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# Origin identification of dried distillers grains with solubles using attenuated total reflection Fourier transform mid-infrared spectroscopy after *in situ* oil extraction

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ABSTRACT

The ban on using processed animal proteins in feedstuffs led the feed sector to look for other sources of protein. Dried distillers grains with solubles (DDGS) could be considered as an important source in this regard. They are imported into Europe mainly for livestock feed. Identifying their origin is essential when labelling is missing and for feed safety, particularly in a crisis situation resulting from contamination. This study investigated applying attenuated total reflection Fourier transform mid-infrared spectroscopy (ATR-FT-MIR) to the oil fraction extracted from samples *in situ* in order to identify the origin of DDGS. The use of spectroscopic and chemometric tools enabled the botanical and geographical origins of DDGS, as well as the industrial process used to produce them, to be identified. The models developed during the study provided a classification higher than 95% using an external validation set.



## 1. Introduction

The ban on using processed animal proteins in feedstuffs led the feed sector to look for other sources of protein. Among the various possibilities, and apart from soybean meal (the main protein source in feed), it was thought that dried distillers grains with solubles (DDGS) could be a source worth considering. As noted in Commission Regulation (EU) No. 575/2011, which deals with feed materials, dried distillers grains are the products of alcohol distillation, obtained by drying the solid residues of fermented grains (e.g., corn, wheat). DDGS are those grains to which syrup from the fermentation or evaporated spent wash has been added.

The process involved in producing ethanol by fermenting yeasts from starch-containing plants is essentially the same as that for producing bioethanol and alcoholic beverages. The expansion of bioethanol production as a renewable energy source has led to increased availability of many co-products as livestock feed and greater variability in their composition. There are many reasons for the variability in DDGS composition, including differences in grain source (i.e., species, varieties) and composition, the process scheme and parameters, the amount of condensed solubles added to wet distiller grains, the effect of fermentation yeast, and analytical methodologies (Liu, 2011).

The first factor of variability is grain source. The starch used to produce ethanol comes mainly from 2 sources: corn and wheat (Cooper & Weber, 2012). Fermenting wheat concentrates the fat and protein content from about 2% to 6% and 13% to 38%, respectively. Fermenting corn concentrates the fat and protein content from about 4% to 11% and 9% to 26%, respectively (Beltranena & Zijlstra, 2008). The main differences between wheat and corn fat are explained by a higher rate of long fatty acid chain in corn (86% of C18) than in wheat (78% of C18) as well as a lower rate of saturated fatty acids in corn (12% of fatty acid total) than in wheat (18% of fatty acid total) and a higher rate of mono-unsaturated fatty acids in corn (28% of fatty acid total) than in wheat (15% of fatty acid total) (Morand-Fehr & Tran, 2001). Variety is also important. Some cereal breeding programs focus on breeding cultivars that will have a higher ethanol yield and improved DDGS composition. For example, the fat content of soft/hard, red/white and spring/winter wheat cultivars can differ significantly (Davis et al., 1980).

The second factor of variability is the industrial process used to produce ethanol and co-products and the various ways of optimising it. Processing methods used for raw grain can have a great impact on protein, fat, fibre and minerals rates and feeding characteristics. For example, the fractionation processes performed prior to fermentation are used to separate hulls/straw, germ and endosperm in order to produce co-products such as grain bran rich in fibre, grain germ rich in oil and distillers grains with high protein and low fat/fibre content. Another process that involves separating

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the gluten from the starch produces co-products such as grain gluten and low protein/high fat distillers grains. In recent years, several processes have been developed to enhance the use of DDGS products. Separating fibre from DDGS via sieving and elutriation produces enhanced DDGS with increased fat and protein content (Srinivasan et al., 2006). Oil extraction from DDGS using centrifugation to produce biodiesel gives low-fat DDGS products. Diaz-Royon, Garcia, and Rosentrater (2012) published a review of the nutrient composition of DDGS that indicated great variability in fat content depending on the process. Moreau, Liu, Winkler-Moser, and Singh (2011) showed that the free fatty acid content depends on the ethanol plant and the process used.

The US produces 50% of the world's ethanol and exports 25% of the DDGS for livestock feed (Cooper & Weber, 2012). The use of antibiotics or fermentation supplements to improve the ethanol production process can result in these products being present in the feed chain. The presence of mycotoxins in grain can lead to the enrichment of this contaminant in DDGS (Zhang, Caupert, Imerman, Richard, & Shurson, 2009). It is crucial to develop tools that detect mislabeled and suspicious samples that, after appropriate confirmation of the adulterant/contaminant, can therefore be removed from the feed chain. Origin identification, particularly in a crisis situation caused by contamination or adulteration, can be essential for feed safety, especially when there is mislabeling or when analysis of the contaminant is difficult or too expensive. Studies on the traceability of feed and feed materials are rare, although feed and feed materials are based on plant material that has many uses in the food sector (e.g., olive oil, wine, juice, water, honey, meat) (Kelly, Guillou, & Brereton, 2010).

