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Authentication of the Botanical and Geographical Origin of Distillers Dried Grains and Solubles (DDGS) by FT-IR Spectroscopy

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Supporting Information

ABSTRACT: Distillers dried grains and solubles (DDGS) were investigated with attenuated total reflection FT-IR spectroscopy both directly in their solid state and as the isolated oils (fat fractions). The collected spectra were evaluated in a first step with principal component analysis (PCA) according to the botanical origin (corn, rice, wheat) and the geographical origin (Canada, China, European Union, India, United States) of the DDGS. In a second step, statistical models were constructed for the characterization of the botanical and geographical origin using linear discriminant analysis (LDA) and soft independent modeling of class analogy (SIMCA). For this purpose, the botanical origin was investigated more deeply for corn and wheat as the most important raw materials used for DDGS production. Also, the geographical origin was investigated exemplary for corn DDGS, derived from China and the United States. Models were validated by a randomized batchwise procedure and showed satisfactory classification rates, in most cases better than 80% correct classification.

KEYWORDS: *distillers dried grains and solubles, Fourier transform infrared spectroscopy, traceability, authenticity, geographical origin, botanical origin, feed material, multivariate data analysis*

INTRODUCTION

The complexity of food and feed production systems is steadily increasing, and in particular the trade in animal feed has become global. In addition, feed materials are obtained from new sources or are produced by new technologies. These facts may contribute to new and unforeseen risks for animal and human health. In a crisis situation due to contamination or adulteration of feed materials, the identification of origin can be essential in regard to feed safety as well as food safety for the following reasons: (a) A risk (e.g., reduced animal welfare) has been associated with a particular product but the reason is not identified. (b) A particular contamination was already identified but the analysis of the contaminant is difficult/impossible and/or expensive. In addition, the level of an assigned risk might be linked to certain areas of origin and a differentiated level of control might be appropriate, which requires that the origin must be traceable and verifiable, not only on the basis of documentation systems provided by the producer or distributor but also by analysis in the laboratory. Thus, "place of origin" will be increasingly linked to the quality of feed materials in a globalized market and will become more and more important. These changing situations underpin the necessity of analytical authentication systems particularly suited to "proof of origin".

Within work package 2 of the European Union (EU) research project Quality and Safety of Feeds and Food for Europe (QSAFFE), distillers dried grains and solubles (DDGS) were chosen to be analyzed—exemplary for feed ingredients—targeting the proof of origin. DDGS are a coproduct of the alcohol-distilling process obtained by drying solid residues of fermented grains (e.g., corn, wheat, barley) to which pot ale syrup or evaporated spent wash was added[1] and are used mainly in feeding of ruminants, poultry, pigs, and fish in aquaculture. As a result of the upgrowth of the fuel−ethanol industry, DDGS became a global commodity and play an even more important role in the feed market due to their high nutrient content (proteins, fat) in relation to the price.[2] Particularly, DDGS were chosen to be analyzed because of the increasing trade of DDGS among different countries and the fact that in the production of DDGS, especially from the fuel−ethanol industry, factors such as the yield of ethanol could possibly become more relevant than quality issues of DDGS.

In addition to these points, DDGS are a feed material showing a relatively high variability. As bioethanol is produced from different botanical raw materials, the quality of the resulting coproduct DDGS depends on the characteristic composition of the cereals. For example, DDGS produced from wheat are usually lower in fat than DDGS obtained from corn[3,4] because the harvested grains show different fat contents themselves. The quality and the nutrient profile of DDGS could even be associated with its geographical origin, as climate conditions for plant growth or soil parameters can be related to the geographical region. Also, variation in the nutrient content of DDGS exists within and among ethanol plants, even within plants using the same fermentation and processing technology.[5,6] In addition, the composition of DDGS can vary in relation to the method of production, too.[2,6] Production factors such as fermentation efficiency, usage of chemicals and enzymes, drying time and temperature, the ratio of distillers wet grains (DWG) to condensed distiller solubles (CDS) in the drying process, and fractionation of raw materials (e.g., removal of germ or pericarp) or fractionation of the coproducts (e.g.,

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extraction of oil or carotenoids) have an impact on the final feed material DDGS. Furthermore, DDGS are produced from many different companies and in different countries worldwide, and therefore production processes are manifold. In addition, ethanol production and production of DDGS might be regulated by national legislation.

However, the variability and particularly the ignorance of the different factors in the product chain could also lead to a situation in which DDGS from particular origins cause further inquiries. Thus, a scenario appears to be reasonable that feed materials from particular regions or countries could be banned for importation, for example. In the case of such an embargo, the proof of the geographical origin is known to be essential in terms of feed and food authenticity issues. In the present study, ATR/FT-IR spectroscopy was therefore used in combination with multivariate data analysis to construct models for the evaluation of the botanical and geographical origin of DDGS. Authentication and traceability approaches of agricultural and food products with this technique already have shown great potential,[7,8] and FT-IR spectroscopy as a fast and robust analytical technique was applied together with chemometrics, for example, in the authentication of olive oil, honey, or wine.[9−13] In principle, an identification of the botanical origin of DDGS could also be possible with other techniques such as microscopy, identifying particles of glumes, kernels, or whole grains of single botanical species. However, the main objective of the present study was to find a procedure that reveals also differences with regard to the geographical origin or other factors such as the production process—besides the botanical origin—of the DDGS samples. The goal was therefore to develop a strategy that could be applied for the proof of origin of feed materials like DDGS. Within work package 2 of the project QSAFFE, ATR/FT-IR spectroscopy was one of the analytical techniques selected for this purpose, and the results of this approach are reported in this study.

