; Kingsly et al., 2010). However, the limited research work on wheat-based DDGS produced from the same bioethanol plant created the lack of knowledge about the variation among different batches of wheat DDGS in ration formulation.

The objectives of this study were to determine the i) differences in nutrient and chemical composition including macro and micro mineral composition, ii) differences in carbohydrate and protein sub-fractions, iii) differences in predicted energy values, iv) in situ rumen degradation kinetics of various nutrients, v) differences in predicted true protein supply to small intestine with three nutrition models (NRC-2001, DVE/OEB-1994 and DVE/OEB-2007), and vi) to detect molecular spectroscopic features with FT/IR-ATR of different batches of feedstock wheat and wheat DDGS from a newly-built bioethanol plant in western Canada to test the possibility of using the molecular spectroscopy technique with multivariate molecular analyses as a fast method to detect batch differences in original feedstock and

wheat DDGS. Wheat DDGS that we studied are mash-type co-products which are different from marble-type co-products.

2.0. LITERATURE REVIEW

2.1. Bioethanol Industry and Animal Feed Industry

The increased demand for energy and the environment related issues with fossil fuels (Environment Canada, 2011) caused a remarkable increase in the production of renewable fuel during past few years. Bioethanol processing is one of the recent trends of producing renewable fuel (RFA, 2011). The global bioethanol production increased from 39,192 million litres in 2006 to 85,763 million liters in 2010 (RFA, 2011). In Canada alone, the production was increased from 569 to 1,350 million liters (APEC, 2008; RFA, 2011). Even though bioethanol can be produced from a variety of feedstocks (Sánchez and Cardona, 2007), the most common feedstocks used are cereal grains (Government of Alberta, 2008; Burden, 2009).

The type of feedstock grain used for bioethanol production differs according to availability and cost of production (Rosentrater and Muthukumarappan, 2006). The most common types of feedstock grains used for the bioethanol production are corn, wheat, sorghum, triticale and barley (Sánchez and Cardona, 2007). Corn is the most widely used grain type for bioethanol production in the USA due to its high starch content and cost effectiveness (Bothast and Schlicher, 2005). For bioethanol production, corn is the main feedstock in Eastern Canada while wheat is the major feedstock in western Canada (CRFA, 2010). A co-product of bioethanol production, distillers dried grain with solubles (DDGS), is a nutrient rich product that can be used in animal feeding (Bothast and Schlicher, 2005). Table 2.1 summarizes the different feedstocks used by Canadian bioethanol plants.

Table 2.1. Different feedstocks used in Canadian bioethanol plants (Adapted from CRFA, 2010)

Plant	Province	FeedStock
Alberta Ethanol and Biodiesel GP Ltd.	Alberta	Wheat
Enerkem Alberta Biofuels	Alberta	Municipal Solid Waste
Growing Power Hairy Hill	Alberta	Wheat
Permolex International, L.P.	Alberta	Wheat, Wheat Starch, Corn, Barley, Rye and Triticale
Husky Energy Inc. Lloydminster	Saskatchewan	Wheat, Corn
NorAmera BioEnergy Corporation	Saskatchewan	Wheat, Corn
North West Terminal Ltd.	Saskatchewan	Wheat, Corn
Pound-Maker Agventures Ltd.	Saskatchewan	Wheat
Terra Grain Fuels Inc.	Saskatchewan	Wheat
Husky Energy Inc. Minnedosa	Manitoba	Wheat and Corn
Amaizeingly Green Products L.P.	Ontario	Corn
GreenField Ethanol Inc. Chatham	Ontario	Corn
GreenField Ethanol Inc. Johnstown	Ontario	Corn
GreenField Ethanol Inc. Tiverton	Ontario	Corn
IGPC Ethanol Inc.	Ontario	Corn
Iogen Corporation	Ontario	Wheat and Barley Straw
Kawartha Ethanol Inc.	Ontario	Corn
Suncor St. Clair Ethanol Plant	Ontario	Corn
GreenField Ethanol Inc. Varennes	Quebec	Corn
Atlantec Bioenergy	Nova Scotia	Sugar Beets
Enerkem Inc. – Sherbrooke Pilot Plant	Quebec	Various Feedstocks
Enerkem Inc.	Quebec	Wood Waste

2.2. Distillers' Grain Composition

The DDGS composition depends on type of feedstock grain used (Drapco et al., 2008), proportion of each grain in a grain blend (Nuez-Ortín and Yu, 2009), grain variety, geographical location of grain growth (Ojowi et al., 1997), proportions of blending condensed distillers soluble with the unfermented fraction of grain (Ileleji et al., 2007; Ileleji and Rosentrater, 2008), processing methods (Belyea et al., 2004). These factors affect the physical, chemical and nutrient profile of DDGS.

2.3. Bioethanol Processes and Co-product Production

Bioethanol can be produced from starch/sugar based feedstocks or from lignocellulosic feedstocks (RFA, 2010). Due to the reason of cost effectiveness and under developed techniques in cellulosic ethanol production, grain/sugar based feedstock usage is more common in bioethanol production (Sticklen, 2008). Production of ethanol with starch based feedstock is based on the following three main commercial processes: dry milling, wet milling and dry grinding (Rausch and Belyea, 2006). Depending on type of processing method used for bioethanol production, the co-product quality varies.

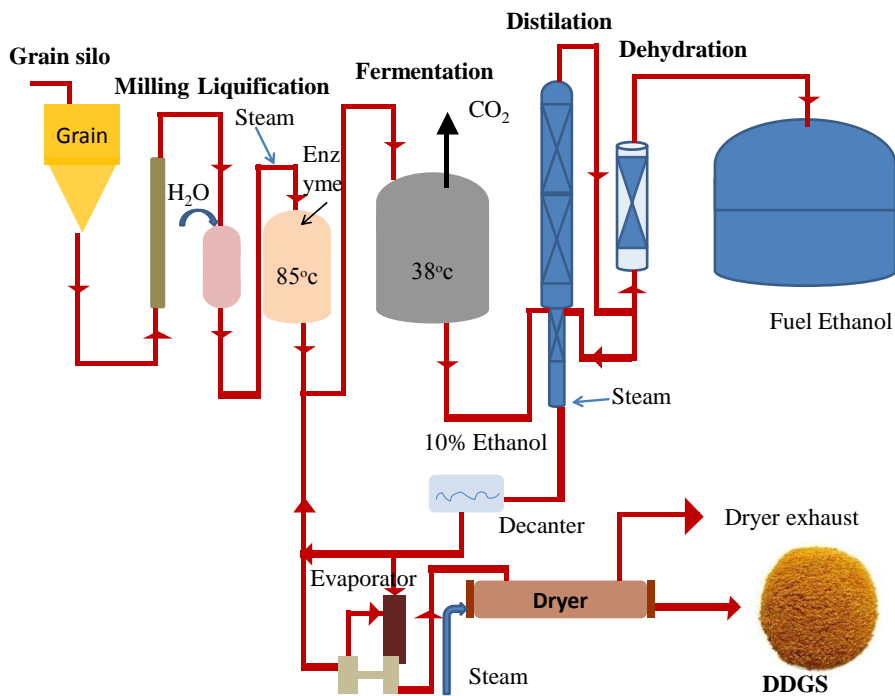


Figure 2.1. Process of generating ethanol from wheat. Adapted from Murphy and Power, 2008

In Canada, most wide spread method of bioethanol ingredient processing prior to fermentation is dry grinding. During dry grinding, whole grain kernel is used for the fermentation. In this process, first feedstock grain is ground and then mixed with water to make slurry which is subjected to cooking. After cooking, the enzyme amylase is mixed with the slurry to liquify the slurry. Glucoamylase and yeast are added to the liquefied slurry to ferment the sugar into “beer” which contains ethanol, water and unfermented solids. After fermentation, the beer is exposed to atmospheric pressure to release CO₂ and then transferred

to a storage tank. The ethanol is recovered from the beer by distillation. Beer is fed to two distillation columns and a stripping column to remove water by transferring through a molecular sieve. Remaining whole stillage is removed and subjected to centrifugation to separate wet grain and the thin stillage. Removal of water from thin stillage results in a concentrated form of condensed distiller soluble (CDS) which is called as “syrup”. DDGS is the combination of CDS (syrup) and the wet grain which dried to make more stable product (Rausch and Belyea, 2006; Murphy and Power, 2008).

2.4. Factors Affecting Distillers Dried Grain with Solubles (DDGS) Quality

The variation in physical, chemical and nutritional quality of DDGS is associated with many factors. Published studies discuss factors affecting the variation, especially, among different bioethanol plants (Spiehs et al., 2002; Belyea et al., 2004; Rosentrater and Muthukumarappan, 2006; Nuez-Ortín and Yu, 2009; Belyea et al., 2010). Since DDGS is a co-product of bioethanol process, any changes to the bioethanol production process gives rise to changes in final DDGS composition and quality. The difference in DDGS quality are primarily due to the variation in grain feedstock used, bioethanol processing conditions or combination of both (Bhadra et al., 2010). Dong and Rasco (1987) found differences in DDGS composition when soft white wheat or hard red wheat was used as the feedstock grain in the bioethanol process. The low concentration of seven essential amino acids for monogastrics was noted when soft white wheat was used. The DDGS quality associated with the feedstock of six spring wheat and winter wheat cultivars, triticale and hybrid corns were documented by Gibreel et al. (2011).

The weak relationship between the chemical compositions of feedstock grain corn to the DDGS was described by Belyea et al. (2004). According to their studies there was no significant correlation between components of corn to the corn DDGS. However, differences in nutrient profiles are possible with different varieties within the same grain type (Liu, 2011). Furthermore the compositional and nutrient availability differences between wheat, corn and blend DDGS were documented in details (Nuez-Ortín and Yu, 2009).

The method of processing has a major impact on DDGS quality. In the traditional dry grinding process, the whole grain kernel is used, which contains the starch, protein, germ and fibre fractions (Liu, 2011). There has been improvement in DDGS quality as well as in

bioethanol process that were observed with the new modified methods such as fractionation of grain to remove non fermentable fraction (hull) (Singh et al., 2005; Wang et al., 2005; Khullar et al., 2009).

Sometimes, the addition of chemical substances during the fermentation procedure (addition of sulfuric acid to control the pH) cause compositional changes in DDGS (Liu, 2011). With the same processing method, variation in nutritional and chemical composition of DDGS was observed due to the changes in the process (Belyea et al., 2004, Rausch and Belyea, 2006; Kingsly et al., 2010). The various factors related to bioethanol and DDGS production process such as extent of starch extraction, type of fermentation (continuous, batch), fermentation time, type and quantity of enzyme addition, centrifugation, extent and temperature of drying and the type of dryers are discussed (Klopfenstein, 1996; Spiels et al., 2002; Kleinschmit et al., 2007; Bhadra et al., 2010). Of these production process associated factors, the drying temperature and proportion of condensed distiller syrup added back to unfermented grain fraction may cause main changes in chemical, nutrient and physical characteristics of DDGS (Ileleji et al., 2007; Ileleji and Rosentrater, 2008; Kingsly et al., 2010) .

2.5. Feed Evaluation

Feed evaluation plays a major role in accessing the feed value among different feeds for animal nutrition and predicting potential animal production levels. The feed value depends on how effectively any feed in a ration supply required nutrients to the animal. When we evaluate similar type of feed stuffs, cost per kg of nutrient play a major role (Church, 1991). The nutrients essential for animal are water, protein, energy, lipids, minerals and vitamins (Church, 1991). The main contributors for feed value of a feed are carbohydrate, protein and fat. The ultimate objective of feed evaluation is to optimize the feed efficiency, animal performance and increase profitability to the producer (Theodorou and France, 2000). There are several methods involved in feed evaluation.

2.5.1. Chemical Methods

To access the nutrient value of a feed, analysis of its constituent composition is essential. The most common method of nutrient constituent evaluation is proximate analysis

which separates nutrients that are required by the animal. With the proximate analysis, water, crude protein, crude fat, fibre, non-fibre carbohydrate, and minerals can be measured.

When evaluating DDGS quality with chemical methods, the wide variation can be expected in the method of analysis and subsequent interpretation of results. Due to the lack of proper industry guidelines for the analysis of DDGS, any change to the method, temperature, particle size and various other factors, there can be confusion between the values obtained from different laboratories (Thiex, 2008).

The accurate moisture determination is critical since all the other nutrients in a feed are expressed based on dry matter content of the feed. Purchasing and inclusion of animal feed ingredient into ration is mainly based on the weight of the feed. Thus any inaccuracy in moisture determination will lead to economical losses related to production losses.

The most common method to determine moisture is oven drying, while most ethanol plants use the faster gravimetric method which is “thermo balance halogen moisture analyzer”. A comparative study carried out by Ileleji et al. (2010) on moisture loss in DDGS with different laboratory techniques found a strong correlation between drying temperature and moisture loss rather than with drying time. They concluded the National Forage Testing Association (N.F.T.A) 2.2.2.5 method (2 g of ground sample at 105°C for 2 h) as the best method to determine dry matter (DM) content in DDGS. Moisture losses of many drying methods are not accurate in sample analysis of DDGS due to the possibility of degradation of heat sensitive substances and evaporation of volatile components (Thiex, 2008). Loss of these components results in overestimation of moisture. The Karl Fisher method using KF apparatus AOAC 2001.12 (2005) was the best method to analyze DM in DDGS while the (N.F.T.A.) 2.2.2.5 method related closely to it (Thiex, 2008).

Proteins are a major element in body tissues and each protein has distinctive function in the body. All proteins are composed of amino acids and synthesized by plant and animal cells. The two most common methods of analysis of protein are AOAC 990.03 (2005) nitrogen (N) combustion and AOAC 2001.11 (2005) Kjeldhal acid digestion. Both these methods measure the nitrogen content and protein is estimated by multiplying N content by factor 6.25. These two methods are not empirical and can be used for crude protein content analysis in DDGS (Thiex, 2008). The use of factor 5.7 to calculate the wheat protein was based on the detailed study carried out by Osborne (1907) on the nitrogen content and amount

of gliadin and glutenin in wheat (Tkachuk, 1969). The inaccuracy in assuming N content in protein as 16% ($100/16=6.25$) or 17.5% ($100/17.5=5.7$) was revealed by Jones (1931). According to the recommendations of FAO (2002), the most accurate method of measuring protein content of food is by getting the molecular weight of individual amino acids. It further describes the less than 1% of the error associated with factor 6.25 when estimate the energy since average contribution of protein is 15% of the diet. However, the inability to distinguish between N of protein and N of non-protein N is a major disadvantage (Givens et al., 2000).

The oldest fiber analysis method is the Weende system (Van Soest, 1967). Due to the fact that ruminant can digest crude fiber, a more detailed detergent fiber analysis system was introduced by Van Soest (1963). With the detergent fiber analysis system, neutral detergent solubles (i.e. non-fibre carbohydrates), neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) can be determined. Higher NDF values were observed when wheat DDGS was corrected for NDIN without sodium sulphite with this detergent system (Rasco et al., 1989). The values obtained for the corn DDGS in the same study was not changed when sodium sulphite was or was not used for NDF determination (Rasco et al., 1989). The higher protein content in wheat DDGS compared with corn DDGS caused higher protein-N content bound to NDF which was lower if sodium sulphite is used during neutral detergent boiling (Dong and Rasco, 1987). In the detergent fibre analysis, sodium sulphite is used to break down the disulphide bonds associated with many cross-linked proteins (Van Soest et al., 1991). The use of sodium sulphite in ruminant feed is discouraged unless the samples need to be analysed for neutral detergent insoluble protein (NDIP), lignin determination or subjected to in vitro digestion (Van Soest et al., 1991).

The need for proper extraction techniques in starch analysis revealed by Petterson et al. (1999). Incomplete solubility and limited enzyme accessibility have been identified as major problems related to starch analysis (Muller-Harvey, 2004). Removal of low molecular weight sugars by treating with 80% ethanol in boiling water followed by gelatinization and solubilisation before the hydrolysis of starch are the initial steps of analyzing starch (Hall et al., 1997). The enzymatic technique introduced by McCleary et al. (1994) was commercialized (Megazyme kit) and has been accepted as an AOAC method (996.1) (Petterson et al., 1999; Muller-Harvey, 2004). Corn starch which remained unhydrolyzed during dry grind ethanol process has been recovered in DDGS (Sharma, 2010). This

unconverted starch (raw starch) is known as resistant starch and the amount present in DDGS depends on type of raw starch and the bioethanol process associated factors (temperature, enzyme, pH, length of hydrolysis) (Sharma, 2010). The most common procedure of analysing crude fat (CFat) is the EU procedure A in which crude fat is extracted with petroleum ether followed by drying of the residue (Michalet-Doreau and Ould-Bah, 1992). The use of diethyl-ether instead of petroleum ether tends to give higher values due to dissolving of some components in feed (urea, sugars etc.) (Mueller-Harvey, 2004). Due to the risks associated with Soxhlet apparatus with the recycling of solvent between lower heating unit and upper cooling system, separate type of extractor was introduced. The new Soxflo instrument is based on dry column procedure and no heat is associated with fat extraction procedure (Brown and Muller-Harvey, 1999).

2.5.2. Feed Evaluation with Nutrient Models

The total digestible nutrient (TDN) and crude protein (CP) were the two main parameters used to predict the energy and protein availability until year 1970 (Tedeschi et al., 2005). A more accurate prediction system based on the California net energy (NE) system was introduced by the National Research Council (NRC) for beef (NRC, 1970) and for dairy (NRC, 1971) cattle. The development of ruminant nitrogen (N) and metabolizable protein (MP) systems (Burroughs et al., 1974) and the implementation with NRC 1989 and 2001 models was a major improvement in model development and application. The Cornell Net Carbohydrate and Protein System (CNCPS), first published in 1992 and continuously updated thereafter, became a more practical model in use due to its validation under a variety of feeding conditions.

2.5.2.1. Cornell Net Carbohydrate and Protein System (CNCPS)

The CNCPS is a mathematical model developed based on animal, feeds, management conditions and different environmental conditions. This model evaluates cattle rations to predict requirements, feed utilization, animal performance and nutrient excretion of dairy and beef cattle (Cornell University, 2010). With the CNCPS system, feed composition is described in relation to their digestion rates (Tylutki et al., 2008). The CNCPS has different sub-models to predict the requirement for growth, maintenance, pregnancy, lactation, body reserves, feed

intake rumen fermentation, intestinal digestion, metabolism and nutrient excretion (Sniffen et al., 1992).

According to the CNCPS, protein is partitioned into three main fractions and carbohydrate is partitioned into four fractions (Sniffen et al., 1992). The three main fractions of feed protein are non-protein N (PA), potentially degradable CP (PB), and unavailable protein (PC). Fraction PB is further subdivided into three fractions based on their rate of ruminal degradation (Krishnamoorthy et al., 1982; Van Soest, 1994). These fractions are named PB1, PB2 and PB3. The PA fraction consists of ammonia, peptides and amino acids and chemically it is buffer soluble (Roe et al., 1990). The rapidly degradable true protein PB1 fraction is also buffer soluble but can be precipitated with tri-chloro acetic acid (TCA) (Krishnamoorthy et al., 1982; Van Soest et al., 1991). Fraction PB2 is intermediary degraded in the rumen and determined by buffer insoluble protein minus the protein insoluble in neutral detergent solution. Fraction PB3 is associated with cell walls and degrades slowly in the rumen and it is soluble in acid detergent but not in neutral detergent (Krishnamoorthy et al., 1982). The ruminal degradation rate constant (K_d) for the three true protein fractions are 120-400 %/h, 3-16 %/h, 0.06-0.55 %/h for PB1, PB2 and PB3, respectively. Fraction PC contains protein insoluble in acid detergent and is unavailable to the animal (Krishnamoorthy et al., 1982).

Carbohydrates are partitioned into four fractions according to the inherent nature of their rumen degradation (Sniffen et al., 1992). Fraction CA contains sugar which degrades fast in the rumen. Fraction CB1 represents starch and soluble fiber which is intermediary degradable in the rumen. The available cell wall fraction CB2 degrades slowly in the rumen. Fraction CC is undegradable and it represents the lignin and resistant starch present. Fractions CA and CB1 are associated with non-structural carbohydrates (NSC), while fraction CB2 and CC are associated with structural carbohydrates. The K_d for carbohydrate fractions were 200-350 %/h, 20-50 %/h, 2-10 %/h for CA, CB1 and CB2, respectively. The CNCPS predicts the microbial protein synthesis (MCP) based on available CHO which is further divided into structural and non-structural CHO (Russell et al., 1992).

The updated version of CNCPS was published with improved model organization and with increased accuracy. The major improvement was that it elaborates the CHO pool which includes sugar, soluble fibre, organic and volatile fatty acids (Tylutki et al., 2008). The

CNCPS V6 consists with two levels. The energy and protein availability for the animal predicts with level 1 for the feed that are not fully categorized and level 2 for feed with sufficient information (ex: dry matter intake) (Tylutki et al., 2008).

Since the bioethanol co-product DDGS contains negligible amount of volatile acids and sugars, in this study CNCPS V5 was used to sub-fractionate the carbohydrate and protein pools. An overview of CNCPS v5 and v6 is attached to the appendix (Figure A1).

2.5.2.2. NRC-2001 Model

The NRC-2001 is a feed evaluation model used in North America to predict the nutrient requirement of dairy cattle. In the NRC-2001 model, energy requirements for the maintenance and milk production are described in premise of metabolic body size (NE_m), tissue (NE_g) and milk (NE_L) composition. The feed energy values are also expressed based on feed NE_m , NE_g and NE_L values.

According to NRC-2001, estimation of TDN at a maintenance level (TDN_{IX}) is based on a chemical summative approach described by Weiss et al. (1992). To calculate truly digestible nutrient components, feeds are partitioned into potentially available NDF, CFat (EE), CP and non-fibre carbohydrate (NFC) and multiplied with their true digestibility. By summing up the obtained values, TDN is calculated. The chemical composition of feed and processing adjustment factor are used to calculate the digestible energy (DE) at a maintenance level. The TDN value of the diet and intake are used to calculate discount factor for DE at a production level. The metabolizable energy at a production level (ME_p) is calculated based on discounted digestible energy and then converted to net energy at a production level. Therefore the NE_L of feed vary, depending on any variation in feed composition, intake and diet composition (Weiss, 2002). From the early publications of NRC, it was found that with the increase of intake there is tendency of reduction in digestible energy concentration. With the today's high producing dairy cattle, this phenomenon plays an important role in calculating DE. The NRC 1978 and 1988 used the constant depression value of 4% for the each increase of maintenance level (NRC, 2001). The NRC 2001 model proposes a variable discount based on TDN_{IX} value and level of intake.

In NRC-2001 model CP is portioned into three fractions based on the in situ rumen incubation procedure. The immediately available fraction (fraction A) which disappears

during the initial soaking period is assumed to be completely degraded in the rumen. The fraction B is assumed to be degraded gradually and disappear with prolonged exposure to rumen fermentation. The fraction C is assumed to be undegraded in the rumen and can be recovered as in situ residues at the end of rumen incubation. The calculation of rumen undegraded feed protein (RUP) and rumen degraded feed protein (RDP) are based on degradation rate of fraction B (K_d) determined with the different time points of in situ rumen incubation. The estimate of the passage rate (K_p) for undigested feed takes the dry matter intake (DMI), amount of concentrate in diet dry matter and amount of forage NDF into account. The microbial protein synthesis is predicted for both heifers and cows with a constant value of 130 g for a Kg of discounted TDN. If RDP is less than $1.18 \times \text{TDN}$, microbial protein yield is estimated as $1.18 \times \text{RDP}$ intakes. The endogenous CP losses are estimated with diet dry matter intake.

There are limitations and draw backs associated with NRC-2001 model when estimating energy values in feed. The NRC-2001 considers the decrease in NE_L density of diet as the NE_L of output increases, but it does not take into account whether the cows with high genetic potential of producing milk have the ability to digest and absorb nutrients more efficiently (Robinson, 2007). In addition the feed factors are used to predict the digestibility, not the feed intake. Therefore when predicting same energy availability for milk production with a high grain diet and high forage diet the model itself assumes the suitability of high forage diet under least cost formulation which is impractical under real farming situation. Also there is no adjustments were made for the cows carrying twin foetuses. The processing adjustment factor (PAF) applies only for the starch digestibility of the processed feed but there is no accountability for other non-neutral detergent carbohydrates (Hall, 2001).

2.5.3. In Situ Nylon Bag Technique as Feed Evaluation Method

In situ incubation is a widely used method of evaluating ruminant feed according to the rate and extent of degradation. This involves suspension of test feed material in the rumen allowing for contact between the test feed and actual rumen environment (Nocek, 1988). The rate and extent of rumen degradation of particular test feed is evaluated by the analysis of in situ residue samples after incubation. This fractionates nutrients into three fractions depending on their rumen availability. These fractions are soluble, potentially degradable and

undegradable fractions, named A, B and C or S, D and U respectively. Also it measures the rate of digestion in potentially degradable fraction (B). The rate of digestion of the soluble fraction cannot be measured due to its possibility of escaping from nylon bags before incubation. The high solubility in rumen fluid makes it rapidly escape the rumen by passage (Nocek, 1988). The questions have been raised about the insoluble small solid particles that escape through the nylon bag and their degradation behaviour. Some studies were suggestive of values for the S fraction and also there was new fractionation method developed to separate W (washable) fraction into insoluble washable (ISWF) and soluble washable fraction (SWF) (Melin et al., 2001; Yang et al., 2005). Azarfar et al. (2007) developed a new method to quantify and to evaluate the chemical composition of the W fraction with a procedure much similar to conventional washing at 0 h rumen incubation. With this method soluble and insoluble fractions at 0 h can be estimated. As for the in situ incubation, 7 g of samples are weighed into each nylon bag, tie rapped and put into polypropylene centrifuge bottles. Then distilled water is added to reach the dilution ratio of 20 ml of water per gram of feed. Then the bottles are placed on a shaker and allowed for shaking for 1 h. Then nylon bags are removed from bottles and the bags are rinsed with a small quantity of water. Then the bottles are centrifuged and supernatant decant through a fast filter paper and the decant is placed into pre-weighed aluminum containers and freeze dried to determine the weight of the soluble fraction (SWF). The pellet is quantitatively collected into pre-weighed aluminum containers and freeze dried to determine the weight of the washable insoluble fraction (ISWF).

Many studies pointed out the potential sources of variation associated with the in situ nylon bag technique (Ørskov et al., 1980; Michalet-Doreau and Ould-Bah, 1992; Nocek, 1985, 1988; Vanzant et al., 1998). Factors associated with variation and repeatability of the in situ nylon bag technique were the factors that related to animal, bag, substrate, temporal, procedural aspects and mathematical component (Vanzant et al., 1998). Furthermore the effect of bag pore size, sample to bag ratio, particle size, the bag incubation sequence, relative location of nylon bags suspended in the rumen, feeding frequency with associated changes in rumen environment, diet composition, type of animal use, washing after incubation and selection of mathematical model to handle in situ residue data were discussed by Ørskov et al. (1980) and Vanzant et al. (1998).

According to “ring test” results, variation in nylon bag data achieved by different laboratories is one of the main practical limitations associated with the method (Madsen and Hvelplund, 1994). The low repeatability of the method, influence of different particle sizes of the sample, the assumption of passage rates for concentrate and forages are among the some of the shortcomings of the method (Ørskov, 1980; Preston, 1995; Noziere and Michalet-Doreau, 2000). The indication of particle size distribution of the test sample instead of grinding screen size is suggested as more suitable in determining degradation behaviour (Noziere and Michalet-Doreau, 2000).

2.5.4. Estimation of Degradation Kinetics with Mathematical Models

Many studies reviewed the requirement of proper mathematical model to fit the fermentation or disappearance curves and goodness of fit (Nasri et al., 2006). Both the nonlinear and logarithmic-linear mathematical models have been used. The most widely used model is the nonlinear first order kinetic model (Ørskov and McDonald, 1979) which was later modified to include lag time (Robinson et al., 1986; Dhanoa, 1988).

$$R(t) = U + D \times e^{-K_d \times (t - T_0)},$$

where, $R(t)$ = residue (%) present at time (t) h of incubation; U = undegradable fraction (%); D = potentially degradable fraction (%); T_0 = lag time (h); and K_d = degradation rate constant (%/h). Estimation of effective degradable fraction (ED) is based on the nonlinear parameters (S , U , D , K_d) obtained with the above equation as:

$$ED(\%) = S + (D \times K_d) / (K_p + K_d),$$

where, S = soluble fraction (%); K_p is assumed to be 4.5 %/h for forages and 6 %/h for concentrates (Tamminga et al., 1994; Yu et al., 2003a, 2003b). The rumen undegradable fraction (RU) can be calculated for each nutrient component as,

$$RU(\%) = 100 - ED(\%).$$

2.5.5. Fourier Transform Infrared Spectroscopic Analysis

Fourier transform infrared (FTIR) spectroscopy is one of the developed methods in identifying different functional groups in a feed or food (Kong and Yu, 2007). It measures the wave intensity of IR light absorbed by a sample. The IR spectra help to identify the structure

of a molecule since it is the plot of the amount of IR radiation that passes through the sample against the wave length or wave number (frequency) (Chalmers and Griffiths, 2002). The FTIR spectrometer (Figure 2.2) is an interferometer in which the light is emitted by a source directed to beam splitter. The beam splitter divides the IR radiation into two, where it allows half of the light to pass through while reflecting the other half bouncing off a moving mirror. This creates a constructive and destructive interference. When two halves of the beams recombine on the beam splitter, they are with path length difference or optical retardation. The recombined light forms an interferogram which resulted from the displacement of the moving mirror. The data obtained from interferogram can be converted to a spectrum with mathematical operation called Fourier Transformation (FT). The FT determines the frequency component and gives rise to a continuous wave form (Herres and Gronholz, 1984; van de Voort and Ismail, 1991; Wade, 2003). There are many advantages related to interferometry (Perkins, 1987). The substantial reduction in scanning time (few seconds) increased accuracy in wave length, enhanced spectrum resolution and progressed in signal to noise ratio (Perkins, 1987; Allison, 2011). The spectral libraries are used to store the analysed spectrum data and in the absence of a particular spectrum for the desired material, spectra from several relevant materials can be used to identify the desired spectrum (van de Voort and Ismail, 1991; Allison, 2011; Singh et al., 2011). With the invention of a new FTIR microscope detector, there is a substantial reduction in the time taken for the acquisition of spectra (Allison, 2011). Moreover the absence of prerequisite in pre-treatment and availability of entire spectrum within few seconds considerably increases the speed of analysis (Han and Faulkner, 1996; Trafford et al., 1999; Allison, 2011).

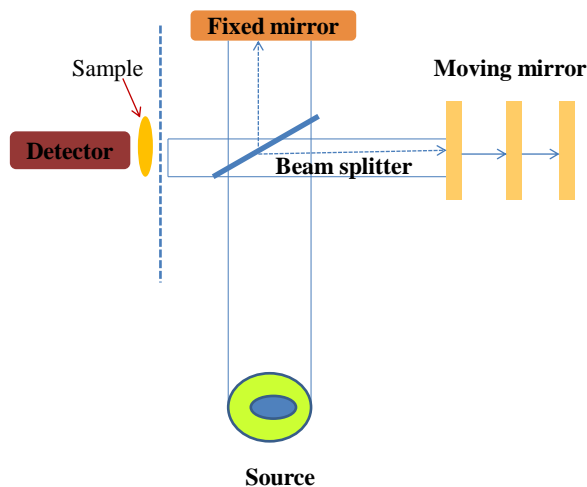


Figure 2.2. Simplified layout of typical FTIR spectrometer. Adapted from Handbook of Instrumental Techniques for Analytical Chemistry, pp 254

The detailed molecular structure information obtained by studying the bands in the spectrum is unique to certain functional groups. The IR spectrum can be used as a “finger print” in identifying molecules. The final identity can be made with a computer by tallying the spectra of “unknown” with a digital spectral database of spectra and by visually comparing the closely matching spectra (Chalmers and Griffiths, 2002). The IR spectroscopy produces different bands of the spectra as a result of vibration of different molecules. Certain functional groups such as OH, NH, CH₃, C=O and C₆H₅ produce bands in exclusive IR frequency ranges irrespective of the type of molecule that contains those functional groups. Furthermore the position of the functional group and frequency within particular range creates more information about functional group environment.

2.5.5.1. Importance of FTIR spectroscopy in Feed Evaluation

With the traditional wet chemical analysis, samples need to be homogenized and then separate the components from the complex matrix (Budevskaa, 2002). This makes it impossible to determine feed intrinsic structure and biological components (Yu, 2006b). The

incompatibility between biological approach and NRC 2001 nutrient modelling in determining total digestible nutrients and energy values further reveals the importance of biological component matrix in identifying the feed digestive behaviour (Yu et al., 2004a). The relevance of FTIR application in food science studies have been identified (Barnett and Ismail, 1989). The analysis of milk samples with FTIR is one of the major advancement in food industry. There are evidences for the successful application of FTIR technique in combination with multivariate spectral analysis, for example, identification of wood from two different plant species, estimation of lignin, hydroxycinnamic acids, nitrogen and alkali index in samples of grasses (Huang et al., 2008; Allison et al., 2011). With the hierarchical cluster analysis, there is a possibility of distinguishing different varieties of the same feed stuffs and also the different treatments of the same variety (Yu, 2005c).