Fourier transform mid-infrared (FT-MIR) spectroscopy is a potential technology for feed authentication based on molecular structure. The ratio between saturated and unsaturated groups, or the ratio between free fatty acid and triglyceride, can be used to identify the botanical origin of wheat/corn DDGS and the process used to create them (Baeten & Dardenne, 2002). Two variants of FT-MIR can be used: diffuse reflectance infrared Fourier transform spectroscopy (DRIFTS) and attenuated total reflectance Fourier transform spectroscopy (ATR-FT-MIR).

The DRIFTS method involves using diffuse reflectance absorbance as a rapid sample fingerprinting method that generates a chemical profile of the sample by presenting the dried samples to an FT-MIR spectrometer. The promising results obtained with grain flour using this technique to predict protein content in ground wheat (Reeves & Delwiche, 1997) led to it being applied to DDGS. DRIFTS has been used to compare the structural characteristics of carbohydrates in wheat and corn DDGS (Yu, Damiran, Azarfar, & Niu, 2011) and provided evidence of significant differences in cellulosic compounds and total carbohydrate, depending on the wavenumber range. DRIFTS has also been used to detect differences between the protein molecular structures in amide I and amide II in wheat DDGS and mixed wheat/corn DDGS (Azarfar, Jonker, & Yu, 2013).

The ATR-FT-MIR method involves using ATR in conjunction with a diamond and an FT-MIR spectrometer. It is useful for analysing materials that are too thick or too opaque for transmission (Karoui, Fernández Pierna, & Dufour, 2008). It has been shown, for example, to be a useful tool for discriminating grains from various hulled wheat species (Suchowilska, Kandler, Wiwart, & Krska, 2012), for determining the *trans* fatty acids in ground cereals products without oil extraction (Yookyung, Himmelsbach, & Kays, 2007), for authenticating the botanical and geographical origins of edible oils based on fatty acid composition (Vermeulen, Abbas, Dardenne, Baeten, & Fernández Pierna, 2010) and for adulteration detection of vegetable oils (Abbas, Dardenne, & Baeten, 2012).

Since DDGS contain up to 15% fat, this technique was used to analyse the fat composition of wheat and corn DDGS. Nietner, Pfister, Glomb, and Fauhl-Hassek (2013) obtained a correct classification of more than 80% in identifying botanical origin and discriminating between DDGS originating from China and the US applying ATR-FT-MIR to DDGS in solid state and to oil extracted by solvent.

This study focused on using ATR-FT-MIR to identify the botanical and geographical origins of wheat and corn DDGS, as well as the industrial process used to produce them (i.e., corn DDGS produced in Jilin or in Heilongjiang companies in China; wheat DDGS produced in Canadian or French companies). The authentication was based on analysing only the composition of the DDGS oil fraction, without taking into account the fat, fibre and protein content. The originality of the study lies in the *in situ* extraction of the oil, without solvent or chemical transformation, thus preventing possible influences on the composition of the oil and reducing drastically the analytical time.

## 2. Materials and methods

### 2.1. Samples

A set of 125 DDGS samples grouped into 5 batches according to production periods between 2011 and 2013 was collected within the framework of the Qsaffe European project (Qsaffe, 2011). The samples were by-products from the production of biofuel and alcoholic beverages from corn, wheat or a mixture of both, mainly from the US, Canada, China and Europe. The set consisted of 23 samples of wheat DDGS and 102 samples of corn DDGS. 4 wheat DDGS samples were from Canada, 8 from France and 11 from other known and unknown sources. For the corn DDGS, 45 samples were from China (28 from Heilongjiang and 17 from Jilin, 29 from the US and 13 from Europe. The corn DDGS from the US came from biofuel or beverage production sites, including 11 samples from beverage production in the Mississippi area, 10 from beverage production in other areas and 8 from biofuel production. 9 European DDGS samples came from the Czech Republic. The 19 remaining corn DDGS samples came from other known and unknown sources.

An additional batch of 30 DDGS was collected and used as an external validation set. The selection of these test samples was based on ensuring representative botanic (corn and wheat), geographical (USA, China, Europe (Austria, Czech Republic, Poland, The Netherlands) and process origins (Chinese (Heilongjiang, Jilin), French, Canadian) in the calibration set.

All the samples were ground to 0.5 mm and homogenised in plastic containers for 6 h using a drum hoop mixer. Only 50 g of the ground sample was distributed and analysed. The botanical origin as labelled by the provider was checked using the isotope ratio mass spectrometry (IRMS). This is the proven technique in food authenticity, to identify the plant origin from the estimation of C3/C4 (wheat/corn) plant material composition based on $\delta^{13}C$ values of DDGS. In addition, some doubtful samples were analysed by polymerase chain reaction (PCR) to confirm the botanical origin. These results will be published in a further paper aiming to compare the different methods developed in the framework of the Qsaffe project.

### 2.2. ATR-FT-MIR analysis

The ATR-FT-MIR spectra of the DDGS were acquired using a Bruker Vertex 70 Fourier transform spectrometer equipped with an ATR golden gate accessory. The spectra (4000–600 $cm^{-1}$) were acquired at a resolution of 4 $cm^{-1}$ with 64 co-added scans/spectrum. The spectral acquisition was done using OPUS 6.5 (Bruker) software.

