Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy 118 (2014) 407–421

Contents lists available at ScienceDirect

Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy

journal homepage: www.elsevier.com/locate/saa

# Non-destructive analysis of the conformational differences among feedstock sources and their corresponding co-products from bioethanol production with molecular spectroscopy

I.H. Gamage [a], A. Jonker [a,b], X. Zhang [c], P. Yu [a,c,*]

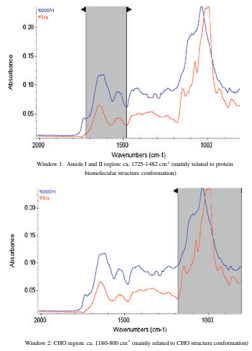
[a] Department of Animal and Poultry Science, College of Agriculture and Bioresources, University of Saskatchewan, 51 Campus Drive, Saskatoon, SK S7N 5A8, Canada
[b] Grasslands Research Centre, Tennent Drive, Private Bag 11008, Palmerston North 4442, New Zealand
[c] Department of Animal Science, Tianjin Agricultural University, 22 Jinjin Road, Xiqing District, Tianjin 300384, China

## HIGHLIGHTS

- Molecular spectroscopy revealed the molecular structural differences.
- Multivariate analyses distinguished the molecular structure differences.
- Structural differences were associated with chemical and nutrient profiles.

## GRAPHICAL ABSTRACT

Biomolecular spectrum of feedstock (wheat) for bioethanol production (red) and co-products from bioethanol processing (blue) at different windows, which were used for multivariate molecular spectral analyses (PCA and CLA) to show batch different.




*Abbreviations:* ADF, acid detergent fibre; 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; ARUP, truly absorbed bypass feed protein in small intestine; AHCA, agglomerative hierarchical cluster analysis; 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); CHO, carbohydrate; CFat, crude fat; CP, crude protein; Cu, copper; D, potentially degradable fraction of in situ rumen incubation; $DE_{3x}$, digestible energy at a production level when intake is 3 times maintenance intake; 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 intestne; DVME, microbial protein synthesized in the rumen and absorbed in small intestine; DVMFE, endogeneous protein in the faeces associated with the digestion; ECP, correction for endogenous protein losses associated with digestion process; ED, effective degradability; EDCP, effective degradability of crude protein; FA, fatty acids; FOM, fermentable organic matter in the rumen; $K_d$, rate constant for in situ rumen degradation of D fraction; $K_p$, rate of passage; NDF, neutral detergent fibre $NE_L$, Net energy for lactation; NPN, non protein nitrogen; NSC, non structural carbohydrate; $MCP_{FOM}$, 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; nNDF, nitrogen corrected neutral detergent fibre NDICP, neutral detergent insoluble crude protein; NDICP, neutral detergent insoluble crude protein; NE, net energy; $NE_m$, net energy for maintenence; $NE_g$, net energy for gain; $NEL_{3x}$, net energy for lactation when intake is 3 times maintenance intake; NFC, non-fibrous carbohydrate; OM, organic matter; PCA, principal component analysis; PB1, rapidly degradable true protein; PB2, intermediately degradable true protein; PB3, slowly degradable true protein; PC, undegradable protein; RDP, rumen degraded protein; SCP, soluble crude protein; S, soluble fraction; $T_0$, lag time; tdCP, truly digestible crude protein; tdFA, truly digestible fatty acids; $TDN_{1x}$, total digestible nutrient at a maintenance level; tdNDF, truly digestible NDF; tdNFC, truly digestible non-fibrous carbohydrate; TP, true protein; U, undegradable fraction.

* Corresponding author. Address: 6D10 Agriculture Building, 51 Campus Drive, 20 Saskatoon, SK S7N 5A8, Canada. Tel.: +1 306 966 4132.
*E-mail address:* peiqiang.yu@usask.ca (P. Yu).