2.5.5.2. Identifying Feed Related Important Functional Groups with FTIR

The organic molecules in a feed (protein, CHO, lipids, etc.) are composed of specific bonds and functional groups. In the absence of electromagnetic field, these functional groups vibrate independently at their equilibrium and have weak interactions between each other. When there is electromagnetic radiation (IR radiation), these functional groups in organic molecules break down the molecular equilibrium and transit the energy between rotational and vibrational movements. This process facilitates the molecule to absorb IR radiation depending on the specific molecular vibrational frequency. The unique molecular structures in biological components give rise to IR spectrum exclusive for different functional groups (Yu, 2004b). The peptide bond is a characteristic of protein since it holds the link between amino acids in polypeptides and protein. The peptide bond contains C = O, C = N and N = H linkages and creates two important bands in IR region. These two bands are the amide I and amide II bands. The amide I band absorbs the IR radiation at ca. 1650 cm^{-1} and is due to the stretching vibrations of C = O (80%) and C = N stretching vibrations. The amide II absorbs IR radiation at ca. 1550 cm^{-1} as a result of N-H bending vibrations (60%) and C = N stretching vibrations (40%) (Jackson and Mantsch, 1995, 2000). The lipids are characteristic of carbonyl C = O ester and CH₂ and CH₃ functional groups. Lipids give rise to IR profile at ca. 1738 cm^{-1} (Carbonyl C = O), ca. 1470 cm^{-1} (CH bending), ca. 2961 cm^{-1} (CH₃ asymmetric stretch), ca. 2925 cm^{-1} (CH₂ asymmetric stretch) and ca. 2871 cm^{-1} (CH₂ symmetric stretch). Since CHO

contains a lot of sugars it comprises of many OH and CO bonds. The CHO creates an IR spectrum between ca. 1180 cm^{-1} and 950 cm^{-1} depending on the type of sugar and bond linkages (Mathlouthi and Koenig, 1987; Yu, 2004b). Both structural and non-structural CHO peak between ca. 1550 and 800 cm^{-1} . The moderate intensity bands at ca. 1420, 1370 and 1335 cm^{-1} are used to identify structural CHO while a band that arises close to 1025 cm^{-1} is used to identify as non-structural CHO in grains. (Wetzel et al. 1998; Wetzel, 2001).

2.5.5.3. Spectrum Analysis with Multivariate Statistical Approach.

Multivariate statistical approach helps to compare the spectral data between each other at once. The comparison can be done with the whole set of spectral data or using part of it irrespective of band identity (Yu, 2006b). Agglomerative hierarchical cluster analysis (AHCA) and principal component analysis (PCA) are the two main methods of comparing spectra under multivariate procedure. Agglomerative Hierarchical Cluster Analysis first calculates the distance matrix depending on similarity of the spectra. Subsequently it searches for the distance matrix for the two most similar IR spectra with the minimal distance to each other. According to agglomerative hierarchical cluster analysis, these spectra combine together to form a new “cluster” or “hierarchical group”. Then the distance for the remaining spectra and for the new cluster are recalculated (Jain and Dubes, 1988; Yu, 2005c).

Principal Component Analysis transforms original set of variables to new uncorrelated set of variables which is called “principal component”. The PCA helps to maintain small number of data set variables with linear combinations by securing the most original information. From this multiple variable system one, two or more “PC”s are extracted and these components are independent (orthogonal) of each other. The first extracted factor shows highest variability and with the extraction of more factors variability becomes less. Eigenvector, is the score that is assigned for each spectrum depicts the relationship of each principal component. This relationship can be presented in either two-dimensional (2D) or three-dimensional (3D) scattered plots (Jolliffe, 1986; Dunteman, 1989; Yu, 2005c).

2.5.5.4. Limitations in FTIR technique

The higher initial cost associated with the technique limited its usage mainly in universities, government and industrial research laboratories. With the substantial reduction of equipment price during past few years made the potential use of technique in many areas (van de Voort and Ismail, 1991). In addition, the requirement of expertise in the field for calibration is another limiting factor in its vast usage.

2.6. Feed Evaluation Based on Predicted Truly Absorbable Protein Supply to Small Intestine

With the modern protein evaluation systems, predictions can be made on true protein value of a feed and the requirement for dairy cattle. These predictions are mainly based on the amount of true protein truly digested and absorbed in small intestine. The two models of protein evaluation are most widely used, TDN-based NRC-2001 model in North America and non-TDN based model DVE/OEB model (Tamminga et al., 1994, 2007) used in Europe. These models have been developed based on the many previously developed nutritional models and in cooperating some of the new approaches (Yu et al., 2000, 2003b; Yu, 2005a). There is an updated version of DVE/OEB protein evaluation system which is DVE-2007.

According to NRC-2001, true protein absorbed in the intestine is the contribution of rumen undegraded feed protein (DRUP), microbial protein synthesized in the rumen (DMCP) and endogenous CP from the rumen (ECP).

Protein degradation balance (PDB) is calculated as Potential MCP synthesized based on ruminally available feed CP – MCP synthesized based on TDN as available energy

Rumen degraded protein (RDP) and rumen undegraded protein (RUP) are calculated as;

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

$$\text{RDP (g/kg DM)} = \text{CP (g/kg DM)} - \text{RUP (g/kg DM)},$$

For rumen microbial protein synthesis (MCP) estimation,

when RDP exceeded 1.18TDN, predicted microbial crude protein (MCP_{TDN}) is calculated as:

$$\text{MCP (g/kg DM)} = 0.13 \times \text{TDN (discounted)},$$

where, factor 0.13 assumes that per kg of TDN 130g of MCP is synthesized.

When RDP is less than 1.18TDN, predicted MCP is calculated as,

$$\text{MCP} = 0.85 \times \text{RDP},$$

For intestinal digestibility of feed and microbial protein:

NRC 2001 assumes that the intestinal digestibility and true protein of MCP as 80%.

Truly absorbed MCP is calculated as $AMCP = 0.80 \times 0.80 \times MCP = 0.64 MCP$

Truly absorbed rumen undegraded true feed protein (ARUP) (g/kg DM) = %dRUP x RUP

where, %dRUP is the intestinal digestibility of RUP which can be estimated using mobile bag technique data or using the longest in situ rumen incubation time point residues (Tamminga et al., 1994) or three-step invitro method (Calsamiglia and Stern, 1995).

For rumen endogenous protein (ECP) estimation:

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

where, NRC-2001 assumes that 80% of ECP as true protein and 50% of ECP pass to the duodenum.

Therefore the truly absorbed endogeneous protein in small intestine,

$AECP = 0.50 \times 0.80 \times ECP$

The total intestinal absorption of true protein is considered as metabolizable protein (MP).

$MP = ARUP + AMCP + AECP$

The DVE/OEB system was developed in 1994 in the Netherlands to replace digestible crude protein (DCP) system (Tamminga et al., 1994). The main objectives with this DVE/OEB protein evaluation system were to prevent the avoidable N losses from the rumen, identify the exact requirement of dairy cattle (Tamminga et al., 1994). In this system, each feed has a metabolizable protein (DVE) and rumen degraded protein balance (OEB) value. The DVE value is a contribution of rumen undegraded feed protein digested and absorbed in small intestine (DVBE), microbial protein synthesized in the rumen and absorbed in small intestine as amino acids (DVME) and endogenous protein losses in the faeces that are associated with digestion (DVMFE).

The DVE value of a feed is calculated as: $DVE = DVBE + DVME - DVMFE$

The OEB value of a feed is the balance between microbial protein synthesized from ruminally degradable intake protein (MREN) and microbial protein synthesized from energy extracted during the anaerobic fermentation procedure (MREE).

The OEB value of a feed is calculated as: $OEB = MREN - MREE$

The optimal synchronization ratio of rumen available N and energy for microbial growth has identified as 25 g N/kg of DM degraded (Beever et al., 1986), 25 g N/kg of organic matter degraded or 32 g N/kg of CHO degraded (Tamminga et al., 1990; Sinclair et al., 1991).

The updated version of DVE/OEB system was published by Tamminga et al. (2007). One of the main differences between DVE/OEB-1994 and DVE/OEB-2007 protein evaluation systems is the use of a modified in situ nylon bag fractionation scheme. The fractions are named as washable soluble (W), washable insoluble (WI), non-washable potentially degradable (D) and non-washable undegradable (U) fraction. The new system assumes that there is no washable fraction in NDF, the starch and residual non-starch polysaccharides (RNSP) contain washable insoluble fractions and the ethanol soluble sugars are the soluble fraction of CHO.

If in situ nylon bag residues cannot be directly analysed for the CFat, the new system suggests correction factor of 65, 44, 17 and 3% of original feed Cfat at 0, 2, 6 and 12h of incubation time points, respectively (Van Duinkerken et al., 2011). The DVE/OEB-2007 system describes both DVE value and the OEB value of a feed. As in DVE/OEB-1994 system, the contributors for the DVE value are the same. But, DRUP and DMCP are calculated in a completely different way.

The main difference between DVE 1994 and DVE 2007 models in estimating DRUP and DMCP are,

In DVE 1994 system there is a correction factor of 1.11 when calculating RUP. The DVE 2007 model does not consider such factor since it assumes that 5% of washable soluble fraction and major portion of washable insoluble fraction escape the rumen degradation which then compensates the 1.11 factor.

When calculating MCP in DVE 1994 system it assumes that per kg of organic matter fermented a fixed amount of microbial crude protein is synthesized (150 g/kg FOM). The DVE 2007 considered the contribution of each component (CP, NDF, RNSP, STARCH, SUGARS) to the fermentable organic matter ($FOM^{DVE2007}$). More over the contribution from each rumen available in situ fractions (S, W-S, and D) of each component is considered. The amount of feed component effectively degraded in the rumen is summerized in Table 2.2.

Table 2.2. Contribution of each fraction of each component for fermentable organic matter (Adapted from Tamminga et al. 2007)

Item	Fraction	Equation
(g/kg FOMr)		
CP	S	$CP \times (S \times K_{ds} / (K_{ds} + K_{ps}))$
CP	W-S	$CP \times ((W-S) \times K_{d(w-s)} / (K_{d(w-s)} + K_{p(w-s)}))$
CP	D	$CP \times D \times K_d / (K_d + K_p)$
NSP	S	$NSP \times ((W-S) \times K_{d(w-s)} / (K_{d(w-s)} + K_{p(w-s)}))$
NSP	D	$NSP \times D \times K_d / (K_d + K_p)$
STARCH	W	$STA \times ((W-S) \times K_{d(w-s)} / (K_{d(w-s)} + K_{p(w-s)}))$
STARCH	D	$STA \times D \times K_d / (K_d + K_p)$
SUGARS	S	$(SU-GOS) \times (S \times K_{ds} / (K_{ds} + K_{ps}))$
NDF	W	$NDF \times ((W-S) \times K_{d(w-s)} / (K_{d(w-s)} + K_{p(w-s)}))$
NDF	D	$NDF \times D \times K_d / (K_d + K_p)$

The total effectively rumen degradable FOMr can be calculated by summing up the contribution from each component.

The microbial protein synthesis from FOMr is calculated with Pirt (1965) equation as follows:

MCP synthesis based on the amount of adenosine triphosphate (ATP) yield depending on contribution from each component of the feed. The microbial growth yield is calculated with the Pirt (1965) equation.

$$1/Y = M/GR + 1/Y_{max},$$

where, Y = yield of microbial dry matter (in g per mole of ATP), M = maintenance requirement of the microbes (mole of ATP * h/ g microbial material), GR = fractional growth rate (h⁻¹), Y_{max} = maximum microbial growth yield without losses in maintenance (g per mole of ATP).

The intestinal available MCP (DMCP) is calculated as follows:

$$DMCP = 0.85 \times 0.75 \times MCP^{FOMr},$$

where, 0.85 is the true protein content of MCP and 0.75 is the intestinal digestibility of MCP (Tamminga et al., 1994).

Both the DVE/OEB 1994 and 2007 systems identify the protein requirement for various metabolic functions of the dairy cow metabolism namely maintenance, milk protein production, changes in body protein balance and pregnancy. These requirements are predicted in DVE values and express in DVE grams per day units (Tamminga et al., 2007; Van Duinkerken et al., 2011). With the DVE/OEB system, evaluation of individual feed stuffs is required. The dairy cattle rations are formulated based on tabulated values of DVE and OEB of the ingredients in the formula.

There are some limitations and draw backs associated with the DVE/OEB system. The DVE, OEB values are calculated based on input values generated by fractionating chemical constituents of feeds from their inherent fractions received with in situ rumen incubation of feed stuffs. Therefore any shortcomings associated with the in situ technique effect the calculations of DVE/OEB system. One other shortcoming is DVE value does not indicate any information about the amino acid composition that can be truly digested and absorbed in the small intestine.

2.7. Summary

Due to the expansion of bioethanol industry increased availability of DDGS created a good market in animal feed industry especially in ruminant feeding. In western Canada, main feedstock for bioethanol production is wheat and the co-product is wheat DDGS. The inconsistency of DDGS is a major concern when incorporating these co-products into diets. The variation in chemical composition and nutrient availability is related to many factors. Compositional variation in original grain, different fermentation conditions, amount of condensed syrup added back, different drying conditions in dryer are the some of the factors related to inconsistency in DDGS. Irrespective of the cause, determination of variability in DDGS is a useful tool in diet formulation. There are several methods that can be used for evaluation of feed.

The chemical analysis determines the composition of a feed. Input of chemical analysis results into CNCPS and NRC 2001 systems assists the estimation and prediction of nutrient availability and rumen degradation behaviour of a feed. In situ rumen incubation trials provide useful information of actual rumen degradation behaviour with the test feed. The values obtained by analyzing in situ fractions are useful input values for mathematical models in predicting rumen degradation kinetics. The FTIR technology facilitates the identification of

different molecular structure characteristics associated with different chemical compounds of a feed. The possibility of identifying different varieties of the same feed with multivariate spectral analysis method is advantageous in feed evaluation. The true protein supply to the small intestine of the host animal is a major concern with modern high producing dairy systems. The prediction of true protein supply to the animal can be determined with the nutrient models when lots of treatment sample need to be screened.

Through literature review, there is extensive information on corn and there is lack of information on wheat. There is lack of studies on batch effect of original feedstock wheat and its co-product wheat DDGS. There is lack of information on magnitude of differences between batches of wheat DDGS. No study has been found to use molecular spectroscopy as a fast tool to detect batch difference in original feedstock and wDDGS. Therefore this project aimed to study 1) nutrient and chemical composition including macro and micro mineral composition, carbohydrate and protein sub-fractions, predicted energy values with NRC-2001 summative approach, 2) in situ rumen degradation kinetics of various nutrients, 3) predicted true protein supply to small intestine with NRC-2001, DVE/OEB-1994 and new DVE/OEB-2007 systems, and 4) differences in molecular spectroscopic features with FT/IR-ATR of different batches of feed stock wheat and wheat DDGS. The hypothesis of this project was that there is significant difference among batches of feedstock wheat and wheat DDGS in structural, chemical and nutrient profiles. These differences can be detected by molecular spectroscopy of FTIR-ATR.

3.0. BATCH EFFECT OF ORIGINAL FEEDSTOCK WHEAT AND WHEAT DDGS ON CHEMICAL AND NUTRIENT PROFILES, IN SITU RUMEN DEGRADATION KINETICS OF NUTRIENTS AND INTESTINAL DIGESTIBILITY OF RUMEN UNDEGRADED PROTEIN.

3.1. Introduction

North America is one of the major producers of grain based renewable fuels. With the existing 28 renewable fuel plants, 2.25 billion litres of renewable fuel are released into the market annually (CRFA, 2010). Concomitantly greater production of co-products, dried distillers grains with solubles (DDGS), has resulted from the increased biofuel production. The high protein and high energy content of DDGS make it a good source of feed nutrients for ruminant diets (Nuez-Ortín and Yu, 2009). However the importance of maintaining consistency for the chemical and nutrient profile of DDGS is one of the major concerns in achieving economic stability in marketing and predicting more accurate ruminant production parameters. Inconsistency in DDGS chemical profiles between bioethanol plants has been revealed (Spiehs et al., 2002; Belyea et al., 2004; Shurson and Noll, 2005; Clemenston et al., 2009; Nuez-Ortín and Yu, 2009; Belyea et al., 2010). Most of these studies were carried out using corn DDGS or corn-wheat blend DDGS. The western Canada is a major producer of wheat and it is also the main feedstock used for the bioethanol production in western Canadian plants. Bioethanol plants use different batches of grain for fermentation which may have different chemical and nutrient profiles. This could lead to differences in chemical and nutrient profiles of DDGS among the batches (Rausch and Belyea, 2005, 2006; Belyea et al., 2010; Liu, 2011). The variation among batches of grain may be due to the variety of grain and variation in particle size distribution variation with the dry grinding process (Rausch and Belyea, 2005; Belyea et al., 2010; Liu, 2011). The mineral composition of a feed is crucial in meeting cattle nutrient requirement as well as the environmental aspect of mineral accumulation (Gould, 1998; Niles et al., 2002; Spiehs et al., 2002; Spiehs and Varel, 2009).

Since chemical composition and nutrient availability cannot be directly correlated to in situ rumen degradation characteristics (Mayer and Mackie, 1986; Cherney et al., 1993), rumen degradation kinetics of nutrients and intestinal digestion are needed.

The objective of this study was to determine the batch difference among the batches of feedstock wheat and among the batches of wheat DDGS from the same wheat-based bioethanol plant in western Canada. The differences among batches of wheat and wheat DDGS were analyzed for chemical and nutrient profiles, carbohydrate and protein sub-fractionation and energy values, rumen degradation kinetics of various nutrients, intestinal digestion of rumen undegraded protein (RUP), and the hourly effective degradation ratio of N to organic matter. The magnitude of variation among the different batches of wheat and wDDGS could be quantified.

Therefore the hypotheses were that nutrient profile and supply significantly differed among batches of feedstock and co-products from the same bioethanol plant; and this variation of nutrient profile and supply were observable to be high among the bioethanol co-products.

3.2. Materials and Methods

3.2.1. Sample Collection

All the samples were obtained from a newly-built bioethanol plant with current bioethanol processing technology in western Canada. A total of thirty samples were collected during year 2009 from five batches (1, 2, 3, 4, 5) of feedstock grain (wheat: 3 samples per batch) and from five batches (I, II, III, VI, V) of wheat DDGS (3 samples per batch). The three sub samples were randomly collected from each batch at different times. The samples were collected every two weeks and an attempt was made to match the corresponding time of wheat and wDDGS according to the standard operating procedure (SOP) of the bioethanol plant. However, in sampling there could be some differences between corresponding batches of wheat and wDDGS.

3.2.2. In Situ Rumen Incubation

Four non lactating Holstein Frisian cows fitted with a rumen cannula with an internal diameter of 10 cm were used for the in situ rumen incubation. The cows were individually fed at 0800 and 1600 twice daily at a maintenance energy level in accordance to NRC nutrient requirement. The animals were cared according to the guidelines of the Canadian Council on

Animal Care (CCAC, 1993). Rumen degradation characteristics of nutrients were measured with the method described by Yu et al. (2000). Approximately seven grams of sample was weighed into each 10 x 20 cm numbered nylon bag (Nitex 03-41/31 monofilament open mesh fabric, Screentec Corp., Mississauga, ON, Canada) with the pore size of 40 μ m. Before incubation, wheat samples were coarsely rolled through 0.203 mm roller gap (Sven Grain Mill, Apollo Machine and products Ltd, Saskatoon, Canada) in the College of Engineering at the University of Saskatchewan (Yu et al., 2003) and wheat DDGS were incubated as is. The study was conducted in two experimental runs and in each run, the treatments were randomly assigned among the four cows. The incubation time points were 0, 2, 4, 8, 12, 24 48 and 72 h. The 'gradual addition/all out' method was followed when introducing and removing samples. The 0 h samples were treated specially at the laboratory according to the procedure described by Azarfar et al. (2007). After incubation, bags were removed from rumen and washed under a cold stream of tap water to remove excess ruminal contents. Subsequently bags were dried at 55°C for 48 h. Dried samples were stored in a refrigerated cool room (4°C) until analysis.

3. 2.3. Chemical Analysis

The feedstock wheat and wheat DDGS samples were passed through 1 mm screen (Retsch ZM-1, Brinkmann Instruments LTD, Ontario, Canada). For the analysis of starch, samples were passed through 0.5 mm screen with the same Restech ZM-1 grinder. Samples were analysed for dry matter (DM: AOAC 930.15), ash (AOAC 942.05), crude protein (CP: 984.13), crude fat (CFat: or ether extract (EE) AOAC 954.02) according to the procedure of AOAC (1990). Analysis of neutral detergent fiber (NDF: Ankom A200 Filter Bag Technique, Ankom Technology, Fairport, NY, USA) and starch (Megazyme total starch assay kit; Megazyme International Ltd, Wicklow, Ireland) (McCleary et al., 1997) were carried out according to the methods mentioned. The NDF was analysed with heat stable α -amylase without sodium sulphite. The acid detergent insoluble crude protein (ADICP) and neutral detergent insoluble crude protein (NDICP) were determined according to the Licitra et al. (1996). The N adjusted NDF (NDFn) was calculated as NDF-NDICP. Non-protein nitrogen (NPN) was analyzed by precipitating true protein with Tungstic acid and calculated as the difference between total N and N content of the residue after filtration. Total soluble crude protein (SCP) was determined by incubating sample with bicarbonate-phosphate buffer and

filtering through Whatman #54 filter paper (Roe et al., 1990). Ethanol soluble carbohydrate (ESC) was analysed according to Hall et al. (1997). All the samples were analysed in duplicates. When analysed error was larger than 5%, chemical analysis was repeated.

Macro and micro mineral analyses were carried out in Central Testing Laboratory Ltd in Nisku, Alberta and the analysis of sulfur and chloride (Cl) in Saskatchewan Research Council (SRC, Saskatoon, Saskatchewan). The 30 samples of five different batches of wheat and corresponding five wheat DDGS batches were subjected to analysis. Each sample was analysed in triplicate.

Residues of each sample were pooled according to incubation time, experimental runs and the treatments and passed through 1 mm screen (Retsch ZM-1, Brinkmann Instruments LTD, Ontario, Canada). The samples were analysed for DM, ash, CP and NDF. The NDF was analysed with heat stable α -amylase and sodium sulfite, since the residues were not analysed further for NDICP.

3. 2.4. Protein and Carbohydrate Profiles.

The Cornell Net Carbohydrate and Protein System (CNCPS) (Sniffen et al., 1992; Chalupa and Sniffen, 1994) was used to partition the protein and carbohydrates of feedstock wheat and wheat DDGS. Protein is fractioned into immediately available non-protein N called PA, true potentially degradable protein (PB) and undegradable protein ADIP called (PC). Fraction PC is associated with lignin, tannin-protein complexes, and Millard products and is unavailable for the animal (Cromwell et al., 1993). Fraction PB was further subdivided into three sub-fractions depending on estimated rumen availability due to the rumen degradation rates.

In the CNCPS system (Sniffen et al., 1992) feed carbohydrates are categorized into four sub-fractions according to rumen degradation rates: Rapidly degradable carbohydrate fraction A (CA) consists of water soluble sugars, intermediately degradable fraction B1 (CB1) consists of starch and soluble fibre, slowly degradable fraction B2 (CB2) consists of available cell walls. Fraction C (CC) consists of completely unavailable cell walls. The fraction CA and CB1 belong to the non-structural CHO (Sniffen et al., 1992). The calculations of CHO sub-fractions recommended by Sniffen et al. (1992) are as follows.

The new CNCPS system (CNCPS_v6) divides CHO into eight sub-fractions, which elaborates the CHO pool into sugars, soluble fibre, organic and volatile fatty acids (Lanzas et al., 2007a; Tylutki et al., 2008). Since the sugars and volatile fatty acids are not significant in wDDGS the CNCPS_v5 which divides CHO into four sub-fractions was used in this study.

3.2.5. Energy Values Estimated Using the NRC-2001 Dairy and NRC-1996 Beef Summative Approach

Energy values were estimated based on the chemical analysis with the NRC (1996, 2001) summative approach. The concentration of truly digestible crude protein (tdCP), fatty acid (tdFA), neutral detergent fiber (tdNDF), and non-fiber carbohydrates (tdNFC) were calculated as follows,

$$\text{tdCP (\%DM)}_{\text{concentrate}} = [1 - (0.4 \times (\text{ADICP}/\text{CP}))] \times \text{CP}$$

If the EE is more than 1, tdFA (%DM) = FA, where, FA = Cfat-1

$$\text{tdNFC (\%DM)} = 0.98 \times (100 - [(\text{NDF} - \text{NDICP}) + \text{CP} + \text{EE} + \text{Ash}]) \times \text{PAF}$$

where, 0.98 is digestibility of NFC and PAF is the processing adjustment factor.

$$\text{tdNDF (\%DM)} = 0.75 \times (\text{NDFn} - \text{ADL}) \times [1 - (\text{ADL}/\text{NDFn})^{0.667}]$$

where, NDFn = NDF-NDICP

Net energy for maintenance (NE_m) and net energy for growth (NE_g) were determined using NRC beef (NRC, 1996).

With the values obtained for truly digestible nutrients, net energy lactation at a production level of intake (NE_{L3x}) was estimated, with summative approach (Weiss et al., 1992) from the NRC 2001 dairy (NRC, 2001).

3.2.6. Particle Size Distribution

For the particle size analysis, wheat DDGS samples were used as it is due to its mash type co-product. To determine the in situ degradation kinetics of wheat, samples were coarsely rolled with the roller gap 0.203 mm (Sven products, Apollo Machine and products Ltd. Saskatoon, Canada). The particle size analysis was carried out with Tyler RoTap (Mentor, OH) at Faculty of Engineering, University of Saskatchewan, which consists of same time sieving and tapping system. The six standard USA sieve numbers were used and the pan numbers were 6, 8, 12, 16, 20, 30 and the bottom pan, with the sieve diameter opening of

3.36, 2.38, 1.68, 1.19, 0.84 and 0.59 mm, respectively. The particles less than 0.59 mm accumulates in the bottom pan. Approximately 100 g of sample was used at each time of sieving and for each sample three replicate were sieved. The sieve stack was arranged in a way where the coarsest particles were at the top and finest in the bottom. The weighed sample was placed in the top pan. The shaker was run for 10 minutes for each sieving and weighed the samples remain in each sieve were weighed separately. After each sieving, the stack of sieves was thoroughly cleaned.

The average particle size of the sample retained on a sieve was determined using the geometric mean of the diameter openings for the two adjacent sieve stacks (Pfof and Headley, 1976).

3.2.7. Rumen Degradation Kinetics of Various Nutrients

Rumen degradation characteristics of nutrients were determined for DM, OM, CP, NDF, and NFC. The in situ data were fitted in the modified first order kinetic equation (Ørskov and McDonald, 1979; Robinson et al., 1986; Dhanoa, 1988).

$$R(t) = U + D \times e^{-k_d \times (t-T_0)}$$

where, R (t) = residue at t h incubation (%); U = undegradable fraction (%); D = potentially degradable fraction (%); T₀ = lag time (h); and K_d = degradation rate of potentially degradable fraction (%/h). In situ parameters were estimated with NLIN (nonlinear) procedure of SAS (SAS, 2009) via Gauss-Newton method.

The effective degradability (ED) and extent of degradation of each nutrient were predicted according to NRC-2001 based on nonlinear parameters (U, D, K_d). The K_p was assumed as 6% (Tamminga et al., 1994).

3.2.8. Hourly Effective Degradation Ratio

The effective degradation was calculated according to Sinclair et al. (1993).

$$ED = W + [(D \times K_d)/(K_d + K_p)] \times [1 - e^{-(K_d + K_p)t}]$$

The difference of cumulative amounts degraded between successive hours regarded as the amount of nutrient degraded each hour. The ratio of N to OM was calculated based on hourly degraded N and OM.

The hourly effective degradation was calculated as,

Hourly ED ratio of $N/OM_t = (HEDN_t - HEDN_{t-1}) / (HEDOM_t - HEDOM_{t-1})$;

where, N/OM_t = ratio of N to organic matter at time t (g N/kg OM); $HEDN_t$ = hourly effective degradability of N at time t (g/kg DM); $HEDN_{t-1}$ = hourly effective degradability of N 1 h before t (g/kg DM); $HEDOM_t$ = hourly effective degradability of OM at time t (g/kg DM); and $HEDOM_{t-1}$ = hourly effective degradability of OM 1 h before t (g/kg DM). According to Czerkawski (1986) the optimum requirement of N to OM in diet formulation was 25g N/kg OM to maximize the microbial protein synthesis efficiency.

3.2.9. Estimated Intestinal Digestibility of Rumen Undegraded Protein

Intestinal digestion of rumen undegraded feed protein was determined using the three-step in vitro procedure described by Calsamiglia and Stern. (1995). The dried ground residues of 12 h rumen incubation time point containing approximately 15 mg of N were mixed with 10 mL of 0.1 mol/L HCL solution containing 1 g/L of pepsin. Then samples were incubated in a shaking water bath at 38 °C for 1 h. The pH was neutralized with 0.5 mL of 1mol/L NaOH and 13.5 mL of pH 7.8 phosphate buffer containing 37.5 mg pancreatin and further incubated at 38°C for 24 h. After incubation, to stop the enzymatic reaction, 3 mL of 100% (w/v) trichloroacetic acid (TCA) solution was added to precipitate undigested proteins. Samples were centrifuged and supernatant (soluble N) was analyzed for N (Kjeldahl method, AOAC 984.13). The blanks were used to get rid of the background effect. Calculation of intestinally digested protein was determined by dividing TCA soluble N from the amount of N in the rumen residue sample.

3.3. Statistical Analysis

Statistical analyses were performed using the Mixed procedure of SAS 9.2 (2009). The model used for the analysis was:

$$Y_{ij} = \mu + t_i + e_{ij}$$

where, μ is the overall mean; t_i is the fixed effect of treatment ($i=2$ wheat vs. wDDG; $i=1-5$ in feedstock, or $i= I-V$ in wDDGS); e_{ij} is the associated error.

The model used for the analysis of in situ residue was:

$$Y_{ijk} = \mu + B_i + R_j + e_{ijk}$$

where, μ is the overall mean; B_i is the fixed effect of treatment ($i=2$ wheat and wDDGS, $i=1-5$ batches in feedstock, or $i=I-V$ in batches wDDGS); R_j is the experimental run as a random effect; e_{ijk} is the random error of observation ijk . Normality test was carried out using Proc Univariate with plot and normal options. For all the statistical analyses significance, was declared at $P<0.05$. The multi-treatment comparison was carried out with Tukey method. Means with different letter groups were obtained with SAS pdmix800 macro (Saxton, 1998).

3.4. Results

3. 4.1. Variation in Chemical Profiles among Different Batches of Original Feedstock and among Different Batches of Wheat DDGS

The detailed chemical profiles of five batches of original feedstock wheat and five batches of wheat DDGS (wDDGS) are presented in Table 3.1. Basic chemical profiles, carbohydrate and crude protein profiles were significantly different between wheat and wDDGS. Wheat and wDDGS differed ($P<0.05$) in DM, ash and CFat with the differences of DM of 4.1 %; ash of 3.8 % of DM; Cfat of 3.7 % of DM, in wDDGS compared to wheat. As expected, wheat contained higher ($P<0.05$) starch (63.4 vs. 1.8 %DM in wDDGS), while wDDGS contained higher ($P<0.05$) NDF, ADF and ADL. The NDF and ADF values obtained for wDDGS were 39.8 and 11.6 %DM vs. 13.9 and 3.0 %DM in wheat. In protein profile, there was significant difference between wheat and wheat DDGS. Higher CP was obtained with wDDGS (42.8 vs. 14.3 %DM) and the soluble crude protein (SCP) of wDDGS was mainly from NPN (91.5 vs. 30.4 %SCP in wheat). Higher ADICP and NDICP values were obtained in wDDGS compared to its parent feedstock wheat. Among the batches of wheat, NDF values varied by 2.5 % of DM. The variation (range) of NPN among the batches of wheat was large and represents 25.4 % on SCP basis. There were significant differences ($P<0.05$) between the batches of wDDGS in DM, ash, NDF, and ADF profiles, but no significant difference ($P>0.05$) in NPN on a SCP basis and ADICP on a CP basis. Among the batches of wDDGS, the range of CP was 3.9% of DM. The NDF varied within the batches of wDDGS by 5.2 %DM. The ADICP values were not significantly different among the batches of wDDGS. Compared to original feedstock grain, ADICP level in wDDGS was higher, irrespective of the batch effect. The wDDGS resulted from original feedstock wheat did not

show similar pattern of variation among the batches of wDDGS compared to their parent feedstock wheat.

3.4.2. Variation in Mineral Profiles among Different Batches of Original Feedstock and among Different Batches of Wheat DDGS

According to the literature review, it was found out that there was a lack of detailed micro and macro mineral profile information for wDDGS. The mineral profiles of original feedstock wheat and wheat DDGS are summarized in Table 3.2. The average values of Ca for wheat and wDDGS were 0.05 and 0.10 (%DM). The phosphorus (P), magnesium (Mg) and sulfur contents were significantly different ($P<0.05$) among the batches of both wheat and wDDGS. Among the batches of wheat, the range of P was 0.09 % of DM while the range among batches of wDDGS was 0.06 % of DM. The range of sulfur content among the batches of wheat was 0.03 %, while the range among the batches of wDDGS was 0.34 %. The average value obtained for sulfur content in wheat DDGS was 1.09 % of DM.

Micro mineral (trace mineral) composition of different batches of feedstock wheat and wDDGS were analysed for copper (Cu), zinc (Zn), manganese (Mn) and iron (Fe). There were significant differences ($P<0.05$) between wheat and wDDGS in micro mineral composition. Among the batches of wheat, Mn and Fe contents ranged by 14.2 and 40.6 mg/kg DM, respectively. Among the batches of wDDGS, Zn, Mn and Fe contents ranged by 18.2, 9.6 and 100 mg/kg DM, respectively.