In order to analyse the DDGS fat composition and to reduce sample preparation time, the DDGS oil was extracted *in situ* on the ATR crystal. This original and innovative sample presentation

(called '*in situ* oil extraction') involves spreading, after homogenisation, a few grams of the sample (DDGS powder in this case) inside a small ring placed on a filter over a diamond crystal. After removing the ring, pressure using the bridge of the golden gate is exerted for 3 min. The filter is then removed and the thin film of extracted oil in contact with the diamond is analysed using FT-MIR. Fig. 1 shows a cross-section of the sample on the ATR golden gate and the sample preparation steps. A beam of infrared light passes through the ATR crystal in such a way as to interact with the sample. This procedure was repeated in triplicate for each sample, taking care to clean the ATR crystal and press between each analysis. The ATR-FT-MIR technique needs just a small sample quantity because the wave penetrating the sample is only a few microns (0.5–5 μm).

### 2.3. Chemometric tools

Once the spectra had been acquired, they were converted from OPUS into MATLAB format using MATLAB version 7.5 (The Mathworks Inc., Natick, MA, USA) and data treatment was performed using PLS-toolbox version 7.0.2 (Eigenvector Research, Inc. 2012). Replicates of the ATR-FT-MIR analyses were averaged. All the spectra were pre-processed using first derivative Savitzky–Golay (window = 7, polynomial = 2) in order to smooth and separate overlapping bands.

Various chemometric methods were applied in order to extract the maximum amount of information from the DDGS data. As a first step, the unsupervised statistical tool principal component analysis (PCA) was applied to the calibration set to get some indication about the natural grouping of the DDGS. Based on this information, a dichotomist classification tree could be built where each node of the tree corresponded to a discrimination model for a specific group of DDGS (Fernández Pierna et al., 2012). These models were developed using the supervised technique partial least squares discriminant analysis (PLS-DA) (Wise et al., 2006) and allowed classifying new samples as within a certain group of DDGS. Moreover and in order to characterise the DDGS origin with a minimum set of variables, the Fisher coefficient was used to select the wavelengths where between-group variation was higher than within-group variation (Welling, 2009). PLS-DA models on these Fisher selected variables were also developed.

For the optimisation of the PLS-DA model, leave-one-out cross-validation (LOOCV) was carried out in order to find the number of latent variables showing the lowest classification error. Using this method, each sample was, in turn, left out of the model formulation and independently predicted. The models built with the 3 selected regions or the selected wavenumbers were then applied to the external validation set of 30 DDGS samples and interpreted in terms of their suitability for the identification/discrimination of botanical, geographical or industrial process origins. The performance of the models was expressed in terms of sensitivity or true positive results (percentage of samples from the group studied that had been correctly classified by the model), and specificity or true negative results (percentage of samples, not from the group studied, that had been correctly classified by the model). Classification error was then calculated from the sum of the false positive results (percentage of samples predicted as belonging to the group studied when they did not) and false negative results (percentage of samples predicted as not belonging to the group studied when they did).

## 3. Results and discussion

### 3.1. Spectral characteristics

Typical spectra from oil extracted *in situ* from wheat and corn DDGS that were obtained using the ATR-FT-MIR spectrometer are presented in Fig. 2, which clearly shows that they correspond to characteristic spectra of fatty acids (Baeten, Aparicio, Marigheto, & Wilson, 2000).

Before model construction, some wavenumber ranges (4000–3200 $cm^{-1}$; 2600–1800 $cm^{-1}$; 1690–1470 $cm^{-1}$; 700–600 $cm^{-1}$) were removed. These ranges were known not to have fat-related information, as well as being likely to cause interference via $H_2O$ vapour, $CO_2$ and noisy signals.

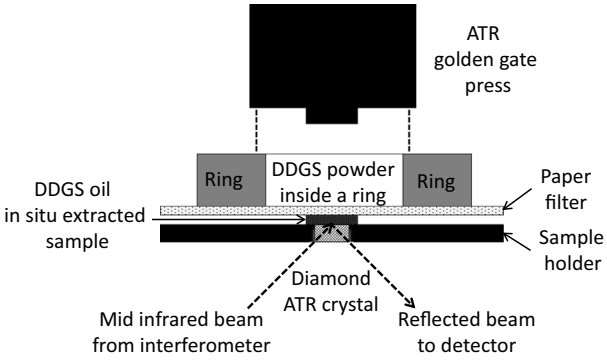
The selected wavenumber ranges (3200–2600 $cm^{-1}$, 1800–1690 $cm^{-1}$ and 1470–700 $cm^{-1}$) were used to develop the models. In the first region (3200–2600 $cm^{-1}$), 3 bands were observed around 3009, 2923 and 2853 $cm^{-1}$. The band around 3009 $cm^{-1}$ were close to the band around 3006 $cm^{-1}$ described by Guillen and Cabo (1997) as due to the C—H stretching vibration of the *cis* —CH=CH— double bonds. van de Voort, Ismail, and Sedman (1995) underlined the fact that spectral features change with the degree of unsaturation. The peak centered near 3006 $cm^{-1}$ shifted to higher frequency as the degree of unsaturation rises. The band around 3009 $cm^{-1}$ was characteristic of fatty acid monounsaturated or polyunsaturated. The bands around 2923 and 2853 $cm^{-1}$ were characteristic of the length of the fatty acid chain (Riaublanc, Bertrand, & Dufour, 2006) and were related to the C—H stretching absorptions found in the acyclic $CH_2$ groups of triglycerides. This wavenumber was also affected by the degree of unsaturation of the fatty acids (Riaublanc et al., 2006).

