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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

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