3.4.3. Variation in Protein and Carbohydrate Sub-Fractions among Different Batches of Original Feedstock and among Different Batches of Wheat DDGS

Protein and carbohydrates were fractionated according to CNCPS (Sniffen et al., 1992; Chalupa and Sniffen, 1994). The CNCPS protein and carbohydrate fractions are closely related to the rumen degradation behavior and indicate the nutrient availability to the animal. In carbohydrate and protein subfractions, there were significant differences between wheat and wDDGS ($P<0.05$) (Table 3.3). The rapidly degradable non-protein N fraction (PA) was higher in wDDGS and the obtained value was 31.8 vs. 10.5 % of CP in wheat. Wheat contained high rapidly degradable true protein fraction PB1 which was 16.5 compared to wDDGS 3.0 % of CP.

Table 3.1. Batch effect of original feedstock wheat and their co-products-wheat DDGS on chemical profiles composition

Item	Batches of wheat										Batches of wDDGS						
	Wheat	wDDGS	SEM	1	2	3	4	5	SEM	CV	I	II	III	IV	V	SEM	CV
	(n=15)	(n=15)		(n=3)	(n=3)	(n=3)	(n=3)	(n=3)		%	(n=3)	(n=3)	(n=3)	(n=3)	(n=3)		%
Basic chemical profile (%DM)																	
DM(%)	88.3b	92.4a	0.17	88.2ab	88.3ab	88.7a	87.9b	88.3ab	0.13	0.32	92.7ab	93.2a	91.6b	92.9ab	91.6b	0.33	1.92
Ash	1.9b	5.7a	0.08	1.8	1.8	1.8	2.0	2.0	0.07	5.83	5.3c	6.2a	5.4c	6.1a	5.7b	0.03	0.47
OM	98.1a	94.3b	0.08	98.2	98.2	98.2	98.1	98.0	0.07	0.09	94.7a	93.8c	94.6a	93.9c	94.4b	0.03	1.94
Cfat	1.4b	5.1a	0.05	1.2	1.4	1.6	1.5	1.5	0.11	10.53	5.2	5.1	5.2	5.2	5.0	0.08	0.37
Structural carbohydrate profile (%DM)																	
NDF	13.9b	39.8a	0.45	14.1ab	13.3bc	12.6c	15.1a	14.1ab	0.30	7.62	38.3b	37.2b	39.3b	41.9a	42.4a	0.53	4.74
ADF	3.0b	11.6a	0.32	2.7bc	2.5c	2.7bc	3.5a	3.4ab	0.16	13.04	10.1b	10.8b	10.6b	12.9a	13.9a	0.44	8.89
ADL	0.8b	3.1a	0.16	0.5b	0.5b	0.5b	1.1a	1.1a	0.07	27.27	2.15c	2.9bc	2.6c	3.5b	4.4a	0.17	12.87
*Hemi	11.0b	28.2a	0.27	11.5ab	10.9ab	9.9b	11.9a	11.0ab	0.39	7.91	28.2ab	26.4b	28.7ab	29.0a	28.5ab	0.50	4.09
Non-structural carbohydrate profile (%DM)																	
Starch	63.4a	1.8b	0.54	61.6	66.4	62.9	63.0	63.4	1.65	3.24	1.6bc	1.4c	2.6a	1.5bc	1.7b	0.06	32.71
Crude protein profile (%CP)																	
CP	14.3b	42.8a	0.31	15.1a	14.4b	13.0c	14.7ab	14.4b	0.64	6.34	44.9a	43.9a	42.0b	41.0b	42.2b	0.26	4.20
SCP	27.0b	34.8a	0.59	27.2bc	28.3ab	30.7a	23.5d	25.0cd	0.44	11.99	34.6a	35.3a	32.3b	36.5a	35.5a	0.47	4.98
NPN	30.4b	91.5a	2.00	41.1a	31.3ab	15.7b	33.4ab	30.2ab	3.87	35.27	87.3	94.1	94.8	91.3	90.1	1.67	3.78
NDICP	10.3b	37.3a	0.65	13.3a	11.1ab	11.0ab	9.2bc	7.1c	0.52	23.64	35.4b	39.6a	43.3a	41.3a	42.5a	0.81	7.90
ADICP	1.1b	3.5a	0.40	0.2c	0.2c	0.1c	3.7a	1.4b	0.21	126.24	3.4	5.1	2.7	4.1	2.3	0.85	44.53

SEM = standard error of mean. *a-d* Means with the different letters in the same row for wheat and wheat DDGS, for the batches of wheat and for the batches of wheat DDGS are significantly different ($P < 0.05$). Multi-treatment comparison by Tukey method. OM, organic matter; Cfat, crude fat or ether extract (EE); NDF, neutral detergent fibre with heat stable α -amylase without sodium sulphite; ADF, acid detergent fibre; ADL, acid detergent lignin; *Hemi, hemicellulose-calculated (Hemi = NDF-ADF); CP, crude protein; SCP, bicarbonate phosphate buffer soluble CP; NPN, nitrogenous compounds soluble in water and not precipitated by Tungstic acid; NDICP, neutral detergent insoluble CP; ADICP, acid detergent insoluble CP. CV; coefficient of variation

Table 3.2. Batch effect of original feedstock wheat and their co-products wheat DDGS on macro and micro mineral profiles.

Item	Wheat	wDDGS	SEM	Batches of wheat					SEM	CV %	Batches of wDDGS					SEM	CV %
				1 (n=3)	2 (n=3)	3 (n=3)	4 (n=3)	5 (n=3)			I (n=3)	II (n=3)	III (n=3)	IV (n=3)	V (n=3)		
Macro minerals (% DM)																	
Ca	0.05b	0.10a	0.004	0.05	0.04	0.03	0.07	0.07	0.010	34.40	0.11a	0.11ab	0.10b	0.10b	0.10a	0.003	5.27
P	0.31b	0.90a	0.008	0.32a	0.33a	0.25b	0.32a	0.34a	0.010	11.42	0.87c	0.93a	0.89bc	0.92ab	0.91a	0.006	2.66
S	0.16b	1.09a	0.024	0.18a	0.15b	0.16ab	0.15b	0.15b	0.006	8.25	1.09b	1.32a	1.03bc	1.03bc	0.98c	0.019	12.33
Mg	0.14b	0.37a	0.004	0.14	0.14ab	0.14ab	0.16a	0.15ab	0.008	6.13	0.35c	0.38a	0.36b	0.38ab	0.38a	0.003	3.82
Cl	0.12b	0.22a	0.005	0.13	0.12	0.12	0.12	0.11	0.009	5.89	0.21b	0.25a	0.21b	0.22ab	0.21b	0.008	7.87
K	0.43b	1.08a	0.010	0.44	0.43	0.42	0.43	0.45	0.020	2.63	0.99d	1.11ab	1.04c	1.14a	1.10b	0.009	5.60
Na	0.02b	0.41a	0.030	0.03	0.01	0.01	0.01	0.01	0.020	63.89	0.27c	0.56a	0.30c	0.54a	0.39b	0.006	32.45
Micro minerals (mg/kg DM)																	
Cu	5.9b	12.8a	1.22	5.6	8.7	6.7	3.9	4.7	1.82	31.61	9.8	18.3	11.4	14.4	10.1	3.33	27.91
Zn	30.4b	75.4a	1.64	30.7	37.0	27.8	27.1	29.3	2.97	13.01	76.2b	87.6a	69.8c	69.4c	74.2b	0.93	9.79
Mn	35.3b	73.3a	7.00	36.8a	37.7a	25.7b	36.6a	39.9a	1.17	15.69	92.8a	100.0a	90.8bc	90.4c	92.6b	0.49	4.16
Fe	57.3b	153.3a	9.90	51.1b	51.8b	41.3b	60.5ab	81.9a	5.71	26.75	200.0	200.0a	100.0b	133.1ab	133.1	21.08	29.22

SEM: Standard error of the mean. a-c Means with different letters at the same row for wheat and wheat DDGS, for the batches of wheat and for the batches of wheat DDGS are significantly different (P<0.05). Multi treatment comparison by Tukey's method. CV; Coefficient of variation.

There were significant differences among the different batches of original feedstock of wheat in protein sub-fractions ($P<0.05$). Original feedstock wheat Batch 3 contained the highest PB1 fraction (25.9 % of CP) while the feedstock Batch 5 contained lowest (5.4 % of CP) where large variation among original feedstock exist. Feedstock wheat Batches 1-3 had the lower intermediately degradable fraction PB2 (60.6-58.3 % of CP) compared with Batches 4 and 5. Batch 4 had the highest unavailable fraction PC (3.7 vs. 0.1 % of CP in Batch 3). There were significant differences among the batches of wDDGS in protein sub-fractions. The wDDGS were lower in rapidly degradable PB1 fraction from 1.7 in Batch III to 4.4 % of CP in Batch I. Among the batches of wDDGS, PB2 fraction varied from 25.2 to 32.6 % of CP and the slowly degradable fraction PB3 varied from 29.5 to 36.9 % of CP.

Carbohydrate sub-fractions between wheat and wDDGS, among different batches of feedstock wheat, and among different batches of wDDGS were significantly different ($P<0.05$) (Table 3.3). Wheat contained higher total carbohydrate fraction compared to wDDGS (82.4 vs. 46.4 % of DM). Wheat was lower in calculated sugars CA fraction 9.9 vs. 45.0 % of CHO, higher in starch and pectin CB1 fraction (74.7 vs. 3.6 % of CHO). Wheat DDGS was higher in available cell wall CB2 fraction (35.4 vs. 13.0 % of CHO) and unavailable fibre CC fraction (16.0 vs. 2.3 % of CHO). There were significant differences among the feedstock batches of wheat in carbohydrate sub-fractions of CB2 and CC. Five feedstock batches were similar in CB1 fraction with average of 74.7 % of CHO. Among the batches of wheat, the range for available cell wall (CB2 fraction) was 2.0 % of CHO and for the unavailable fibre (CC fraction) was 1.8 % of CHO. Among the batches of wDDGS, carbohydrate sub-fractions were significantly different ($P<0.05$). The calculated sugars (CA fraction) varied among the batches of wDDGS by 8.7 % of CHO. The Batch III was highest in CB1 fraction (5.2 % of CHO) and the range among the batches of wDDGS was 2.2 % of CHO. The available cell wall CB2 fraction was highest in the Batch I (41.1 % of CHO) compared to the lowest CB2 fraction in Batches II and III with the variation of 9.6 % of CHO. The unavailable fibre CC fraction was significantly varied among the Batches of wDDGS by 10.8 % of CHO.

3.4.4. Variation in Energy Content among Different Batches of Original Feedstock and among Different Batches of Wheat DDGS

The estimated energy values based on NRC-2001 Dairy and NRC-1996 Beef are presented in Table 3.4. There was significant difference between the original feedstock wheat and wDDGS in total digestible nutrients ($P<0.05$). Wheat was higher in tdNFC while wDDGS was higher in tdCP, tdFA and tdNDF. The obtained value for the tdNFC in wheat was 72.4 vs. 24.3 %DM in wDDGS and tdCP, tdFA and tdNDF of wDDGs was 42.2, 4.1 and 10.7 vs. 14.3, 0.4 and 6.7 %DM in wheat. There were significant differences ($P<0.05$) among the batches of original feedstock wheat in tdNFC, tdCP, and tdNDF. The highest tdNFC was in the feedstock Batches 2 and 3. The Batch 1 was higher in tdCP. Among the batches of wDDGS, there were significant differences in tdNFC, tdCP, and tdNDF, but no significant difference in tdFA values. The higher tdNFC was in wDDGS Batches II and III while Batch I contained higher tdCP and tdNDF values. Among the batches of wDDGS, a tdNDF value varied by 2.8 %DM. Wheat was higher in TDN1x values (87.3 %DM) compared to wDDGS (79.5 %DM). Among the batches of original feedstock wheat, the variation for TDN1x values was 1.5 %DM. The TDN1x values varied among batches of wDDGS by 4.2% of DM.

3.4.5. Particle Size Distribution among the Batches of Wheat and Wheat DDGS

The results for particle size distribution among the batches of wheat and wheat DDGS was shown in Table 3.5. When compared the retention of particles at each sieve, there were a significant differences ($P<0.05$) between wheat and wheat DDGS in particle size distribution. The roller-milled wheat retained in sieves with larger diameter openings than wheat DDGS (percentage retention at sieve # 6, 8, 12 were 5.2, 25.6 and 31.3 % compared to wheat DDGS 0.1, 0.1 and 0.4 %, respectively). Geometric mean of particle size varied among the batches of roller milled wheat from 1629 – 1756 μm and among the batches of wheat DDGS 650 – 690 μm . In wDDGS Batch I, 49 % of the particles remained at the bottom pan while in Batch V 36 % of the particles remained at the bottom pan. The significant differences ($P<0.05$) in particle size distribution among the batches of wDDGS in different particle size categories were observed. In wDDGS, around 75% of particles are less than 0.84 mm which indicates a high level of fine particle size in this mash type of wDDGS.

Table 3.3. Batch effect of original feedstock wheat and their co-products of wheat DDGS on CNCPS carbohydrate and protein subfractions that associated with different rumen degradation.

Item	Wheat	wDDGS	SEM	P	Batches of wheat					SEM	P	Batches of wDDGS					SEM	P
					1	2	3	4	5			I	II	III	IV	V		
Protein sub-fractions (%CP)																		
PA	10.5b	31.8a	1.00	<0.0	11.1b	8.9b	4.8c	7.9bc	19.6a	0.75	<0.01	30.2b	33.2a	30.6b	33.3a	32.0ab	0.49	<0.01
PB1	16.5a	3.0b	1.32	<0.0	16.2b	19.5b	25.9a	15.7b	5.4c	1.18	<0.01	4.4	2.1	1.7	3.2	3.5	0.60	0.06
PB2	62.7a	27.9b	0.95	<0.0	59.5b	60.6b	58.3b	67.3a	67.9a	0.60	<0.01	32.6a	27.8b	28.1b	25.2b	25.6b	0.74	<0.01
PB3	9.2b	33.79a	0.87	<0.0	13.1a	10.9a	10.8a	5.5b	5.7b	0.51	<0.01	29.5b	31.8ab	36.9a	34.2ab	36.6a	1.30	0.01
PC	1.1b	3.51a	0.40	<0.0	0.22c	0.17c	0.12c	3.7a	1.4b	0.21	<0.01	3.37	5.1	2.7	4.1	2.3	0.85	0.21
TP	88.4a	64.6b	1.11	<0.0	88.7b	91.0b	95.1a	88.5b	79.0c	0.68	<0.01	66.4ab	61.7c	66.7a	62.6bc	65.8ab	0.84	<0.01
Carbohydrate sub-fractions (%CHO)																		
CA	9.9b	45.0a	0.6	<0.01	10.3	8.6	11.6	9.3	9.4	1.37	0.47	44.0b	50.1a	47.2ab	42.0b	41.4b	1.27	<0.01
CB1	74.7a	3.6b	0.1	<0.01	74.8	76.6	75.0	73.4	74.4	1.53	0.57	3.3b	3.0b	5.2a	3.1b	3.4b	0.12	<0.01
CB2	13.1b	35.4a	0.5	<0.01	13.4ab	13.1ab	11.9b	13.9a	13.2ab	0.12	0.02	41.1a	31.5c	34.4bc	37.4ab	32.9bc	1.07	<0.01
CC	2.3b	16.0a	0.6	<0.01	1.5b	1.7b	1.6b	3.3a	3.1a	0.05	<0.01	11.5c	15.4bc	13.3c	17.6b	22.3a	0.84	<0.01
CHO (%DM)	82.4a	46.4b	0.3	<0.01	81.9b	82.4b	83.7a	81.8b	82.1b	0.16	<0.01	44.7b	44.8b	47.5a	47.6a	47.2a	0.26	<0.01

SEM= standard error of mean. a-c Mean with different letters at the same row for wheat and wheat DDGS, for the batches of wheat and for the batches of wheat DDGS are significantly different (P<0.05). Multi-treatment comparison by Tukey method. True protein (TP) = PB1+ PB2+ PB3; Total carbohydrates (CHO) = 100 – CP – Cfat - ash

Table 3.4. Batch effect of original feedstock wheat and their co-products-wheat DDGS on total digestible nutrient (TDN) and energy values estimated using NRC-2001 and 1996 summary approach

Item	Wheat	wDDGS	SEM	P	Batches of wheat					SEM	P	Batches of wDDGS					SEM	P
					1	2	3	4	5			I	II	III	IV	V		
Digestible nutrients (%DM)																		
tdNFC	72.40a	24.26b	0.401	<0.01	71.29b	72.18ab	74.05a	71.98b	72.49ab	0.367	<0.01	22.71c	25.46ab	26.91a	23.11bc	23.11bc	0.589	<0.01
tdCP	14.25b	42.18a	0.300	<0.01	15.09a	14.36b	12.96c	14.50b	14.34b	0.087	<0.01	44.30a	43.00b	41.50cd	40.31d	41.80bc	0.263	<0.01
tdFA	0.43b	4.13a	0.047	<0.01	0.21	0.40	0.55	0.52	0.48	0.112	0.27	4.16	4.08	4.18	4.24	3.98	0.084	0.30
tdNDF	6.67b	10.74a	0.252	<0.01	7.49a	7.25a	6.83a	5.91b	5.90b	0.178	<0.01	12.00a	9.21c	10.39bc	11.78ab	10.30bc	0.589	<0.01
Total digestible nutrient at a maintenance level (%DM)																		
TDN _{IX}	87.30a	79.47b	0.358	<0.01	87.34abc	87.70ab	88.08a	86.56c	86.83bc	0.191	<0.01	81.37a	79.86b	81.23ab	77.75c	77.17c	0.318	<0.01
Predicted energy values (Mcal/kg DM) (NRC-2001 Dairy)																		
NE _{L3X}	2.01b	2.07a	0.011	<0.01	2.02ab	2.02ab	2.02ab	1.99c	2.00bc	0.005	<0.01	2.14a	2.09b	2.11ab	2.01c	2.00c	0.010	<0.01
Predicted energy values (Mcal/kg DM) (NRC-1996 Beef)																		
NE _m	2.17b	2.21a	0.011	0.02	2.17ab	2.18a	2.18a	2.15c	2.15bc	0.004	<0.01	2.28a	2.23b	2.25ab	2.14c	2.14c	0.010	<0.01
NE _g	1.49b	1.52a	0.010	0.03	1.49ab	1.50a	1.50ab	1.47c	1.48bc	0.004	<0.01	1.58a	1.54b	1.56ab	1.46c	1.46c	0.008	<0.01

SEM= standard error of mean. a-c Mean with different letters at the same row for wheat and wheat DDGS, for the batches of wheat and for the batches of wheat DDGS are significantly different (P<0.05). Multi-treatment comparison by Tukey method

3.4.6. Batch Effect of on In Situ Rumen Degradation Characteristics of Dry Matter

The effect of batch on in situ rumen degradation characteristics of dry matter is summarized in Table 3.6. There were significant differences ($P<0.05$) between wheat and wDDGS in rumen degradation of DM of W (S), D, U, K_d , ED and RU fractions other than T0 which was similar across the treatments. Wheat was lower in W (S) but higher in D. The W (S) for wheat and wDDGS was 7.3% vs. 35.0% and D fraction was 81.7% and 49.6% for wheat and wDDGS, respectively. The U of wDDGS was higher than wheat (15.5 vs. 11.0%). The K_d of wheat was two times higher than wDDGS (25.3 vs. 10.6 %/h). The effective degradability of wheat was 72.7 vs. 66.2% in wDDGS. Rumen undegraded dry matter (RUDM) in wheat and wDDGS was 27.3% and 33.8% respectively. The lower W (S) fraction of wheat may be due to its particle size when processed coarsely with the roller gap 0.203 mm. The particle size distribution of wheat is summarized in Table A1 in Appendix. The mash type wDDGS had finer size of particles (~ 43% of the sample less than 0.5 mm) which resulted in higher W (S) fraction. The lower K_d of wDDGS may be partially due to the production of Millard products during the drying process of wDDGS (Weiss et al., 1986) and high fibre content.

There were also significant differences ($P<0.05$) among the batches of feedstock wheat in D, U, K_d , ED and RUDM fractions. Feedstock Batch 3 was higher ($P<0.05$) in D (83.5 vs. 80.3% in Batch 5) while Batch 5 was higher in U (12.7 vs. 9.6% in Batch 3). The ED of DM in the Batch 3 was higher ($P<0.05$) which was 76.5% compared to 67.7% in Batch 5. Rumen undegraded dry matter (RUDM) was higher ($P<0.05$) in the Batch 5 (32.4 vs. 23.5% in Batch 3).

The ranges in W (S) fraction and K_d were 2.4% and 3.8%/h, respectively. Among the batches of wDDGS there were no significant difference in D, U, EDDM, and RUDM.

Table 3.5. Particle size distribution among different batches of wheat and wheat DDGS used for in situ study

USA Sieve	Sieve size (mm)	Wheat (n=6) (%)	wDDGS (n=6) (%)	SEM	P value	Batches of wheat			SEM	CV	Batches of wDDGS			SEM	CV
						1 (n=2) (%)	3 (n=2) (%)	5 (n=2) (%)			I (n=2) (%)	III (n=2) (%)	V (n=2) (%)		
# 6	3.36	5.2a	0.1b	0.23	<0.01	6.2a	4.1b	5.2ab	0.45	0.20	0.1	0.1	0.0	0.03	0.87
# 8	2.38	25.6a	0.1b	0.48	<0.01	24.3	25.8	26.7	1.18	0.20	0.1a	0.1ab	0.0b	0.02	0.87
# 12	1.68	31.3a	0.4b	0.47	<0.01	27.9b	32.9a	33.2a	0.60	0.20	0.3	0.4	0.3	0.02	0.17
# 16	1.19	16.2a	5.4b	0.36	<0.01	15.6	16.9	16.0	0.73	0.20	4.4b	6.5a	5.3ab	0.36	0.20
# 20	0.841	5.1b	17.0a	0.38	<0.01	5.1	5.0	5.1	0.19	0.20	14.6b	18.0a	18.4a	0.59	0.12
# 30	0.594	4.5b	34.2a	0.62	<0.01	4.6	4.6	4.2	0.26	0.20	31.3b	32.4b	38.8a	0.59	0.12
Bottom pan	<0.594	10.9b	42.7a	1.16	<0.01	13.8a	10.0b	9.02b	0.95	0.20	49.0a	42.3b	36.6c	1.53	0.15
GM_mean (µm)		1695.3a	674.4b	16.43	<0.01	1628.5	1701.8	1755.7	34.76	0.20	649.8b	683.0a	690.3a	6.64	0.03

SEM: Standard Error of Mean. a-c Mean with the different letters in the same row were significantly different ($P<0.05$). Multi treatment comparison by Tukey Method.

Table 3.6. Batch effect of original feedstock wheat and their co-products-wheat DDGS on in situ rumen degradation kinetic of dry matter.

Item	Wheat (n=6)	wDDGS (n=6)	SEM	P value	Batches of feed stock			SEM	P value	Batches of wDDGS			SEM	P value
					1 (n=2)	3 (n=2)	5 (n=2)			I (n=2)	III (n=2)	V (n=2)		
In situ rumen degradation kinetics of dry matter														
W (S) (%)	7.3b	35.0a	2.00	<0.01	8.0	6.9	7.0	2.09	0.03	33.8b	36.2a	34.9ab	1.93	0.02
D (%)	81.7a	49.6b	2.21	<0.01	81.3ab	83.5a	80.3b	2.66	0.02	50.7	49.2	48.8	1.84	0.19
K _d (%/h)	25.3a	10.6b	1.45	<0.01	25.9ab	31.0a	18.9b	2.22	0.01	10.2ab	8.9b	12.7a	1.79	0.05
U (%)	11.0b	15.5a	0.42	<0.01	10.7b	9.6b	12.7a	0.67	<0.01	15.6	14.6	16.2	0.49	0.12
EDDM (%)	72.7a	66.2b	0.96	<0.01	73.9a	76.5a	67.7b	1.03	<0.01	65.6	65.5	67.5	1.12	0.16
RUDM (%)	27.3b	33.8a	0.96	<0.01	26.1b	23.5b	32.4a	1.03	<0.01	34.4	34.5	32.5	1.12	0.16

SEM= standard error of mean. a-b Mean with different letters at the same row for wheat and wheat DDGS, for the batches of wheat and for the batches of wheat DDGS are significantly different ($P<0.05$). Multi-treatment comparison by Tukey method.

W (S), truly soluble washable fraction (0 h incubation); D, potentially degradable fraction calculated as: $100 - (S+U)$; U, undegradable fraction; K_d, fractional degradation rate; EDDM, effective degradability of DM calculated as: $S + D \times K_d / (K_d + K_p)$, where passage rate assumed to be 6 %/h (Tamminga et al., 1994). K_d and U were estimated from the first order exponential model: $R(t) = U + D \times e^{-k_d \times (t-T_0)}$; RUDM, rumen undegraded dry matter.

3.4.7. Effect of Batch on In Situ Rumen Degradation Characteristics of Organic Matter

The rumen degradation characteristic of OM is summarized in Table 3.7. Compared to wDDGS, wheat was higher in D and K_d values of OM. The degradation rate of K_d in wheat was two times higher than in wDDGS. The obtained values were 82.1%, 25.6 %/h in wheat vs. 51.9%, 10.5%/h in wDDGS for D and K_d , respectively. Wheat was higher in EDOM (72.8 vs. 65.0 % in wDDGS) and lower in RUOM (27.2 vs. 35.0 % in wDDGS). Among the batches of wheat there were significant differences ($P<0.05$) in D, K_d , U, EDOM and RUOM. The range of EDOM was 8.8 % among the batches of wheat, while the range of the RUOM was 8.7 %. Wheat Batch 5 was lowest in EDOM and highest in RUOM. Other than the W (S) and K_d values among the batches of wDDGS, there were no significant differences detected in other rumen degradation parameters of OM.

3.4.8. Effect of Batch on In Situ Rumen Degradation Characteristics of Crude Protein

The effect of batch on in situ rumen degradation characteristics of CP is presented in Table 3.8. When compared to feedstock wheat, wDDGS was significantly different ($P<0.05$) in W (S), D, U, effective degradability of CP (EDCP) and rumen undegraded CP (RUP). Wheat DDGS was higher ($P<0.05$) in W(S) (34.3 %) and lower in D (54.6 %) compared to 12.7% and 79.8% in wheat for W and D fractions, respectively. The EDCP of both wheat and wDDGS were 67% of CP. Among the batches of feedstock, there was significant difference in D, U, K_d , EDCP and RUP. The range among the batches of wheat in D fraction was 6.1% of CP and range in fraction U was 5.5% of CP. The range of K_d among the batches of wheat was 11.5%/h. The EDCP content was higher in both Batches 1 and 3 (103, 96 vs. 87 g/kg DM in Batch 5) and the Batch 5 was higher in RUP (65 vs. 38 g/kg DM in Batch 3). Among the batches of wDDGS, there were significant differences in W (S), D, EDCP and RUP. The range of W (S) among the batches of wDDGS was 5.9% of CP and the range of D among wDDGS batches was 7.4% of CP. Batch III was higher in D (59.1 vs. 51.7% of CP in Batch V).

Table 3.7. Batch effect of original feedstock wheat and their co-products-wheat DDGS on in situ rumen degradation kinetic of organic matter.

Item					Batches of Wheat			Batches of wDDGS						
	Wheat (n=6)	wDDGS (n=6)	SEM	P value	1 (n=2)	3 (n=2)	5 (n=2)	SEM	P value	I (n=2)	III (n=2)	V (n=2)	SEM	P value
In situ rumen degradation kinetic of organic matter														
W (S) (%)	6.9b	32.4a	2.08	<0.01	7.7	6.4	6.5	2.21	0.05	31.3b	33.6a	32.2ab	1.99	0.03
D (%)	82.1a	51.9b	2.29	<0.01	81.6ab	84.0a	80.8b	2.78	0.03	52.9	51.5	51.2	1.90	0.26
K _d (%/h)	25.6a	10.5b	1.47	<0.01	26.3ab	31.3a	19.2b	2.31	0.02	10.2ab	8.9b	12.6a	1.75	0.04
U (%)	11.0b	15.8a	0.43	<0.01	10.8ab	9.6b	12.7a	0.68	0.01	15.8	14.9	16.6	0.51	0.11
EDOM (%)	72.8a	65.0b	0.96	<0.01	74.0a	76.6a	67.8b	1.05	<0.01	64.4	64.2	66.3	1.16	0.17
RUOM (%)	27.2b	35.0a	0.96	<0.01	26.0b	23.5b	32.2a	1.05	<0.01	35.6	35.8	33.7	1.16	0.17

SEM= standard error of mean. a-b Mean with different letters at the same row for wheat and wheat DDGS, for the batches of wheat and for the batches of wheat DDGS are significantly different (P<0.05). Multi-treatment comparison by Tukey method

W (S), truly soluble washable fraction (0 h incubation); D, potentially degradable fraction calculated as: $100 - (S+U)$; U, undegradable fraction; K_d, fractional degradation rate; EDOM, effective degradability of OM calculated as: $S + D \times K_d / (K_d + K_p)$, where passage rate assumed to be 6 %/h (Tamminga et al., 1994). K_d and U were estimated from the first order exponential model: $R(t) = U + D \times e^{-k_d \times (t-T_0)}$; RUOM, rumen undegraded OM.

Table 3.8. Batch effect of original feedstock wheat and their co-products-wheat DDGS on in situ rumen degradation kinetic of crude protein

Item	Wheat (n=6)	wDDGS (n=6)	SEM	P value	Batches of Wheat			SEM	P value	Batches of wDDGS			SEM	P value
					1 (n=2)	3 (n=2)	5 (n=2)			I (n=2)	III (n=2)	V (n=2)		
In situ rumen degradation kinetics of crude protein														
W (S) (%)	12.7b	34.3a	7.55	<0.01	12.9	11.7	13.5	10.53	0.23	36.2a	30.4b	36.3a	4.54	<0.01
D (%)	79.8a	54.6b	6.77	<0.01	82.1a	81.3a	76.0b	10.02	<0.01	53.1b	59.1a	51.7b	3.50	<0.01
K _d (%/h)	14.2	9.8	2.23	0.06	12.5ab	20.8a	9.3b	2.19	0.02	8.3	7.9	13.3	3.25	0.06
U (%)	7.5b	11.0a	0.94	<0.01	5.0b	7.0b	10.5a	0.68	<0.01	10.7	10.5	12.0	1.39	0.57
EDCP (%)	67.3a	66.8b	4.33	<0.01	68.2a	74.0a	59.6b	2.06	<0.01	66.9ab	63.3b	70.4a	1.53	<0.01
RUP (%)	32.8	33.2	1.60	0.86	31.8b	26.1b	40.4a	2.06	<0.01	33.1ab	36.7a	29.6b	1.53	<0.01

SEM= standard error of mean. a-c Mean with different letters at the same row for wheat and wheat DDGS, for the batches of wheat and for the batches of wheat DDGS are significantly different ($P<0.05$). Multi-treatment comparison by Tukey method

W (S), truly soluble washable fraction (0 h incubation); D, potentially degradable fraction calculated as: $100 - (S+U)$; U, undegradable fraction; K_d, fractional degradation rate; EDCP, effective degradability of CP calculated as: $S + D \times K_d / (K_d + K_p)$, where passage rate assumed to be 6 %/h (Tamminga et al., 1994). K_d and U were estimated from the first order exponential model: $R(t) = U + D \times e^{-k_d \times (t-T_0)}$; RUCP, rumen undegraded CP.

Table 3.9. Batch effect of original feedstock wheat and their co-products-wheat DDGS on in situ rumen degradation kinetic of neutral detergent fiber

Item	Wheat (n=6)	wDDGS (n=6)	SEM	P value	Batches of Wheat				P value	Batches of wDDGS				
					1 (n=2)	3 (n=2)	5 (n=2)	SEM		I (n=2)	III (n=2)	V (n=2)	SEM	
In situ rumen degradation kinetic of neutral detergent fiber														
W (S) (%)	12.7b	34.3a	7.55	<0.01	12.9	11.7	13.5	10.53	0.23	36.2a	30.4b	36.3a	4.54	0.00
D (%)	50.4a	70.2a	1.00	<0.01	52.7	51.7	46.8	2.07	0.16	70.4	69.6	70.6	0.85	0.71
K _d (%/h)	19.3b	32.0a	3.50	0.02	12.4	21.3	24.3	7.60	0.54	23.2b	34.9ab	37.7a	3.28	0.03
U (%)	47.7a	29.8b	1.65	<0.01	43.5b	46.4ab	53.2a	3.34	0.03	29.7	30.4	29.4	0.85	0.71
EDNDF (%)	37.4b	58.4a	1.07	<0.01	37.6ab	40.8a	33.8a	1.61	0.04	55.1b	59.2ab	60.9a	1.04	0.01
RUNDF (%)	62.6a	41.6b	1.07	<0.01	62.5ab	59.8b	66.2a	1.61	0.04	44.9a	40.8ab	39.2b	1.04	0.01

SEM= standard error of mean. a-c Mean with different letters at the same row for wheat and wheat DDGS, for the batches of wheat and for the batches of wheat DDGS are significantly different (P<0.05). Multi-treatment comparison by Tukey method

W (S), truly soluble washable fraction (0 h incubation); D, potentially degradable fraction calculated as: $100 - (S+U)$; U, undegradable fraction; K_d, fractional degradation rate; EDNDF, effective degradability of NDF calculated as: $S + D \times K_d / (K_d + K_p)$, where passage rate assumed to be 6 %/h (Tamminga et al., 1994). K_d and U were estimated from the first order exponential model: $R(t) = U + D \times e^{-k_d \times (t-T_0)}$; RUNDF, rumen undegraded NDF.

