Quality and SAfety of Feeds and Food for Europe

ATR-FT-MIR spectroscopy after in situ fat extraction as a control tool for the identification of DDGs origin

Philippe Vermeulen*, Juan Antonio Fernández Pierna, Ouissam Abbas, Pierre Dardenne, Vincent Baeten

Walloon Agricultural Research Centre (CRA-W), Valorisation of Agricultural Products Department,
Chaussée de Namur, 24, B-5030 Gembloux (Belgium), *p.vermeulen@cra.wallonie.be

Introduction

The ban for using processed animal protein in the feeding stuffs led the feed sector to prospect other possible protein sources. Among the different possibilities, Dried Distillers Grains with Solubles (DDGs), which is a sub product of some industrial processes, could be also considered as an important source to take into account. In USA, 30 % of the produced corn is dedicated to ethanol generation and the DDGs obtained as residue of the process are largely exported to Europe. However, the possible use of antibiotics or fermentation supplements to improve ethanol production could include some risks in the feed chain. To reduce this risk and to identify quickly the origin of DDGs, the current study proposes a method based on the fatty acid composition, using the Attenuated Total Reflection Fourier Transform Mid Infrared Spectroscopy (ATR-FT-MIR), directly by extracting in situ the fat fraction of the DDGs sample.

Material

For this experiment, 184 samples of DDGs were collected and analysed. These are residues from two different processes: alcoholic beverage or biofuel production derived from corn or wheat, mainly from USA, China and Europe. **Table 1** presents a summary of the samples used in this study and their fat content estimated using calibration equations constructed with NIR (Near Infrared) historical databases from the PROVIMI company. This table puts into evidence the large fat variability in DDGs samples (from 2 % to 12.4 %) and the slightly high variability inside each group (from 0.16 % to 1.5 %).

Table 1: Predicted fat content by NIR for the available samples.

		Fat content (%)			
Group	Nb	Min	Max	SD	Mean
Corn China Heilongjiang (biofuel)	30	2.0	5.2	0.65	3.1
Corn China Jilin (biofuel)	18	6.6	10.4	1.1	8.0
Corn EU Czech Republic (biofuel)	21	7.5	9.4	0.60	8.4
Corn USA (biofuel)	15	7.5	12.3	1.5	9.4
Corn USA Mississippi (beverage)	10	8.3	11.9	1.2	10.0
Corn USA other (beverage)	26	6.6	12.0	1.5	10.1
Corn other source	33	6.1	12.4	1.3	10.9
Wheat France (biofuel)	13	3.5	5.2	0.43	4.4
Wheat other source	13	4.4	7.2	0.72	5.1
Wheat Canada (biofuel)	5	5.1	5.4	0.16	5.2

Nb = number of samples; Min = minimum value; Max = maximum value; SD = standard deviation; Mean = average value

Method

ATR-FT-MIR spectra of DDGs samples were acquired using a Bruker Vertex 70 Fourier Transform spectrometer equipped with a golden gate Attenuated Total Reflectance accessory. Samples were spread and the measure has been done directly on the fat extracted in situ by pressing during 3 minutes, on a diamond crystal, the DDGs powder inside a ring and over a paper filter (**Figure 1**). This original protocol allows to reduce the sample preparation time and is performed without solvent extraction neither chemical transformation, which could have an influence on the fat composition.