In the second region (1800–1690 $cm^{-1}$), one band around 1745 $cm^{-1}$ and the shoulder around 1712 $cm^{-1}$ were observed. These frequencies were correlated with the vibration of the fatty acid carbonyl group of the fatty acid glycerol ester linkage. The band around 1745 $cm^{-1}$ was characteristic of the C=O ester double bond of the triglyceride (Riaublanc et al., 2006) and the shoulder around 1712 $cm^{-1}$ was characteristic of the C=O double bond of the free fatty acid (Ismail, van de Voort, Emo, & Sedman, 1993; Riaublanc et al., 2006).

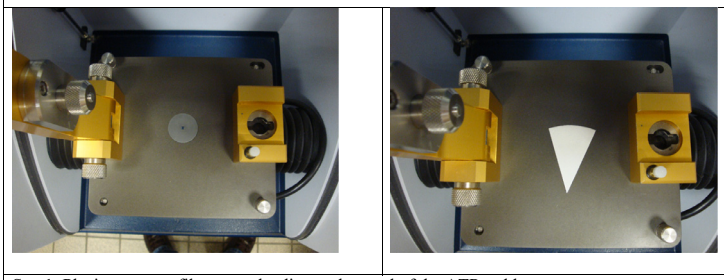
In the third region (1470–700 $cm^{-1}$), bands around 1419, 1398 and 1379 $cm^{-1}$ were related to $CH_3$ and $CH_2$ symmetric and asymmetric deformations and bands around 1167 and 1099 $cm^{-1}$ were related to C—O or C—C stretch. This region also included a *trans* band of the unsaturated group —CH=CH— around 966 $cm^{-1}$ (Guillen & Cabo, 1997; Socrates, 1998; Yookyung et al., 2007).

### 3.2. Principal component analysis (PCA) – exploratory analysis

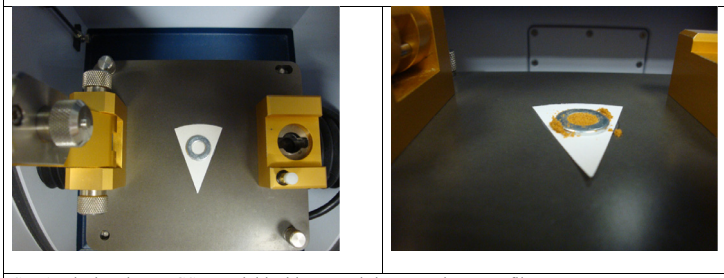
As a first step, an exploratory analysis using PCA was performed in order to provide an overview of the full dataset, including the 125 DDGS samples, and to get some indication about the natural grouping and, therefore, the possible discriminant equations to construct. Fig. 3 shows the scores for PC1 vs. PC2. PC1 explains 99.48% of the variation, which is related mainly to the 1735–1753 $cm^{-1}$ region corresponding to the C=O ester double bond of the triglyceride and to the 2840–2940 $cm^{-1}$ region corresponding to the acyclic $CH_2$ groups of the triglyceride. PC2 explains 0.31% of the variation, which is related mainly to the 1701–1718 $cm^{-1}$ region corresponding to the C=O double bond of the free fatty acid and to the 1735–1753 $cm^{-1}$ region corresponding to the C=O ester double bond of the triglyceride. Based on the botanical origin information, PC1 and PC2 enabled an easy distinction to be made between the DDGS produced from wheat (G1) and corn (G2). The difference between DDGS from China and other sources was explained mainly by PC2. Within the corn group (G2), 3 main subgroups were identified: DDGS from biofuel production by Heilongjiang in China (G2a); DDGS from biofuel production by Jilin in China (G2b); and DDGS from biofuel or beverage production by companies located mainly in USA and Europe (G2c). In this G2c subgroup, the number of companies involved in the delivery of the DDGS they produce is high, and so the concerned industrial



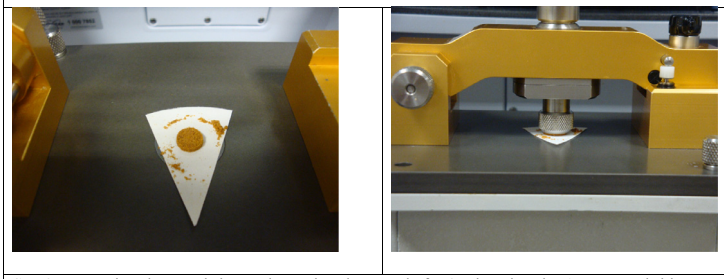

Cross-section of the sample on the ATR golden gate



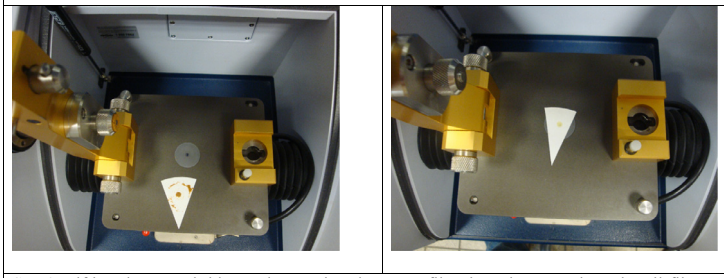
Step1: Placing a paper filter over the diamond crystal of the ATR golden gate