3.4.9. Effect of Batch on In Situ Rumen Degradation Characteristics of Neutral Detergent Fibre

The effect of batch on rumen degradation characteristic of NDF is summarized in Table 3.9. Compared to wheat, wDDGS was higher in EDNDF (58.4 vs. 37.4%). Among the batches of wheat, RUNDF varied by 6.4 %. Among the batches of wDDGS, W (S) fraction varied by 5.9 % of NDF, K_d varied by 15 %/h, EDNDF varied by 5.8 %, and RUNDF varied by 5.7%. Table 3.9 shows 30-36% of NDF washed out from nylon bags indicating fine particle size of this type of wDDGS that we studied.

3.4.10. Effect of Batch on Hourly Effective Degradation Ratios between Nitrogen and Organic Matter

The effect of batch on hourly effective degradation ratios of N and OM is shown in Table 3.10. There were significant difference ($P<0.05$) between wheat and wDDGS in hourly effective degradation ratio of N and OM at all incubation time points. The higher ratios of EDN and EDOM were observed at longer incubation time points due to the small differences among consecutive incubation time points. Among the batches of wheat, there was a significant difference ($P<0.05$) at 6 h incubation time point, in which 5% variation observed among the batches of wheat. There were significant differences ($P<0.05$) among the batches of wDDGS at 0, 6 and 12 h time points and the ranges of hourly effective degradation ratio of EDN to EDOM were 24.2, 8.6 and 15.6% respectively.

3.4.11. Effect of Batch on Estimated Intestinal Digestibility of Rumen Undegraded Protein

The effect of batch on estimated intestinal digestibility of rumen undegraded feed protein in small intestine is presented in Table 3.11. Wheat was higher in intestinal digestibility of rumen undegraded feed protein (79 vs. 67 % in wDDGS). Among the batches of wheat, the intestinal digestibility of RUP varied by 7.3 % while among the batches of wDDGS it varied by 6.8 %. For wheat the highest digestibility of RUP was in Batch I (83.3%) while for wDDGS the highest digestibility of RUP was in Batch III (71 %) which disagrees with 85% assumed in NRC-1996.

Table 3.10. Batch effect of original feedstock wheat and their co-products-wheat DDGS on the hourly effective degradation ratio of N to OM

Item	Wheat (n=6)	wDDGS (n=6)	SEM	P value	Batches of wheat			SEM	P value	Batches of wDDGS			SEM	P value
					1 (n=2)	3 (n=2)	5 (n=2)			I (n=2)	III (n=2)	V (n=2)		
Hourly effective degradation ratio of EDN to EDOM														
Syn_0	35.4b	77.3a	15.15	<0.01	35.4	30.9	40.1	24.6	0.27	87.8a	63.6c	80.5b	5.51	<0.01
Syn_2	14.3b	69.6a	6.46	<0.01	14.5	15.8	12.5	2.8	0.10	64.0	74.0	71.0	10.39	0.20
Syn_6	22.3b	71.0a	4.24	<0.01	24.7a	23.5a	18.6b	2.5	0.013	68.6b	76.5a	67.9b	6.16	0.02
Syn_12	45.9b	74.2a	3.84	<0.01	57.3	46.6	33.9	7.5	0.14	76.4a	80.8a	65.2b	2.71	0.01
Syn_24	253.3a	84.4b	70.91	0.04	384.9	260.0	114.9	153.0	0.41	95.1	92.6	65.3	13.35	0.06

SEM=standard error of mean. a-c Means with different letters at the same row for wheat and wheat DDGS, for the batches of wheat and for the batches of wheat DDGS are significantly different ($P<0.05$). Multi treatment comparison by Tukey method.

Syn_0, effective degradation ratio of N to OM at 0 h incubation time point; Syn_2, hourly effective degradation ratio of N to OM at 2 h; Syn_6, hourly effective degradation ratio of N to OM at 6 h; Syn_12, hourly effective degradation ratio of N to OM at 12 h, Syn_24, hourly effective degradation ratio of N to OM at 24 h.

Table 3.11. Batch effect of original feedstock wheat and their co-products-wheat DDGS on estimated intestinal digestibility of rumen undegraded protein (%dRUP)

Item	Wheat (n=6)	wDDGS (n=6)	SEM	P value	Batches of wheat			SEM	P value	Batches of wDDGS			SEM	P value
					1 (n=2)	3 (n=2)	5 (n=2)			I (n=2)	III (n=2)	V (n=2)		
Intestinal digestibility of RUP (%)														
%dRUP	78.7a	66.8b	3.27	<0.01	83.3a	76.0b	76.4b	4.19	<0.01	65.5b	70.9a	64.1b	2.23	<0.01

SEM=Standard Error of the Mean. a-b Mean with different letter value at the same row for wheat and wheat DDGS, for the batches of wheat and for the batches of wheat DDGS are significantly different (P<0.05). Multi treatment comparison by Tukey Method

3.5. Discussion

The values of original feedstock wheat obtained for the DM, CP, and NDF are in agreement with the values reported in NRC 2001. Also the values obtained for chemical profile of wDDGS are with the close agreement of the values reported by published studies (Widyaratne and Zijlstra, 2007; Thacker and Widyaratne, 2007; Nuez-Ortín and Yu, 2009). Among the batches of feedstock wheat, ADF values are lower than the previously reported values. The obtained ADF values for wDDGS were in close agreement with values previously reported (Boila and Ingalls, 1994a, b; Widyaratne and Zijlstra, 2007; Gibb et al., 2008; Nuez-Ortín and Yu, 2009; Azarfar et al., 2011). The similar NDF variability was reported by Nuez-Ortín and Yu (2009). Higher ADICP levels in cotton seed meal (Arieli et al., 1989) and canola meal (Nia and Ingalls, 1992) were reported due to high temperature treatment in the processing, which can be related to higher observed values of ADICP among batches of wDDGS. Belyea et al. (2004) reported that it was not the original feedstock grain but there were other factors that cause for the variation in DDGS, such as factors associated with production process. Also Ham et al. (1994) and Lodge et al. (1997) reported the differences among the wet and dry co-products from bioethanol production. Therefore the processing plays a significant role in causing large variation in co-products from bioethanol production.

Mineral composition in dairy cattle diet is very critical since excess or deficit of required amount of minerals can lead to various metabolic disorders. Other than the adverse impacts on animal, excessive mineral consumption leads to increase excretion of minerals to the environment (Spiehs and Varel, 2009). Both Ca and P were in agreement with the values given by NRC 2001 for wDDGS. Furthermore NRC identifies P as the most likely mineral to be in excess for the animal with the much higher possibility to contaminate the environment specially the surface water. The highest proportion of absorbed P in dairy cattle is directed for the milk P (NRC, 2001). Thus NRC recommends the P content of a diet as 0.30 – 0.40% of the diet DM depending on the milk production. The average value obtained for sulfur content in wDDGS was 1.09 % of DM, higher than the value given by NRC 2001 for wheat DDGS. The NRC 2001 value and the obtained average value for sulfur was 0.44 and 1.09 %DM, respectively. The excess intake of sulfur by beef cattle fed with diet containing 0.5 % sulfur resulted in the occurrence of polioencephalomalacia revealed by McAllister et al. (1997). The addition of various chemicals during bioethanol production process cause increased mineral

content and high variation in mineral profiles (Batal and Dale, 2003; Belyea et al., 2006; Liu and Han, 2010). Thin stillage, obtained after centrifugation of whole stillage, was found to have a concentrated mineral content (Liu and Han, 2010). The uneven mixing of different proportions of thin stillage with unfermented distillers grain might be one of the reasons for the variation among different batches of wheat DDGS. The study carried out by Rausch and Belyea (2006) observed the changes in mineral composition during the dry grinding process due to the addition of exogenous minerals in the bioethanol production process. Other than the adverse impacts on animal, excessive mineral consumption leads to increase excretion of minerals to the environment (Spiehs and Varel, 2009). The exposure of ruminants to a higher level of iron (Fe) take place with water, feed and via soil (Spears, 2003).

The increased PB2 and PB3 fractions in wDDGS may be due to the denaturation of protein during the drying process of DDGS (Goelema, 1999). The increased intermediately and slowly degradable fractions in wDDGS made it less available for microbial degradation in the rumen (Kleinschmit et al., 2007; Nuez-Ortín and Yu, 2009). The higher unavailable fraction in wDDGS compared to its original feedstock wheat may be partially due to the Millard reaction that might take place with temperatures applied during the final drying processing (Cromwell et al., 1993). The estimated values for NE_{L3x} closely agree with the values reported by Nuez-Ortín and Yu (2009) and Azarfar et al. (2011).

The mash type wDDGS used in this study has a fine particle size (650 μ m). The most common type of DDGS is ball shape which has higher mean particle size. The small particle sizes of DDGS are considered to have detrimental effects on rumen digestive characteristics and handling and storage of DDGS (Belyea et al., 2004). The lower K_d of wDDGS may be partially due to the production of Millard products during the drying process of wDDGS (Weiss et al., 1986) and high fibre content. These results are in close agreement with the results published by Nuez-Ortín and Yu (2009) except the W (S) fraction. The method we used to separate the 0h fraction was different from the method used by Nuez-Ortín and Yu (2009) and that may be attributed to the differences in W (S) fraction. Among the batches of wDDGS, there were significant differences ($P<0.05$) in W (S) and K_d . Nuez-Ortín and Yu (2009) indicated that the sources of wDDGS, and differences in particle size and texture of wDDGS may cause the difference in values observed in situ study. The higher effective

degradation content of NDF of the wDDGS may be partially due to the escape of small particles through the nylon bags giving higher washable fraction.

With modern high yielding dairy cow feeding systems, accurate prediction of true protein availability for the rumen microbial protein synthesis and for the true digestibility in small intestine is important. There were evidences of correlation between increased milk yield and decreased rumen degradation of CP from various sources and intestinal available RUP (Ørskov et al., 1981; Netemeyer et al., 1982; Sahlu et al., 1984). The provision of feed to optimize the ratio between effective degradability of N and organic matter is one way of achieving maximum microbial yield and making N and energy to synchronize. According to published studies, 25 g N/kg OM truly degraded in the rumen maximize the microbial protein synthesis (Czerkawski, 1986; Tamminga et al., 1990; Sinclair et al., 1993). Higher ratios indicate potential N loss or shortage of energy from the rumen and lower ratios indicate inadequacy of N or excess energy for microbial growth. In both cases, there is a negative impact for animal or the environment. Moreover for the economic viability of production systems, the measurement of effective degradation ratios between N and OM is a useful tool in provision of feed ingredients in ration formulation.

Even though these variation among batches of wDDGS were statistically significant, these variations are not strongly supportive of biological significance when include into total mixed rations (TMR). A study by Shurson et al. (2002) with DDGS from ten different bioethanol plants in South Dakota reported the consistency within plant DDGS. The variation within plant DDGS was less than 10% for CP, CFat, Crude fibre and nitrogen free extract (NFE) compared to among plant variation. Many published studies highlighted the inclusion of higher percentages of wDDGS (20-40%) in feedlot cattle diets replacing the barley grain. The increased dry matter intake and the similar fat levels achieved with barley grain further supports the possibility of including higher amount of wDDGS in beef cattle diet (McKinnon and Walker, 2008; Beliveau and McKinnon, 2009; Gibb et al., 2009; Walter, 2010). Penner and Christensen (2009) found out that possibility of formulating dairy cattle diets with 19% wDDGS in diet DM replacing the concentrate without any negative impact to milk yield. Chibisa et al. (2010) replaced canola meal with 20% wDDGS without any change to milk yield or milk fat content.

3.6. Conclusions and Implication

In conclusion, there were significant differences ($P < 0.05$) among the batches of feedstock wheat and wheat DDGS from the same bioethanol plant in terms of chemical composition, macro and micro mineral profiles, carbohydrate and protein sub-fractions, estimated energy values, rumen degradation kinetics and hourly effective degradation of N to OM ratio. The sources of variation were not only due to the variation among the batches of feedstock wheat but also may be due to the various factors associated with bioethanol processing. Even though, the variation among batches of wDDGS was relatively low, at very high inclusion rates in cattle diet, these variations should be considered as higher variations among the batches which may cause some biologically significant effect.

4.0. STUDY POSSIBILITY OF USING MOLECULAR SPECTROSCOPY AS A FAST METHOD TO DETECT BATCH EFFECTS OF ORIGINAL FEEDSTOCK WHEAT AND CO-PRODUCTS WHEAT DDGS FROM BIOETHANOL PRODUCTION

4.1. Introduction

With the results observed in the previous chapter, it is evident that there were significant differences among the batches of original feedstock of wheat and among the batches of co-products of wheat DDGS (wDDGS). The fast detection of these batch differences is a question. The traditional chemical analysis, in situ rumen and in vitro studies are labor intensive and time consuming. Moreover, it is practically impossible to perform in vivo animal trials for a large number of feed treatments. Thus, it is a need to have a technique which allows rapid identification of batch differences. Near Infrared Spectroscopy (NIR) technique is one of the most commonly used physical techniques in feed evaluation (Adesogan, 2002; Yu, 2004b). However, NIR technique is unable to identify functional groups in feeds. These functional groups are closely related to the nutrient availability. Previous studies by Yu and Nuez-Ortín (2010) and Yu et al. (2011) revealed the possibility of using Fourier transformed infrared vibration spectroscopy (FT/IR) as a potential method to detect structural differences of DDGS in relation to feed quality and nutrient utilization in animal. The importance of endogenous structure in identifying feed micro-structure further enhances the FT/IR as a promising method in feed evaluation. The requirement of small sample volume and visually distinguishable graphical changes make the technique more user friendly.

The infrared (IR) spectra produced with FT/IR spectrometers help to determine the information of molecular structure conformation of biopolymers. The IR spectrum is demonstrated as a plot in which the IR radiation passes through the sample, plots against the wave length or wave number of the radiation. Detailed molecular structure information of the spectrum is obtained by analyzing the specific bands in the spectrum which characterize the chemically important functional groups (Chalmers and Griffiths, 2002). The multivariate techniques of spectral analysis methods, agglomerative hierarchical cluster analysis (AHCA) and principal components analysis (PCA) can be used to classify and discriminate the spectral data associated with matrix conformation in relation to chemical and structural makeup. With

AHCA, the spectroscopic data at a fingerprint region are clustered according to similarity of spectra and the results are interpreted based on different clusters or linkage tree (Chalmers and Griffiths, 2002).

The objective of this study was to study possibility of using molecular spectroscopy (FT/IR) as a fast method to detect batch difference among original feedstock wheat and co-products wheat DDGS from the same bioethanol processing plant.

Therefore the hypothesis was that the changes induced by bioethanol processing and differences between batches are observable with Fourier Transform Infrared Spectroscopy with Attenuated Total Reflectance (FTIR-ATR).

4.2. Materials and Methods

4.2.1. Sample Preparation

Samples from feedstock wheat batches 1, 3 and 5 and wheat DDGS batches I, III and V were ground through 0.25 mm screen twice (Retsch ZM⁻¹, Brinkmann Instruments LTD, Ontario, Canada).

4.2.2. Molecular Spectral Analyses by Fourier Transformed Infrared Spectroscopy with Attenuated Total Reflection

Inherent molecular spectral analysis of samples was carried out in the University of Saskatchewan with JASCO FT/IR- 4200 with a ceramic IR light source and a deuterated L-alanine doped triglycine sulphate detector (JASCO Corporation, Tokyo, Japan) made out of MIRacleTM attenuated total reflectance accessory module and equipped with a ZnSe crystal and pressure clamp (PIKE Technologies, Madison, WI, USA). The spectra were generated from the mid IR (4000 – 800 cm⁻¹) portion of the electromagnetic spectrum with 256 co-added scans and a spectral resolution of 4 cm⁻¹ with JASCO SpectraManager II software. Each sample was exposed to five analyses creating five separate spectrums. The OMNIC 7.2 software (Spectra Tech, Madison, WI, USA) was used for detection, identification and measurement of specific band by comparing with the reference spectrum data published (Jackson and Mantsch, 1995, 2000, 2002; Wetzal et al., 1998; Stuart, 2004; Marinkovic and Chance, 2005). The brief summaries of most important IR spectrum bands (regions) and

functional groups related to nutritive values of a concentrated feed published in feed research are summarized in Table 4.1.

Table 4.1. Some important functional groups and IR bands in feed research.

Item	Wave number (cm ⁻¹)	References
Amide I and Amide II bands (mainly related to protein)	ca. 1650, 1565	Jackson and Mantsch, 1995; 2000; Miller, 2002; Stuart, 2004; Yu, 2004b; Marinkovic and Chance, 2005.
Carbonyl C=O ester (mainly related to lipids)	ca. 1738	Jackson and Mantsch, 2002; Yu, 2004; Miller, 2002.
CH ₂ CH ₃ asymmetric and symmetric stretching band (mainly related to lipid molecular structure conformation)	ca. 3022-2800	Wetzel et al., 1998.
Band mainly related to carbohydrates	ca. 1180 - 800	Wetzel et al., 1998; Yu, 2004b.
Band mainly related to cellulosic compounds	ca. 1420, 1370, and 1335	Wetzel et al., 1998.

4.2.2.1. Using Multivariate Statistical Analysis for FT/IR Spectral Data

Multivariate analysis of spectra with AHCA and PCA was carried out using Statistica 7.0 software. The different batches of original feedstock wheat and different batches of co-products wDDGS were compared in FT/IR-ATR spectral data. For AHCA and PCA analyses the spectral regions of ca. 1800 – 800 cm⁻¹ (fingerprint region), ca. 1800 – 1725 cm⁻¹ (carbonyl C=O ester, mainly related to lipid structure confirmation), ca. 1725 – 1482 cm⁻¹ (amide I and amide II region mainly related to protein structure confirmation), ca. 1482 – 1180 cm⁻¹ (mainly associated with structural carbohydrate) and ca. 1180-800 cm⁻¹ (mainly related to carbohydrates) of the mid IR region were selected since most of the chemically important functional groups in feeds generate spectra at this regions (Wetzel et al., 1998; Yu, 2005b).

4.2.2.2. Agglomerative Hierarchical Cluster Analysis (AHCA)

In AHCA analysis results are display as “dendrograms”, which calculates the distance matrix for two most similar IR spectra by algorithm searches. The similar spectra collectively are called “cluster” or “hierarchical group”. Then the distances for the remaining spectra are calculated from the newly formed cluster (Cytopec, 2004; Yu, 2005b). The Ward’s algorithm method and Elucidian Distance were used in this study.

4.2.2.3. Principal Component and Classification Analysis (PCA)

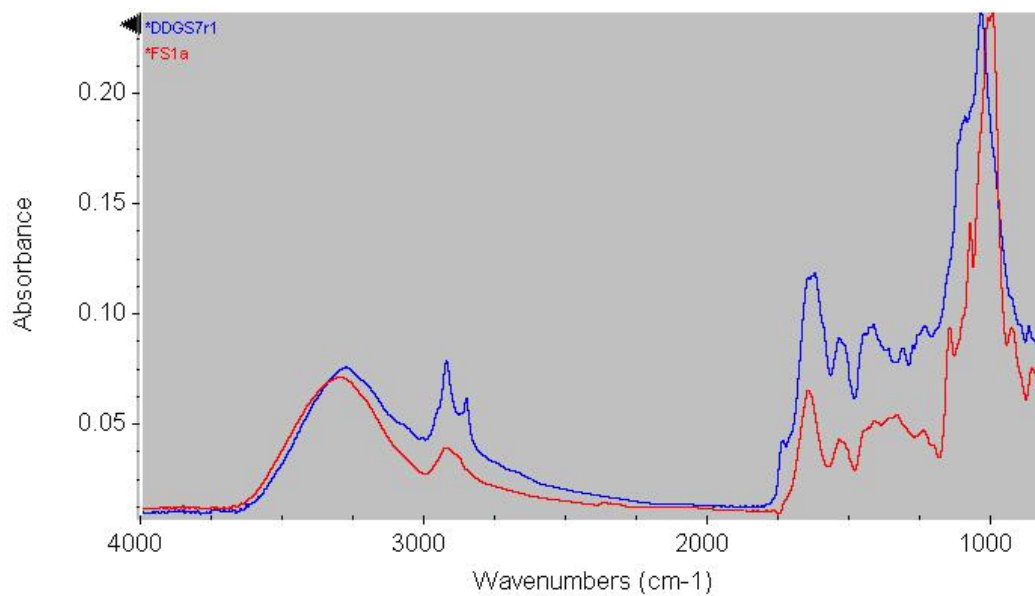
Principal component analysis is a statistical data reduction method which converts the original set of variables to a new set of variables. These new variables are called Principal Components (PCs). The first derived PCs contain more variability and with the extraction of more factors, variability becomes less. These factors are independent from each other (Sockalingum et al., 1998; Yu, 2005b). The results of PCA analysis can be presented as two-dimensional (2D) or three-dimensional (3D) scattered plots.

4.3. Results

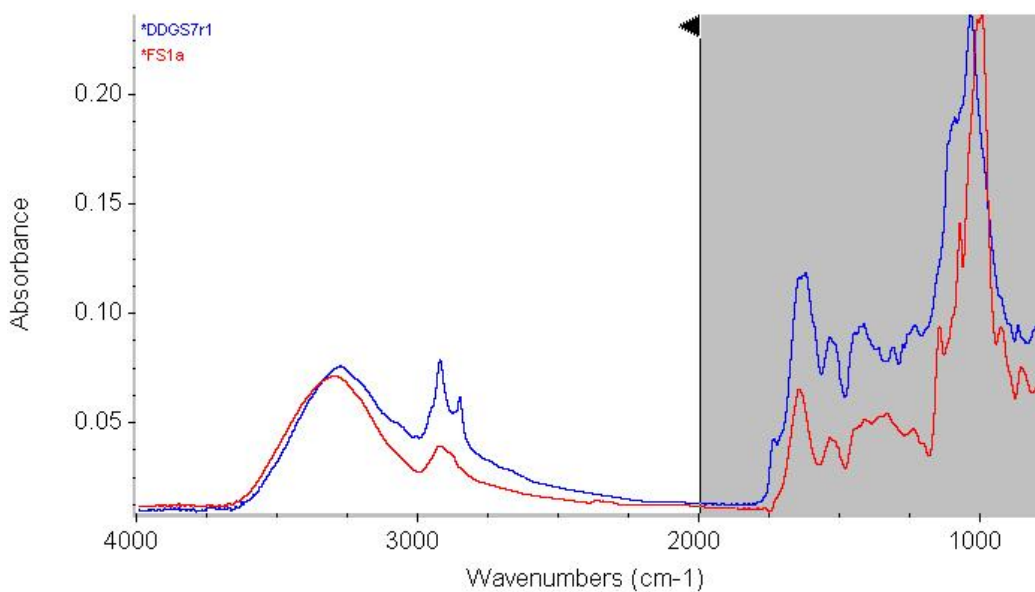
The typical FT/IR molecular spectrum of original feedstock of wheat and co-products of wDDGS in the whole mid IR region (ca. 4000 – 800 cm^{-1}) are presented in Figure 4.1 The whole spectrum contains unique absorption peaks at different wave numbers which can be correlated with the chemical make-up of the wheat and wDDGS. Figure 4.1 highlights different spectral regions which are related to different functional groups in wheat and wDDGS.

4.3.1. Variation in IR Spectra of In the Region ca. 1880 – 800 cm^{-1} in Different Batches of Original Feedstock Wheat and Different Batches of Wheat DDGS

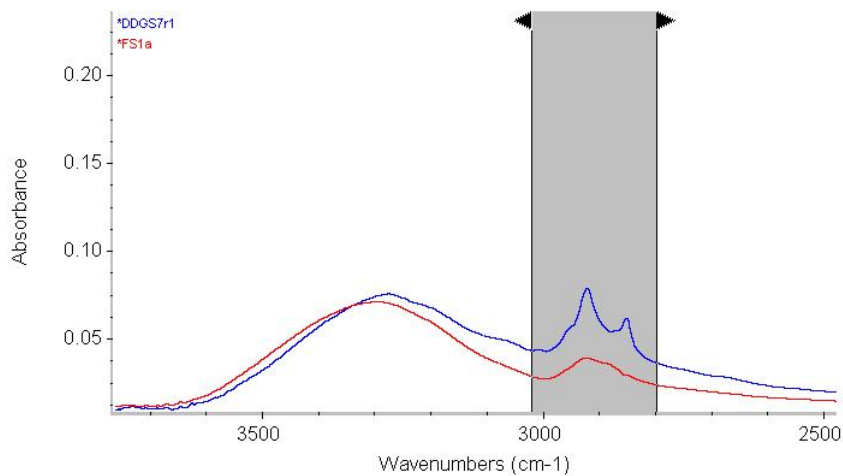
Figures 4.1 and 4.2 display the variation between wheat and wDDGS, batches of wheat and wDDGS in the region (ca. 1800 – 800 cm^{-1}). The figures clearly show distinguish spectral pattern between wheat and wDDGS. It also clearly shows differences in spectral pattern between different batches of wheat and between different batches of wDDGS.



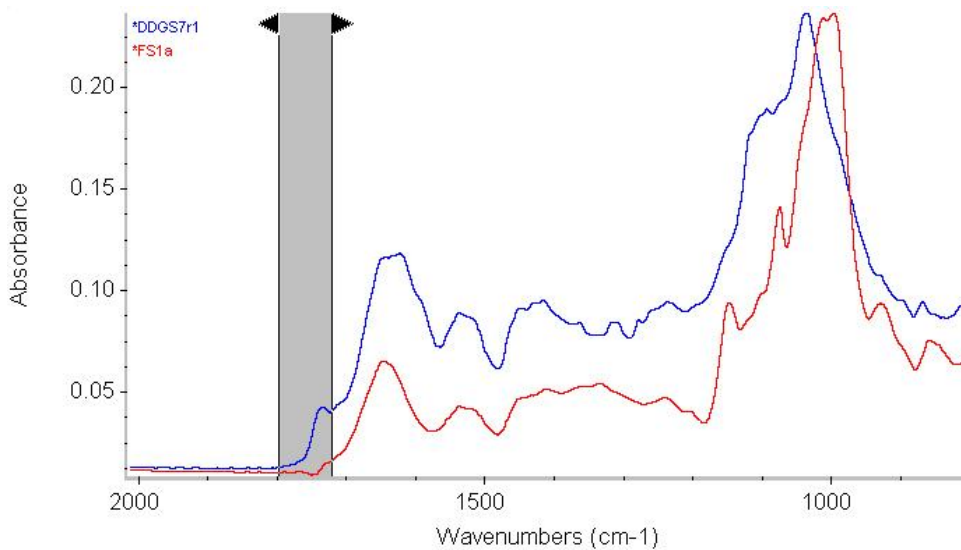
(a) Region (Window 1): Whole mid-IR region: ca. 4000-800 cm^{-1}



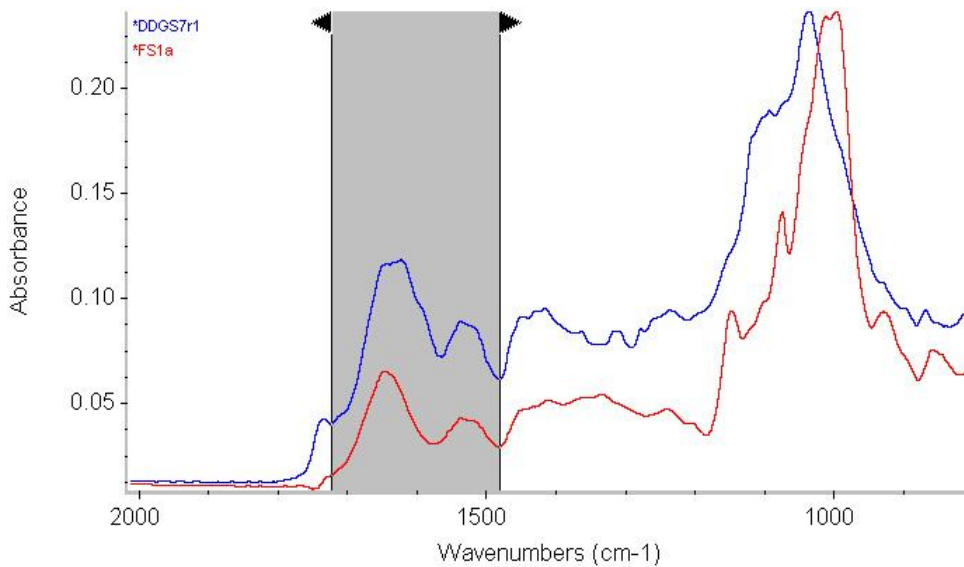
(b) Region (Window 2): Fingerprint region: ca. 2000-800 cm^{-1} (various functional groups in this region)



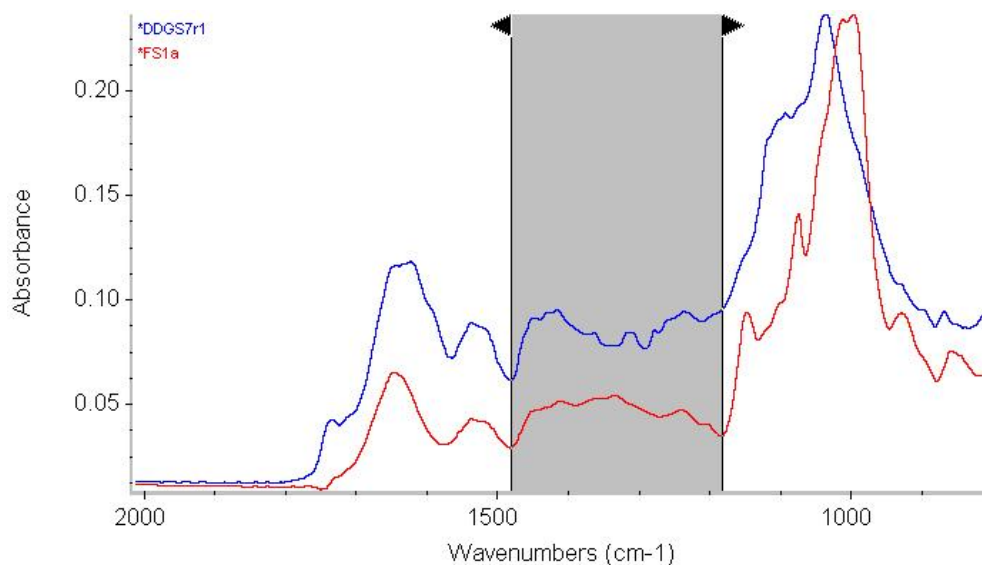
(c) Region (Window 3): CH₂ CH₃ asymmetric and symmetric stretching region: ca. 3022-2800 cm⁻¹ (mainly related to lipid molecular structure conformation)



(d) Region (Window 4): Carbonyl C=O ester region: ca. 1800-1725 cm⁻¹ (mainly related to lipid molecular structure conformation)



(e) Region (Window 5): Amide I and II region: ca. 1725-1482 cm^{-1} (mainly related to protein molecular structure conformation)



(f) Region (Window 6): structural CHO region: ca. 1482-1180 cm^{-1} (mainly related to structural conformation)

Figure 4.1. FTIR molecular spectrum of original feedstock of wheat (red) and its co-product of wheat DDGS (blue) at different regions (windows), which were used for multivariate molecular spectral analyses (PCA and CLA).

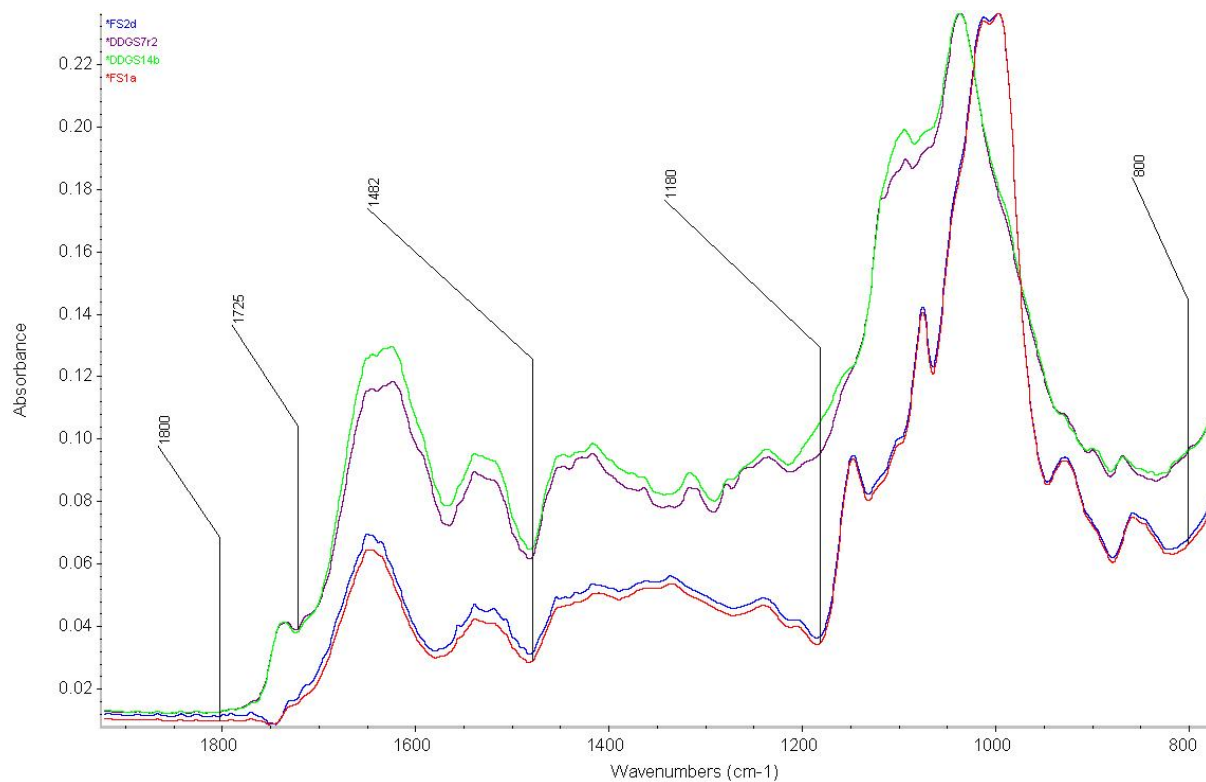


Figure 4.2. Batch effect on FTIR molecular spectrum of original feedstock of wheat (bottom two spectra) and co-products of wheat DDGS (top two spectra), showing variation of molecular spectral features which indicate the structural difference.