MATERIALS AND METHODS

DDGS Samples and Chemicals. Eighty-eight samples of DDGS were collected in the dry state in the context of the EU project QSAFFE. DDGS samples in this study are defined as feed materials according to the numbering of the EU feed catalogue:[1] either (a) number 1.12.10 (distillers' dried grains) or (b) number 1.12.11 (distillers' dried grains and solubles/distillers' dark grains). The DDGS samples used for the study derived from different botanical raw materials and were collected from different geographical origins as stated by the producer and summarized in Table 1. Detailed information on the botanical and geographical origin of the DDGS samples as well as on the production process is provided in the Supporting Information. All DDGS samples were stored at 4 °C in the dark until analysis.

Table 1. Botanical and Geographical Origin of the DDGS Samples

	Canada	China	EU	India	USA	unknown
corn		31	3		23	12
wheat	2		6			7
mixed			2			1
rice				1		

Petroleum benzine for fat extraction was of p.a. grade with boiling range of 40−60 °C (AnalR Normapur, VWR Prolabo, Fontenay-sous-bois, France); hexane and methanol for cleaning of the ATR diamond surface were of p.a. grade (AnalR Normapur, VWR Prolabo); glass fiber filters for accelerated solvent extraction (ASE) were 30 mm in diameter and obtained from Dionex (Thermo Fisher Group, Waltham, MA, USA); 2 mL glass vials and Teflon/silicone/Teflon septa were used from WICOM (Heppenheim, Germany).

Sample Preparation. The DDGS samples were investigated both directly in their solid state and as their isolated fat fractions (subsequently called oils). For the solid DDGS approach, samples were preground with a centrifugal mill (ZM 200, Retsch, Germany; mesh size = 0.5 mm) in a first step and subsequently homogenized in plastic containers (filling level approximately two-thirds) for 6 h using a drum hoop mixer (RRM 100, Engelsmann, Germany). Finally, 0.5 g of the homogenized preground sample was finely ground with a ball mill (MM 301, Retsch; 5 mL steel cylinders and 2 steel balls with 7 mm diameter, 30 Hz) for 4 min. The particle size of the finely ground DDGS was determined via microscopy with 150× magnification using a Continuμm IR microscope in visible light mode in combination with a Cassegrain lense and digital camera (Thermo Fisher Scientific). More than 99% of the particles ranged from 10 to 200 μm, and only single particles of a size up to 500 μm were detected.

The oils (fat fractions) were isolated from the preground (homogenized) DDGS samples with an ASE 300 accelerated solvent extractor (Dionex Corp., Thermo Fisher Group) using petroleum benzine as solvent. Extraction conditions were chosen according to their applicability under consideration of methods of previous studies dealing with oil extraction from DDGS.[14−17] The samples were extracted in 33 mL ASE cells, equipped with two glass fiber filters (one on the top and one on the bottom of the cell), at 80 °C and 150 bar without a preheat step, a 5 min heating period, three 5 min static extraction cycles, a 50% flush volume, and a 60 s purge time. ASE cells were disassembled and visually checked for leakage after extraction. The solvent was removed directly from the extracts in 250 mL ASE glass bottles (30 min at 300 mbar followed by 30 min at 50 mbar) with a Multivapor P-6 (Büchi, Flawil, Switzerland) at 40 °C, and the remaining oil was dried directly in the ASE glass bottles for 60 min at 103 ± 2 °C. The gravimetric fat content was determined (d = 0.1 mg, analytical balance Sartorius ME254S, Goettingen, Germany) after 60 min of storage in the desiccator, and oil samples were subsequently transferred in 2 mL glass vials and sealed with Teflon/silicone/Teflon septa for further storage at −20 °C in the dark. Oil samples (in the glass vials) were defrosted for 10 min at 50 °C, homogenized with an overhead shaker for 10 min, and centrifuged for 10 min (3112g) prior to FT-IR analyses.

FT-IR Spectroscopy. FT-IR spectra were collected with a Nicolet 6700 series spectroscope (Thermo Fisher Scientific) equipped with a Smart Performer Accessory (single ATR with diamond crystal), a KBr beamsplitter, and a deuterated triglycine sulfate (DTGS) detector. The optics were continuously flushed with dried nitrogen gas (purity 5.0). Spectra were recorded within the wavenumber range of 4000 and 525 cm^{-1} for each sample in triplicates with a spectral resolution of 4 cm^{-1} (data spacing of 1.928 cm^{-1}, Happ−Genzel apodization). The finely ground solid DDGS samples were placed on the diamond using a micrometric pressure device (Thermo Fisher Scientific) with a standardized pressing force (unit 4 of 20 on the scale of the micrometric pressure device). The oils were measured at 25 ± 0.1 °C, maintained with a DC-50-K10 temperature control unit (Thermo Fisher Scientific), by pipetting 1 μL of the oil on the ATR crystal. Prior to acquisition of each sample, a background spectrum (laboratory air) was collected, and each sample was subsequently measured against its particular background spectrum. The number of scans per spectrum (n = 64 for solid DDGS and n = 32 for oils) was selected with regard to a sufficient signal-to-noise ratio (of the main signals in comparison to the baseline) and an acceptable acquisition time. After each measurement, the diamond was cleaned thoroughly with hexane and methanol in a two-step procedure and dried with a soft tissue afterward. Background spectra were controlled visually for remaining solvent or sample residues. The spectrometer was checked regularly for spectral resolution, signal-to-noise-ratio, and wavenumber accuracy (with a polystyrene film) to ensure the stability of the instrument during the measurements.

