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Fast determination of acidity index and chlorophyll in soybean grains through an Ultra-Compact Near-infrared spectrometer

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ABSTRACT

This work aims to propose an alternative methodology to measure acidity index and chlorophyll content in ground soybean grains using an ultra-compact near-infrared (NIR) spectrometer. The experiment was conducted using 592 soybean samples for acidity index and 348 for chlorophyll content, harvested in southern Brazil. A partial least squares (PLS) model was developed for both parameters, being optimized by outlier detection and validated through its merit figures. For the acid index model the root mean squared error for calibration (RMSEC) was 0.1974 and validation (RMSEP) 0.1694, correlation coefficient (Rcal) 0.8589. Moreover, residual prediction deviation for calibration (RPDcal; 1.7661) and validation (RPDval; 1.8525) were also determined. The results for chlorophyll content were RMSEC (623.2972 mg kg^{-1}) and RMSEP (621.9778 mg kg^{-1}), Rcal (0.8709), RPDcal (1.5956) and RPDval (1.6295). Results suggest that the NIR spectroscopy can be used to provide a suitable tool for the measurement of the acidity index and the chlorophyll content of the soybean, bringing a significant improvement mainly on the speed of analysis, what is an important goal to industrial routine purposes, besides advantages as non-invasively and non-destructive characteristics, exempt from waste generation and chemical reagents, being a potential alternative methodology for online monitoring in the soybean processing industries.

KEYWORDS: fast analysis; ultra-compact NIR; PLS; chemometric methods; industrial improvements; food analysis.

INTRODUCTION

Soybean (*Glycine max* (L.) Merrill) is a very important cultivar worldwide, mainly due to the wide range of possibilities for its derivative products in human and animal food, making it very attractive for the design of dietary food products, which directly influences in its economic value (Lopes, Lima, Leal, & Nelson, 2017; Silagadze *et al*., 2017). After harvesting, the grains must be stored until the industrialization process, and therefore, considering the conditions, changes in its composition may happen (Ribeiro et al., 2014; Kamizake et al., 2016; Liu et al., 2017).

A very effective way to measure soybean grain quality is through its acidity index, once it evidences the state of conservation of the oil present into the grain. It is known that light and heating accelerates the decomposition of glycerides, and rancidity is almost always accompanied by the formation of free fatty acids (Clemente and Cahoon, 2009; Yang et al*.,* 2015). The storage in common silos can compromise the integrity of the grains due to oxidative processes and diverse enzymatic actions, which can be intensified depending on the storage time: the longer the storage time, the higher the acidity index. The elevated acidity generates a higher cost for the refining process of the degummed oil, which demands more inputs and increases the loss of raw material (oil) (Bazoni, Ida, Barbin, & Kurozawa, 2017; Pal, Patra, Sahoo, Bakhara, & Panda, 2015).

During formation until the physiological maturation phase, this acidity index in soybean grains is around 0.3% to 0.5%. When the grains are in harvest conditions, the degradative process starts. Levels of up to 0.7% of free fatty acids are considered tolerable since the tolerance of the soybean oil market establishes a maximum of 0.05% (Oliveira et al., 2016). Therefore, when the grains have high acidity indexes, they necessity neutralization, a process in which products of significant cost are added (Bischoff et al., 2016; Hammond, Johnson, Su, Wang, & White, 2005).

Another problem that the industry faces continually is related to greenish of the grains, said to be caused due to the presence of chlorophyll in grain when it is immature harvested, which affects the quality of the extracted oil (Werner et al., 2017). Chlorophylls are natural pigments found in plants, responsible for capturing light, being present in the chloroplasts of leaves and other photosynthetic plant tissues (Scheer, 2013; Roca et al., 2016). Its incidence in soybean seeds depends on several factors such as genotype, stage of maturity, weather conditions; stress conditions (drastic temperature fluctuations, occurrence of intense frosts, diseases affecting the root, stems and plant leaves), hydric deficit during the reproductive stage, presence of pests, among other (Teixeira, Ligterink, França-Neto, Hilhorst, & da Silva, 2016).

Immature grains have a percentage of 2% to 3% less oil when compared to the mature grain, resulting in a higher acidity, also having a higher refining cost because it requires a greater volume of clarifying agents and so reducing their commercial value (Borrmann et al., 2009; Nguyen, Kisiala, Andreas, Emery, & Narine, 2016).

The oil greening or oil darkness is given during the oil extraction from green oleaginous grains, which have elevated chlorophyll, providing to the final product commercially undesired characteristics. Moreover, besides being visually

undesired it is a pro-oxidant molecule: chlorophyll reduces the oxidative stability of the oil, leading to the so-called rancification, which reduces the efficiency of the hydrogenation process (Borrmann et al., 2009).