4.3.2. Multivariate Spectral Analyses

4.3.2.1. Determination of Molecular Structure Differences in the Fingerprint Region (1800-800 cm⁻¹)

4.3.2.1.1 Comparison between Feedstock Wheat and Wheat DDGS

Comparison between feedstock of wheat and co-products of wDDGS in the fingerprint region are shown in Figure 4.3A. In both AHCA and PCA analyses, results of wheat and wDDGS show similar variation. The dendrogram of AHCA analysis shows two distinct groups at the linkage distance ~ 2 for wheat and wheat DDGS. The similar results were observed with scattered plot of PCA in which two clearly demarcated ellipses represent the wheat and wheat DDGS. The PC₁ and PC₂ explain 83.28 and 15.62% of variation of spectral data, respectively. This supports the results obtained with chemical analysis and nutrient availability studies that the significant differences between feedstock of wheat and co-product of wDDGS reported in Chapter 3.

4.3.2.1.2. Comparison between Different Batches of Feedstock of Wheat

The spectral variation between wheat Batch 1 vs. Batch 3, Batch 1 vs. Batch 5 and Batch 3 vs. Batch 5 was studied using AHCA and PCA. The results are summarized in Figure 4.3B. The obtained results show some overlapping between Batch 1 vs. Batch 3 and Batch 1 vs. Batch 5 both in dendrograms and scattered plots of PCA. This may be due to the similar structural characteristics linked to the chemical makeup of wheat grains in Batch 1 to the Batches 3 and 5. The feedstock Batch 3 and Batch 5 forms two different groups below the linkage distance 0.01 in AHCA. The PCA analysis shows two clearly distinguishable ellipses in which the PC₁ and PC₂ explain 94.80% and 5.06% of variation in spectral data, respectively.

4.3.2.1.3. Comparison between Different Batches of Wheat DDGS

Batch I vs. Batch III, Batch I vs. Batch V and Batch III vs. Batch V show some overlapping both in dendrograms of AHCA and scattered plots of PCA. The results were

shown in Figure 4.3C. The molecular structure similarities among the different batches of wDDGS may create the obtained results in the figure.

4.3.2.2. Determination of Molecular Structure Differences in Protein Amide I and Amide II Region (ca. 1725-1482 cm^{-1})

4.3.2.2.1. Comparison between Wheat and Wheat DDGS

The comparison was made between wheat and wheat DDGS for the spectra difference in Amide I and Amide II region and the results are summarized in Figure 4.4A. The wheat and wDDGS form two distinguishable groups just under the linkage distance of 1 in dendrogram of AHCA. In PCA, the wheat and wDDGS grouped into two distinct ellipses with 88.64% of total variation explained by PC1 and 8.82% of total variation explained by PC2. This clearly indicates that protein conformation differ between wheat and wDDGS.

4.3.2.2.2. Comparison between Different Batches of Feedstock of Wheat

When compared the different batches of wheat for the molecular structure spectral profile, some overlapped results were obtained. The results were summarized in Figure 4.4B. Feedstock wheat Batch 1 vs. Batch 3 and Batch 3 vs. Batch 5 contained structure similarities as shown in both AHCA and PCA. Between wheat Batch 1 and Batch 5, the dendrogram shows some overlapping but two distinct ellipses can be identified in scattered plots of PCA.

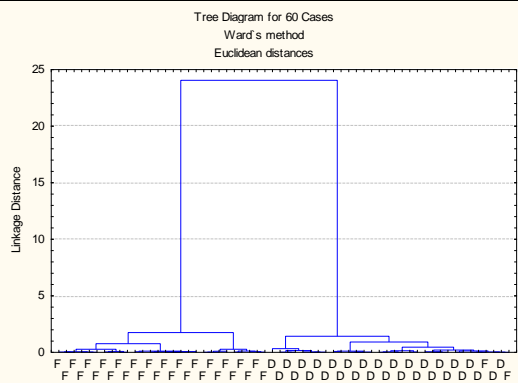
4.3.2.2.3. Comparison between Different Batches of Wheat DDGS

When comparison was made among the batches of the co-product of wDDGS in relation to protein molecular structure, wDDGS Batch I vs. Batch III, Batch I vs. Batch V and Batch III vs. Batch V were not grouped into distinguishable ellipses in PCA scattered plots. The AHCA analysis did not show two distinguishable clusters. The results were summarized in Figure 4.4C. The results indicate similar spectral pattern in protein amide I and amide II region among the batches of co-products of wDDGS.

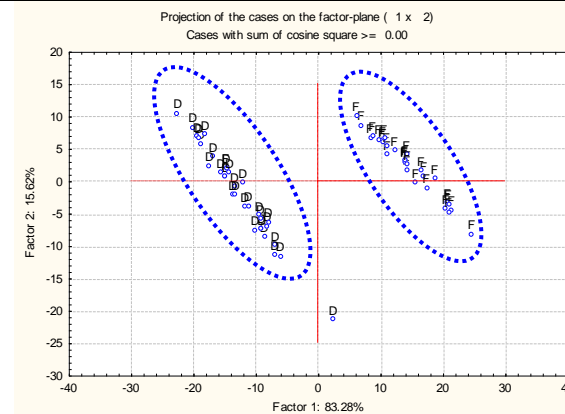
I: Cluster Analysis (AHCA): Fingerprint region ca. 1800-8000 cm⁻¹

II: Principal component analysis (PCA): Fingerprint region ca. 1800-800 cm⁻¹

(A) Comparison: Original feedstock of wheat vs. Co-product of wheat DDGS

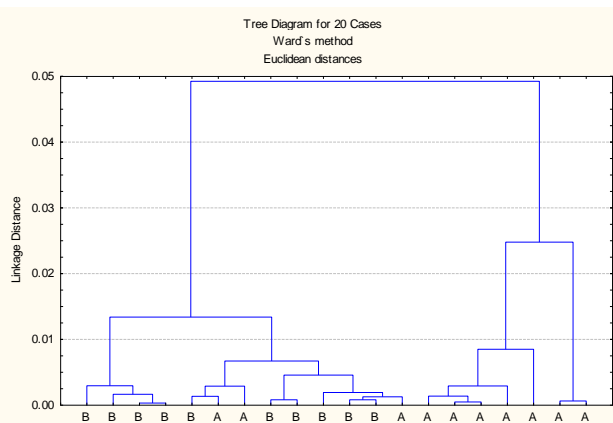


(1) CLA: Comparison of wheat (F) vs. wDDGS (D)

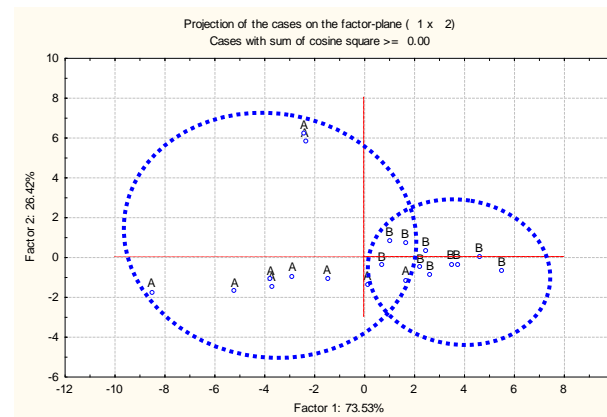


(2) PCA: Comparison of wheat (F) vs. wDDGS (D): PC1 and PC2 explain 83.28 and 15.62% of the variation of spectral data, respectively.

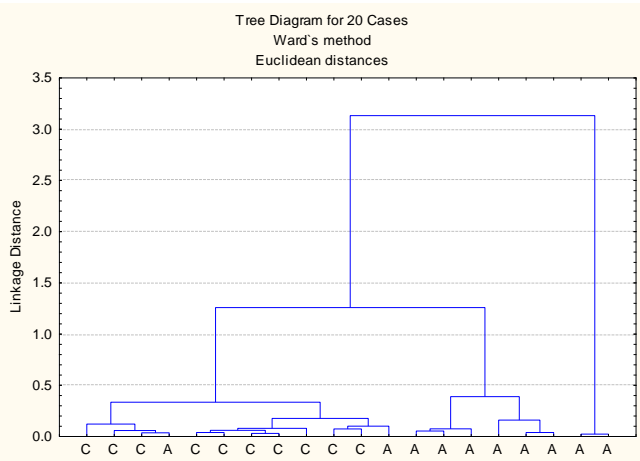
(B) Comparison between different batches of original feedstock wheat



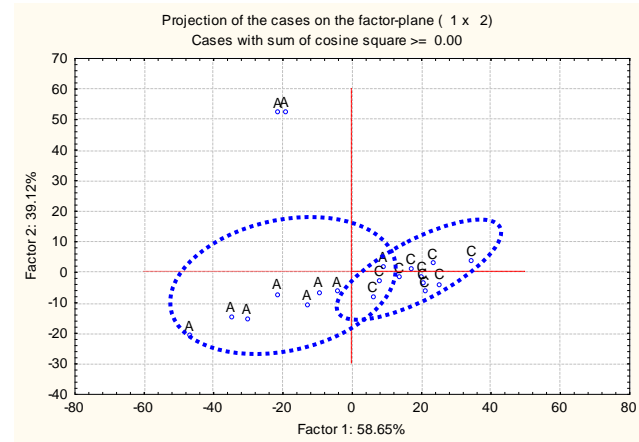
(3) CLA: Feedstock Wheat Batch 1 (A) vs. Batch 3 (B)



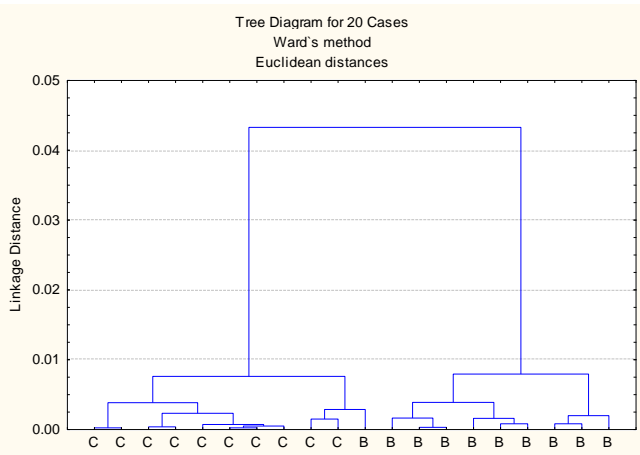
(4) PCA: Feedstock Wheat Batch 1 (A) vs. Batch 3 (B)



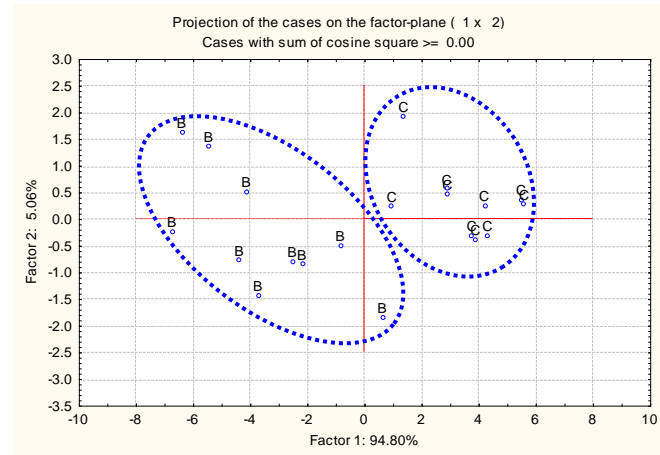
(5) CLA: Feedstock Wheat Batch 1 (A) vs. Batch 5 (C)



(6) PCA: Feedstock Wheat Batch 1 (A) vs. Batch 5 (C)



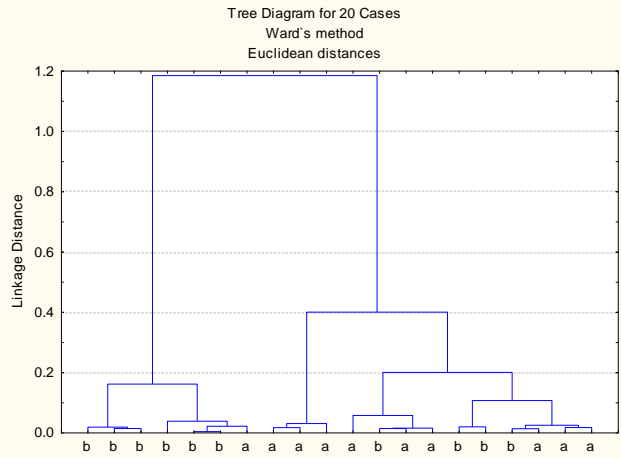
(7) CLA: Feedstock Wheat Batch 3 (B) vs. Batch 5 (C)



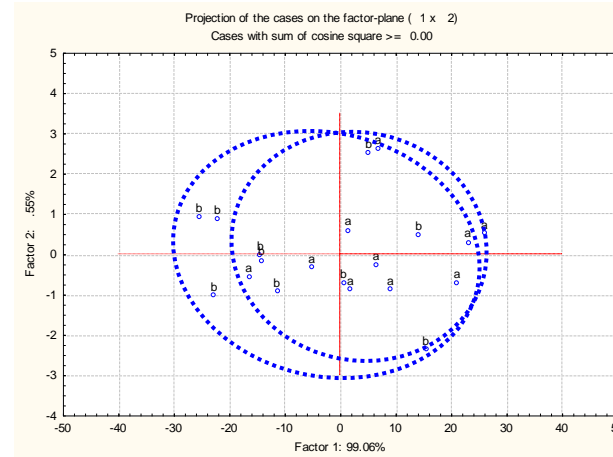
(8) PCA: Feedstock Wheat Batch 3 (B) vs. Batch 5 (C)

(C) Comparison between different batches of wheat DDGS

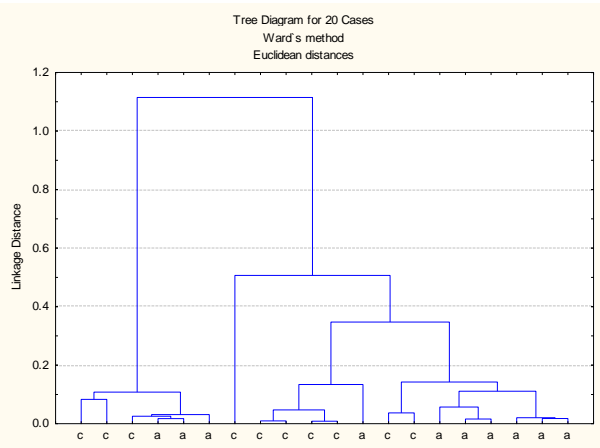
89



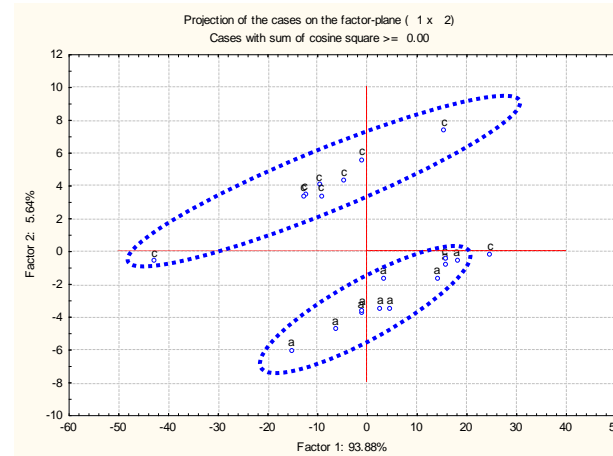
(8) CLA: wDDGS Batch I (a) vs. Batch III (b)



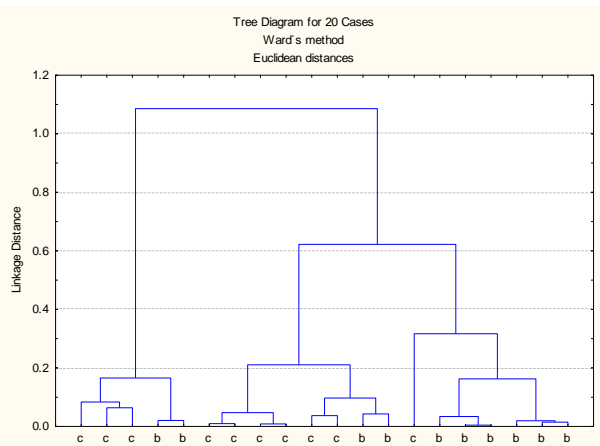
(9) PCA: wDDGS Batch I (a) vs. Batch III (b)



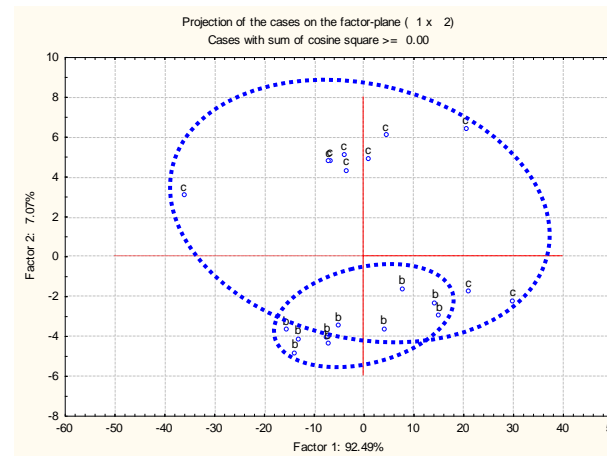
(11) CLA: wDDGS Batch I (a) vs. Batch V (c)



(12) PCA: wDDGS Batch I (a) vs. Batch V (c)



(13) CLA: wDDGS Batch III (b) vs. Batch V(c)



(14) PCA: wDDGS Batch III (b) vs. Batch V (c)

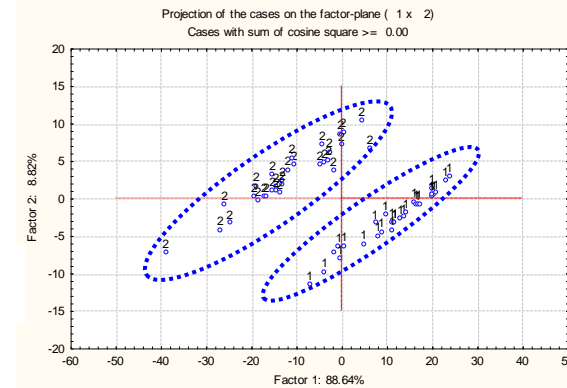
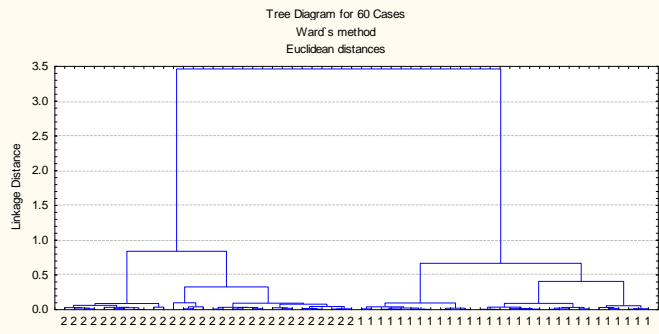
69

Figure 4.3 Multivariate molecular spectral analyses in the fingerprint region (ca. 1800-800 cm^{-1}): (A) Comparison of original feedstock wheat and wheat DDGS; (B) Comparison of three different batches (Batch 1, 3 and 5) of original feedstock wheat; (C) Comparison of three different batches of wheat DDGS (Batch I, III, and V). I: Cluster analysis (1) Select spectral region: ca. 1800 to 800 cm^{-1} ; (2) Distance method: Euclidean; (3) Cluster method: Ward's algorithm]; II: Principal component analysis: Scatter plots of the 1st principal components (PC1) vs. the 2nd principal components (PC2)

I: Cluster Analysis (CLA): Amide I and II region ca. 1725 – 1482 cm⁻¹
(mainly related to protein structure conformation)

II: Principal component analysis (PCA): Amide I and II region ca. 1725 – 1482 cm⁻¹
(mainly related to protein structure conformation)

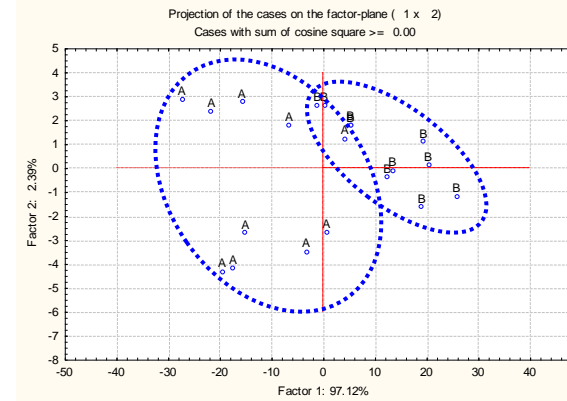
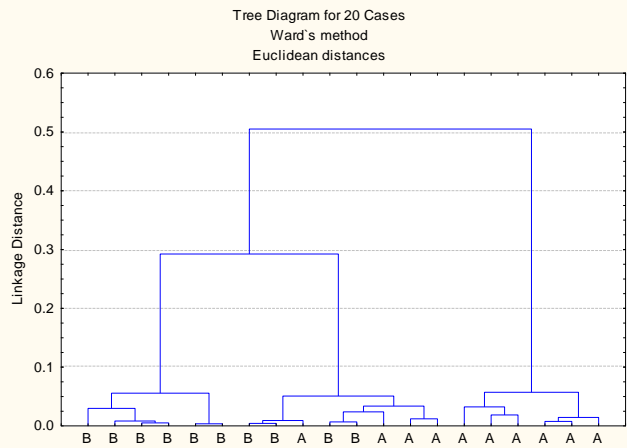
(A) Comparison: Original feedstock of wheat vs. Co-product of wheat DDGS



(1) CLA: Comparison of wheat (1) vs. wDdGS (2)

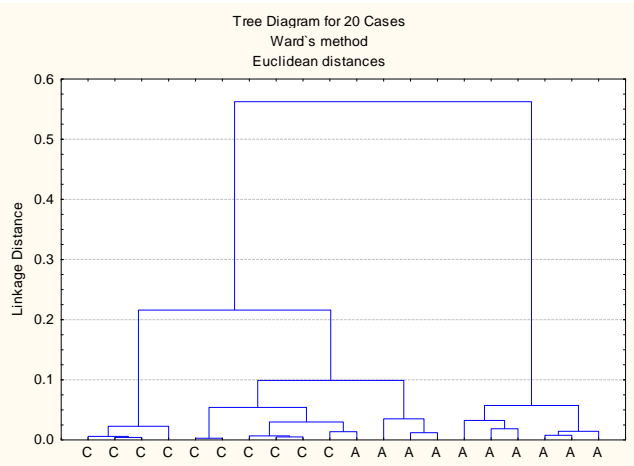
(2) PCA: Comparison of wheat (1) vs. wDdGS (2); PC1 and PC2 explain 88.64 and 8.82% of the variation of spectral data, respectively.

(B) Comparison between different batches of original feedstock wheat

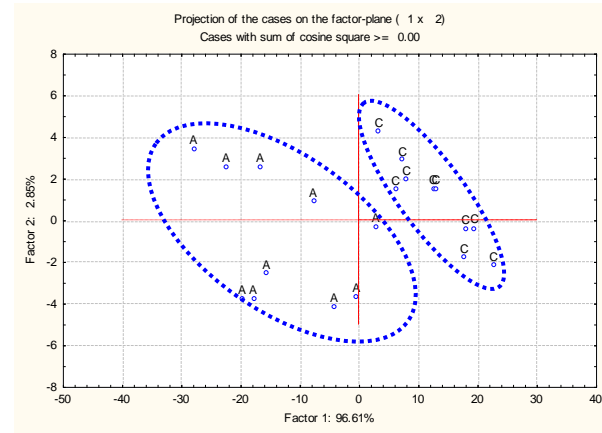


(3) CLA: Feedstock Wheat Batch 1 (A) vs. Batch 3 (B)

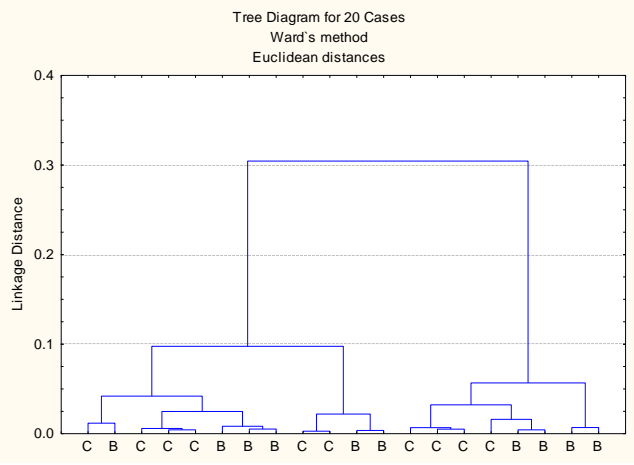
(4) PCA: Feedstock Wheat Batch 1 (A) vs. Batch 3 (B)



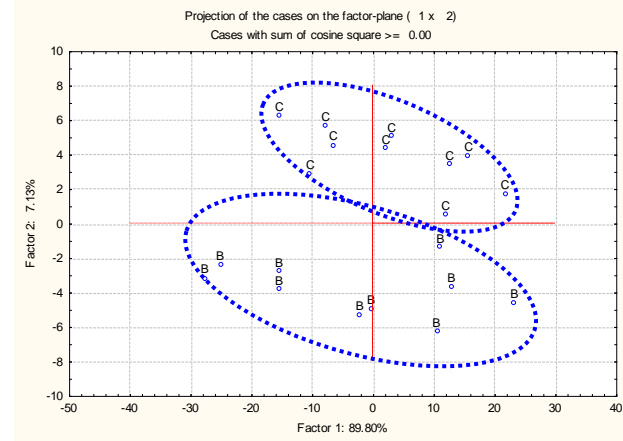
(5) CLA: Feedstock Wheat Batch 1 (A) vs. Batch 5 (C)



(6) PCA: Feedstock Wheat Batch 1 (A) vs. Batch 5 (C)

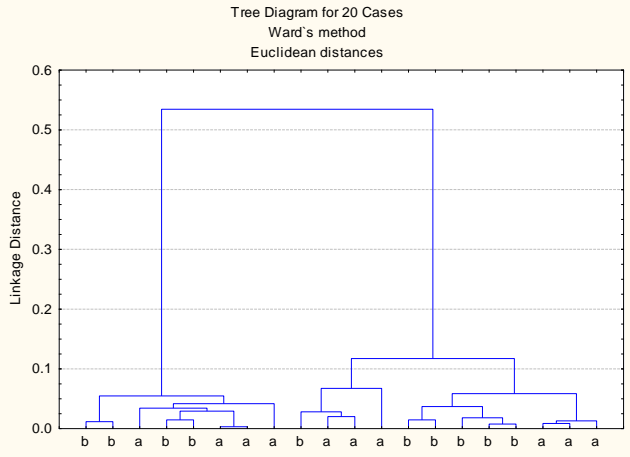


(7) CLA: Feedstock Wheat Batch 3 (B) vs. Batch 5 (C)

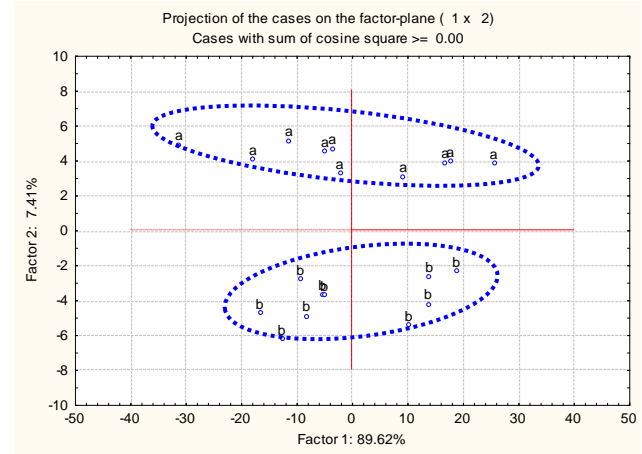


(8) PCA: Feedstock Wheat Batch 3 (B) vs. Batch 5 (C)

(C) Comparison between different batches of wheat DDGS

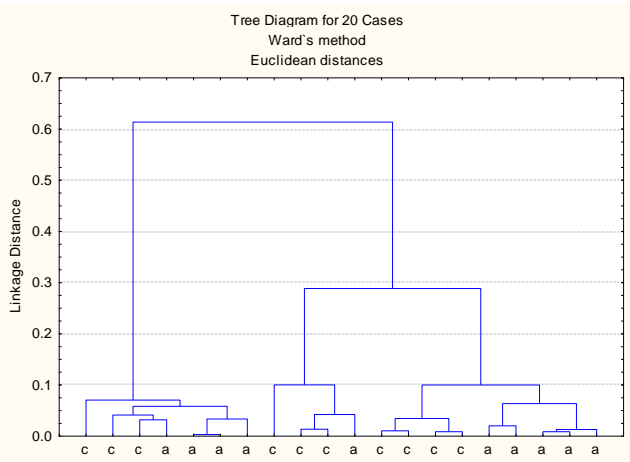


(9) CLA: wDDGS Batch I (a) vs. Batch III (b)

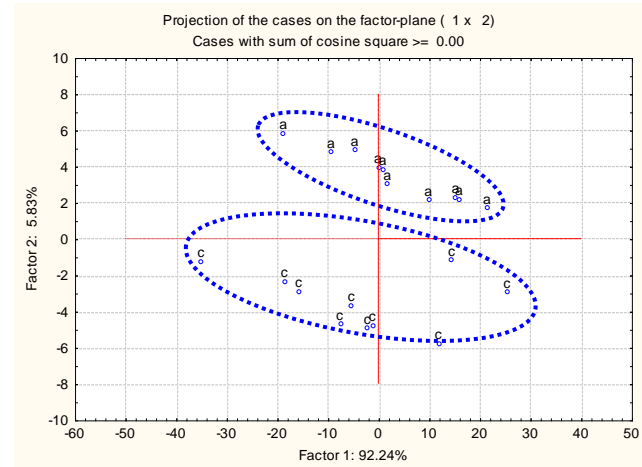


(10) PCA: wDDGS Batch I (a) vs. Batch III (b)

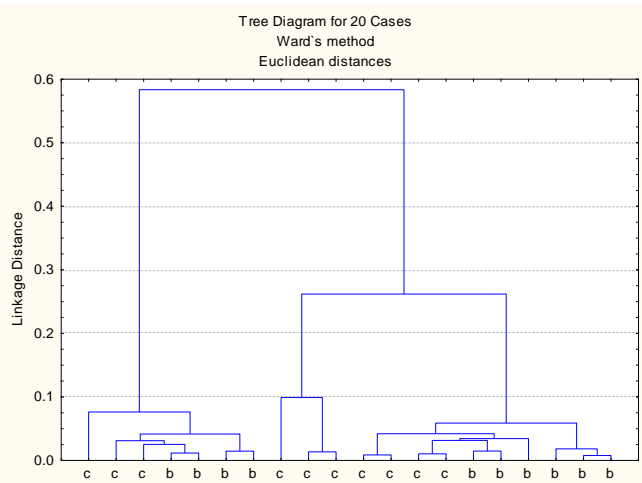
72



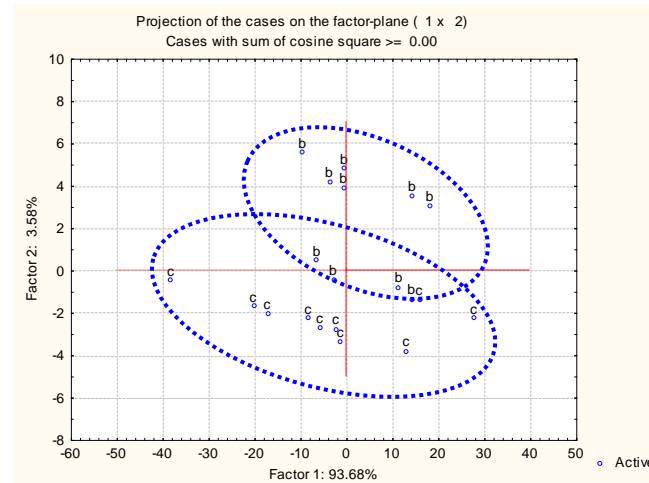
(11) CLA: wDDGS Batch I (a) vs. Batch V (c)



(12) PCA: wDDGS Batch I (a) vs. Batch V (c)



(13) CLA: wDDGS Batch III (b) vs. Batch V(c)



(14) PCA: wDDGS Batch III (b) vs. Batch V (c)

Figure 4.4 Multivariate molecular spectral analyses in protein amide I and II region (ca. 1725-1482 cm^{-1}): (A) Comparison of original feedstock wheat and wheat DDGS; (B) Comparison of three different batches (batch 1, 3 and 5) of original feedstock wheat; (C) Comparison of three different batches of wheat DDGS (batch I, III, and V).