Step2: Placing the DDGS material inside a metal ring over the paper filter



Step3: Removing the metal ring and pressing the sample for 3 min using the ATR press bridge



Step4: Lifting the press bridge and removing the paper filter in order to analyze the oil film on the diamond crystal using ATR-FT-MIR

**Fig. 1.** *In situ* oil extraction sample preparation protocol using ATR-FT-MIR.

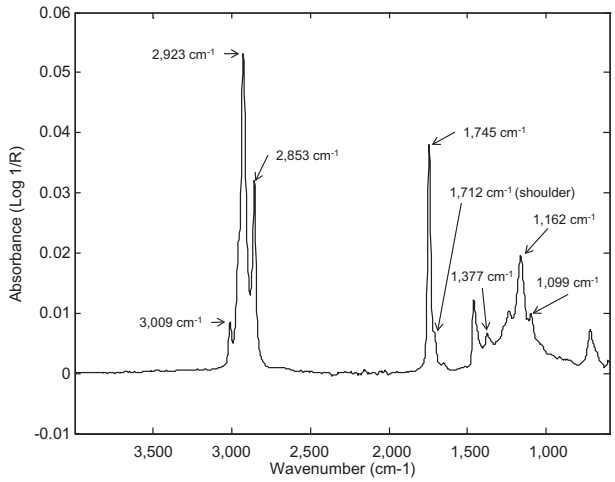



**Fig. 2.** Typical ATR-FT-MIR spectra collected after *in situ* oil extraction of DDGS.

processes are more various in comparison to the G2a and G2b groups. Within the wheat group, the number of companies involved in delivering the DDGS they produce is also high and the number of the samples is quite low. Based on the available geographical information, however, 2 subgroups were defined: DDGS from biofuel production in 2 countries, Canada and France.

The identification of these groups was confirmed by the fat content of the samples that had been predicted using a specific DDGS calibration equation based on NIR spectra acquired on the DDGS powder samples using a FOSS XDS NIR spectrometer active in the 400–2500 nm range This calibration equation was characterised by a coefficient of determination of 0.98 and a standard error of cross validation of 0.34%. The fat content was lower for the wheat DDGS (4.6%) than for the corn DDGS (7.6%). The corn DDGS from Heilongjiang in China was characterised by very low fat content (3.1%) compared with the corn DDGS from Jilin in China (8.1%) and other corn DDGS from the US (9.6% and 9.9% for biofuel and beverage production, respectively) and Europe (8.8% for Czech DDGS). The 2 groups of DDGS from the US were also characterised by high variability in fat content (SD = 1.8 and 1.4 for biofuel and beverage production, respectively). This variability was low for the French wheat DDGS (SD = 0.31), Canadian wheat DDGS (SD = 0.61) and Czech corn DDGS (SD = 0.28), probably reflecting the lower number of ethanol production companies involved in the delivery of their DDGS. These results show the large variability of this property in DDGS, reflecting what has been reported in the literature (Diaz-Royon et al., 2012).

### 3.3. Partial least squares discriminant analysis (PLS-DA) – supervised analysis

Based on the information obtained from the PCA, several groups were defined: wheat DDGS (23 samples) including 8 from France and 4 from Canada; corn DDGS (102 samples); corn DDGS from Europe and the US (42 samples); corn DDGS from China (45 samples) including 28 from Heilongjiang and 17 from Jilin.

A dichotomist classification tree was then built in which each node included a PLS-DA discrimination model built on the 3 selected wavenumbers ranges (3200–2600 $cm^{-1}$, 1800–1690 $cm^{-1}$ and 1470–700 $cm^{-1}$). PLS-DA models were also built on variables selected using the Fisher coefficient. The equations built were: (Eq1) corn vs. wheat DDGS; (Eq1.1) China vs. US–EU corn DDGS; (Eq1.1.1) Heilongjiang vs. Jilin China corn DDGS; and (Eq1.2) Canada vs. France wheat DDGS. Table 1 summarises the performance of the discrimination models in terms of sensitivity, specificity and classification error, in calibration, using LOOCV and applied to the external validation set, with the 3 wavenumber ranges or with the selected wavenumbers.

#### 3.3.1. Botanical origin of DDGS (Eq1)

With regard to the PLS-DA model for discriminating between the botanical origin of wheat and corn DDGS (Eq1), Table 1 shows that 100% of the samples were correctly classified in both the calibration set and LOOCV. When working only with the 3 variables selected by the Fisher coefficient (1699, 1716 and 2923 $cm^{-1}$), good sensitivity (99%) and specificity (100%) for both calibration and cross-validation were obtained.