Quality Assurance Samples. One additional sample of DDGS was used as quality control sample (described as reference in all

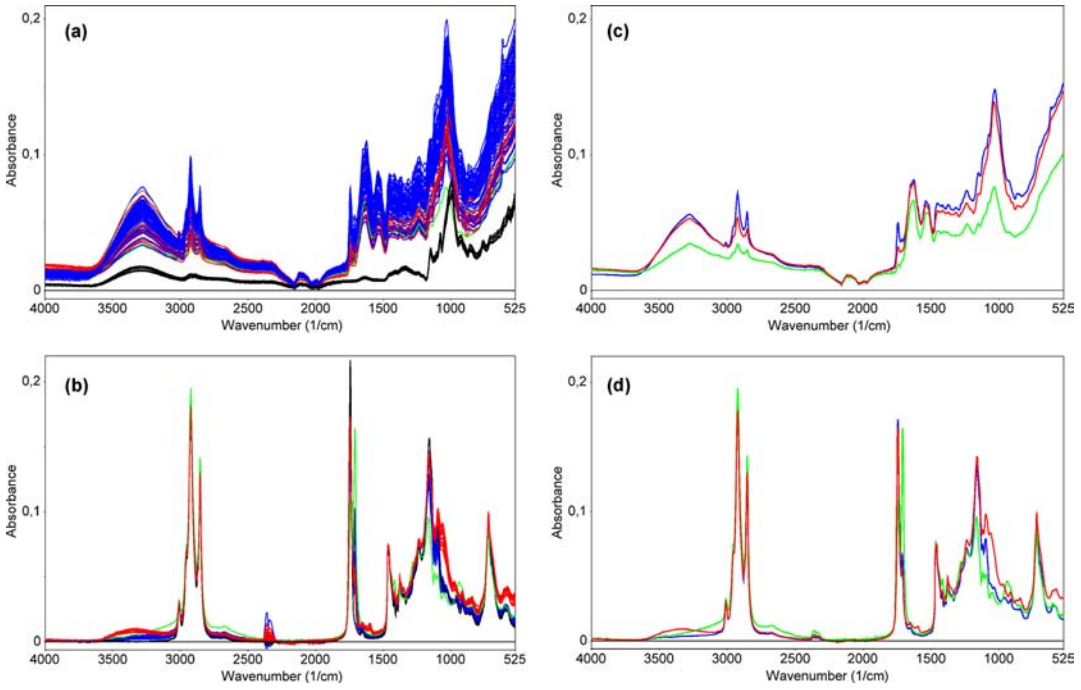

Figure 1. Raw spectra of (a) the solid DDGS samples and (b) the oil extracts of the DDGS samples; mean (averaged) spectra of (c) the solid DDGS samples and (d) the oil extracts of the DDGS samples: blue lines, corn DDGS; red lines, wheat DDGS; green lines, rice DDGS; black lines in (a), starch as quality assurance sample for solids; black lines in (b), sunflower oil as quality assurance sample for oils. All spectra were analyzed with FT-IR spectroscopy between 4000 and 525 cm^{-1}.

figures). As well, starch (starch, soluble extra pure; MerckKGaA, Darmstadt, Germany) was used as quality assurance sample for the solid DDGS measurements, and one sample of sunflower oil (blend of three different sunflower oil samples, produced in-house) was used as quality assurance sample for the oil DDGS measurements. Quality assurance samples were included in FT-IR spectroscopy for each measurement day and were subsequently evaluated in data analysis together with the DDGS samples. Also, six samples of cereals ground from the whole grains (two samples of corn, wheat, and barley, respectively) were included in the study to monitor the quality of the data analysis procedure.

Chemometric Data Analysis. Multivariate data analysis was carried out using The Unscrambler X 10.2 software (CAMO Software, Oslo, Norway). For all multivariate approaches, the triplicate spectra of each sample were averaged prior to data analysis. Wavenumber regions that did not provide relevant spectral information (baseline areas) or which were assigned to disturbing signals (e.g., CO_2 absorption) were cut off prior to the data analysis. Thus, spectra of the solid DDGS samples were evaluated in the regions 3700−2700 and 1800−550 cm^{-1}; spectra of the oils extracted from the DDGS were analyzed in the regions 3700−2600 and 1850−525 cm^{-1}.

Principal component analysis (PCA) was performed for explorative data analysis to recognize potential clustering (similarities and differences) of the DDGS samples. For this purpose raw spectra as well as spectra that had been preprocessed by standard normal variate (SNV) transformation, mean normalization, first or second Savitzky−Golay derivative, Savitzky−Golay smoothing, and also combinations thereof were evaluated. SNV transformation was finally applied to the spectra of the solid DDGS samples and describes a method that calculates the mean and standard deviation of a single spectrum (row-wise). Each absorbance value x_{ij} (at a wavenumber j) is corrected according to eq 1 with $\bar{x}_i$ being the mean of all absorbances x_{ij} in spectrum i.

$$x_{ij}(\mathrm{SNV}) = \frac{(x_{ij} - \bar{x}_i)}{\sqrt{\frac{\sum_{j=1}^{k}(x_{ij} - \bar{x}_i)^2}{k-1}}} \quad (1)$$

PCA was based on seven components, applying the nonlinear iterative partial least-squares algorithm (NIPALS). For detection of possible outliers, Hotelling's T^2 95% confidence ellipse was included in the respective score plots (enabled by The Unscrambler software) during data analysis. Hotelling's T^2 probability distribution is a generalized Student's t distribution, which was described first by Harold Hotelling[18] and is frequently used for outlier detection in multivariate statistics.