I: cluster analysis (1) Select spectral region: protein amide I and II regions: ca. 1725 – 1482 cm^{-1} ; (2) Distance method: Euclidean; (3) Cluster method: Ward's algorithm; II: principal component analysis: Scatter plots of the 1st principal components (PC1) vs. the 2nd principal components (PC2).

4.3.2.3. Determination of Molecular Structure Differences in Carbonyl C=O Region: (ca. 1800-1725cm⁻¹) Mainly Related to Lipid Structure Conformation

4.3.2.3.1. Comparison between Feedstock of Wheat and Co-products of Wheat DDGS

The results obtained with analyzing feedstock of wheat and wDDGS for carbonyl C=O lipid structure related changes demonstrated in Figure 4.5A. Wheat and wDDGS were clearly separated by AHCA dendrograms. This result shows significant differences in carbonyl C=O spectra between wheat and wDDGS, indicating different lipid conformation.

4.3.2.3.2. Comparison between Different Batches of Feedstock Wheat

When compared the feedstock Batch 1 vs. Batch 3, Batch 1 vs. Batch 5 and Batch 3 vs. Batch 5, the results (Figure 4.5B) shows that the analyzed batches of wheat were different from one another in spectral profile related to lipid structure conformation in carbonyl C=O region. The PCA scattered plots demonstrated distinguishable grouping of each batch in separate ellipses. The different wheat batches formed different clusters.

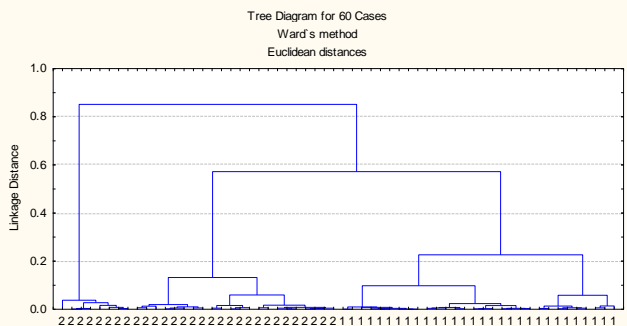
4.3.2.3.3. Comparison between Different Batches of Wheat DDGS.

Wheat DDGS Batch I vs. Batch V demonstrated two distinguishable grouping of Batch I and Batch V in two ellipses (Figure 4.5C). Wheat DDGS Batch I vs. Batch III displayed structural similarities between the two batches by indistinguishable clustering at AHCA and some overlapping of ellipses of PCA. The wheat DDGS Batch III vs. Batch V also had some overlapping in cluster formation and grouping in PCA. This emphasizes that the similarity between wDDGS Batches I and III and between wDDGS Batches III and V in the molecular structure.

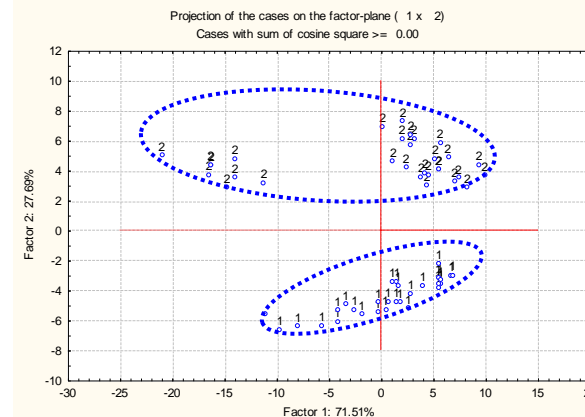
I: Cluster Analysis (AHCA): Carbonyl C=O ester region: ca.1800-1725 cm⁻¹ (mainly related to lipid structure conformation)

II: Principal component analysis (PCA): Carbonyl C=O ester region: ca.1800-1725 cm⁻¹ (mainly related to lipid structure conformation)

(A) Comparison: Original feedstock of wheat vs. Co-product of wheat DDGS



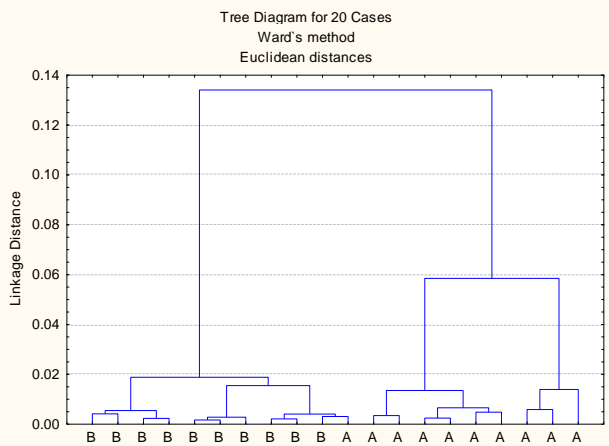
(1) CLA: Comparison of wheat (1) vs. wDDGS (2)



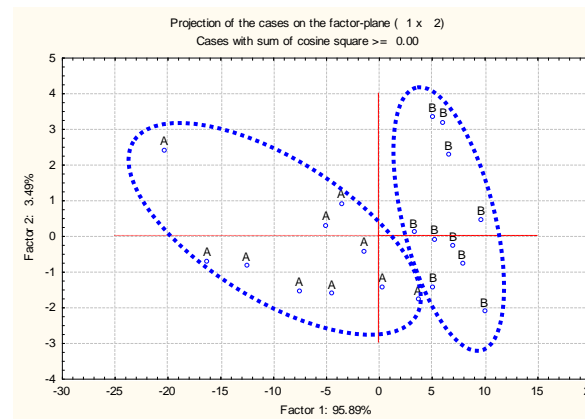
(2) PCA: Comparison of wheat (1) vs. wDDGS (2): PC1 and PC2 explain 88.64 and 8.82% of the variation of spectral data, respectively.

75

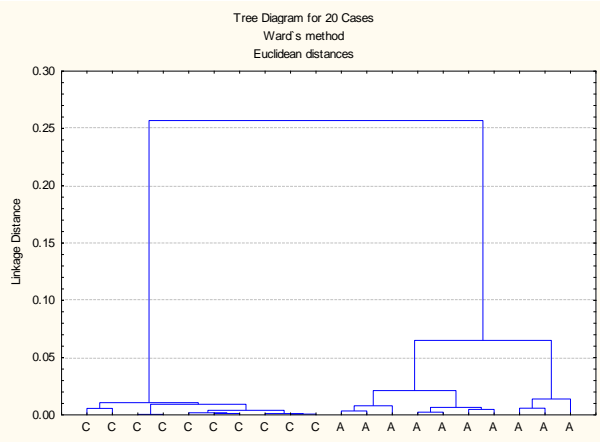
(B) Comparison between different batches of original feedstock wheat



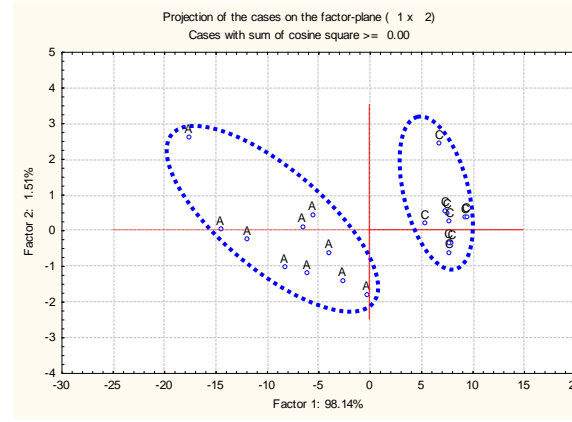
(3) CLA: Feedstock Wheat Batch 1 (A) vs. Batch 3 (B)



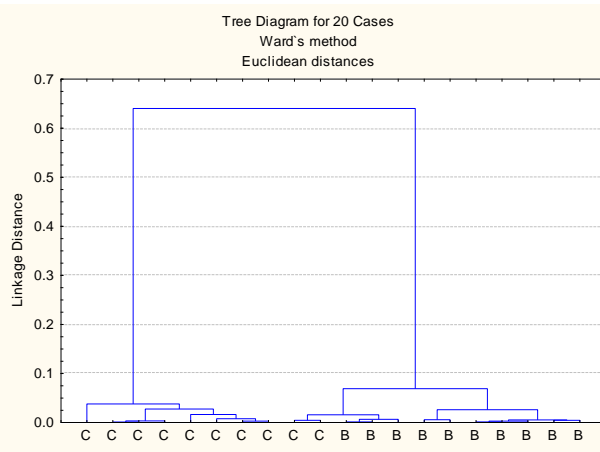
(4) PCA: Feedstock Wheat Batch 1 (A) vs. Batch 3 (B)



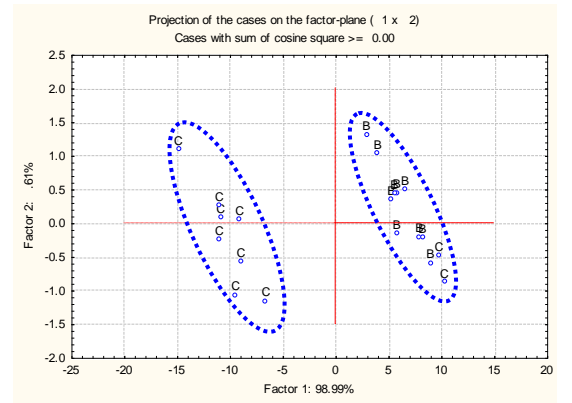
(5) CLA: Feedstock Wheat Batch 1 (A) vs. Batch 5 (C)



(6) PCA: Feedstock Wheat Batch 1 (A) vs. Batch 5 (C)

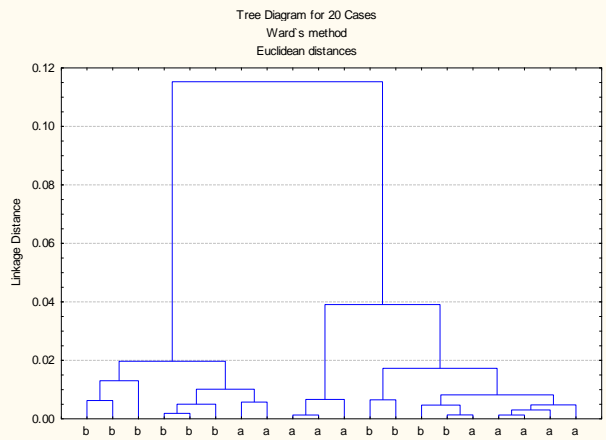


(7) CLA: Feedstock Wheat Batch 3 (B) vs. Batch 5 (C)

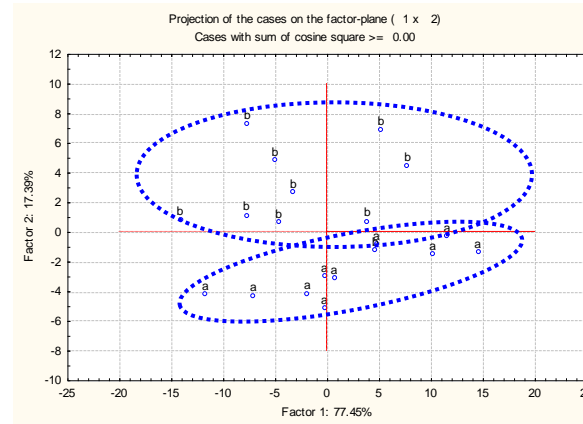


(8) PCA: Feedstock Wheat Batch 3 (B) vs. Batch 5 (C)

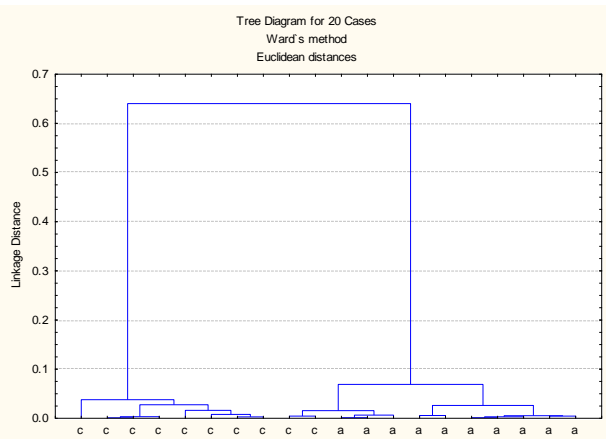
(C) Comparison between different batches of wheat DDGS



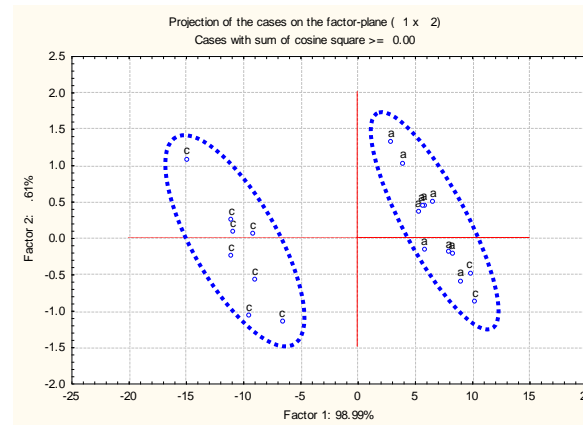
(9) CLA: wDDGS Batch I (a) vs. Batch III (b)



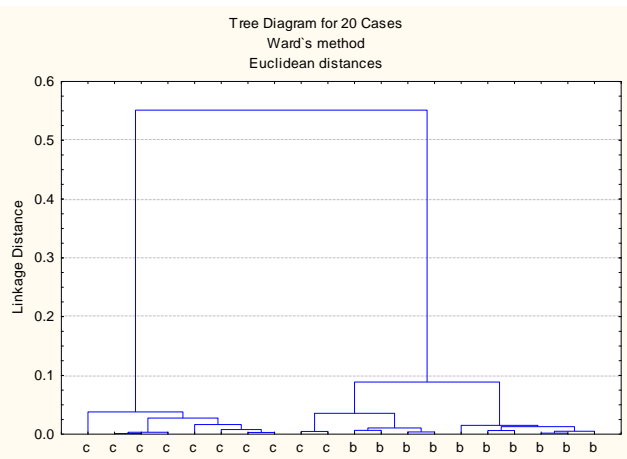
(10) PCA: wDDGS Batch I (a) vs. Batch III (b)



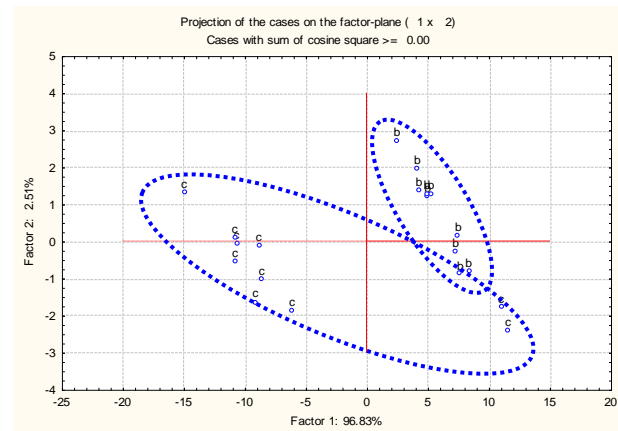
(11) CLA: wDDGS Batch I (a) vs. Batch V (c)



(12) PCA: wDDGS Batch I (a) vs. Batch V (c)



(13) CLA: wDDGS Batch III (b) vs. Batch V(c)



(14) PCA: wDDGS Batch III (b) vs. Batch V (c)

Figure 4.5. Multivariate molecular spectral analyses in carbonyl C=O ester region (mainly related to lipid structure conformation): (A) Comparison of original feedstock wheat and wheat DDGS; (B) Comparison of three different batches (batch 1, 3 and 5) of original feedstock wheat; (C) Comparison of three different batches of wheat DDGS (batch I, III, and V).

I: cluster analysis (1) Select spectral region: carbonyl C=O ester region: ca. 1800 -1725 cm^{-1} ; (2) Distance method: Euclidean; (3) Cluster method: Ward's algorithm]; II: principal component analysis: Scatter plots of the 1st principal components (PC1) vs. the 2nd principal components (PC2).

4.3.2.4. Determination of Molecular Structure Differences Mainly Associated with Structural Carbohydrate:

4.3.2.4.1 Comparison between Feedstock of Wheat and Co-products of Wheat DDGS

As shown Figure 4.6A, compared with feedstock of wheat, the co-product wDDGS showed distinct difference by forming two clusters at the linkage distance ~ 1 in AHCA, indicate different structural chemical makeup between wheat and wDDGS in CHO compounds.

4.3.2.4.2. Comparison between Different Batches of Feedstock of Wheat

When comparison was made among the batches of feedstock of wheat, wheat Batch 1 vs. Batch 3 and Batch 3 vs. Batch 5 the two classes cannot be clearly distinguished (Figure 4.6B). The wheat Batch 1 vs. Batch 5 made two distinct classes at the linkage distances 0.1 and 0.2. In PCA of the wheat Batch 1 vs. Batch 5, the each batch was grouped into separate ellipses while Batch 1 vs. Batch 3 and Batch 3 vs. Batch 5 had some overlapping. The structure difference among different wheat varieties or among different source may be the reason for observed difference in molecular spectral pattern mainly associated with structural carbohydrate.

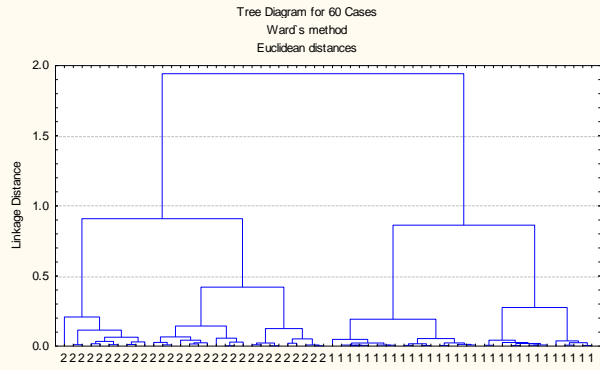
4.3.2.4.3. Comparison between Different Batches of Wheat DDGS.

No clear distinguishable cluster or groups can be identified in AHCA dendrograms and PCA scatter plots when compared the batches of wheat DDGS as in Figure 4.6C, indicate the similarity of structural chemical makeup among the batches of WDDGS.

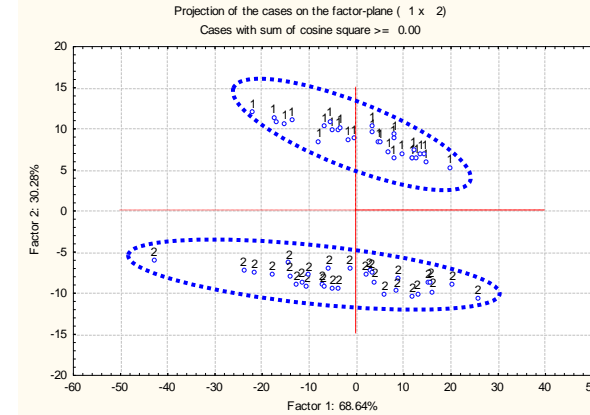
I: Cluster Analysis (CLA): Region mainly associated with structural CHO ca. 1482-1180 cm^{-1}

II: Principal component analysis (PCA): Region mainly associated with structural CHO ca. 1482-1180 cm^{-1}

(A) Comparison: Original feedstock of wheat vs. Co-product of wheat DDGS



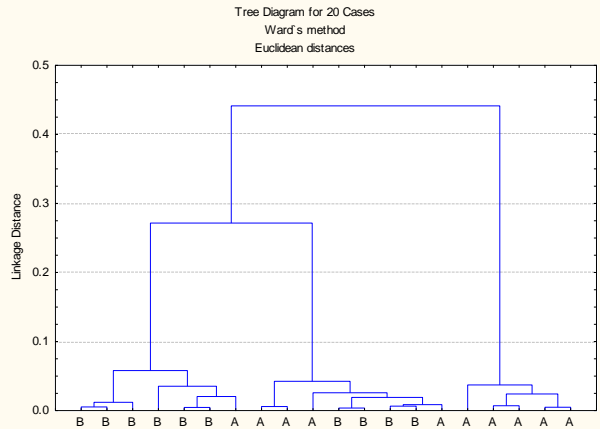
(1) CLA: Comparison of wheat (1) vs. wDDGS (2)



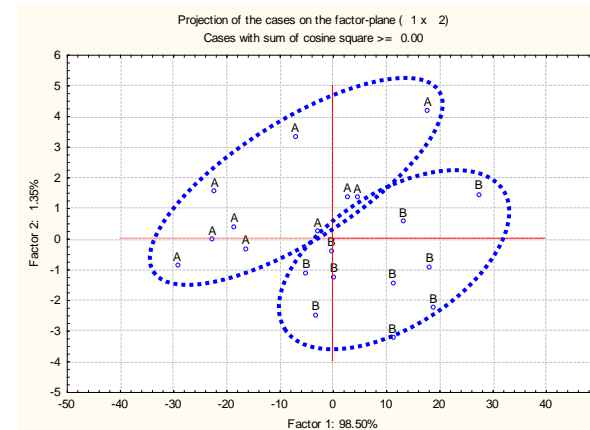
(2) PCA: Comparison of wheat (1) vs. wDDGS (2); PC1 and PC2 explain 68.64 and 30.28% of the variation of spectral data,

88

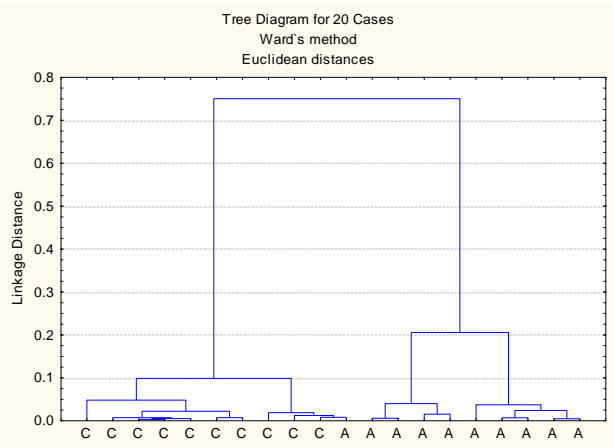
(B) Comparison between different batches of original feedstock wheat



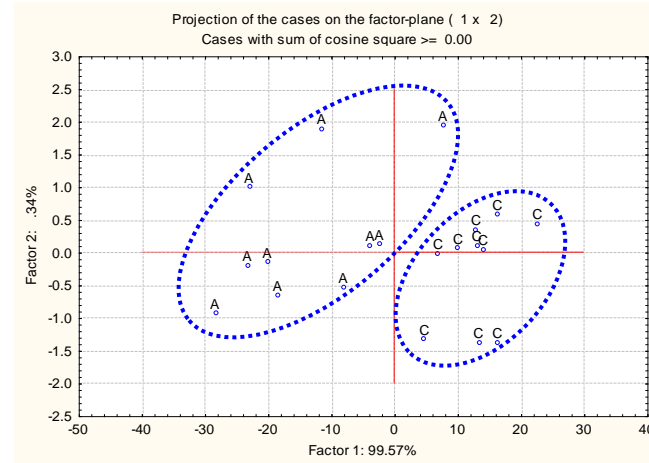
(3) CLA: Feedstock Wheat Batch 1 (A) vs. Batch 3 (B)



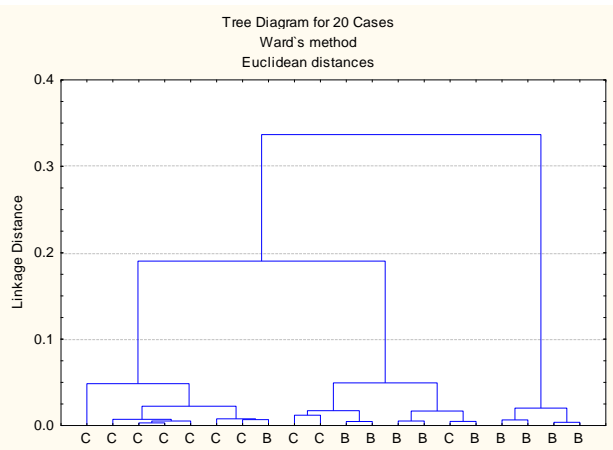
(4) PCA: Feedstock Wheat Batch 1 (A) vs. Batch 3 (B)



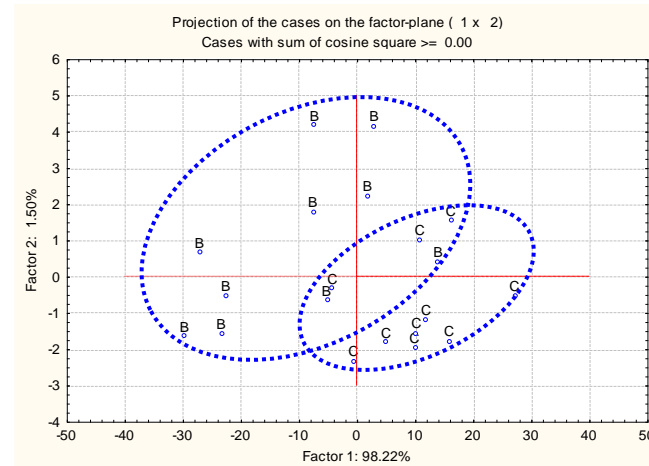
(5) CLA: Feedstock Wheat Batch 1 (A) vs. Batch 5 (C)



(6) PCA: Feedstock Wheat Batch 1 (A) vs. Batch 5 (C)



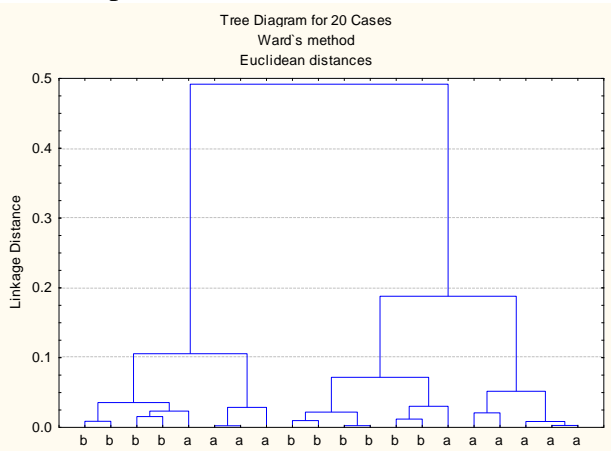
(7) CLA: Feedstock Wheat Batch 3 (B) vs. Batch 5 (C)



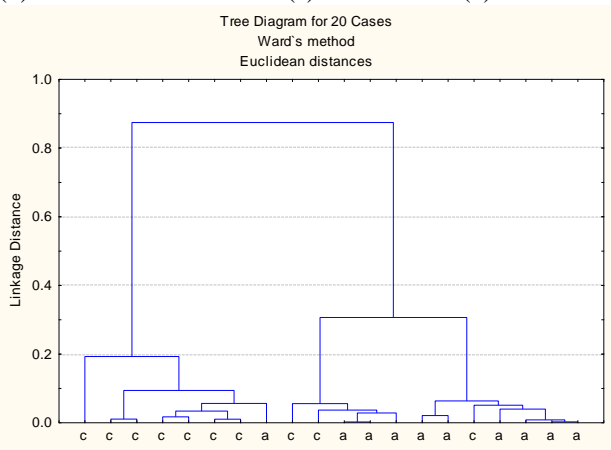
(8) PCA: Feedstock Wheat Batch 3 (B) vs. Batch 5 (C)

(C) Comparison between different batches of wheat DDGS

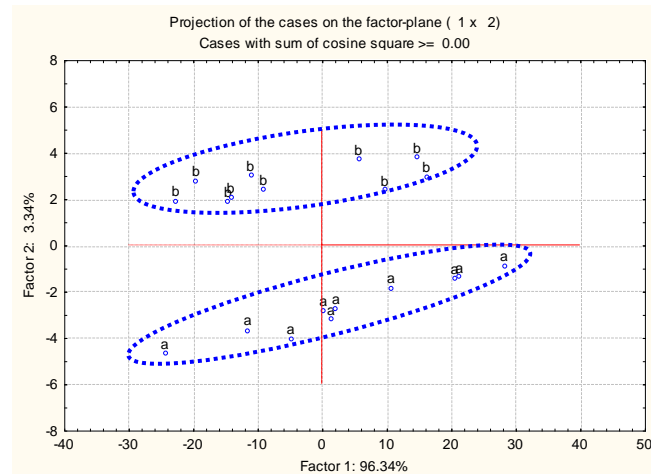
82



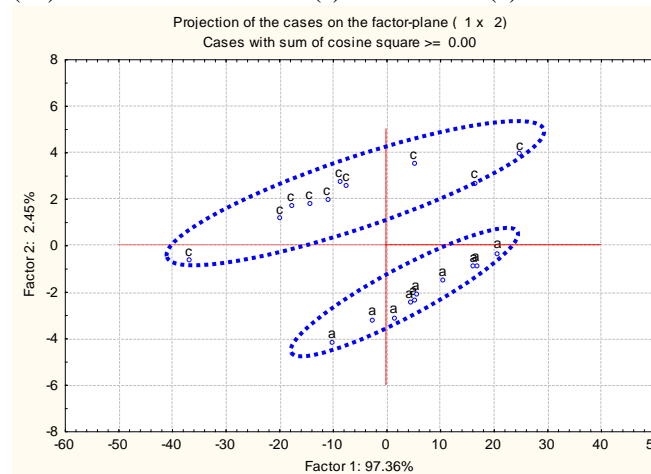
(9) CLA: wDDGS Batch I (a) vs. Batch III (b)



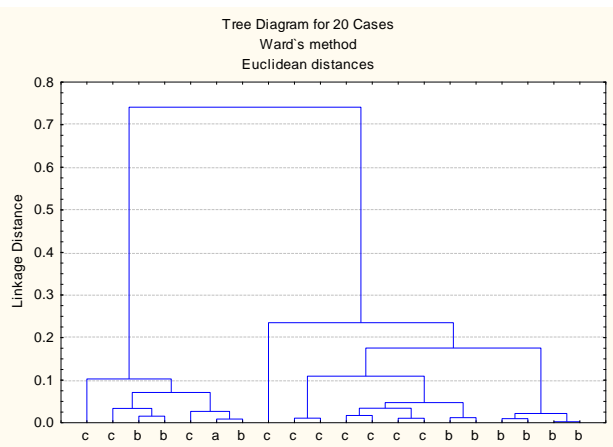
(11) CLA: wDDGS Batch I (a) vs. Batch V (c)



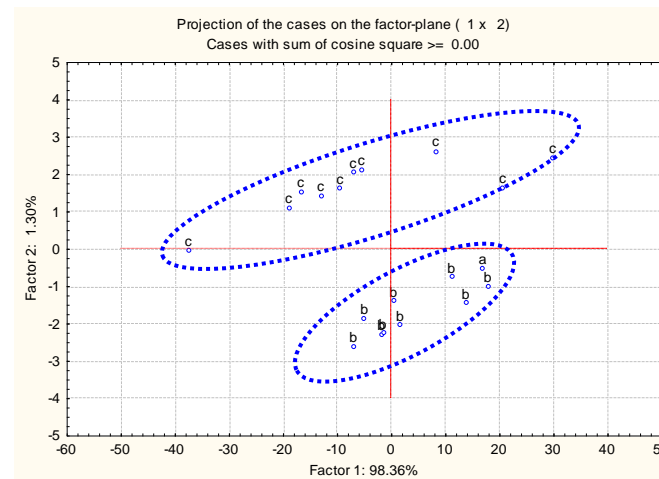
(10) PCA: wDDGS Batch I (a) vs. Batch III (b)



(12) PCA: wDDGS Batch I (a) vs. Batch V (c)



(13) CLA: wDDGS Batch III (b) vs. Batch V(c)



(14) PCA: wDDGS Batch III (b) vs. Batch V (c)

Figure 4.6. Multivariate molecular spectral analyses in the region mainly associated with structural carbohydrates (ca. 1482-1180 cm^{-1}): (A) Comparison of original feedstock wheat and wheat DDGS; (B) Comparison of three different batches (batch 1, 3 and 5) of original feedstock wheat; (C) Comparison of three different batches of wheat DDGS (batch I, III, and V).

I: Cluster analysis (1) Select spectral region: ca. 1482 to 1180 cm^{-1} ; (2) Distance method: Euclidean; (3) Cluster method: Ward's algorithm; II: principal component analysis: Scatter plots of the 1st principal components (PC1) vs. the 2nd principal components (PC2)

4.3.2.5. Determination of Molecular Structure Differences Mainly Associated with Carbohydrate

4.3.2.5.1. Comparison between Feedstock of Wheat and Co-products of Wheat DDGS

Wheat and wheat DDGS were separated into two distinguishable groups both with AHCA and PCA (Figure 4.7A). In bioethanol production, the non-structural carbohydrates (eg. starch component) of the cereal grains are utilized for fermentation which in turn changes the CHO molecular characteristics of unfermented grain fraction. Wheat and wheat DDGS were separated into two ellipses with 96.88% of total variation explained by PC1 in PCA Analysis.

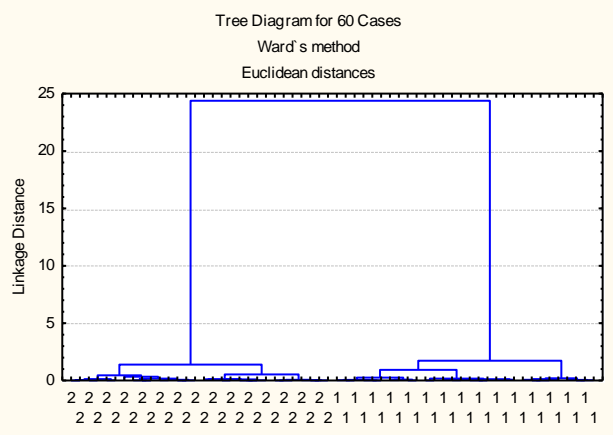
4.3.2.5.2. Comparison between Different Batches of Feedstock Wheat

When comparison was made among the three batches of wheat (Figure 4.7B), none of the comparisons was able to make distinguishable differences among the batches of wheat in this carbohydrate region ($1180 - 800 \text{ cm}^{-1}$). There was overlapping among the batches of wheat which indicate structural similarity.