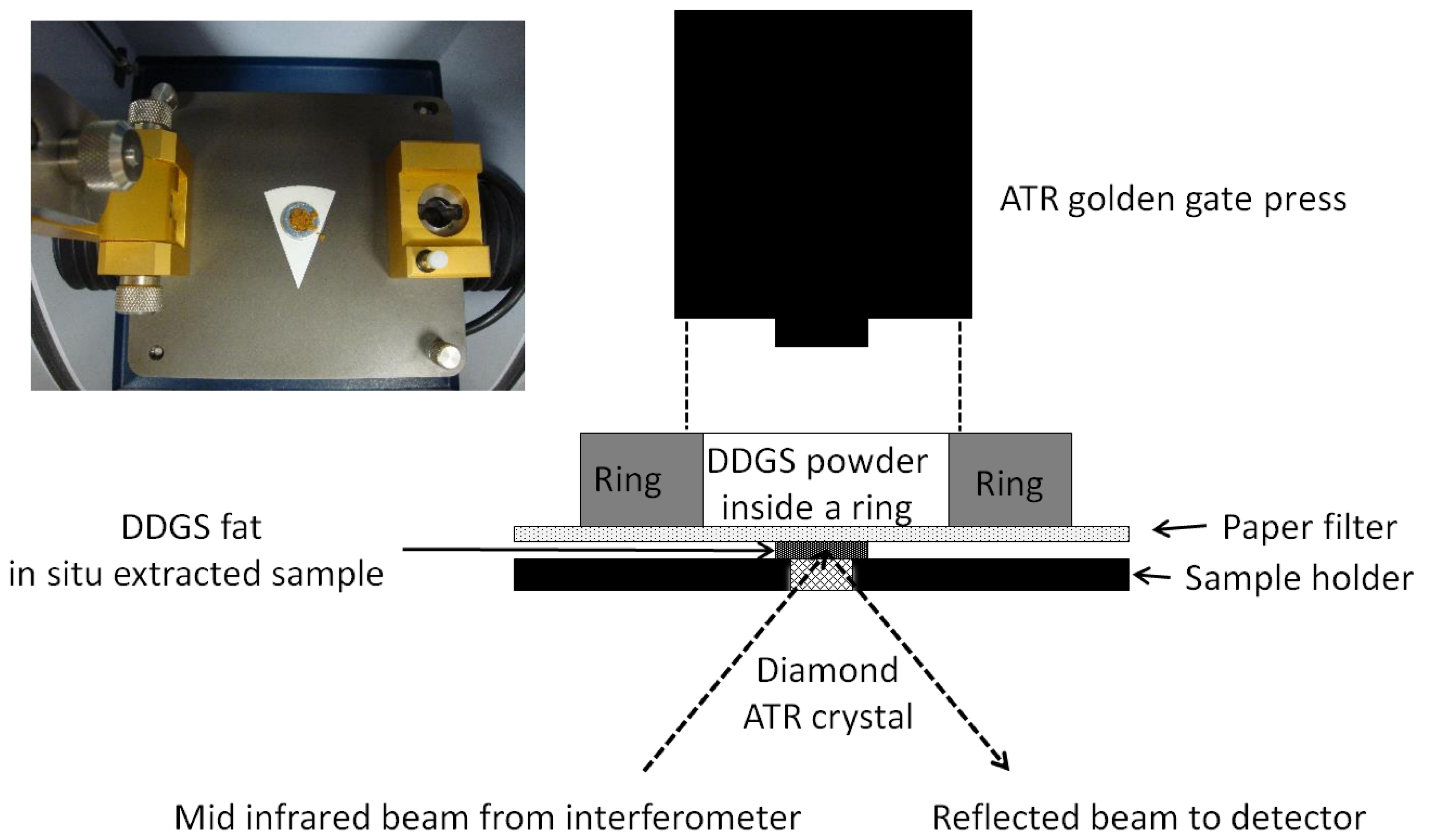

Figure 1: In-situ fat extraction sample preparation for ATR-FT-MIR analysis.

Principal Component Analysis (PCA): exploratory study

The use of chemometric tools as PCA for exploratory analysis (**Figure 2**) and PLS-DA (Partial Least Squares Discriminant Analysis) as supervised classification method allowed to discriminate the DDGS spectra, in a dichotomist way, according to their botanical (corn/wheat DDGs) and their geographical origin (corn DDGs from China/USA-EU) as well as to their industrial process origin (corn Chinese processes, corn Czech process, wheat French process) (**Figure 3**).