According to the grouping of the DDGS samples at the PCA level, classification approaches were performed to analyze the indicated geographical and botanical origin of the DDGS samples. Classification was performed with linear discriminant analysis based on PCA and the Mahalanobis distance (PCA-LDA) and with soft independent modeling of class analogy (SIMCA) based on a 5% significance level. Classification of DDGS samples was evaluated according to their botanical origin of corn (n = 69) or wheat (n = 15) and their geographical origin, exemplary conducted with corn DDGS from China (n = 31) and the United States (n = 23).

For both questions (botanical and geographical origin), models were constructed in the first instance with all data (100% of the samples were used as training set), and all samples were predicted in the generated model (100% prediction procedure). In a second step, the models were validated in a randomized batchwise procedure. For this purpose, the data sets of the botanical and geographical origin, respectively, were randomized (with the software The Unscrambler) in a training set, which consisted of two-thirds of the samples and a test set (internal validation set), which consisted of the remaining one-third of the samples, separately for each modeled class. This randomization was performed three times, and the modeling was repeated afterward. Therefore, validation results are based on average classification results concerning three different randomly selected test sample sets. Depending on the randomization of the data sets, PCA-LDA models were based on three to six principal components (PCs) for the solid DDGS and two or three PCs for the oils. Accordingly, for class modeling within SIMCA models, three to six PCs have been used for the solid DDGS and two to six PCs for the oils. To ensure a direct comparability between the solid DDGS and the oils, the randomized training and test sets consisted of the same samples (in each of the

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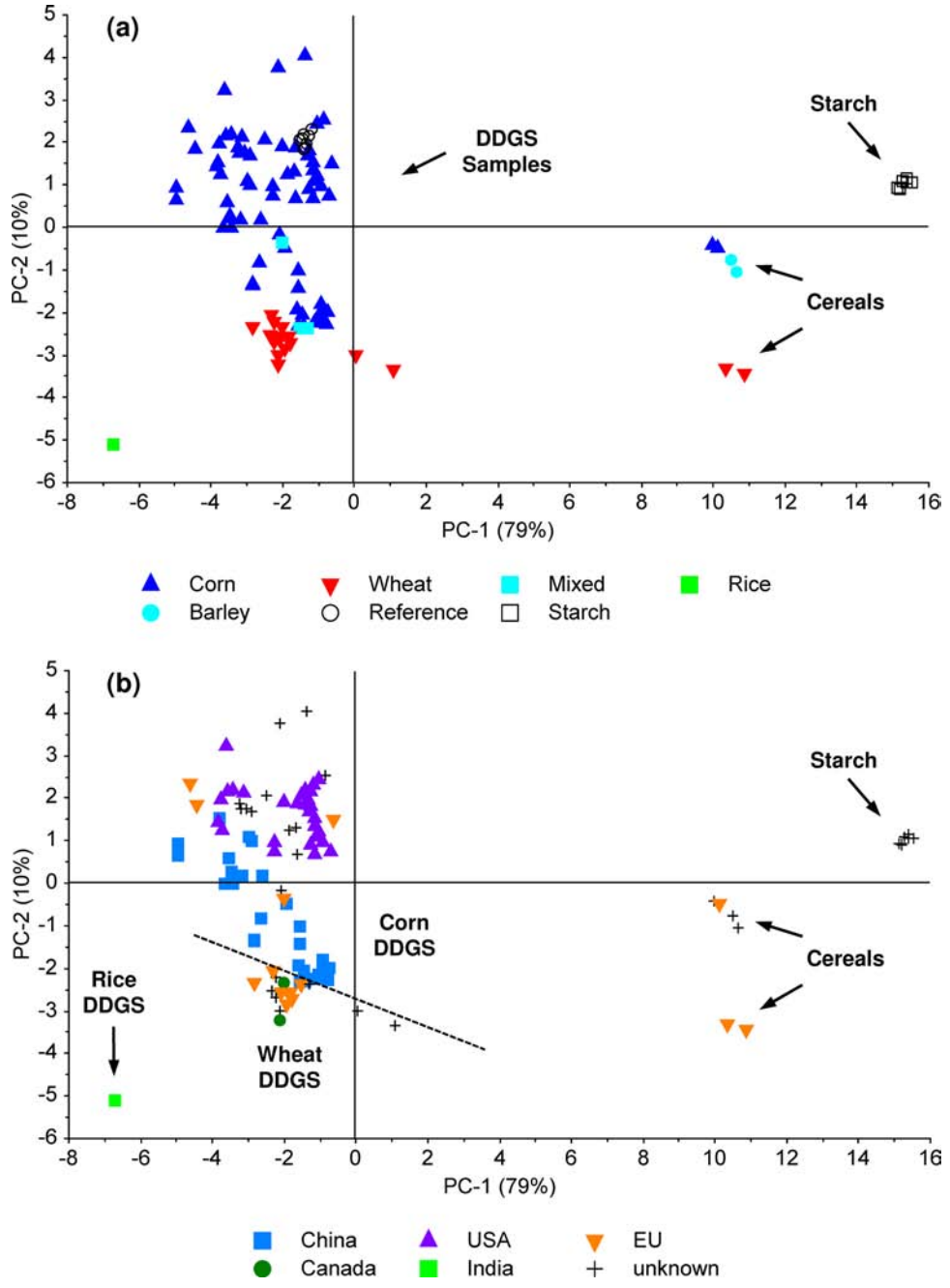

Figure 2. PCA scores plot of FT-IR spectra of solid DDGS samples colored according to (a) botanical origin and (b) geographical origin including quality control samples (starch samples and whole grain samples of corn, wheat, and barley). Standard normal variate (SNV) preprocessing of the solid DDGS spectra was performed. The broken line illustrates the two clusters of corn DDGS and wheat DDGS and does not represent statistical results. PCA was based on seven components. Explained variance for each component is given in parentheses.

three repetitions) for the evaluation of the respective solid DDGS and oils approach.