The 2923 $cm^{-1}$ band corresponded to the stretching vibrations of acyclic $CH_2$ groups of the fatty acids in relation to fatty acid content and length of the fatty acid chain. This wavenumber is also

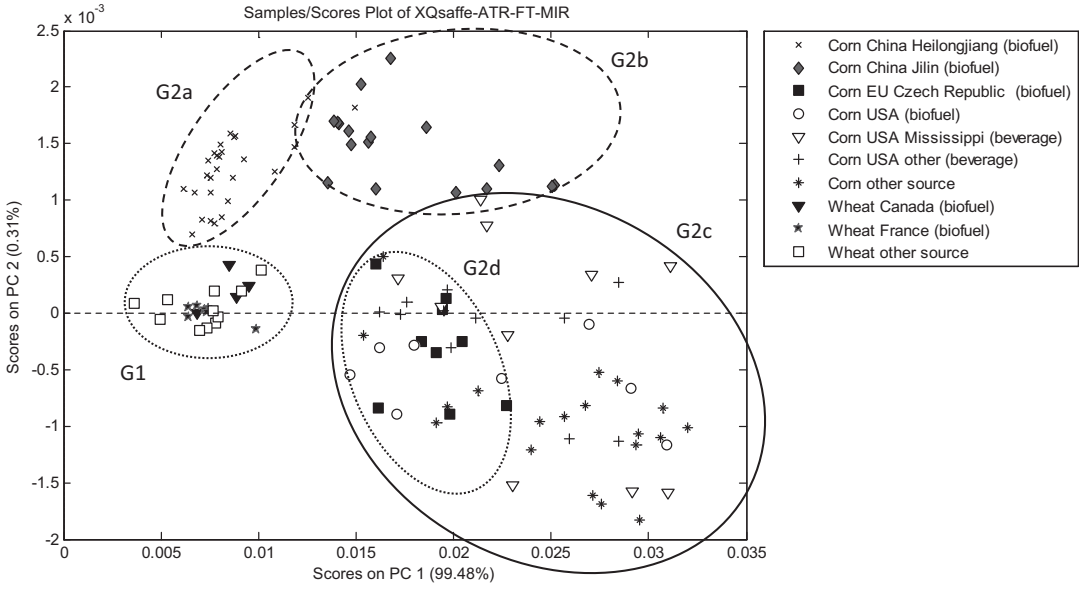



**Fig. 3.** PC1 and PC2 scores of PCA performed on pre-processed ATR-FT-MIR spectra (Savitzky–Golay window = 7, polynomial = 2) indicating the differences between the main groups of DDGS.

**Table 1**
Performance of the PLS-DA discrimination models with the three wavenumber ranges or with the selected wavenumbers.

| | Eq1 | | Eq1.1 | | Eq1.1.1 | | Eq1.2 | |
|---|---|---|---|---|---|---|---|---|
| | Corn vs. wheat | | Corn China vs. USA/EU | | Corn China Heilongjiang vs. Jilin | | Wheat Canada vs. France | |
| | 6 LV | 1 LV | 5 LV | 1 LV | 5 LV | 1 LV | 2 LV | 1 LV |
| | 3 wavenumber ranges | Selected wavenumbers 1699, 1716, 2923 $cm^{-1}$ | 3 wavenumber ranges | Selected wavenumbers 1164, 1716 $cm^{-1}$ | 3 wavenumber ranges | Selected wavenumbers 1153, 2999 $cm^{-1}$ | 3 wavenumber ranges | Selected wavenumbers 818, 1088, 2764 $cm^{-1}$ |
| Calibration | 102 corn, 23 wheat | | 45 China, 42 USA/EU | | 28 Heilongjiang, 17 Jilin | | 4 Canada, 8 France | |
| Sensitivity | 100.0 | 99.0 | 100.0 | 91.1 | 100.0 | 92.9 | 100.0 | 100.0 |
| Specificity | 100.0 | 100.0 | 100.0 | 92.9 | 100.0 | 100.0 | 100.0 | 100.0 |
| Classification error | 0.0 | 0.5 | 0.0 | 8.0 | 0.0 | 3.6 | 0.0 | 0.0 |
| Cross-validation (LOOCV) | 102 corn, 23 wheat | | 45 China, 42 USA/EU | | 28 Heilongjiang, 17 Jilin | | 4 Canada, 8 France | |
| Sensitivity | 100.0 | 99.0 | 100.0 | 91.1 | 89.3 | 89.3 | 0.0 | 100.0 |
| Specificity | 100.0 | 100.0 | 100.0 | 92.9 | 94.1 | 100.0 | 62.5 | 87.5 |
| Classification error | 0.0 | 0.5 | 0.0 | 8.0 | 8.3 | 5.4 | 68.8 | 6.3 |
| External validation set | 26 corn, 4 wheat | | 2 China, 24 USA/EU | | 1 Heilongjiang, 1 Jilin | | 1 Canada, 3 France | |
| Sensitivity | 96.2 | 96.2 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 |
| Specificity | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 |
| Classification error | 1.9 | 1.9 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |

affected by the degree of unsaturation of the fatty acids (Riaublanc et al., 2006). Morand-Fehr and Tran (2001) showed that the main differences between corn and wheat fat are explained by higher fatty acid content and a higher rate of long fatty acid chain in corn, as well as a lower rate of saturated fatty acids and a higher rate of mono-unsaturated fatty acids in corn.