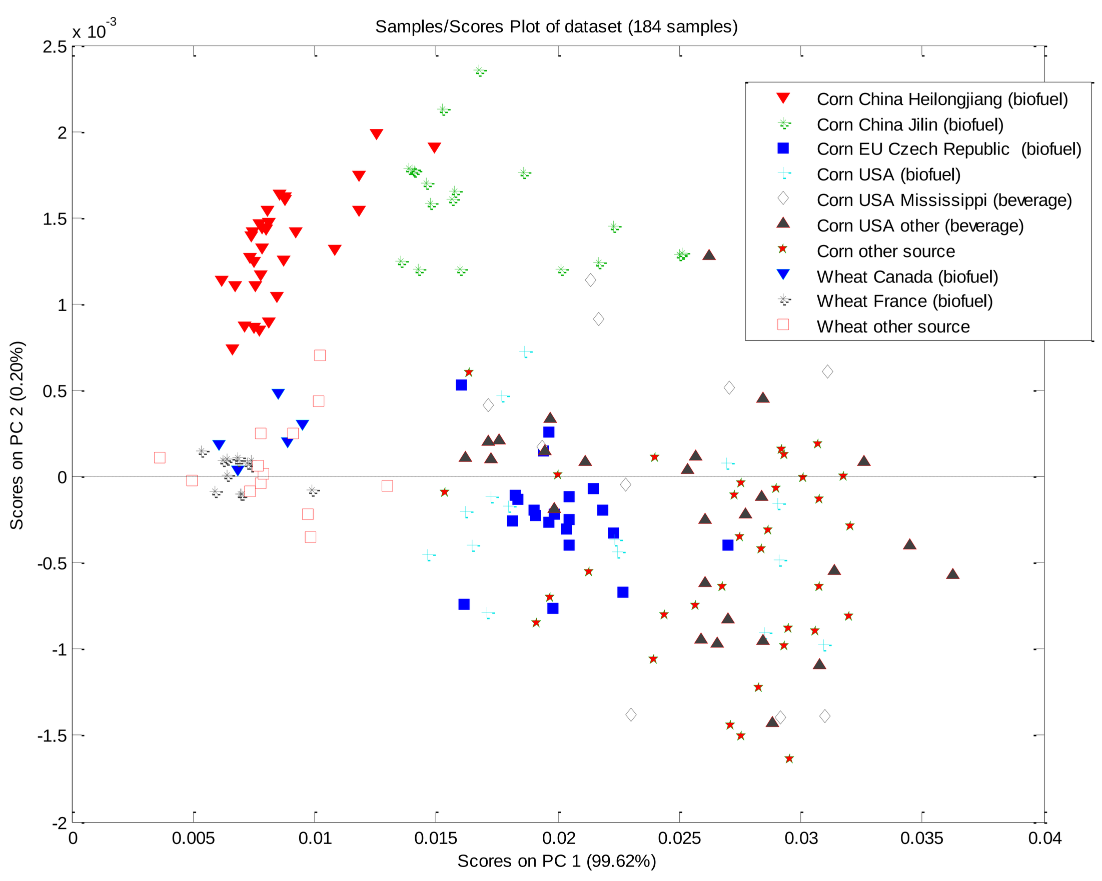

Figure 2: PC1 vs PC2 score plot on pre-processed ATR-FT-MIR spectra (1st Derivative Savitzky-Golay (window = 7, polynomial=2)) putting into evidence several geographical/process origin groups.

Partial Least Squares Discriminant Analysis (PLS-DA): classification

PLS-DA models were developed following two different approaches. Firstly, the full dataset was used as calibration set and validated by using the leave-one-out cross-validation method. With the second approach, a training set of 80% of the samples randomly selected was used to build the model, which has been validated using the remainder 20 % of the samples. **Figure 4** shows the performance of the models.