RESULTS AND DISCUSSION

ATR/FT-IR Spectra of Solid DDGS and Oils Extracted from DDGS. The mid-infrared spectra of solid DDGS samples and starch as the respective quality assurance sample are shown in Figure 1a, and the mid-infrared spectra of oils extracted from the DDGS and sunflower oil as the respective quality assurance sample are shown in Figure 1b. In addition, mean (averaged) spectra for corn DDGS, wheat DDGS, and rice DDGS are shown for the solid DDGS (Figure 1c) and the oils (Figure 1d). For the solid DDGS a clear difference of the DDGS spectra in comparison to the starch spectra is observed (Figure 1a), but the visual differences in the original infrared spectra between the botanical species rice, wheat, and corn DDGS are quite small and obvious only after the spectra of each botanical species have been averaged (Figure 1c). Overall, the spectra of the solid DDGS are similar to previously published mid-infrared (ATR) spectra of DDGS,[19,20] except for the wavenumber regions between 2300 and 1900 cm^{-1} (disturbing signals), which were excluded for data analysis. Within the spectra of the oils extracted from the DDGS (Figure 1b) differences of the respective botanical species can already be

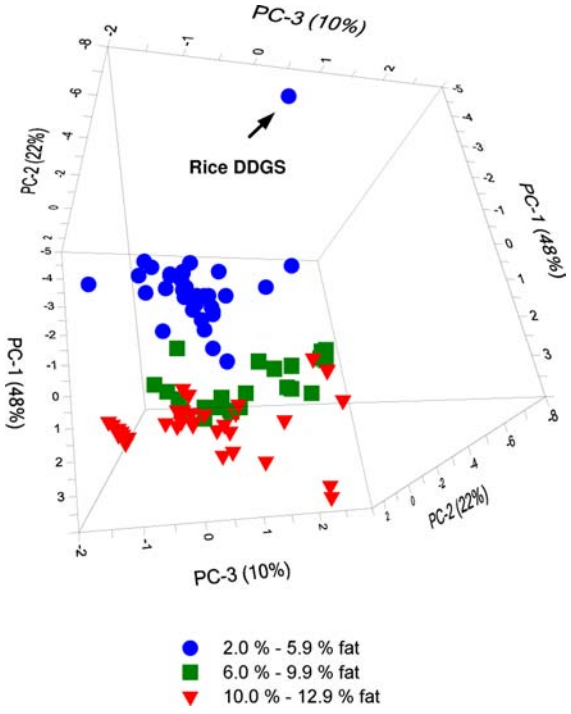

Figure 3. 3D-PCA scores plot of FT-IR spectra of solid DDGS samples colored according to different fat content classes. Explained variance for each component is given in parentheses.

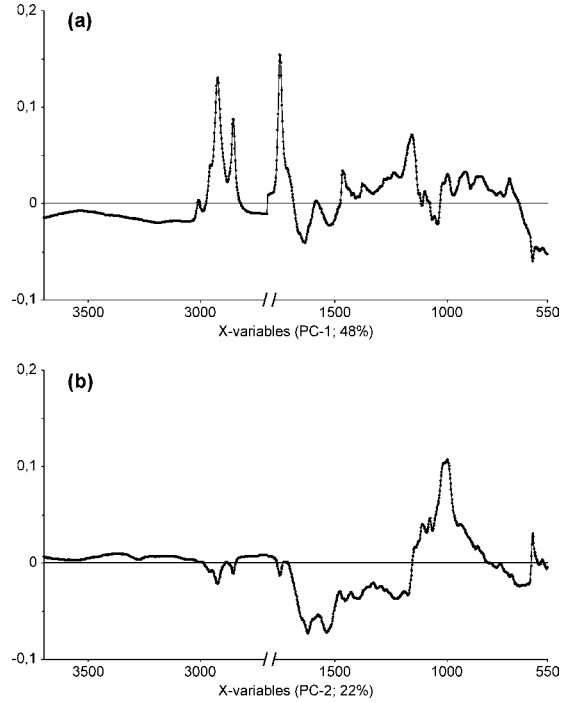

Figure 4. PCA loadings plot of FT-IR spectra of solid DDGS samples for (a) PC-1 and (b) PC-2 indicating the influence of single-wavenumber regions on the variance explained by the respective PC.

observed by visual inspection, especially in the region between 2000 and 800 cm^{-1}. Here, considerable differences for the stretching vibrations of the C═O carboxylic group of esters at 1743 cm^{-1} and the C═O carboxylic group of the free fatty acids at 1711 cm^{-1}, stated as 1746 and 1711 cm^{-1}, respectively, by Guillén and Cabo[21] for edible oils and fats, can be observed. The presence of free fatty acids in DDGS oil is a known fact, reported especially for corn DDGS produced in the dry-grind process in levels up to 22% free fatty acids in the oil.[22−24] Besides differences in the C═O bands, differences in the fingerprint region, especially in the wavenumber range of 1000−800 cm^{-1}, are noticeable at the level of visual spectrum inspection. Furthermore, the oils extracted from corn DDGS were consistently of orange color, the oils extracted from wheat DDGS of brown color, and the oils extracted from the rice DDGS of ocher color. Hence, a first indication for the determination of the botanical origin of the DDGS samples could be obtained by visual inspection of the oils extracted from the DDGS.