The bands near 1699 and 1716 $cm^{-1}$ selected by the Fisher coefficient were close to the 1708 $cm^{-1}$ band corresponding to free fatty acid content. Fig. 4 shows, within the 1800–1690 $cm^{-1}$ range for the free fatty acid/triglyceride ratio, the differences in spectral profile between corn and wheat DDGS from several geographical origins. The band around 1712 $cm^{-1}$ showed that Chinese corn DDGS samples were characterised by more free fatty acids than corn DDGS from the US and Europe and wheat DDGS from Canada and France. The band around 1745 $cm^{-1}$ showed that the corn DDGS samples were characterised by a triglyceride fraction higher than the wheat DDGS samples, except for the corn DDGS from Heilongjiang in China. Based on these 2 peaks around 1712 (free fatty acid) and 1745 $cm^{-1}$ (triglyceride), several groups were defined.

By applying these models to the external validation set, only one corn DDGS sample was misclassified as wheat DDGS. This sample was from a company in Austria, a country not represented in the calibration set. It was characterised by a fat content of 7.8%, which is lower than other DDGS from Europe or the US. This affiliation to a new DDGs group not yet studied could explain this misclassification. Based on the 3 wavenumbers selected by the Fisher coefficient, a graph was built using the X axis for pre-processed data near 2923 $cm^{-1}$ and the Y axis for the sum of the pre-processed data near 1699 and 1716 $cm^{-1}$. Fig. 5 shows the discrimination between corn and wheat DDGS from the calibration set. The validation samples fall within or near the ellipse corresponding to a 90% of confidence limit. All the wheat samples are classified as wheat DDGS, the others as corn DDGS.

### 3.3.2. Geographical and process origin of corn DDGS (Eq1.1, Eq1.1.1)

In order to determine the geographical and process origin of corn DDGS, a dichotomist classification tree of 2 PLS-DA models from the node of the corn/wheat PLS-DA model was built in order to discriminate the Chinese DDGS from US and European DDGS, as well as to discriminate between the 2 Chinese processes.

With regard to the PLS-DA model for discriminating between DDGS from Chinese processes and other processes (Eq1.1), Table 1 shows that 100% of samples were correctly classified in both the calibration set and LOOCV. When working only with the 2 variables selected by the Fisher coefficient (1164 and 1716 $cm^{-1}$), lower sensitivity (91.1%) and specificity (92.9%) for both calibration and LOOCV were obtained. The main differences between Chinese and US–EU corn DDGS were explained by the 1716 $cm^{-1}$ band corresponding to the free fatty acid content and the C—O stretching in the 1190–1075 $cm^{-1}$ region. By applying these models to the external validation set, the 2 Chinese DDGS were correctly discriminated from the others.

Within the Chinese group, with regard to the PLS-DA model determining the Chinese process origin of corn DDGS (Eq1.1.1), Table 1 shows that 100% of the samples were correctly classified in the calibration set and 8% of them were misclassified in LOOCV. When working only with the 2 variables selected by the Fisher coefficient (1153 and 2999 $cm^{-1}$), 3.6% and 5.4% of the samples were misclassified in calibration and LOOCV, respectively. The main differences between the 2 Chinese companies lay in the high fatty acid content compared with the acyclic $CH_2$ groups (2999 $cm^{-1}$) for the DDGS from Jilin and in the C—O stretching in the 1190–1075 $cm^{-1}$region. The very low fat content for the DDGS from Heilongjiang could be explained by an oil extraction step in the ethanol process. By applying these models to the external val-

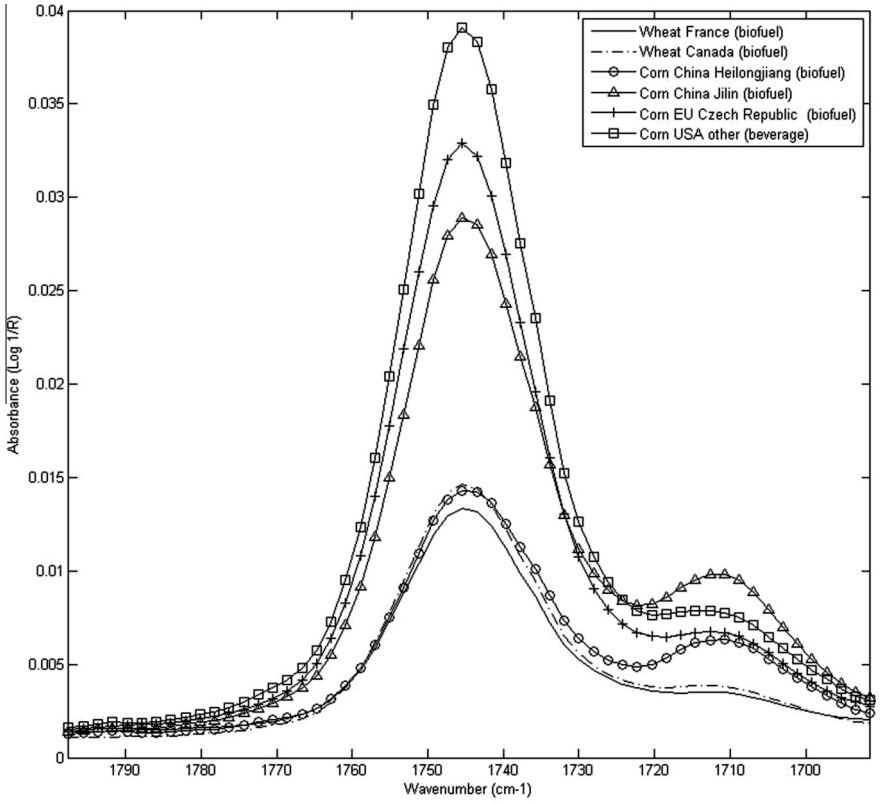



**Fig. 4.** Differences in spectral profile in the 1800–1690 $cm^{-1}$ range between corn and wheat DDGS with several geographical origins.

idation set, the 2 Chinese samples were correctly classified according to their process origin.