Due to the economic importance of the extraction and marketing of soybean oil, the index of acidity and chlorophyll content are essential parameters in the assessment of grain quality. However, traditional analytical methods used in quality control are time-consuming, expensive, demands for skilled labor, being sample destructive most of the times, besides generating waste (Costa, Morgano, Ferreira, & Milani, 2017; Ma, Wang, Chen, Cheng, & Lai, 2017). In addition, the traditional analytical methodologies difficult the rapid and economical evaluation of produced soybeans grains for an effective quality control (Lee et al., 2013).

To food analysis, in order to face the high demand for quality control, the development of fast analytical routine methods to quantify different analytes is being each time more required, mainly due to the increasing demand. In this sense, optical methods can provide a set of important features to fit the needs. Among them, the near-infrared (NIR) spectroscopy is one of the most used tools for calibration purposes (Bevilacqua, Bucci, Materazzi, & Marini, 2013; Ellis, Muhamadali, Haughey, Elliott, & Goodacre, 2015; Lima, Andrade, da Silva, & Honorato, 2018), especially when associated with chemometrics (Mees et al., 2018; Sánchez et al., 2017), being considered as a reliable technique to deal with quantification *(*Restaino et al., 2011; Ma et al., 2017). The NIR technique offers advantages such as rapidity, non-destructive and non-invasive character and therefore, is sample preparation free or demands for minimal sample preparation (Qu et al., 2015; Núñez-Sánchez et al., 2016; Revilla et al., 2017; Grassi and Alamprese, 2018) To use it for quantitative determinations, NIR spectra must be correlated with results produced by conventional methods by using multivariate calibration models, establishing a relationship between the two datasets (spectral and conventional) (Fodor et al., 2011; Porep et al., 2015).

Partial least squares regression (PLS) is considered the most applied regression method for the construction of multivariate calibration models to first order data since it obeys linear behavior (Geladi and Kowalski, 1986; Gatius et al., 2017). This method correlates two data matrices, one containing the instrumental measures, X (independent variables), and one with the values of the property of interest, measured by the reference method, y (dependent variables) (Al-Harrasi et al., 2017; Genisheva et al., 2018). Several multivariate models proposed to food analysis cannot be used to the routine analysis due to the lack of validation, which requires a huge amount of sample to be sufficiently representative and allow for routine laboratory analysis (Huang, Yu, Xu, & Ying, 2008).

Due to the advantages of the technique, some studies investigating soy quality parameters such as crude protein, lipids, moisture and ashes (Ferreira et al., 2013; Santos et al., 2018; Zhu et al., 2018) were performed, demonstrating good performance as a rapid method for these determinations. Near-infrared spectroscopy combined with multivariate analysis has also been used to quantify leaf chlorophyll content in plants, such as the study by Xie et al. (2007) that used spectroscopic techniques to quantify the chlorophyll content in tomato leaves and to distinguish whether it was transgenic. Fernández-León et al. (2010) proposed for the simultaneous determination of chlorophyll in broccoli and cabbage, using the photometric properties of these compounds. In soybean leaves these pigments were studied by Singh et al. (2013) and Tang et al. (2011), which

quantified foliar pigments based on the wavelet decomposition of hyperspectral characteristics. In spite of having studies on chlorophyll in soybean leaves, there are no reports in the literature on research of chlorophyll content in ground soybean grains, much less on acidity index. Based on this, the objective of this study is to investigate the capacity of NIR spectroscopy coupled to PLS to determine acidity index and chlorophyll content in soybeans grains by constructing and validating a calibration model. In this sense, the sampling is reflecting the real demand situation in a routine analysis laboratory.

MATERIALS AND METHODS

SAMPLES AND REFERENCE METHODOLOGY FOR ACIDITY INDEX AND CHLOROPHYLL CONTENT

A total of 592 soybean samples for the acid index parameter and 348 for chlorophyll content were acquired from a cooperative agroindustry located in the Paraná state, south of Brazil. These samples were harvest from the northwest region of Paraná state in the period from March 29 to June 7, 2016. Approximately 100 g of the sample was dried in a stove with air circulation at 130ºC during 1 hour and then remained for 12 hours in a desiccator. After this period, the dried samples were powdered in rotor mill (Marconi, model MA-090CFT), with mesh 0.85 mm aperture, and sent to be analyzed through the reference method in the industry.

