Calibration Model for a Near Infrared Spectroscopy (NIRS) System to Control Feed Quality of Soy Cake Based on Feed Value Assessments In-Vitro

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When harvested from the field, soybeans and products derived thereof may contain significant amounts of trypsin inhibitors. In order to reduce these substances in livestock feeding different processes (e.g. heat treatment) may be used. However, exposing the soybean to an excessive over treatment could result in glycosylation of amino acids (especially lysine) which impairs feed protein utilization. The goal of the present project was to optimize the treatment of soybeans in decentral processing plants by implementing a near infrared calibration system. Therefore, two different batches of soybeans where processed into partly de-oiled soy cake using four different approaches. To receive a robust near infrared calibration, each way of processing was adapted in order to produce over treatment, under treatment and optimal treatment of soybeans. The feed quality of the soy cake variants was assessed using laboratory analyses. The near infrared spectra, recorded along with the processing of soybeans into partly de-oiled soy cake, were combined with the laboratory analyses to be able to establish a near infrared spectroscopy (NIRS) calibration for the trypsin inhibitor activity (TIA) in soy cake. Therefore, for a sample size of 50 samples, 200 spectra were recorded and analyzed. After pre-treatment of the spectra and partial least square (PLS) regression analysis the calibration was automatically tested with a leave-one-out validation. The result showed that the method of NIRS combined with pre-treatment and PLS offered a good accuracy (R² = 74.48 %) and allowed fast detection of TIA in processed soy cake.

1. Introduction

Locally produced feed is gaining more importance in monogastric livestock feeding especially when regarding protein sources. Due to its amino acid composition with high amounts of essential amino acids like lysine, soy is a very valuable component in monogastric livestock feeding. Nevertheless, the raw untreated soybean contains significant amounts of trypsin inhibitors. Heat treatment is commonly used to reduce the activity of these substances to a minimum tolerable (Kraft et al. 2013). It was shown by (Ahmed 2001) and (Heger et al. 2016) that the intensity of heat treatment had a significant influence on the feed’s digestibility and therefore also on the quality of the feed. Soybeans fed to fast growing monogastric animals should contain a trypsin inhibitor activity (TIA) not higher than 5 mg/g (Monari et al. 1993; Clarke and Wiseman 2005, 2007; Batterham et al. 1993). The latest studies show that a decrease in growth performance of fast growing monogastric animals for full fat soy cake can be seen at a TIA below 8 mg/g (Heger et al. 2016). If heat treatment is applied in excess it may also lower the feed’s quality due to amino acid glycosylation (Faldet et al. 1992; Adrian 1974). Therefore heat treatment of raw soybeans and products derived thereof like full fat soy cake or partly de-oiled soy cake needs to be optimized. Near infrared spectroscopy (NIRS) is often used in the agricultural sector especially for feed quality control or biogas processes control (Delwiche et al. 2006; Krapf et al. 2011; Fontaine et al. 2001). Different soybean batches, different temperatures and different sizes of soybean particles might affect the final calibration (Kessler 2007; Martens und Martens 2001; Martens and Naes 2001; Zhang and Zhang 2015). Evonik Industries AG already established a NIRS calibration for grounded soy
products in laboratory use. Therefore, the aim of this study is to test a first calibration for processed soybeans that can be used for optimizing the heat treatment at decentral soybean processing plants.

2. Material and Methods

2.1 Soybean processing / sample generation

At the beginning of the project the focus was put on the different processing techniques for soybeans. Therefore, two homogenous batches of soybeans Sultana (conventional farming, harvested in Bavaria, Germany) and Merlin (organic farming, harvested in Romania) were chosen based on their TIA (Sultana: 37.3 mg/g; Merlin: 40.5 mg/g; (Deutsches Institut für Normung - Normenausschuss Lebensmittel und landwirtschaftliche Produkte 2002). Both batches were processed as follows (table 1):

**Thermal treatment:** The raw untreated soybeans were slightly moistened and then toasted (temperature 115 °C, duration 40 s). Following the thermal treatment the soybeans were cooled down and de-oiled by mechanical force. The thermal treatment was conducted at the facilities of Gerauer OHG (Kirchham, Germany), the deoiling at MEIKA Tierernährung GmbH (Großaitingen, Germany). Four final samples and 16 NIR spectra could be established.

**Hydrothermal treatment:** The raw, untreated soybeans were treated with steam (temperature 103 °C, duration 40 min). After the hydrothermal treatment the soybeans were cooled down and de-oiled by mechanical force. The hydrothermal treatment was conducted at the facilities of Amandus Kahl GmbH & Co. KG (Reinbeck, Germany), the de-oiling at MEIKA. Eight final samples and 32 NIR spectra could be established.