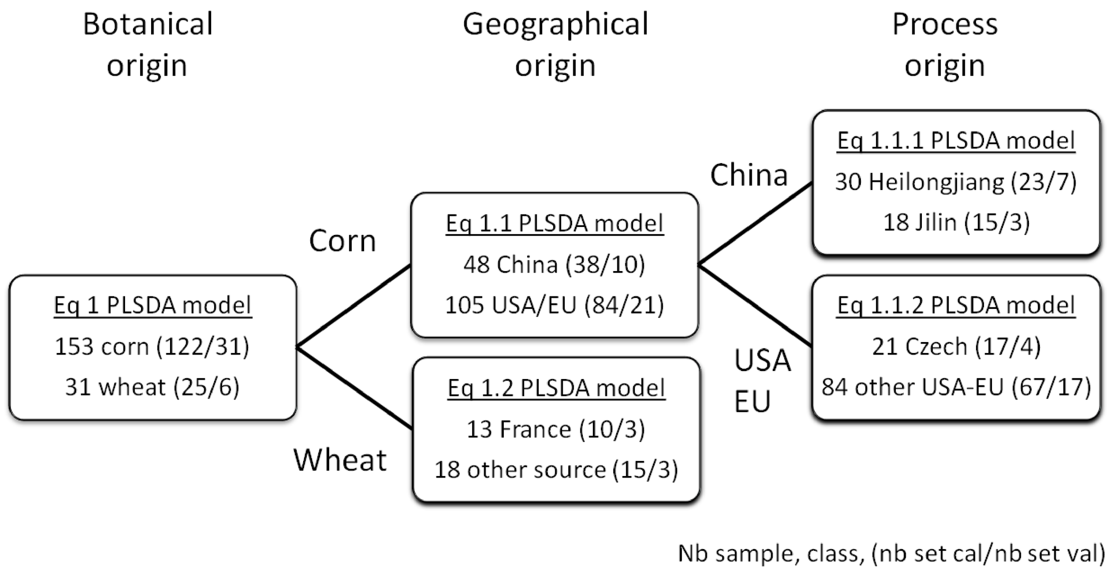

Figure 3: Datasets used to build the PLS-DA equations. Total number of samples and samples used for calibration and validation (c/v) are included in the boxes.

	Eq1 Corn vs wheat	Eq1.1 Corn China vs USA/EU	Eq1.1.1 Corn China Heilongjiang vs Jilin	Eq1.1.2 Corn Czech Republic vs Other	Eq1.2 Wheat France vs other
Approach 1	**5 LV**	**6 LV**	**4 LV**	**5 LV**	**4 LV**
Calibration	**153 corn, 31 wheat**	**48 China, 105 USA/EU**	**30 Heilongjiang, 18 Jilin**	**21 Czech Republic, 84 other**	**13 France, 18 other**
Sensitivity	100.0	100.0	100.0	100.0	100.0
Specificity	100.0	100.0	94.4	96.4	100.0
Classification error	0.0	0.0	2.8	1.8	0.0
Cross-validation	**153 corn, 31 wheat**	**48 China, 105 USA/EU**	**30 Heilongjiang, 18 Jilin**	**21 Czech Republic, 84 other**	**13 France, 18 other**
Sensitivity	99.3	100.0	93.3	71.4	92.3
Specificity	100.0	99.0	94.4	92.9	77.8
Classification error	0.3	0.5	6.1	17.9	15.0
Approach 2	**4 LV**	**5 LV**	**6 LV**	**9 LV**	**5 LV**
Calibration	**122 corn, 25 wheat**	**38 China, 84 USA/EU**	**23 Heilongjiang, 15 Jilin**	**17 Czech Republic, 67 other**	**10 France, 15 other**
Sensitivity	100.0	100.0	100.0	100.0	100.0
Specificity	100.0	100.0	100.0	100.0	100.0
Classification error	0.0	0.0	0.0	0.0	0.0
Cross-validation	**122 corn, 25 wheat**	**38 China, 84 USA/EU**	**23 Heilongjiang, 15 Jilin**	**17 Czech Republic, 67 other**	**10 France, 15 other**
Sensitivity	99.2	100	87.0	70.6	70.0
Specificity	96.0	97.6	86.7	82.1	86.7
Classification error	2.4	1.2	13.2	23.7	21.7
Validation set	**31 corn, 6 wheat**	**10 China, 21 USA/EU**	**7 Heilongjiang, 3 Jilin**	**4 Czech Republic, 17 other**	**3 France, 3 other**
Sensitivity	100.0	100.0	100.0	100.0	100.0
Specificity	100.0	100.0	100.0	94.1	100.0
Classification error	0.0	0.0	0.0	2.9	0.0

Figure 4: Calibration and validation performance of the discrimination models, in terms of sensitivity, specificity and classification error.

Conclusion

This study has shown the potential of ATR-FT-MIR to discriminate DDGs according to different origins (botanical, geographical and industrial process) and using an in-situ fat extraction. In comparison to other vibrational spectroscopy methods performed on DDGs powder, this method allows analyzing only the fat fraction and the composition of the fatty acids.

www.qsaffe.eu

The research leading to these results has received funding from the European Union Seventh Framework Programme (FP7/2007-2013) under grant agreement n° 265702.