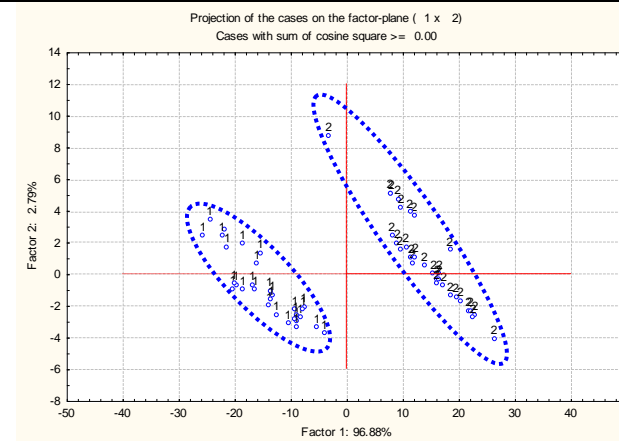
4.3.2.5.3. Comparison between the Different Batches of Wheat DDGS

When the wheat DDGS Batch I vs. Batch V, Batch I vs. Batch V and Batch III vs. Batch V, none of the AHCA and PCA were able to form distinguishable clusters and ellipses among each other (Figure 4.7C) in this region ($1180 - 800 \text{ cm}^{-1}$). In recent publication (Yu, 2011) it was found that different functional groups which are related structural and non-structural carbohydrate response differently to bioethanol processing. Some of them are highly correlated to nutrient utilization and availability.

(A) Comparison: Original feedstock of wheat vs. Co-product of wheat DDGS



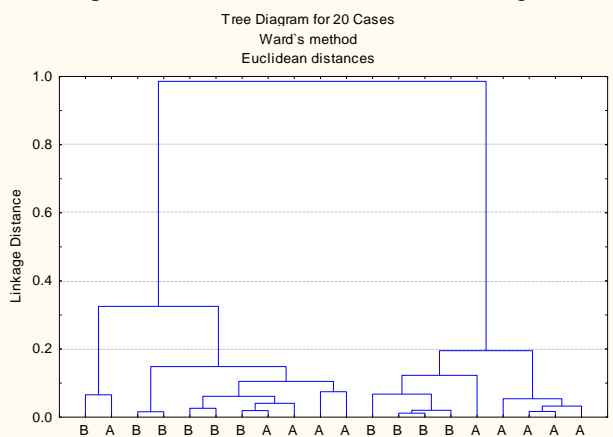
(1) CLA: Comparison of wheat (1) vs. wDDGS (2)



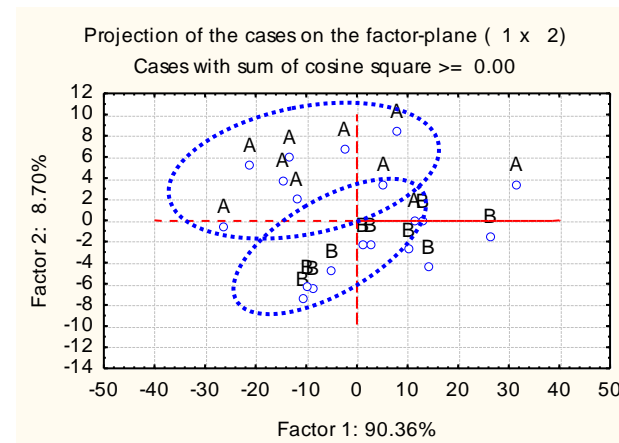
(2) PCA: Comparison of wheat (1) vs. wDDGS (2): PC1 and PC2 explain 96.88 and 2.79% of the variation of spectral data, respectively.

85

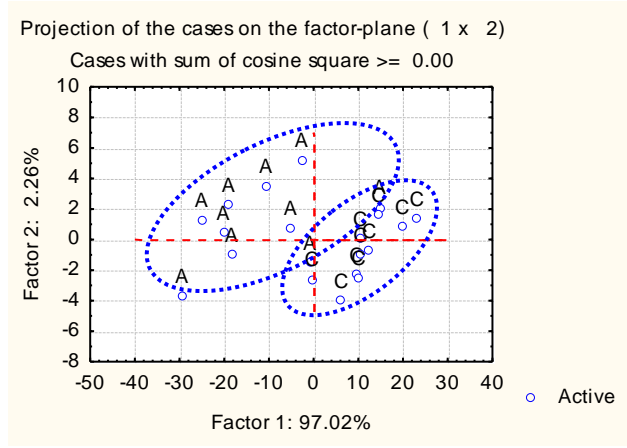
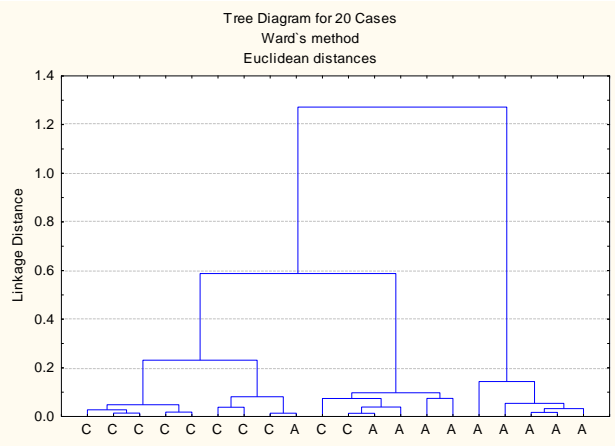
(B) Comparison between different batches of original feedstock wheat



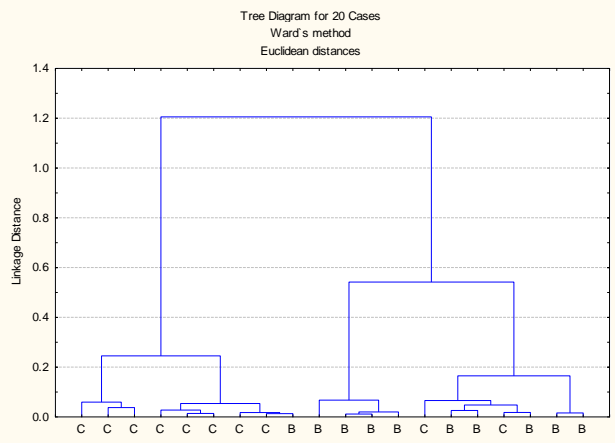
(3) CLA: Feedstock Wheat Batch 1 (A) vs. Batch 3 (B)



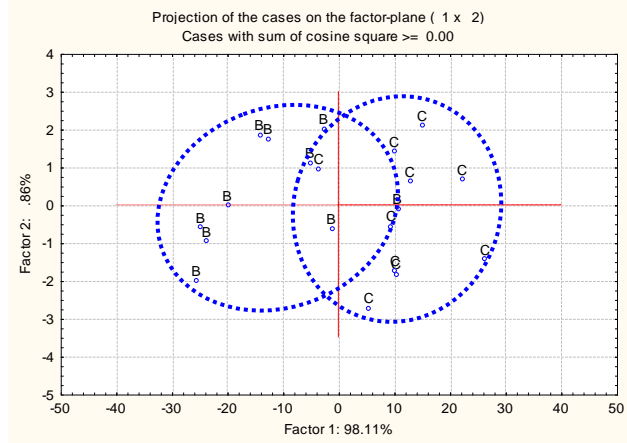
(4) PCA: Feedstock Wheat Batch 1 (A) vs. Batch 3 (B)



(5) CLA: Feedstock Wheat Batch 1 (A) vs. Batch 5 (C)



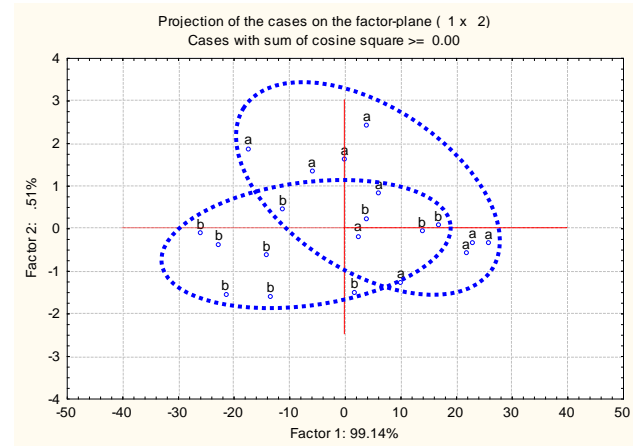
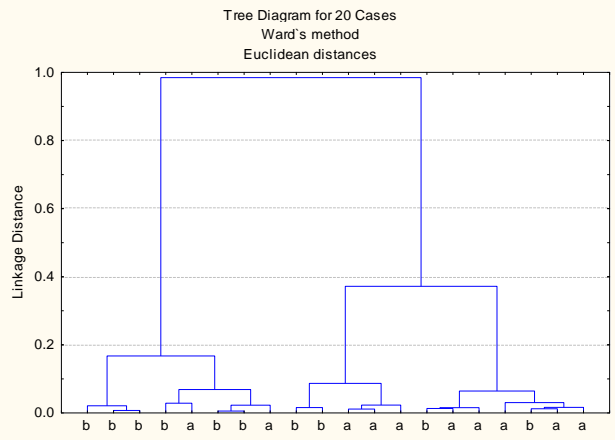
(6) PCA: Feedstock Wheat Batch 1 (A) vs. Batch 5 (C)



(7) CLA: Feedstock Wheat Batch 3 (B) vs. Batch 5 (C)

(8) PCA: Feedstock Wheat Batch 3 (B) vs. Batch 5 (C)

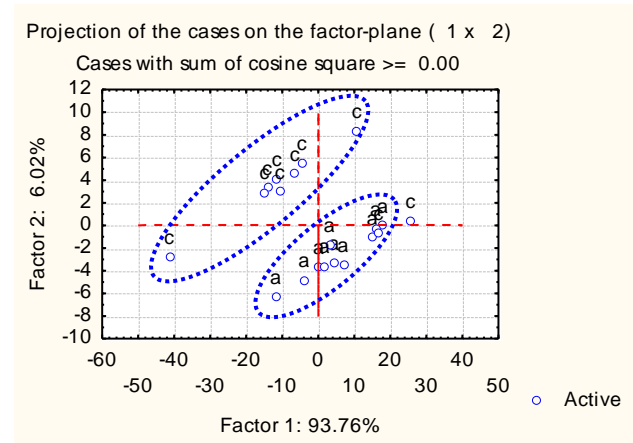
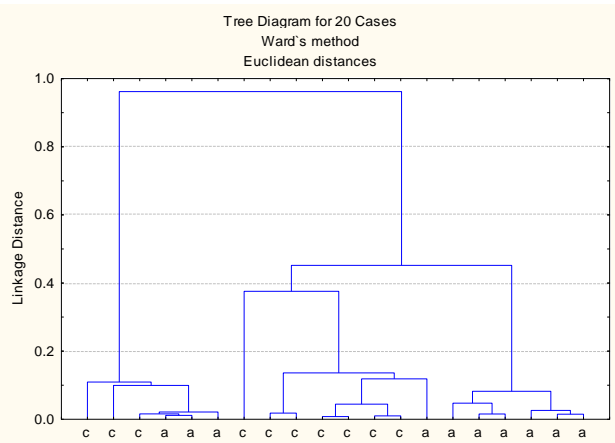
(C) Comparison between different batches of wheat DDGS



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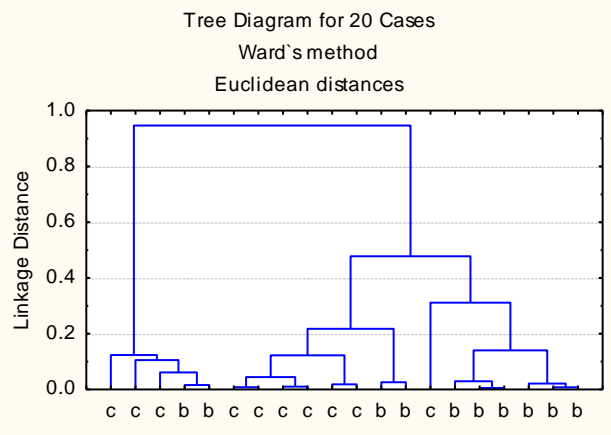
(9) CLA: wDDGS Batch I (a) vs. Batch III (b)

(10) PCA: wDDGS Batch I (a) vs. Batch III (b)

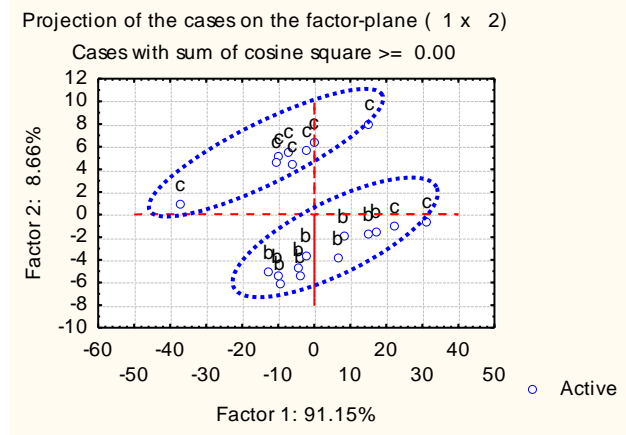


(11) CLA: wDDGS Batch I (a) vs. Batch V (c)

(12) PCA: wDDGS Batch I (a) vs. Batch V (c)



(13) CLA: wDDGS Batch III (b) vs. Batch V(c)



(14) PCA: wDDGS Batch III (b) vs. Batch V (c)

Figure 4.7. Multivariate molecular spectral analyses in carbohydrate region (ca. 1180-800 cm^{-1}): (A) Comparison of original feedstock wheat and wheat DDGS; (B) Comparison of three different batches (batch 1, 3 and 5) of original feedstock wheat; (C) Comparison of three different batches of wheat DDGS (batch I, III, and V).

I: cluster analysis (1) Select spectral region: CHO region: ca. 1180 to 800 cm^{-1} ; (2) Distance method: Euclidean; (3) Cluster method: Ward's algorithm]; II: principal component analysis: Scatter plots of the 1st principal components (PC1) vs. the 2nd principal components (PC2).

4.4. Discussion

The obtained results for the chemical composition and nutrient availability studies support clearly distinguishable molecular structure differences detected by molecular spectroscopy FT/IR-ATR between feedstock of wheat and the co-product of wDDGS. The AHCA and PCA analysis results of Amide I and Amide II region were in agreement with the preliminary study carried out by Yu et al. (2010) which indicated that bioethanol processing alter the molecular structure conformation of protein compared to the original grain. The findings of carbohydrate structural characteristic were closely in agreement with Yu et al. (2011) in which a detailed study was performed to identify how the bioethanol processing affected the carbohydrate structural characteristics of co-products. They revealed that different cereal grains response differently to bioethanol processing and different functional groups response differently to bioethanol processing within the same type of cereal grain. Different functional groups which are related structural and non-structural carbohydrate response differently to bioethanol processing. Some of them are highly correlated to nutrient utilization and availability.

In this study, the FT/IR multivariate spectral analysis revealed molecular spectral characteristics related to chemical and nutrient components in the feed. As an example in Figure 4.6. with FTIR-ATR with multivariate molecular spectral analysis (CLA, PCA), there was clear demarcation between wheat batch 1 and batch 5 at the region of $1482-1180\text{cm}^{-1}$. This region of the spectrum contains functional groups mainly related to structural carbohydrate. When these results compared with the wet chemical analysis results (Table 3.1.) of NDF data (cell wall material related to structural CHO). The NDF values were same in both batch 1 and batch 5 (14.1%DM). The NDF value only indicates the total cell wall content but not its constituent components. Therefore these results revealed the enhanced sensitivity of FTIR molecular spectral analysis in identifying feed treatment difference compared to conventional wet analysis. These spectral differences which are mainly related to structure conformation may be the reason for large variation of Kd values numerically between the wheat batches 1 and 5 (12 vs. 24%/h).

The multivariate spectral analysis with CLA and PCA were used as opposed to univariate analysis. With the multivariate spectral analysis ability to determine molecular

structural differences associated with all the chemical components is an advantage. It helps for better qualitative separation of molecular structure characteristics. However, multivariate spectral analysis is able to identify spectral difference between feedstock and wDDGS, but it does not show exact difference. Therefore in future, we should try univariate spectral analysis to find out which chemical functional groups are different.

4.5 Conclusion and Implication

The ease of traditional time consuming and laborious wet chemical analysis further confirms the appropriateness of this FT/IR technology in feed evaluation. The results demonstrated the multivariate molecular spectral analysis with AHCA and PCA in detecting difference not only visually but with statistical evidences. In future, univariate molecular spectral analysis which is able to detect functional group intensity difference will be considered to identify batch difference and to analyse the response of each functional group to bioethanol processing.

5.0. GENERAL DISCUSSION AND CONCLUSION

The main purpose of this study was to identify the variation among different batches of feedstock wheat and among different batches of new mash-type wheat DDGS (wDDGS) produced from the same bioethanol plant with current processing technology in terms of chemical and mineral composition, carbohydrate and protein sub-fractions, estimated energy values with NRC-2001 summative approach, in situ rumen degradation kinetics, in vitro intestinal digestibility and molecular spectral differences with FTIR/ATR analysis.

The chemical and mineral composition profiles were significantly different among wheat and wDDGS, among the batches of wheat and among the batches of wDDGS. The removal of starch during the bioethanol production process leads to concentration of other nutrients into DDGS. The high protein and high fibre compared to wheat is the most desirable character in wDDGS as a ruminant feed ingredient. In mineral profiles higher sulphur content associated with wDDGS is a concern in this mash-type of wDDGS. The higher P content should be taken into account in balancing the rations with calcium. Excess mineral consumption has negative impact on both the animal and the environment. Since yeast proteases cannot degrade wheat protein completely the protein in wDDGS is mainly from wheat. The CNCPS carbohydrate and protein sub-fractionation shows variation among different batches of wheat and among different batches of wDDGS. The higher slowly degradable fibre fraction (PB3) compared to wheat favours wDDGS as a by-pass protein. The higher unavailable protein fraction (PC) among the batches of wDDGS may be due to the Millard reaction associated with higher drying temperature of DDGS. Since CNCPS protein and carbohydrate sub-fractions were calculated based on chemical composition data, any variation associated with chemical composition of wheat and wDDGS batches reflect in calculated CNCPS sub fractions.

According to particle size distribution around 40% of the wDDGS particles remained in the bottom pan. The particle size of wDDGS among the batches varied from 650-690 μ m. This indicates the smaller particle size (<0.59mm) of the mash type wDDGS. Compared to other types of DDGS which has ball shape with much larger particle size, these mash type wDDGS may behave differently in in situ rumen degradation. The higher effective degradation content of NDF may be due to the higher washable fraction associated with smaller particle size of wDDGS used in this study. An in situ degradability trial was

conducted in the purpose of identifying different degradation kinetics associated with different batches of wheat and wDDGS. The results of this study demonstrated significant difference in effective degradability (ED) of DM, EDOM and EDCP among the batches of wheat. The batches of wDDGS significantly vary in ED of CP and NDF content. The EDCP varied by 7% while EDNDF varied by 6% among batches of wDDGS. The variation among batches of wDDGS in rumen undegradable protein RUP was 7%. Even though these in situ rumen degradation kinetics are statistically significant among batches of wDDGS, further studies are required to determine whether this difference cause biological significance when formulate rations with wDDGS. The use of in situ nylon bag technique in determining the in situ rumen degradation kinetics of samples with smaller particle size may be not very desirable method. The higher washable/soluble fraction of wDDGS is due to the escape of small particles via in situ nylon bags. This reflects the possibility of rumen by pass of these small particles when cattle fed rations with this type of wDDGS. The sample size (7g) compared to TMR fed to the cow may not reflect the actual degradation behavior of these wheat and wDDGS. Therefore if we could test the differences of wDDGS batches by formulating TMR with different batches of wDDGS, it would have been an ideal study to find out the differences in situ rumen degradation kinetics. When analyse wDDGS as a single feed ingredient, the nitrogen (N) to energy ratio revealed the possibility of excess N supply in rumen which can be a loss. In the actual situation TMR are balanced with animal nutrient requirement in which case the balancing feed rations with correct amount of wDDGS may not be a problem with excess N supply. However, the cows normally eat every 4 h and N/OM effective degradation beyond the 10 h incubation may not reflect biological significance.

The predicted true protein supply to small intestine with three nutrient models revealed the wDDGS (data in Appendix) as rich truly digestible and absorbable source of protein. With all three models, variation in true protein supply to small intestine was observed with different batches of wDDGS.

Expansion of grain based bioethanol production creates a continuous availability of DDGS to the market. There is a demand for development of rapid analytical tool in identifying chemical composition data of DDGS in related to the molecular structure differences. Due to the greater sensitivity and faster scan speeds, the FT/IR-ATR technique was tested in this study as a rapid tool of analysis. The use of FT/IR-ATR technique proved

the possibility of identifying different molecular spectral characteristics associated with different chemical functional groups. The multivariate spectral analysis provided the identification of wheat and wDDGS. The spectral difference in Chapter 4 is clearly linked to chemical and nutrient differences in Chapter 2. In determining molecular structure differences associated with different batches of wheat and wDDGS, the results are not very clear. The FT/IR-ATR results observed in this preliminary study are suggestive of possibility of developing molecular spectral characteristics in identifying batch difference. The selection of précised IR band areas and identification of different variables (peak height, peak width) associated with molecular structure differences may be suggestive of as next step of this study. We can do univariate molecular spectral analysis and check functional group intensity between batches of feedstock and wDDGS. For example, Figure 5.1 and 5.2 show different functional groups. Using univariate analysis, we can analyse the difference among batches of wheat and wheat DDGS.

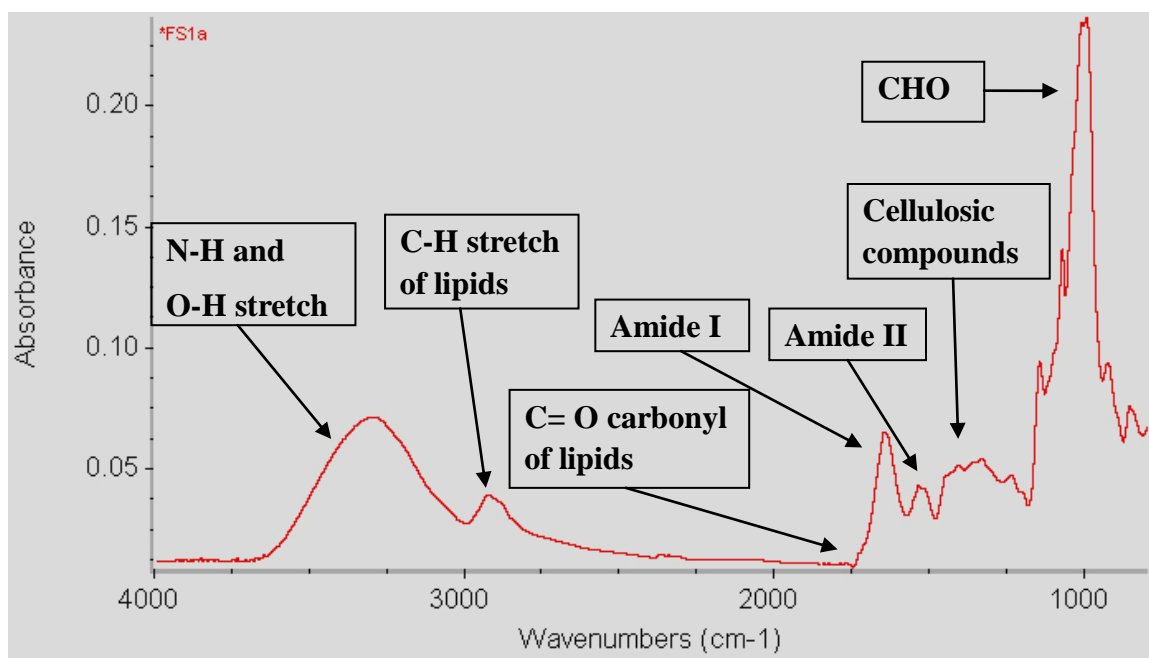


Figure 5.1. Typical spectrum in bioethanol feedstock of wheat in the region ca. 4000-800 cm^{-1} showed function groups of biomolecular and biopolymers: N-H and O-H stretch, C-H stretch, amide I and II, C = O carbonyl, CHO and cellulosic compounds.

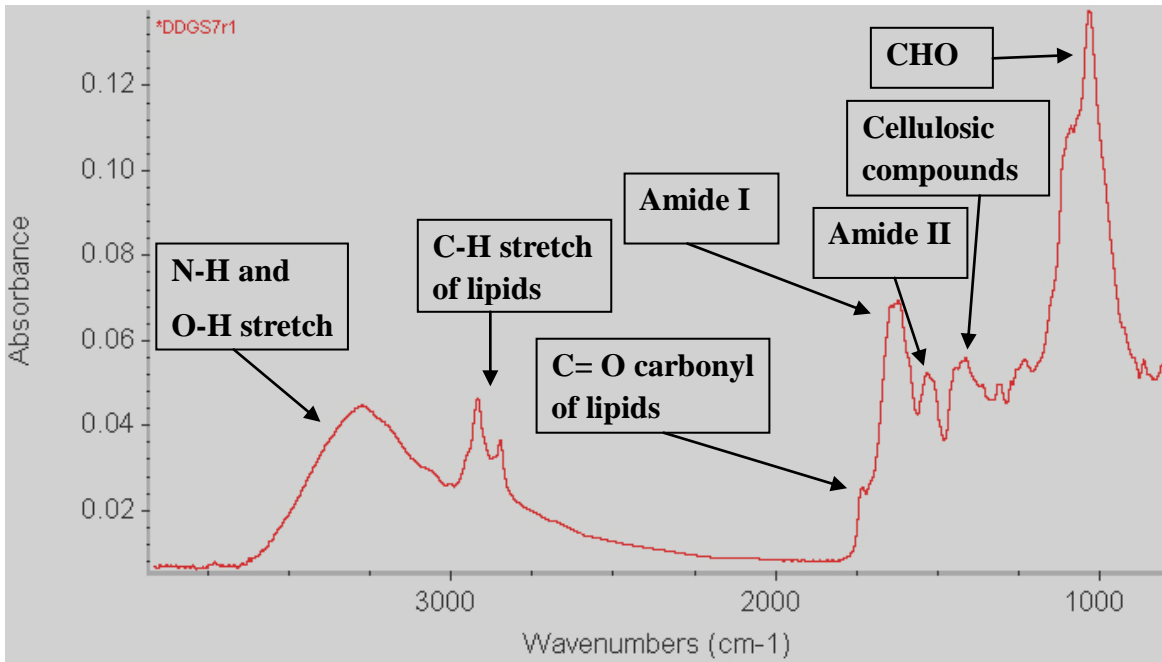


Figure 5.2. Typical spectrum in the co-products of bioethanol processing (wheat DDGS) in the region ca. 4000-800 cm^{-1} showed function groups of biomolecular and biopolymers: N-H and O-H stretch, C-H stretch, amide I and II, C = O carbonyl, CHO and cellulosic compounds.

This study provides the magnitude of differences among the batches of feedstock and wDDGS from the same bioethanol plant. These observations provide insight to the nutritionists to consider the variation occurring among DDGS from the same bioethanol plant, particularly when use a high amount of co-products.

Further research needs to be done in following areas:

- Detect the responses and sensitivity of each functional group to bioethanol processing to understand how the intrinsic structure changes are related to nutrient availability in animals.
- Study the relationship between FTIR functional group intensity and nutrient utilization in animal.

6. 0. REFERENCES

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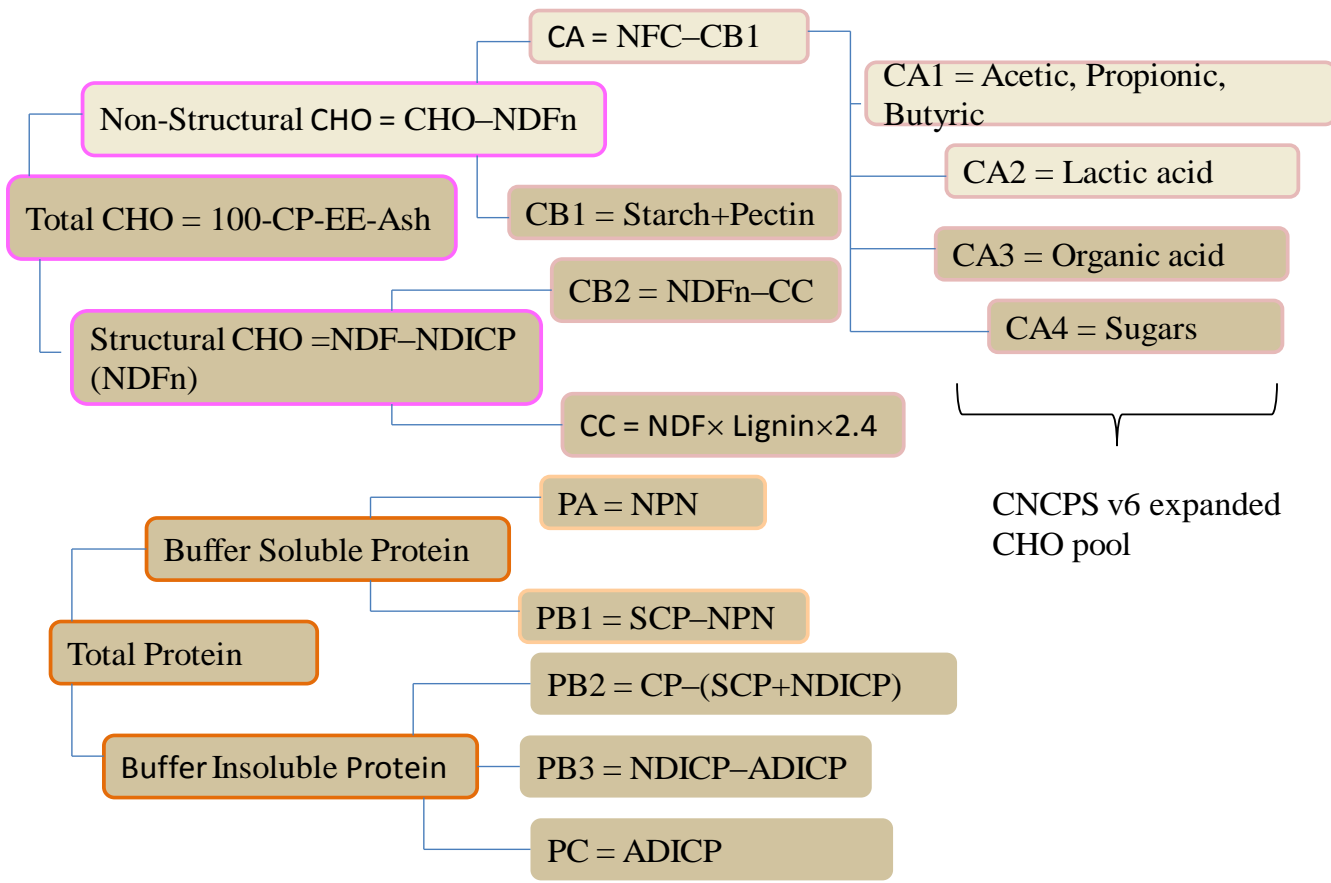
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7.0. APPENDIX



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Figure A1. Schematic representation of Cornell Net Carbohydrate and Protein sub-fractions in CNCPS v5 and v6. Adapted from Fox et al. (2004) and Tylutki et al. (2008).

PREDICTION OF NUTRIENT SUPPLY TO DAIRY CATTLE FROM ORIGINAL FEEDSTOCK WHEAT AND WHEAT DDGS WITH DVE/OEB SYSTEMS AND NRC 2001 MODELS: BATCH EFFECTS

Introduction

Nutrient models facilitate correct prediction and estimation of dairy cattle feeding programmes. With the better understanding of the ruminant digestive system, the integration of values obtained with in vivo and in vitro studies into mechanistic models helps to optimize the animal performance in relation to the changes in ration. When the sample volume is limiting or large number of samples to be evaluated, it is really advantageous for nutritionists to use mathematical models rather than expensive, labor intensive and time consuming in vivo methods to screen treatments. In modern high producing dairy cow production system, the prior estimation of truly absorbable protein in small intestine is vital. Therefore different protein evaluation systems have been developed and only few of them became successful (Tamminga et al., 1994).

Some of the models developed earlier to predict true protein supply to animals were PDI (INRA, 1978; Verité, 1987), AP (NRC, 1985) and MP (AFRC, 1992). TDN based model NRC-2001 and non-TDN based model DVE/OEB system (Tamminga et al. 1994) are two widely used dairy cattle nutrition models. NRC 2001 model is used in North America while DVE/OEB is more common in Europe. The DVE/OEB system discusses the truly absorbable protein supply to small intestine and degraded protein balance in the rumen. The true protein supply to small intestine is discussed in NRC-2001 by means of Metabolizable Protein (MP). The DVE system consider the balance between rumen N and energy supply which in terms determine the contribution of microbial protein supply to the small intestine.