Principal Component Analysis. PCA was performed for explorative data analysis to recognize potential clustering (similarities and differences) of the DDGS samples. A removal of samples from the data set was omitted, as all samples that had been indicated as possible outliers by statistics (Hotelling's T^2 statistics) were found as plausible due to certain characteristics of the respective samples. The FT-IR spectra of the solid DDGS samples were preprocessed by SNV transformation to minimize effects due to light scattering or shifted spectra. The SNV preprocessed spectra of the solid DDGS samples showed superior PCA results compared to data analysis of the original spectra and also compared to differently preprocessed spectra (such as mean normalization, first and second derivative, or Savitzky−Golay smoothing). In contrast, preprocessing of the spectral data of the oils did not improve results after PCA, whereupon raw data were used here.

The PCA results (scores plot) of the solid DDGS samples are shown in Figure 2 with symbols colored according to their botanical origin (Figure 2a) and their geographical origin (Figure 2b), respectively. As expected, DDGS samples are separated from starch samples and whole grain samples of corn, wheat, and barley (cereals), additionally included in the analysis, at the first principal component (PC-1). Furthermore, the DDGS derived from corn and wheat cluster clearly at the second principal component (PC-2), in the same direction as observed for the cereal samples of corn and wheat (Figure 2a), indicating that information of the botanical origin can be associated with PC-2. Also, the rice DDGS sample is clearly separated from other DDGS samples, and the samples of mixed botanical origin are located between the wheat and corn DDGS samples, which emphasizes the separation along PC-2 according to the plant raw material used for production.

With regard to the geographical origin of the solid DDGS samples (Figure 2b), within the corn DDGS samples a separation of samples from China toward samples from United States and Europe is observed in the direction of PC-2. Samples from the EU belonging to the corn DDGS group are not clearly separated yet; possibly separation will be achieved if more samples from Europe will be analyzed in the future. Concerning the geographical origin of the wheat DDGS samples, a first separation of wheat DDGS samples from Europe and wheat DDGS samples from Canada is observed in the direction of PC-3 (data not shown). Furthermore, it appears that the separation of solid DDGS samples in the PCA can be correlated to the crude fat content of the samples (Figure 3). For demonstration purposes, three fat content classes (based on gravimetric results, cf. the Supporting Information) were defined, and samples clustered along PC-1 according to these classes (Figure 3). The corresponding loading plots of PC-1 and also PC-2 (Figure 4) do not indicate single-wavenumber

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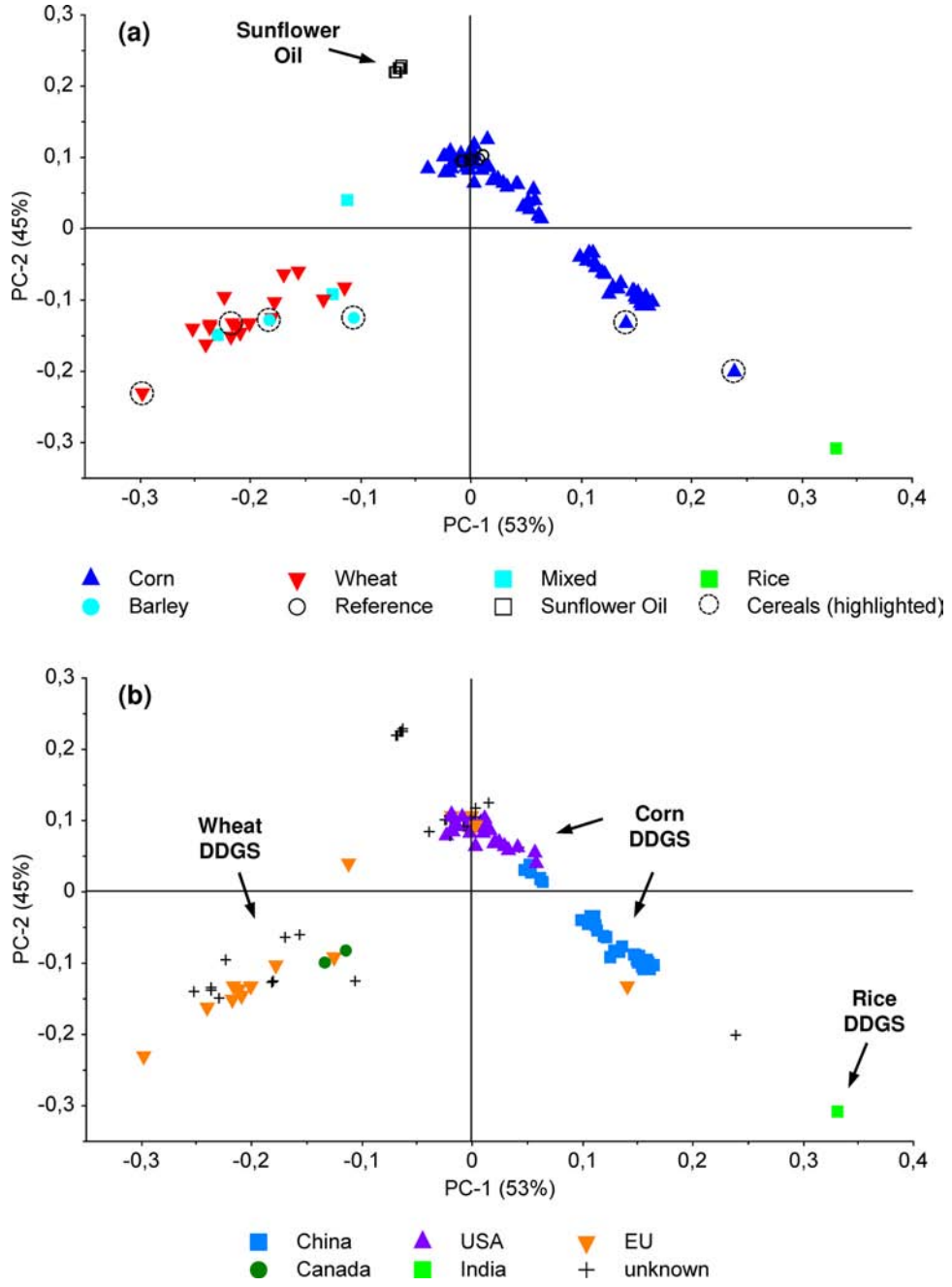