**Pressure and thermal treatment:** At first the raw untreated soybeans were mechanically de-oiled. Afterwards they were treated with steam (temperature 103 °C, duration 10 min) and extruded with an expander (temperature 130 °C, duration 1 – 5 s). The soy cake was then cooled down after processing. The pressure and thermal treatment was conducted at the facilities of Amandus Kahl, the de-oiling at MEIKA. 14 final samples and 56 NIR spectra could be established.

**Kilning and thermal treatment:** The raw, untreated soybeans were first treated with heat. During this process the revoked steam was circulated around the soybeans (temperature 160 °C, duration 30 min). The soybeans were de-oiled by mechanical force after this process. The kilning and thermal treatment as well as the de-oiling was conducted at the facilities of EST GmbH (Mühlberg, Austria). Eight final samples and 32 NIR spectra could be established.

**Field samples:** Since the new calibration should be used at decentral processing plants, different field samples were collected during the processing of various batches of soybeans. 14 samples and 56 NIR spectra could be established at the facilities of Rieder Asamhof GmbH & Co. KG (Kissing, Germany) using hydrothermal treatment parameters as described above.

<table>
<thead>
<tr>
<th>Thermal processing [°C; min]</th>
<th>Hydrothermal processing [ST: min, LT: min, Exp.: °C]</th>
<th>Pressure and thermal processing [ST: 1; LT: 00; Exp.: 110</th>
<th>Kiling and thermal processing [°C; min]</th>
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<tr>
<td>115; 0.6</td>
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<td>ST: 1; LT: 00; Exp.: 110</td>
<td>130; 40</td>
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<tr>
<td>120; 0.6</td>
<td>ST: 1; LT: 03; Exp.: 0</td>
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<td>160; 30</td>
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<tr>
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<td>190; 20</td>
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<td>ST: 1; LT: 48; Exp.: 0</td>
<td>ST: 1; LT: 12; Exp.: 110</td>
<td>190; 30</td>
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<td>ST: 1; LT: 48; Exp.: 130</td>
<td>190; 30</td>
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Parameters chosen for laboratory analysis were TIA, solubility in potassium hydroxide (KOH) and the WEENDER analysis. For the first calibration, presented in this paper, only the TIA was chosen.
2.2 Near infrared spectroscopy calibration

During each step of the soybeans’ processing into soy cake NIR spectra were recorded. In combination with the results of the laboratory analysis (TIA) a calibration model with the calibration software OPUS 7.5 by Bruker Optics GmbH was established.

2.2.1 Near infrared spectrometer

The processed soybeans were recorded by a TANGO FT-NIR Spectrometer (Bruker Optics GmbH 2017). The NIR sensor used in this experiment is capable of recording spectra by reflection measurements at a spectral range between 11500 and 4000 cm\(^{-1}\). A cup, also provided by Bruker Optics GmbH, with a glass bottom was fully filled with processed soybeans. Once the soybean was processed, two warm and two cold NIR spectra were recorded. Therefore the temperature was measured with an infrared thermometer (Testo, 830-T2) and the cup was rotating on the NIR sensor in order to record a higher quality spectra.

2.2.2 Near infrared spectra evaluation

All processed soybeans were chemically analyzed for TIA at the laboratory of the Chair of Animal Nutrition (Technical University of Munich) following DIN EN ISO 14902:2002-02. Since there were two warm and two cold spectra per analysed sample, four spectra per processing step were available for the evaluation using the software OPUS. In total, 200 spectra were available for the calibration (Figure 2, left). At first, data pre-treatment was conducted which included the evaluation of the first derivative, multiple scatter correction (MSC) and 17 smoothing points (Figure 2, right). A Partial Least Square (PLS) regression was performed with OPUS as described by Conzen (2005). The calibration was automatically tested by a leave-one-out cross validation (Martens and Martens 2001). The final calibration was determined from an optimisation routine of OPUS after the removal of the outliers. During the optimisation step, various frequency regions and also spectral pre-treatments were systematically tested to determine the optimal calibration (Krapf et al. 2011).

During the optimisation process the maximum number of PLS Components was restricted to 10. The model performance was assessed by the following statistical parameters (Conzen 2005):

- **Coefficient of determination (R\(^2\)):** \(R^2\) indicates the proportion of the variance in Y, that is accounted for by the model. The higher the value for \(R^2\), the better the correlation between the variance of the concentration and the spectral data.

- **Root Mean Square Error of Cross Validation (RMSECV):** The RMSECV indicates the square root of the mean square error of the cross validation. This indicates how precise the value of the samples is presumed during the internal validation.

- **BIAS:** Indicates the ordinate of a regression line. The closer the bias gets to 0, the better the calibration.

- **RPD value:** This value indicates the suitability of the calibration for the prediction. With a higher RPD value the calibration will more likely be able to predict the right sample values.

- **Offset:** The offset variable is the log of the time period under study with a regression coefficient of 1.

- **Slope:** The slope indicates the steepness of a line. The greater the magnitude, the steeper the line.