1386-1425/$ - see front matter 
http://dx.doi.org/10.1016/j.saa.2013.08.095

ARTICLE INFO

*Article history:*
Received 14 July 2013
Received in revised form 15 August 2013
Accepted 23 August 2013
Available online 5 September 2013

*Keywords:*
Molecular spectral analysis
Bioethanol co-products
Feedstock sources

ABSTRACT

The objective of this study was to determine the possibility of using molecular spectroscopy with multivariate technique as a fast method to detect the source effects among original feedstock sources of wheat and their corresponding co-products, wheat DDGS, from bioethanol production. Different sources of the bioethanol feedstock and their corresponding bioethanol co-products, three samples per source, were collected from the same newly-built bioethanol plant with current bioethanol processing technology. Multivariate molecular spectral analyses were carried out using agglomerative hierarchical cluster analysis (AHCA) and principal component analysis (PCA). The molecular spectral data of different feedstock sources and their corresponding co-products were compared at four different regions of ca. 1800–1725 $cm^{-1}$ (carbonyl C=O ester, mainly related to lipid structure conformation), ca. 1725–1482 $cm^{-1}$ (amide I and amide II region mainly related to protein structure conformation), ca. 1482–1180 $cm^{-1}$ (mainly associated with structural carbohydrate) and ca. 1180–800 $cm^{-1}$ (mainly related to carbohydrates) in complex plant-based system. The results showed that the molecular spectroscopy with multivariate technique could reveal the structural differences among the bioethanol feedstock sources and among their corresponding co-products. The AHCA and PCA analyses were able to distinguish the molecular structure differences associated with chemical functional groups among the different sources of the feedstock and their corresponding co-products. The molecular spectral differences indicated the differences in functional, biomolecular and biopolymer groups which were confirmed by wet chemical analysis. These biomolecular and biopolymer structural differences were associated with chemical and nutrient profiles and nutrient utilization and availability. Molecular spectral analyses had the potential to identify molecular structure difference among bioethanol feedstock sources and their corresponding co-products.



## Introduction

Bioethanol processing results in producing large amount of co-products from bioethanol production [1]. These co-products are good sources as animal feeds. In a recent study [2], it was found that there were significant differences in chemical and nutrient profiles among the original feedstock source and their relevant co-products of wheat DDGS (wDDGS). To test the sources and batch effect on nutrient value of feedstock and co-products, we need to do traditional wet chemical analysis, in situ and in vitro techniques and animal digestibility trial.

The question is how to fast detect batch or sources difference within a short time among feedstock sources of wheat and co-products from bioethanol production. The traditional "wet" chemical analysis and in situ rumen studies are labor intensive and time consuming [3]. It is practically almost impossible to do in vivo animal trials for many feed treatments. Thus there is an urge need to have a technique which enables to identify the sources and batch difference with a quick method.

The most commonly used infrared vibrational spectroscopy in feed evaluation is nearIR spectroscopy technique. However, nearIR technique is unable to identify functional groups (biomolecular and biopolymers) in feeds. But the functional groups are close related to nutrient utilization and availability [4,5]. Published studies [6,7] revealed the possibility of using Fourier transformed infrared vibration (micro)spectroscopy as a potential method to detect chemical–structural difference of co-products and are related to feed quality and nutrient utilization and availability in animals.

The importance of structure or spatial conformation in identifying feed micro-structure further enhances the infrared spectroscopy 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 IR molecular spectra are produced with FT/IR spectrometers and 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 [8]. With, in conjunction with multivariate spectral analysis, many structural data related to biological tissues can be identified and categorized [9]. The multivariate spectral analysis methods of agglomerative hierarchical cluster analysis (AHCA) and principal components analysis (PCA) can be used to classify and discriminate the data related to matrix conformation in relation to chemical and structural makeup [10]. With AHCA, the spectroscopic data can be clustered according to similarity of spectra and the results can be interpreted based on different clusters [8].

The objective of this study was to study possibility of using molecular spectroscopy with multivariate technique as a fast method to detect structural difference among original feedstock sources and their corresponding co-products from bioethanol production. The hypothesis of this study was that the differences in chemical and nutrient profiles were related to molecular structural differences. These differences could be revealed with molecular spectroscopy with multivariate technique.

## Materials and methods

### *Feedstock sources and their corresponding co-product from bioethanol production*

Five sources of feedstock grain (wheat: 3 samples per batch) and their corresponding five batches of the co-product (wheat DDGS: 3 samples per batch) were collected from a newly-built bioethanol plant with current bioethanol processing technology in western Canada. The chemical profiles, nutrient profile, particle size distribution, in situ degradation kinetics, intestinal digestion and modeling nutrient supply were published previously [2]. The variation of range and %CV as well as mean of detailed chemical and nutrient profile affected by sources are summarized in Tables 1–3 and full results in [2].

### *Molecular spectroscopy*

Samples from feedstock wheat sources 1, 3, 5 and their corresponding co-product of wheat DDGS batches I, III, V were finely