Within the US–EU group, the most well-characterised process was the one from the Czech Republic. The PLS-DA model developed to discriminate this Czech process from others in the US and Europe did not give good results. The classification error was high (results not presented in this paper).

### 3.3.3. Geographical and process origin of wheat DDGS (Eq1.2)

Within the wheat group, with regard to the PLS-DA model discriminating between the Canadian and French process origins of wheat DDGS (Eq1.2), Table 1 shows that 100% of the samples were correctly classified in the calibration set and 69% were misclassified in LOOCV. When working only with the 3 variables selected by the Fisher coefficient (818, 1088 and 2764 $cm^{-1}$), 100% of the samples were correctly classified in the calibration set and 6.3% were misclassified in LOOCV. The main differences between the Canadian and French DDGS were explained by the aliphatic unsaturation (—CH=CH—) in the 1000–780 $cm^{-1}$ region. The differences between these geographical origins could be explained by the efficiency of the ethanol production process or by the use of wheat cultivars from different groups (hard or soft, red or white, winter or spring) which could lead to a different fatty acid composition. By applying these models to the external validation set, the 4 wheat samples were correctly classified as being of Canadian or French origin.

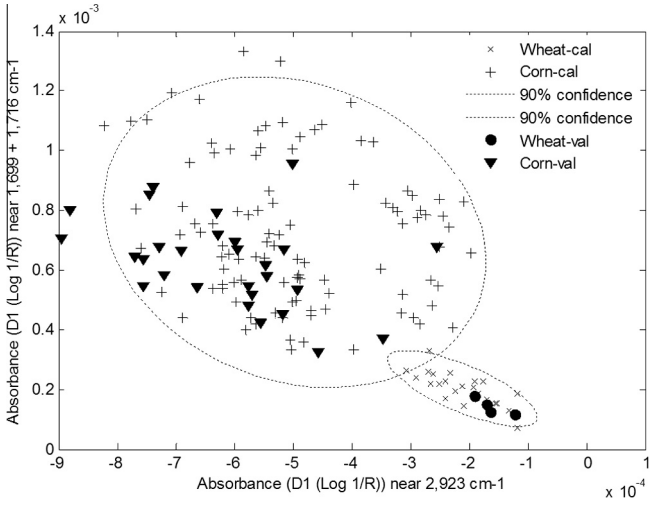



**Fig. 5.** Discrimination between corn and wheat DDGS based on 3 wavenumbers (1699, 1716 and 2923 $cm^{-1}$) showing calibration and validation sets within the 90% confidence ellipse.

## 4. Conclusions

This study illustrates the potential of ATR-FT-MIR for discriminating DDGS oil according to the origin. The originality of the study lies in the innovative way used to measure the oil by extracting it directly *in situ* from DDGS powder samples without using solvent extraction or chemical transformation, thus preventing possible influences on the composition of the oil and reducing drastically the analytical time. The use of chemometric tools such as the Fisher coefficient for wavenumber selection and PLS-DA for supervised classification method allowed the DDGS to be discriminated, in a dichotomist way, according to botanical (corn/wheat DDGS) and geographical (corn DDGS from China/US–EU) origin, as well as the industrial process used (Chinese processes for corn, Canadian and French processes for wheat). The studied models gave a correct classification higher than 95% using the external validation set. The main wavenumbers selected by the Fisher coefficient

explained the free fatty acid content in fat, the length of the fatty acid chains and the degree of unsaturation of the fatty acids, allowing discrimination of the botanical and process origins of the DDGS.

Feed companies and control laboratories need simple and rapid methods. The ATR-FT-MIR method developed in this work is a direct, rapid and non-destructive method that needs a small sample and enables only the oil fraction and fatty acid composition to be analysed. Compared with the ATR-FT-MIR method applied to oil extracted by solvent, using this method for oil extracted *in situ* prevents triglycerides from being hydrolysed and converted into free fatty acid. The method also enables the free fatty acid content to be analysed, which can give an indication of constraints used in the ethanol production process. On-site application is possible using cost-effective miniaturised FT-MIR instruments. The transferability of the methodology has not been studied, but several previous studies using the vibrational spectroscopy technique showed the transferability possibilities of such methods (Azizian & Kramer, 2012; Vermeulen et al., 2013; Von Holst et al., 2008).

This method could be particularly helpful when labelling is missing or during a crisis situation due to contamination, when origin identification could be essential in terms of feed safety. The *in situ* oil extracted could be valorised to detect any contamination of the DDGS with mineral oils or animal fat that is rich in saturated fat.

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