Figure 5. PCA scores plot of FT-IR spectra of oils extracted from DDGS samples colored according to (a) botanical origin and (b) geographical origin including quality control samples (sunflower oil samples and extracted oils of whole grain samples of corn, wheat, and barley are highlighted). PCA was based on seven components. Explained variance for each component is given in parentheses.

regions with outstanding influence on the explained variance of the respective PCs, except for the absorption bands of the CH stretching vibrations between 3000 and 2850 cm^{-1} (Figure 4), indicating the influence of the fat content. As corn and wheat grains have typically different fat contents, 3.2−4.3% for corn and 1.6−2.1% for wheat,[25] a separation of the respective botanical species of DDGS samples according to the fat content could be expected. However, the clear separation of the rice DDGS sample from the corn/wheat DDGS samples (Figure 3) cannot be explained by the fat content alone, as the rice DDGS sample consisted of 3.3% fat and several corn DDGS samples showed fat contents around 3.0% (data not shown). In fact, separation of DDGS samples after chemometric data analysis seems to be dependent on other spectral regions as well (Figure 4) but cannot be explained by specific signals.

The oils extracted from the DDGS samples (Figure 5) reveal a slightly different picture compared to the solid DDGS approach. Corn DDGS samples are clearly separated from wheat DDGS samples at PC-1 (Figure 5a). Again, the rice DDGS sample is well separated from the DDGS samples with different botanical origins, but the oils extracted from the whole grains (quality control samples) fall into the cluster of the DDGS samples. With regard to the geographical origin of the samples, again within the corn DDGS group, samples from China and from the United States are separated into two groups (Figure 5b). This separation becomes even more

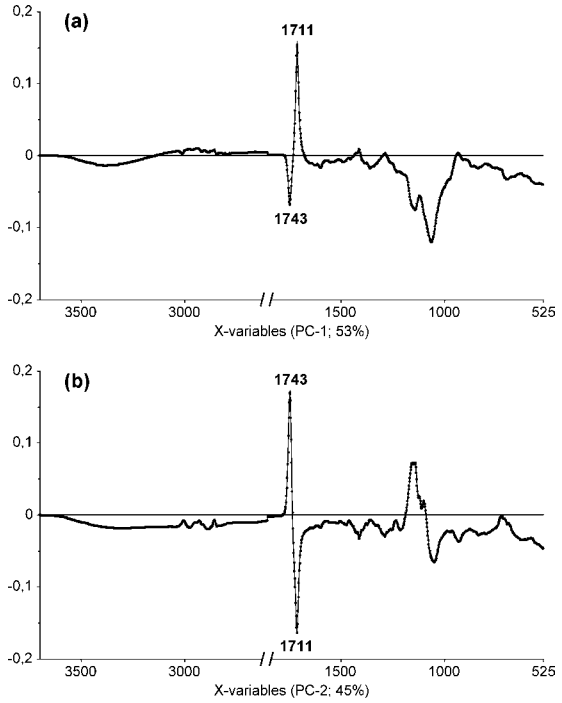

Figure 6. PCA loadings plot of FT-IR spectra of oils extracted from DDGS samples for (a) PC-1 and (b) PC-2 indicating the influence of single-wavenumber regions on the variance explained by the respective PC.

obvious when only these two groups are considered in a separate PCA model (data not shown). Within the corn group the three European corn DDGS samples appear close to the corn DDGS samples from the United States. Here, a separation was not achieved at the PCA level, possibly due to the low number of samples. In general, the separation in both PC-1 and PC-2 is correlated mainly to the absorption of the stretching vibrations of the C═O carboxylic group of esters at 1743 cm^{-1} and the C═O carboxylic group of the free fatty acids at 1711 cm^{-1}, as can be seen in the high loadings on PC-1 and PC-2, respectively (Figure 6). On the one hand, the content of free fatty acids—and therefore also the IR signal for free fatty acids at 1711 cm^{-1}—could depend on the storage of the DDGS samples and continuous hydrolysis of triglycerides by lipases or the analytical method chosen for extraction of the oil from the DDGS.[26] On the other hand, the content of free fatty acids in DDGS was reported to be higher after a dry-grind ethanol process compared to processes preventing germ breakage such as coarse wet-grinding ones.[24] According to Wang et al.,[24] the hydrolysis of the corn oil, resulting in free fatty acids, is presumably caused by the endogenous lipase or the exogenous enzymes secreted by yeast, or both. Therefore, it remains unclear whether the content of free fatty acids can be explained by one of the above-mentioned factors or if various reasons have to be taken into consideration. However, the separation of the oils in PC-1 also depends on spectral absorption in the fingerprinting region, as can be seen especially in the loadings for the wavenumber range of 1200−800 cm^{-1}. Here, characteristic absorption signals such as the asymmetric stretching vibrations of the CO−O−C group[27] can be observed, which influence the clustering of the oil samples accordingly. However, due to the overlapping signals (combination bands) in this region, absorptions at single wavenumbers cannot be assigned to single chemical compounds. In summary, it seems that the different content of free fatty acids is one reason for the separation of the oils, but also other chemical compounds appear to be important in this respect and should be taken into consideration in further research investigations.