Determination of acidity index: 25 g of the ground soybean grain was added to 50 mL of hexane and subjected to constant stirring for 1 hour using a magnetic stirrer to extract the oil. After extraction, the supernatant was filtered, and the liquid was conduct to an air circulating greenhouse at 100 °C, to evaporate the solvent. Then, 7.0 g of the extracted oil was weighed, adding 50 mL of isopropyl alcohol (99%) and 1 mL of phenolphthalein 1%. Titration was carried out using sodium hydroxide (NaOH) 0.25 $mol.L^{-1}$, until persistent pink coloration for approximately 1 minute. The acidity index corresponds to the quantity (mg) of the sodium hydroxide necessary to neutralize the free fatty acids present in 1 g of fat. This reference methodology was adapted from American Oil Chemists Society (AOCS, 2009).

Determination of chlorophyll content: approximately 25 g of the powdered sample was wrapped in a paper filter and arranged to suffer extraction in n-hexane during 5 hours in soxhlet. The solvent was removed by evaporation using a heater plate in a stove with air circulation at 130 ºC by 1 hour and cooled in a desiccator at room temperature. To chlorophyll quantification, the oil obtained was submitted to absorbance measurement in the wavelengths of 630, 670 and 710 nm, using cuvette with an optical path of 50 mm. This methodology is an official method utilized by the industry and proposed by American Oil Chemists' Society (AOCS, 2009).

NIR SPECTRA, MULTIVARIATE CALIBRATION AND FIGURES OF MERIT

Spectra of the ground soybean grain samples were obtained by using an Ultra-compact near-infrared spectrophotometer (JDSU Uniphase Corporation, MicroNIR 1700), acquired at room temperature by diffuse reflectance in the range from 910

to 1676 nm. PLS model was developed in the Matlab R2013a software using the PLS-Toolbox 7.8 package (Eigenvector Research Inc.).

The PLS method has been discussed in detail in relevant references (Geladi and Kowalski, 1986; Kowalski and Beebe, 1987; Otto, 1999; Brereton, 2000; Sampaio et al., 2018). In this study, the data matrix **X** was constituted by the NIR spectra of ground soybean grain samples and the vector **y** contained values for acidity index obtained by the reference method. The model was developed using mean center pre-processing.

Outliers were evaluated through the leverage against spectral residuals plot (ASTM, 2012; da Silva et al., 2014). The validation of the proposed multivariate model was certified by determining its figures of merit, such as accuracy, linearity, sensitivity, analytical sensitivity, adjustment, residual prediction deviation (RPD), limits of detection and quantification. The determination was done based on Silva et al. (2012), Souza *et al.* (2015), Souza et al. (2014), Ferreira et al. (2013) and Valderrama et al. (2007).

RESULTS AND DISCUSSION

Ground soybeans grain Raw NIR spectra and after spectral baseline correction by the first derivative, using the Savitski-Golay algorithm (Savitsky and Golay, 1964), using a 13 points window and first order polynomial are shown in Figure 1.

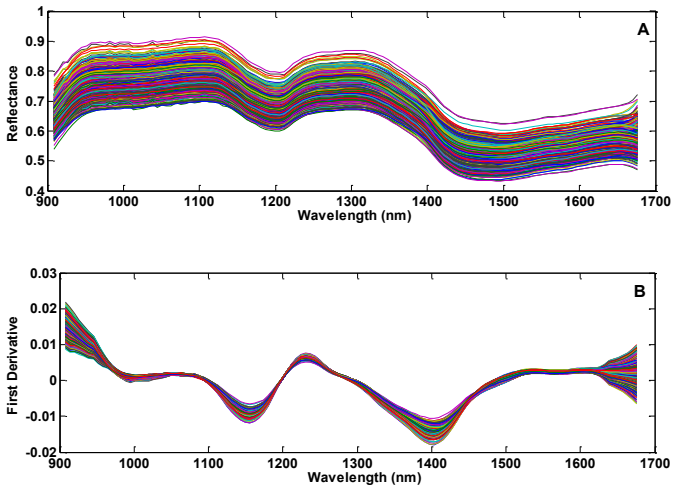

Figure 1. NIR spectra of ground soybeans grains. (**A**) Raw spectra. (**B**) Spectra after baseline correction.

The calibration and validation data sets, selected by the Kennard-Stone algorithm (Kennard and Stone, 1969), were composed of 392 and 200 samples, respectively, for the acid index model. For the chlorophyll content model, 232 samples were used to construct the calibration model, while 116 samples were used for validation. Root mean square error of cross-validation (RMSECV) and percentage of explained variance in the standard method (y block) was used to select the optimum model dimension. In this case, the minimum RMSECV to the calibration samples, obtained by contiguous block cross-validation of ten samples, resulted in the choice of 12 and 18 latent variables for the acidity index and

chlorophyll models, respectively, considering 70% as a minimum variance explained in the y block.