Even though the principles of predicting true protein supply to small intestine is similar between the both models, the concepts used in calculation and prediction differ among each other (Yu, 2005a). In both models three main contributory factors for true protein supply to small intestine are: i) rumen undegraded feed protein, ii) microbial crudeprotein synthesized in the rumen, and iii) endogenous protein. The objective of this study was to evaluate the batch effect on predicted nutrient supply from feedstock wheat and corresponding wheat DDGS with the NRC-2001, DVE/OEB-1994 and DVE/OEB-2007 models. The DVE/OEB-2007 (Tamminga et al., 2007) is a most recently developed model.

Materials and Methods

Sampling

Three batches of feedstock of wheat (Batches 1, 3, 5) and corresponding three batches of wheat DDGs (Batches I, III, V) were used for this experiment. The wheat samples were roller milled (Sven Roller Mill, Apollo Machine and Products Ltd, Saskatoon, SK) with roller gap 0.203 mm. Wheat DDGS samples were used as they were.

Animals and Diets

Animals and diets were as same in Chapter 3.

In Situ Rumen Incubation

In situ rumen incubation procedure was as same in Chapter 3 and the same in situ results were used in nutrient modeling.

Chemical Analysis

For the original feedstock samples of wheat and wheat DDGS, the values obtained with the chemical composition analysis in Chapter 3 were used. For the rumen incubated samples of wheat and wheat DDGS, the values used for the determination of in situ rumen degradation kinetics in the same Chapter 3 were used.

Rumen Degradation Kinetics

In situ rumen degradation kinetics was determined for DM, OM, CP NDF and RNSP. The first order kinetic model originally developed by Ørskov and McDonald (1979) and modified by Robinson et al. (1986) and Dhanoa (1988) was used. The degradation rate K_d of potentially degradable fraction (D) was determined for OM, CP, NDF, and RNSP.

$$R(t) = U + D \times \exp^{-K_d(t-\text{lag})}$$

where, R is the residue (%) of incubated sample at t h incubation time point, U is undegradable fraction, lag is the time taken to initiate the degradation and K_d is the degradation rate (%/h). Since starch degrades more rapidly in the rumen according to Tamminga et al. (1994) lag time and U are assumed to be zero for starch. Non linear

parameters were estimated with PROC NLIN from SAS (2009) with iterative least square regression (Gauss-Newton method).

In Vitro Estimation of Intestinal Digestibility of Rumen Undegraded Protein (%dRUP)

In vitro estimation of intestinal digestibility of rumen undegraded protein was carried out according to the three step in vitro procedure described by Calsamiglia and Stern (1995). The in detailed procedure was described in Chapter 3.

Prediction of true protein supply with NRC - 2001

For accurate diet formulation, the knowledge about rumen feed protein degradation kinetics is a necessity. The adequate supply of RDP for rumen microbes, supply of RUP for host animal are required to maximize the production (NRC, 2001). Rumen degraded feed protein (RDP), rumen undegraded feed protein (RUP), and rumen undegraded starch (only for feedstock samples) were predicted based on the non linear parameters estimated (W (S), U, D, K_d), as follows:

$$\text{RDP (\%)} = S + (D \times K_d) / (K_p + K_d),$$

$$\text{RUP (\%)} = U + (D \times K_p) / (K_p + K_d),$$

$$\text{RUS}_t (\%) = S \times 0.1 + (D \times K_p) / (K_p + K_d),$$

where, $D = 100 - W(S) - U(\%)$; K_p = estimated outflow rate of digesta from the rumen (%/h) and this is assumed to be 6 %/h (Tamminga et al., 1994). The 0.1 is the compensation factor for the difference between in situ and in vivo results which assumes that 10% of the starch escapes the rumen degradation under in vivo situation (Nocek and Tamminga 1991; Tamminga et al. 1994; Yu et al. 2003b)

Prediction of Metabolizable Protein (MP) with NRC (2001) Model

The true protein absorbed as amino acids in the small intestine is the most important part of the host animal nutrition. According to NRC 2001, the metabolizable protein composed of three major contributory protein sources. Total MP can be calculated as follows:

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

where, AMCP is the absorbable microbial protein, ARUP is the truly absorbable rumen undegraded feed protein and AECP is the truly absorbable endogenous crude protein in the small intestine.

To calculate the truly absorbed protein in small intestine, the digestibility of contributory protein should be considered. The NRC (2001) assumes that the microbial crude protein contributed by bacteria and protozoa contains 80% of true protein while the other 20% from nucleic acids. It also assumes that the digestibility of true protein of MCP as 80%. Thus AMCP is calculated as follows:

$$\text{AMCP (g/kg DM)} = \text{MCP (g/kg DM)} \times 0.8 \times 0.8$$

The NRC (2001) assumes that the yield of MCP from kg of TDN as 130 g. Therefore MCP was calculated as follows:

$$\text{MCP (g/kg DM)} = 0.130 \times \text{TDN}$$

If RDP is less than $1.18 \times \text{MCP}$ (TDN predicted yield), the MCP is calculated as follows;

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

where, NRC (2001) assumes that the 85% of the RDP is converted to MCP.

The digestibility of RUP (%dRUP) was determined according to three-step in vitro procedure described by Calsamiglia and Stern (1995) and ARUP was calculated as follows:

$$\text{ARUP (g/kg DM)} = \text{RUP (g/kg DM)} \times \text{dRUP (\%)}$$

The estimation of endogenous crude protein (ECP) was calculated as follows:

$$\text{ECP (g/kg DM)} = 1.9 \times 6.25 \times \text{DM (\%)} / 100$$

where, 1.9 is the NRC (2001) assumption that 1.9 g of endogenous N is produced from a kg of DM and 6.25 is the conversion factor for N to protein.

The NRC (2001) assumes the true protein content in ECP passing to duodenum as 50% and the 80% digestibility in the small intestine. Therefore AECP was calculated as follows;

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

The degraded protein balance (DPB) can be calculated for NRC 2001 as described by Yu et al. (2003). It is the difference between microbial proteins synthesized from rumen degraded feed protein (RDP) and the potential energy from anaerobic fermentation in the rumen. The DPB was calculated as follows:

$$\text{DPB (g/kg DM)} = \text{RDP (g/kg DM)} - 1.18 \times \text{MCP (g/kg DM)}$$

Prediction of true protein supply with DVE/OEB - 1994 System

The DVE/OEB nutrient modelling system was developed in the Netherlands for dairy cattle. The system was developed to describe the digestion and metabolism of N in dairy cows in detail. It also aimed at feeding, required amount of N to prevent the N losses. Accurate prediction of milk production was another aspect with this system (Tamminga et al., 1994).

The DVE/OEB-1994 System

The DVE/OEB system composed of two major parts which describes DVE value and OEB value for a feed (Tamminga et al., 1994). The role of energy balance in protein supply is a new concept introduced by this model (Yu, 2005a). The DVE value consists of rumen undegraded feed protein that absorbs in small intestine as amino acids, the microbial protein synthesized in rumen and absorbed in small intestine as amino acid and correction for endogenous losses resulting from digestion.

$$\text{DVE (g/kg DM)} = \text{AMCP (g/kg DM)} + \text{ARUP (g/kg DM)} - \text{ENDP (g/kg DM)}$$

where, AMCP is the intestinally absorbable microbial protein fraction, ARUP is the truly absorbable fraction of rumen undegraded feed protein and ENDP is the correction factor for endogenous protein losses due to digestion.

The microbial protein production is based on the fermentable organic matter (FOM) content in DVE/OEB - 1994 system and FOM is calculated as:

$$\text{FOM (g/kg DM)} = \text{DOM (g/kg DM)} - \text{EE (g/kg DM)} - \text{RUP (g/kg DM)} - \text{RUST (g/kg DM)} - (0.50) \times \text{FP (g/kg DM)}$$

FOM is calculated from digestible organic matter (DOM) but DOM is corrected for crude fat (EE), rumen undegraded feed protein (RUP), undegraded starch (RUST) and 50% of the fermentation end products in ensiled products.

In the DVE/OEB - 1994 system, AMCP was calculated as follows:

$$\text{AMCP (g/kg DM)} = \text{FOM (g/kg DM)} \times 0.150 \times 0.75 \times 0.85$$

where, DVE/OEB-1994 assumes that 150 g of MCP is produced from a kg of FOM, in which true protein is assumed to be 75% with the 85% digestibility in the small intestine.

In DVE/OEB – 1994 system, ARUP was calculated as follows:

$$\text{ARUP (g/kg DM)} = \text{CP (g/kg DM)} \times (1.11 \times \text{RUP (\%CP)} / 100) \times (\% \text{dRUP} / 100)$$

The correction for endogenous losses in digestion was calculated as follows:

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

where, UDM is the combination of both organic and inorganic indigestible matter. Therefore UDM was calculated as follows:

$$\text{UDM (g/kg DM)} = 1000 - \text{DOM (g/kg DM)} - \text{VRAS (g/kg DM)},$$

where, VRAS is the digestible inorganic matter.

The degraded protein balance is a new concept introduced by the DVE/OEB system where it concerns the balance between microbial protein syntheses in the rumen from available rumen degradable CP and potential energy from anaerobic fermentation in the rumen.

Microbial crude protein based on RDP (MCP_{RDP}) was calculated as;

$$\text{MCP}_{\text{RDP}} \text{ (g/kg DM)} = \text{CP (g/kg DM)} \times [1 - (1.11 \times \text{RUP (\%CP)/100})]$$

Microbial crude protein based on FOM (MCP_{FOM}) was calculated as:

$$\text{MCP}_{\text{FOM}} \text{ (g/kg DM)} = \text{FOM (g/kg DM)} \times 0.15$$

The degraded protein balance value was estimated as:

$$\text{OEB (g/kg DM)} = \text{MCP}_{\text{RDP}} \text{ (g/kg DM)} - \text{MCP}_{\text{FOM}} \text{ (g/kg DM)}$$

The DVE/OEB-1994 system recommends the OEB value not to be negative in dairy cow ration due to the possibility of N shortage for microbial protein synthesis.

Prediction of true protein supply with the DVE/OEB - 2007 system

The DVE/OEB 2007 (Tamminga et al., 2007) is the updated version of DVE/OEB-1994 system. The model describes DVE and OEB values for each feed as in DVE/OEB-1994. The in situ fractions are divided into four fractions since washable fraction is sub-divided into washable insoluble (WI) and washable soluble (WS) fractions. The ethanol soluble carbohydrate (ESC) is the S fraction of the carbohydrate which assumed to be degraded rapidly in the rumen (Tamminga et al., 2007). The model assumes that the washable rumen non starch polysaccharides (RNSP) and starch contain only insoluble material while NDF does not contain any soluble fraction. The model describes the correction factors 65, 44, 17 and 3% for the crude fat at 0, 2, 6 and 12h incubation residues respectively (Tamminga et al., 2007).

The DVE value was calculated as follows:

$$\text{DVE} = \text{DMCP} + \text{DRUP} - \text{DMFP}$$

where, DMCP is the microbial protein synthesized in the rumen and digested in the intestine, DRUP is the feed protein not degraded in the rumen but digested in small intestine and DMFP is the endogenous protein losses associated with digestion.

The microbial protein synthesized in the rumen and digested in the intestine is calculated as follows:

The DVE/OEB-2007 system does not calculate fixed amount of microbial protein synthesis from the fermentable organic matter (FOM) as in the previous model. The DVE/OEB- 2007 system considers that the amount of ATP that can be extracted from the feed differ among the components of a feed. The fermentable fraction of each nutrient was calculated as:

$$FCOMP = COMP \times \{S \times K_{ds}/K_{ds} + K_{ps}\} + (W-S) \times K_{d(w-s)}/K_{d(w-s)} + K_{p(w-s)} + D \times K_{dD}/(K_{dD} + K_{pD})\}$$

where, FCOMP is the effectively rumen degradable component (g/kg DM) and COMP is the amount of relevant nutrient component in the feed stuff.

The microbial protein synthesized based on available rumen fermentable organic matter (FOM) depends on the ATP yield of each component, yield of microbial dry matter (Pirt, 1965; Tamminga et al., 2007) and different bacterial strains and growing conditions (Russel and Strobel, 2005). With the equation of Pirt (1965) the actual microbial growth yield can be calculated as follows:

$$1/Y = M/GR + 1/Y_{max},$$

where, Y = yield of microbial dry matter (in g per mole of ATP), M = maintenance requirement of the microbes (mole of ATP * h⁻¹ per g microbial material), GR = fractional growth rate (h⁻¹), Y_{max} = maximum microbial growth yield without losses in maintenance (g per mole of ATP).

Statistical Analysis

Statistical analysis was carried out with Mixed procedure of SAS 9.2 (2009). The model used was,

$$Y_{ijk} = \mu + B_i + R_j + e_{ijk},$$

where, Y_{ijk} is the observation of the dependant variable ijk; μ is the population mean for the variable; B_i is the fixed effect of the feeds (i = 1 - 3 for wheat and i=I - III for wDDGS), R_j is

the random effect of the run and e_{ijk} is the random error associated with the observation ijk . Normality test was performed using Proc Univariate with plot and normal options. Significance was declared at $P < 0.05$ and Tukey's method was used for the multi treatment comparison. The letter grouping was obtained with SAS pdmix800 macro (Saxton, 1998).

Results

Batch Effect of Wheat and wheat DDGS on Metabolic Characteristics of Protein with NRC 2001

Batch Effect on Microbial Crude Protein Synthesis and Truly Absorbed Microbial Crude Protein (AMCP)

The effect of batches of wheat and wDDGS on protein metabolic characteristics modeled with the NRC 2001 is shown in Table A2. The NRC - 2001 assumes that MCP yield is 130 g/kg TDN (discounted) intake. With the obtained results, RDP from wheat was lesser than the requirement ($1.18 \times \text{MCP}$) while RDP from wDDGS was exceed the requirement. Therefore MCP was used accordingly with MCP_{TDN} and MCP_{RDP} . The microbial crude protein based on RDP was higher in wDDGS than that in wheat and the values were 244 and 81 g/kg DM, respectively. The range among the batches of wheat for MCP based on RDP was 14 g/kg DM while the range among the batches of wheat DDGS was 32 g/kg DM. Based on TDN, the MCP synthesis was higher in wheat compared to wDDGS and the values were 104 vs. 96 g/kg DM in wheat and wDDGS, respectively. The MCP and AMCP were higher in wheat DDGS compared to wheat and the obtained values were 244 vs. 81 g/kg DM and 61 vs. 52 g/kg DM for wDDGS and wheat, respectively. Among the batches of wheat, there was a significant difference ($P < 0.05$) in AMCP with a range of 9 g/kg DM and among the batches of wDDGS the range was 3 g/kg DM.

Table A1. Batch effect of original feedstock wheat and their co-products-wheat DDGS on predicted nutrient supply with NRC-2001 model.

Item					Batches of wheat				Batches of wDDGS					
	Wheat (n=6)	wDDGS (n=6)	SEM	P value	1 (n=2)	3 (n=2)	5 (n=2)	SEM	P value	I (n=2)	III (n=2)	V (n=2)	SEM	P value
Using the NRC-2001 dairy model to predict of the potential nutrient supply														
1. Truly absorbed rumen synthesized microbial protein in the small intestine (g/kg DM)														
MCP _{RDP} (based on RDP)	80.9b	244.3a	3.77	<0.01	87.6a	82.3ab	73.5b	2.71	<0.01	256.2a	224.7b	252.0a	5.59	<0.0
MCP _{TDN} (based on TDN)	104.3a	95.5b	0.58	<0.01	104.3b	105.4a	103.5	0.28	<0.01	97.5a	97.0a	92.1b	0.16	<0.0
RDP	123.1a	112.7b	0.69	<0.01	123.1b	124.4a	122.2	0.32	<0.01	115.0a	114.4a	108.7b	0.19	<0.0
AMCP	51.8b	61.1a	0.99	<0.01	56.1a	52.1a	47.1b	1.57	<0.01	62.4a	62.1a	59.0b	0.11	<0.0
2. Truly absorbed rumen undegraded feed protein in the small intestine (g/kg DM)														
RUP ^{NRC}	46.8b	142.5a	4.02	<0.01	48.1b	33.8c	58.6a	2.83	<0.01	149.1a	153.5a	124.9b	6.50	<0.0
ARUP	35.0b	95.7a	4.13	<0.01	39.9a	20.4b	44.7a	4.19	<0.01	97.7a	109.0a	80.3b	7.06	0.01
3. Endogenous protein in the digestive tract (g/kg DM)														
ECP	10.5b	10.9a	0.02	<0.01	10.5b	10.5a	10.5b	0.01	<0.01	11.0	10.9	10.9	0.03	0.04
AECP	4.2b	4.4a	0.01	<0.01	4.2b	4.2a	4.2b	0.00	<0.01	4.4a	4.3b	4.3b	0.01	0.03
4. Total truly absorbed protein in the small intestine (g/kg DM)														
MP	90.94b	161.16a	4.29	<0.01	100.2a	76.7b	96.0a	3.37	<0.01	164.5a	175.4a	143.6b	7.05	<0.0
5. Protein degraded balance (PDB, g/kg DM)														
DPB	-28.1b	174.7a	4.50	<0.01	-20.0a	-28.5ab	-35.7b	2.92	<0.01	186.4a	149.9b	187.7a	6.55	<0.0

SEM= standard error of mean. a-c Means with different letters at the same row for wheat and wheat DDGS, for the batches of wheat and for the batches of wheat DDGS are significantly different (P<0.05). Multi-treatment comparison by Tukey method. MCP_{RDP}, microbial protein synthesized based on RDP; MCP_{TDN}, microbial protein synthesized based on discounted TDN; RDP, rumen degradable protein; AMCP, truly absorbed microbial protein in small intestine; RUP^{NRC}, rumen undegraded feed protein; ARUP, truly absorbed feed protein in small intestine; ECP, rumen endogenous crude protein; AECP, truly absorbed endogenous protein in the small intestine; MP, truly absorbed total metabolizable protein in small intestine; DPB, degraded protein balance difference between potential microbial protein synthesis based on ruminally degraded feed protein and that based on energy –TDN available for microbial fermentation in the rumen.

Batch Effect on Truly Absorbed Rumen Undegraded Feed Protein in Small Intestine (ARUP)

Wheat DDGS was about three times higher in RUP compared to its parent grain wheat (143 vs. 47 g/kg DM for wDDGS and wheat, respectively). Wheat Batch 5 was highest in RUP (59 g/kg DM) compared to the lowest in Batch 3 (34 g/kg DM). The variation among batches of wDDGS in RUP was 25 g/kg DM. The same trend was observed with ARUP, among the batches of wDDGS (26 g/kg DM). The higher ARUP value in wDDGS compared to wheat makes wDDGS higher in MP value.

Batch Effect on Total Metabolizable Protein (MP)

Due to the higher contribution of MCP and RUP from wDDGS compared to wheat, wheat DDGS was higher in total metabolizable protein (MP) value (161 vs. 91 g/kg DM for wDDGS and wheat, respectively). Among the batches of wheat the range of MP was 24 g/kg DM and among the batches of wDDGS the range was 32 g/kg DM.

Batch Effect on Rumen Degraded Protein Balance (DPB)

The DPB was negative for all wheat samples, while DPB was positive for all wDDGS samples. The negative DPB value for wheat indicates the potential shortage of N for microbial protein synthesis. The positive DPB indicates the potential N loss from the rumen. The average PDB of wheat was -28 g/kg DM and DPB of wheat DDGS was 175 g/kg DM. Among the batches of wheat, PDB varied by 16 g/kg DM and among the batches of wDDGS varied by 38 g/kg DM. There were a significant differences ($P < 0.05$) in DPB among the batches of wheat and among the batches of wDDGS, which indicates the difference in protein metabolism in the rumen. In this study we used single feed ingredient to evaluate DPB in the rumen but in actual situation ration contains more feed ingredients which would be compensate and balance the feed protein degradation in the rumen and potential microbial synthesis. Zero or slightly positive DPB to ensure sufficient N supply for microbial protein synthesis is recommended in a ration.

Batch Effect of Wheat and Wheat DDGS on Metabolic Characteristics of Protein with the DVE/OEB - 1994 System

The predicted values with the DVE/OEB - 1994 system are summarized in Table A3.

Batch Effect on Microbial Protein Synthesis and Truly Absorbed Microbial Protein in Small Intestine

In DVE/OEB-1994 system, microbial protein synthesis is based on FOM. Since wheat was higher in organic matter content than in wDDGS, MCP_{FOM} and $AMCP^{DVE}$ values were higher in wheat. Among the batches of wheat, the range of MCP_{FOM} and $AMCP^{DVE}$ were 17 and 10 g/kg DM, respectively. The ranges among the batches of wDDGS were 5 g/kg DM for both MCP_{FOM} and $AMCP^{DVE}$ respectively. The microbial protein synthesized based on energy released during anaerobic fermentation process (MCP_{FOM}) was lower than the MCP synthesized based on RDP for wDDGS and the average values obtained were 105 vs. 272 g/kg DM for MCP_{FOM} and MCP_{RDP} , respectively. The MCP_{RDP} varied among the batches of wheat by 18 g/kg DM. Among the batches wDDGS, the variation (range) for MCP_{RDP} was 38 g/kg DM.

Batch Effect on Truly Absorbed Rumen Undegraded Feed Protein in Small Intestine ($ARUP^{DVE}$)

The rumen bypass protein (RUP) content was higher in wDDGS compared to the feedstock wheat (Table A3). As a result, $DRUP^{DVE}$ was greater in wDDGS (101 vs. 39 g/kg DM in wheat). The value of wheat DDGS as a bypass protein for the host animal indicates by the higher $ARUP$ content. The RUP^{DVE} content varied among the batches of wheat by 27 g/kg DM and among the batches of wheat DDGS by 32 g/kg DM.

Even though there were significant differences ($P < 0.05$) between wheat and wheat DDGS (3 vs. 4 g/kg DM in wheat vs. wheat DDGS) in endogenous protein losses, there was no significant difference observed among the batches of wheat and wheat DDGS.

Table A2. Batch effect of original feedstock wheat and their co-products-wheat DDGS on predicted nutrient supply using DVE-1994 modeling approach

Item	Wheat (n=6)	wDDGS (n=6)	SEM	P value	Batches of wheat			SEM	P value	Batches of wDDGS			SEM	P Value
					1 (n=2)	3 (n=2)	5 (n=2)			I (n=2)	III (n=2)	V (n=2)		
1. Truly absorbed rumen undegraded feed protein in the small intestine (g/kg DM)														
RUP ^{DVE}	52b	158a	4.5	<0.01	53b	38c	65a	3.1	<0.01	166a	170a	139b	7.2	<0.01
ARUP ^{DVE}	41b	106a	4.7	<0.01	44a	28.9b	49.6a	1.8	<0.01	109a	121a	89b	7.8	<0.01
2. Truly absorbed rumen synthesized microbial protein in the small intestine (g/kg DM)														
FOM	751a	698b	11.3	<0.01	778. a	788a	686b	11.7	<0.01	691b	685b	717a	6.9	<0.01
MCP _{FOM} ^{DVE}	113a	105b	1.70	<0.01	117a	118a	101b	1.8	<0.01	104b	103b	108a	1.0	<0.01
EDCP	95b	287a	4.3	<0.01	103a	95.8a	86.5b	2.9	<0.01	301a	264b	296a	6.6	<0.01
MCP _{RDP} ^{DVE}	90b	272a	4.6	<0.01	98a	92a	80b	3.2	<0.01	285a	247b	283a	7.3	<0.01
AMCP ^{DVE}	72a	67b	1.1	<0.01	74.4a	75.4a	65.6b	1.12	<0.01	66.1b	65.5b	68.6a	0.7	<0.01
TPSI	136b	237a	3.7	<0.01	141a	126b	142a	3.1	<0.01	243a	247a	219b	6.5	<0.01
3. Endogenous protein in the digestive tract (g/kg DM)														
UDM	39	39	1.8	0.96	32	39	45	3.5	0.09	39	38	39	1.5	0.92
ENDP	3b	4a	0.1	<0.01	3	3	4	0.26	0.06	4	4	4	0.1	0.68
4. Total truly absorbed protein in the small intestine (g/kg DM)														
DVE	109.3b	169a	4.3	<0.01	116a	101b	111a	1.5	<0.01	170a	182a	153b	7.3	<0.01
5. Protein degraded balance (OEB, g/kg DM)														
OEB	-23b	167a	4.0	<0.01	-19	-26	-23	3.3	0.13	181a	145b	175a	6.3	<0.01

SEM: standard error of the mean. Means with different letters at the same row for wheat and wheat DDGS, for the batches of wheat and for the batches of wheat DDGS are significantly different ($P<0.05$). Multi treatment comparison Tukey method. RUP, rumen undegraded feed protein; ARUP, rumen undegraded feed protein truly absorbed in small intestine; FOM, organic matter fermented in the rumen, MCP_{FOM}^{DVE}, microbial protein synthesized from rumen available energy; EDCP, effectively degraded feed protein in the rumen; MCP_{RDP}^{DVE}, microbial protein synthesized in the rumen based on rumen degraded feed protein; AMCP^{DVE}, truly absorbed rumen synthesized microbial protein in the small intestine; TPSI, true protein supplied to the small intestine; UDM, undigested dry matter; ENDP, endogenous protein losses in the digestive tract; DVE, truly absorbed protein in the small intestine supplied by RUP, MCP_{FOM} and correction for ENDP; OEB, difference between potential microbial protein synthesis based on rumen degraded feed protein and that based on energy available for microbial fermentation in the rumen.

Batch Effect on DVE and OEB Values

The wDDGS was higher in DVE (169 vs. 109 g/kg DM in wheat) and OEB value (167 vs. -23 g/kg DM in wheat). The range of DVE value among the batches of wheat was 15 g/kg DM while among the batches of wheat DDGS it was 29 g/kg DM. For the feedstock wheat the OEB value was negative which indicates the potential shortage of N for microbial protein synthesis.

Batch Effect of Wheat and Wheat DDGS on Metabolic Characteristics of the Protein Predicted Using DVE/OEB-2007 System.

The predicted values with the DVE/OEB-2007 system are summarized in Table A4.

Batch Effect on Microbial Protein Synthesis and Absorption in Small Intestine (DMCP)

In DVE/OEB 2007 system microbial crude protein synthesis was based on each fraction (S, W-S, D) of each nutrient component that fermented in the rumen. Subsequently the MCP based on FOM was higher in wheat (162 vs. 97 g/kg DM in wDDGS) and MCP based on RDP was higher in wDDGS (280 vs. 94 g/kg DM). The higher non-structural polysaccharide content in wheat (starch) (634 vs. 18 g/kg DM in wDDGS) must have provided the higher ATP yields (Russel and Strobel, 2005; Tamminga et al. 2007). Among the batches of wheat and among the batches of wDDGS, there were significant differences ($P < 0.05$) in MCP based on both FOM and RDP. As a result, the estimated MCP absorption in small intestine varied among the batches of feed stock grain wheat as well as among the batches of wheat DDGS. The range among batches of wheat in MCP based on FOM was 24 g/kg DM while the range among the batches of wDDGS was 15 g/kg DM. The MCP based on RDP varied by 17 g/kg DM among the batches of wheat while the variation (range) among the batches of wDDGS was 35 g/kg DM.

Table A3. Effect of different batches of feed stock wheat and wDDGS on prediction of nutrient supply to dairy cows using the DVE/OEB-2007 system.

	Wheat (n=6)	wDDGS (n=6)	SEM	P value	Batches of wheat			SEM	P value	Batches of wDDGS			SEM	P value
					1 (n=2)	3 (n=2)	5 (n=2)			I (n=2)	III (n=2)	V (n=2)		
1. Truly absorbed rumen synthesized microbial protein in the small intestine (g/kg DM)														
FOM	730a	518b	18.6	<0.01	733ab	785a	673b	46.5	0.02	507 b	508b	539a	46.6	<0.01
MCPE _{FOM}	162a	97b	3.5	<0.01	162ab	174a	150b	6.4	0.02	89b	98a	104a	7.1	<0.01
MCPN _{RDP}	94b	280a	4.3	<0.01	102a	95a	85b	2.3	<0.01	293a	258b	288a	7.4	<0.01
DMCP ^{DVE}	103a	62b	2.2	<0.01	103ab	111a	96b	4.1	0.02	57b	62a	66a	4.5	<0.01
2. Truly absorbed rumen undegraded feed protein in the small intestine (g/kg DM)														
DRUP ^{DVE}	39b	101a	5.6	<0.01	40.8a	28b	46a	2.0	<0.01	103a	114a	85b	7.8	<0.01
3. Endogeneous protein losses in the digestive tract (g/kg DM)														
UDM	145	168	14.8	0.10	164	138	132	30.8	0.58	168	160	177	4.8	0.09
DMFP	11	13	1.1	0.10	12	10	10	2.3	0.58	13	12	13	0.4	0.09
4. Total truly absorbed protein in the small intestine (g/kg DM)														
DVE	128b	150a	4.3	<0.01	132	121	132	5.6	0.21	147b	164a	139b	4.4	<0.01
5. Degraded protein balance (g/kg DM)														
OEB	-68b	183a	4.8	<0.01	-60a	-78b	-64ab	5.0	0.02	204a	160c	184b	4.7	<0.01

SEM: standard error of mean. a-b Means with different letters at the same row for wheat and wheat DDGS, for the batches of wheat and for the batches of wheat DDGS are significantly different ($P < 0.05$). Multi treatment comparison Tukey Method. MCPE_{FOM}, amount of microbial protein synthesized based on rumen available energy from each fraction of nutrient; MCPN_{RDP}, amount of microbial protein synthesized based on rumen available nitrogen; DMCP^{DVE}, truly absorbed rumen synthesized microbial protein in small intestine; DRUP^{DVE}, truly absorbed rumen undegraded feed protein in small intestine; UDM, undigested dry matter; DMFP, endogeneous protein losses in the digestive tract calculated as $DMFP = 0.075 \times (DM - DOM - dASH)$; DVE, total truly absorbed protein in small intestine; OEB, the difference between amount of microbial protein synthesized based on rumen available energy and amount of microbial protein synthesized based on rumen available N.

Batch Effect on Truly Absorbed Rumen Undegraded Feed Protein in Small Intestine (DRUP^{DVE})

The undegraded feed protein absorbed in small intestine was about three times higher in wDDGS than in wheat (101 vs. 39g/kg DM in wheat). There were significant differences ($P < 0.05$) among the batches of wheat and among the batches of wheat DDGS in DRUP values. Among the batches of wheat the variation was 18 g/kg DM and among the batches of wDDGS the DRUP values varied by 28 g/kg DM.

The undegraded dry matter (UDM) and the endogenous losses due to digestion were not significantly different among the wheat and wDDGS, among the batches of wheat and among the batches of wDDGS.

Batch Effect on DVE and OEB Values

The total truly absorbed protein in small intestine (DVE) was significantly different ($P < 0.05$) among the batches of wDDGS with a range of 25g/kg DM. Wheat DDGS was greater in both DVE and degraded protein balance OEB values (150 vs. 128g/kg DM in wheat for DVE and 183 vs. -68g/kg DM in wheat for OEB). The negative OEB values with the wheat indicate, when model nutrient availability of wheat as a single feed ingredient, the possibility of insufficient N supply to microbial production. Since wheat DDGS contain higher values of DVE it is a good true protein source in replacing expensive protein meal in animal diets.

Discussion

In contrast to the results obtained by Nuez-Ortín and Yu (2010) where both wheat and wDDGS were higher in RDP than in $1.18 \times \text{MCP}_{\text{TDN}}$, only wDDGS was higher in RDP than the $1.18 \times \text{MCP}_{\text{TDN}}$ in this study. The negative OEB values of wheat indicates the potential shortage of N for microbial protein synthesis. The negative OEB values of wheat compared to wDDGS is due to the fact that the microbial protein synthesized based on energy released during anaerobic fermentation process (MCP_{FOM}) is lower than the MCP synthesized based on RDP for the wDDGS. The obtained results for OEB values in wheat was not in agreement with the values obtained by Nuez-Ortín and Yu (2010) in which OEB for wheat was in

positive value. The values obtained for DVE was in agreement with the values obtained by Nuez-Ortín and Yu (2010). The results show that the wDDGS contained higher truly absorbable proteins of DVE which could be used to replace expensive protein meal in animal diet.

Conclusion

The three models used in this study (NRC-2001, DVE/OEB - 1994 and DVE/OEB - 2007) were to predict truly absorbable protein supply to small intestine of the host animal. All three models discussed the contribution of types of protein: truly absorbable rumen undegraded feed protein; truly absorbable rumen synthesized microbial crude protein and endogenous protein associated with the digestive process. Even though the three models discuss about the same terminology, the concepts of arriving into final calculated values differ among each other. The true protein supply to small intestine is considered as metabolizable protein (MP) in NRC-2001 model and MP is calculated as the summation of ARUP, AMCP and AECF. In both DVE/OEB models endogenous protein losses associated with digestion process considered as a loss. When predicting MCP synthesis, NRC - 2001 assume a fixed value based on TDN (130 g of MCP per kg of TDN) and DVE/OEB - 1994 assumes fixed value based on fermentable organic matter content (150 g of MCP per kg of FOM). The DVE/OEB 2007 system does not assume any fixed value for MCP synthesis and estimation was done based on contribution of each fraction (S, W-S, D) in each nutrient component in feed for FOM. All three models consider zero or positive protein degradable or OEB value to ensure sufficient supply of N for MCP synthesis. According to the predicted values with the three nutrient modelling systems, there were significant differences ($P < 0.05$) in truly absorbed protein supply to small intestine and degraded protein balance in dairy cattle among the batches of wDDGS.

All three models pointed out that wheat DDGS produced in western Canada is an excellent source of truly absorbable protein supply to dairy cattle.