PCA results were also evaluated with regard to the production process from which the DDGS samples originated (bioethanol production vs alcoholic beverage production). Neither for the solid DDGS samples nor for the oils could a separation according to the production process be achieved. In addition, data analysis revealed no significant impact of the production process with regard to the two questions of botanical or geographical origin.

Reference DDGS Sample for Monitoring of Stability. To monitor the stability of the approach over the time of the FT-IR measurements, one DDGS sample (the reference DDGS sample) was stored over the sampling period and sequentially measured several times. PCA reveals that the scores of this DDGS sample (black circles in Figures 2a and 5a) were very similar, indicating that the FT-IR measurements were fairly comparable over the entire study.

Classification Approaches and Validation of Models. According to the grouping of the DDGS samples at the PCA level, classification approaches were performed to investigate the botanical and geographical origin of the DDGS samples. Classification of DDGS samples was evaluated with both PCA-LDA and SIMCA modeling according to their botanical origin of corn (n = 69) or wheat (n = 15) and their geographical origin, exemplary conducted with corn DDGS from China (n = 31) and the United States (n = 23). Classification results are shown in Tables 2 and 3.

For both questions (botanical and geographical origin), the models were constructed in the first instance with all data (100% of the samples were used as training set) and resulted in a very good classification performance, except for the question of the geographical origin in the analysis of the oils after SIMCA modeling (Table 2). However, it has to be mentioned that a 100% prediction procedure is typically very optimistic, and validation results after a randomized batchwise procedure are judged to be more reliable.[28]

Table 2. Classification Results (Percent) of DDGS Samples According to the Botanical and Geographical Origin Based on 100% Prediction Procedure

		PCA-LDA		SIMCA		
question	sample state	correct classification	misclassification	correct classification	outlier	ambigous
botanical origin (corn vs wheat)	solids	100	0	95	1	4
	oils	100	0	97	3	0
geographical origin (China vs USA)	solids	100	0	98	2	0
	oils	100	0	83	2	15

Table 3. Validation Results (Percent) after Randomized Batchwise Validation Procedure for the Botanical and Geographical Origin of DDGS

		PCA-LDA		SIMCA		
question	sample state	correct classification	misclassification	correct classification	outlier	ambigous
botanical origin (corn vs wheat)	solids	95	5	81	4[a]	14
	oils	100	0	98	2	0
geographical origin (China vs USA)	solids	94	6	68	32	0
	oils	94	6	81	4	15

[a] + misclassifier (1%).

Therefore, in a second step, the models were validated in a randomized batchwise procedure with a training set consisting of two-thirds of the samples and a test set (internal validation set) including the remaining one-third of the samples, for both questions (botanical and geographical origin), respectively. After the randomized batchwise procedure, best classification results of the unknown internal test samples were obtained after PCA-LDA with both the solid DDGS and the oils (Table 3). In summary, the classification results after SIMCA modeling are the best for the oils with correct classification rates of 98% for the botanical origin and 81% for the geographical origin (concerning corn DDGS from China versus corn DDGS from United States). At this point, it should be mentioned that SIMCA modeling must be considered as more suitable for an extension of the models with further classes, that is, botanical raw materials such as barley and sorghum or geographical origins such as the EU, which are not yet included in the model. In fact, the advantage of class modeling (e.g., SIMCA) is that "class spaces defined for different classes may overlap ... and a portion of the global domain may be covered by no class space: samples found in such a region are not therefore compatible with any class studied".[29] In SIMCA models, such samples can also build up new classes outside the present class spaces in contrast to discriminant classification approaches such as PCA-LDA. In conclusion, the extraction of the oils from the DDGS led to slightly superior validation results for the determination of the botanical and geographical origin of DDGS samples, compared to the solid DDGS.

Authentication of the Botanical and Geographical Origin of DDGS. The analytical proof of origin of DDGS—exemplary for feed materials—was the objective of the present study. For this purpose, FT-IR analysis was successfully adapted for the matrix of solid DDGS and coupled with multivariate data analysis of the resulting spectra. Although the most dominant factor after data analysis was the botanical origin of the DDGS, the discrimination of the geographical origin of DDGS was indicated for the example of corn DDGS from China and corn DDGS from the United States. However, the influence of factors such as the production process (e.g., beverage production vs bioethanol production) has to be considered in the data analysis. It is not clarified yet at which step in the development of the statistical models these factors have to be included. For example, if it turns out that another factor, such as the production process, has a stronger influence on the separation of DDGS samples than the geographical origin, models should first be evaluated for this factor (e.g., different production processes) before the geographical origin can be assessed. In the future, it has therefore to be verified with samples from different production systems if the identification of the geographical origin is linked to the production process. Furthermore, it would be of interest to analyze more samples derived from corn to improve the constructed models for the geographical origin and to validate the generated models with iterative bootstrap procedures. However, results obtained so far underpin the suitability of the proposed analytical strategy (ATR/FT-IR spectroscopy combined with multivariate data analysis) for the proof of origin of DDGS. This strategy could be used to verify feed authenticity by the initiation of traceability procedures whenever discrepancies from paper documentation are observed.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Detailed information of investigated DDGS samples with their botanical and geographical origin, the production process, and the gravimetric fat contents. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

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