There were considered as outliers 44 and 36 for the calibration, 25 and 20 samples for validation for the acidity index and chlorophyll models, respectively, datasets by presenting high leverage and spectral residuals simultaneously. Due to this fact, these samples were removed. The figures of merit for the models build are shown in Table 1.

Table 1. Parameters of merit for NIR-PLS model in the quantification of acidity index (%) and chlorophyll content (mg Kg^{-1}).

Parameters of merit			
		Acidity index	Chlorophyll
Accuracy	RMSEC	0.1974	623.2972
	RMSEP	0.1694	621.9778
Fit	R_{cal}	0.8589	0.8709
	R_{val}	0.8541	0.8073
RPD_{cal}		1.7661	1.5956
RPD_{val}		1.8525	1.6295
Analytical $Sensitivity^{-1}$		0.0555	300.6507
Detection limit		0.1832	992.1474
Quantification limit		0.5552	3006.5000

Accuracy, represented by the RMSEC and RMSEP indicates that models dimension was properly chosen, suggesting that the models were not overfitted. Another way to investigate the accuracy is by fitting the reference values against the predicted ones and them by the correlation coefficient. Figure 2 shows the fit to the NIR-PLS model, presented by plotting the reference against estimated values for acidity index (a) and chlorophyll content (b).

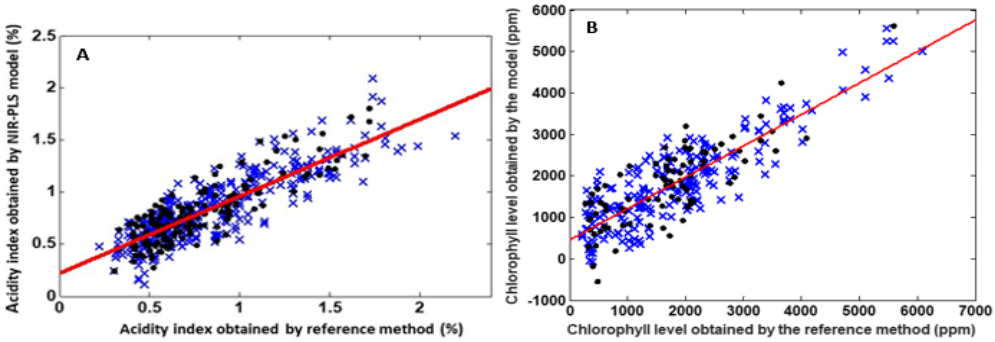

Figure 2. Fit: (x) Calibration samples (●) Validation samples.

For the acidity index model, the correlation coefficient value, higher than 0.85 in the calibration and validation fit, was considered satisfactory when the reference method is titration. Chlorophyll content model presented a correlation coefficient of 0.8709, and these results can be considered adequate for these predictions, since the official method showed a great variation. In the literature, it can be found that previous research reported coefficient value around 0.7 (D. S.

Ferreira, Galão, Pallone, & Poppi, 2014) and 0.8 (D. A. Santos, Lima, Março, & Valderrama, 2016).

Residual prediction deviation, RPD, defined as the ratio of natural variation in the samples to the extent of probable errors occurring during the prediction, is considered more useful for comparing models on different data sets or in absolute terms. It was calculated for the calibration and validation sets of the acidity index model and presented values of 1.7661 and 1.8525 for calibration and prediction, respectively. For the chlorophyll content model, the RPD value for calibration was 1.5956 and 1.695 for validation, both models were considered satisfactory (between 2.4 and 1.5) (Williams and Norris, 2001).

The analytical sensitivity^{-1}, presented in Table 1, is appropriate considering the models analytical range (0.22-4.36% for acidity index and 223-8.454 ppm for chlorophyll content). This parameter of merit is estimated by the inverse of the ratio between sensitivity and standard deviation for the spectral noise. By considering a perfect model fit and that the spectral noise represents the large source of error, analytical sensitivity^{-1} allows for the establishment of a minimum concentration difference which is discernible by the analytical method in the range of concentrations where it was applied. Based on this, it is possible to distinguish samples with acidity index difference of 0.0555% and 300.6507 ppm for chlophyll content.

Residuals plot from calibration and validation samples the acidity index (a) and chlorophyll content (b) models are shown in Figure 3 and were used to evaluate the models linearity. The residuals distribution is randomly distributed, which reinforces the linear behavior.