- **Coefficient of Correlation:** The coefficient of correlation predicts the degree to which changes to the value of one variable predict change to the value of another.

3. Results and discussion

3.1 Soybean processing / sample generation

The processing of the two batches of soybeans resulted in a stepwise degradation of the TIA. As shown in figure 1, samples with over, under and optimum treatment could be generated. The results of the KOH analysis of the processed batches Merlin and Sultana support the gradient from over to under and optimum treatment. A NIR spectra could be recorded for every different step during processing. Soybeans fed to fast growing monogastric animals should contain a TIA not higher than 5 mg/g (Monari et al. 1993; Clarke and Wiseman 2005, 2007). New studies suggest a possible TIA as high as 8 mg/g before growth depressions occur (Heger et al. 2016). The protein dispersibility (KOH) reduces while intensifying the heat treatment (Faldet et al. 1992). KOH should therefore be kept between 78 and 85 % (Van Eys, 2012).
Figure 1: Reducing the activity of trypsin inhibitor (TIA) and the protein solubility in potassium hydroxide (KOH) using different processing types and two different soy batches (table 1). The processing resulted in a stepwise degradation of TIA and KOH.

3.2 Near infrared spectroscopy calibration

Figure 2 (left) shows all of the recorded spectra measured from 11000 to 4000 cm\(^{-1}\). A very similar progression of the spectra can be seen. The final calibration consists of 161 calibration spectra, based on 200 recorded spectra and 50 analyzed TIA values. The data pre-treatment included the evaluation of the first derivative, multiple scatter correction (MSC) and 17 smoothing points (figure 2, right). After pre-treatment the following regions were used for optimisation as indicated by vertical lines in figure 2 (middle): 9400 - 7496 cm\(^{-1}\), 6104 - 5448 cm\(^{-1}\), 4600 – 4248 cm\(^{-1}\). Those regions were chosen, based on the software’s optimization routine. The white areas show the areas included in the calibration (figure 2, middle). The calibration was tested with a leave-one-out cross validation (Conzen 2005; Martens and Martens 2001, Martens and Naes 2001). A test set validation was not conducted at this time due to a lack of samples.

![Figure 2: Left: the untreated spectra as recorded by the NIRS Sensor in the 11000 cm\(^{-1}\) to 4000 cm\(^{-1}\) region. Middle: For PLS regression the following regions were used for the optimisation as indicated by vertical lines: 9400 - 7496 cm\(^{-1}\), 6104 - 5448 cm\(^{-1}\), 4600 – 4248 cm\(^{-1}\). The grey areas indicate those areas excluded from the calibration. Right: the spectra after pre-treatment with the first derivative, multiple scatter correction (MSC) and 17 smoothing points.](image)

Considering the statistical values (\(R^2\): 74.48; RMSECV 5.9; BIAS: -0.235; RPD 1.98; slope: 0.788; offset 2.867; coefficient of correlation 0.8645, figure 3) the established TIA calibration shows that the method of NIRS combined with pre-treatment and PLS offers a good accuracy and allows fast detection of TIA in processed soy cake (figure 3). Also comparing the statistical values to other soybean calibrations or other calibrations in general, the established statistical parameters of this calibration show good results (Delwiche et
al. 2006; Krapf et al. 2011; Zhang and Zhang 2015). The RMSECV (5.9) has to be seen in relation to the concentration range of the calibration data set. Therefore the RMSECV results in an error of 12.1 %. The RPD value (RPD: 1.98) should be higher for a robust calibration but this may also be due to the multiple constitutions of the soybeans after the different processing steps. All spectra were recorded right after the soybeans processing. The soybeans were not grounded but used as they turned out after the process. Different sizes in soybean particles, different batches in general and different processing parameters might have also affected the outcome of the calibration (Fontaine et al. 2001; Kessler 2007; Martens und Naes 2001; Haaland and Thomas 1988). In order to generate a more stable calibration (higher $R^2$ and better RPD value) more processed soybean samples need to be recorded. Furthermore a test set validation should be undertaken for the established calibration for external verification.

Figure 3: NIR estimates of activity of trypsin inhibitor (TIA) validated via leave-one-out cross validation.

4. Conclusions

All different processing types used in this experiment can be applied to process soybeans and to reach the targeted goals for optimum processing (especially for TIA and KOH). In order to reliably reach those goals in decentral processing plants an online process control should be established. The study also showed that it is possible to establish a NIRS calibration for TIA in soy products processed at decentral processing plants. Nevertheless, more samples should be implemented in the calibration to make the calibration model more stable. Therefore, 60 more samples are being chemically analysed at the moment and further samples will be collected at decentral processing plants. Moreover, further calibration factors like crude protein, oil content, water content as well as solubility in potassium hydroxide will be integrated in the main calibration. A further step of this project will be to implement a NIRS sensor in a decentral processing plant and to realize online process control in order to guarantee optimum feed quality.

Acknowledgments

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References