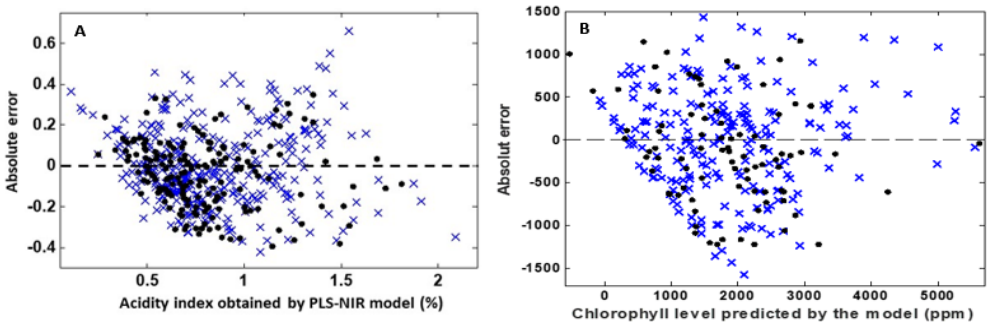

Figure 3. Residuals. (x) Calibration samples (●) Validation samples.

Detection and quantification limits are suitable to the industrial purposes, once the detection limit (close to 0.2%) is lower than the harvest condition index, while quantification limit (0.5%) is lower than the tolerable one (0.7%). The chlorophyll content model detection and quantification limits were 992.1474 mg Kg^{-1} and 3006.5000 mg Kg^{-1}, respectively. Thus, the minimum concentration that can be reliably measured by the method based on NIR-PLS is 992.14 mg Kg^{-1}, which indicates that samples below this level can be incorrectly measured (or measured without reliability). Moreover, the NIR-PLS methodology can detect samples with chlorophyll content of the 3006.5000 mg Kg^{-1}. However, as samples with low chlorophyll content are desired by industry, the results suggest the possibility of using a PLS model as an alternative methodology for determining soybean chlorophyll content bringing the advantages of fast, non-destructive, does not employ reagents/solvents and does not generate toxic wastes.

CONCLUSIONS

NIR spectroscopy and multivariate calibration based on PLS can be used to predict acidity index and chlorophyll content in ground soybean grains. The figures of merit suggest that the models have appropriate sensitivity capacity and accuracy, detection and quantification limits, indicating that it can be used in the industrial routine analysis as an alternative methodology. By comparing to the traditional ones, the NIR-PLS models present advantages as minimal sample preparation, capacity to be employed to measure other parameters, such as protein, moisture, among others, and it is waste-free, being coherent to the green chemistry and economical friendly in the sense that it diminishes the waste storage.

COMPLIANCE WITH ETHICAL STANDARDS

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Conflict of Interest: All the authors involved in this research declares they have no conflict of interest.

Ethical Approval: This article does not contain any studies with human participants or animals performed by any of the authors.

Informed Consent: not applicable.

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Determinação rápida do índice de acidez e clorofila em grãos de soja através de um espectrômetro de infravermelho próximo ultracompacto

RESUMO

Este trabalho tem como objetivo propor uma metodologia alternativa para medir o índice de acidez e o teor de clorofila em grãos de soja moídos usando um espectrômetro ultracompacto de infravermelho próximo (NIR). O experimento foi conduzido com 592 amostras de soja para índice de acidez e 348 para conteúdo de clorofila, colhidas no sul do Brasil. Um modelo de mínimos quadrados parciais (PLS) foi desenvolvido para ambos os parâmetros, sendo otimizado pela detecção de outlier e validado por seus valores de mérito. Para o modelo de índice de acidez, o erro quadrático médio da raiz para calibração (RMSEC) foi de 0,1974 e a validação (RMSEP) de 0,1694, coeficiente de correlação (Rcal) de 0,8589. Além disso, o desvio de predição residual para calibração (RPDcal; 1,7661) e validação (RPDval; 1,8525) também foram determinados. Os resultados para o teor de clorofila foram RMSEC (623,2972 mg kg-1) e RMSEP (621,9778 mg kg-1), Rcal (0,8709), RPDcal (1,5956) e RPDval (1,6295). Os resultados sugerem que a espectroscopia NIR pode ser usada para fornecer uma ferramenta adequada para a medição do índice de acidez e do teor de clorofila da soja, trazendo uma melhora significativa principalmente na velocidade da análise, que é um objetivo importante para fins de rotina industrial, além de vantagens como características não invasivas e não destrutivas, isentas de geração de resíduos e reagentes químicos, sendo uma metodologia alternativa potencial para monitoramento on-line nas indústrias de processamento de soja.

PALAVRAS-CHAVE: análise rápida; NIR ultracompacto; PLS; métodos quimiométricos; melhorias industriais; análise de alimentos.

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