Effect of the calibration model on the correlation between spectral data and nitrogen content in various agricultural objects

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The present study aims to examine and compare the effect of spectral pre-processing, wavelength range and the number of the reflectance spectra selection on the strength of regression relationships between near-infrared reflectance spectra and the nitrogen concentration in dried and ground grasses, undried grasses and maize silage of “as is” moisture, intact rape-seeds, whole wheat grains and soil. The agricultural objects differed in particle size, moisture or organic matter content. Calibration models were developed using modified partial least squares (MPLS) regression. The application of various calibration models had a different impact on the accuracy of the equations subject to the investigated object. The investigated factors had a greater effect on the relationship between spectral data and nitrogen content in undried silage, wheat grains and undried grasses. In most cases spectra derivatisation effect was more important than scatter correction or wavelength range. The accuracy of the equations was improved when pre-treated by derivatisation data of entire spectra (400–2500 nm) were calibrated for dried and undried grasses, silage and rapeseed. The number of calibrated wavelengths in the range 1100–2500 nm did not have any significant effect on the accuracy of the equations.

Key words: NIR spectra, calibration model, MPLS regression, nitrogen content, agricultural objects

INTRODUCTION

The United Nations have recently issued a stern warning regarding the excessive use of nitrogen in agriculture. However, it is an essential nutrient for crop production, therefore determination of nitrogen content may be required for different purposes. The major nutritive element of cattle ration, necessary for meat and milk production, is protein content in forage. Accurate and reliable protein in wheat testing is more important than other single grain evaluation criterion for baking industry. Together with the high demand for the oil, the meal by-product is a significant component of rapeseeds. Protein is commonly measured as crude protein (CP), which is 6.25 times the nitrogen content for forage and rapeseed, and 5.7 times for grain. Determination of nitrogen content in the soil is important due to the vital relation of this nutrient to plant nutrition and growth as well as to the formation of yield and its quality. Nitrogen fertilisation needs to be optimised to avoid the leaching and run-off of nitrogen from soil and to reduce production costs. Fertilising with nitrogen generally increases the crude protein level of plant production.

Conventional methods for protein or nitrogen content measurement are expensive, time-consuming, labour-intensive, and generate hazardous waste. An alternative, near-infrared (NIR) spectroscopy, has been used extensively for grains, seeds and forage for more than 20 years, avoiding these limitations [1–5]. NIR methodologies are approved by the Association of Official Analytical Chemists (AOAC), the International and the American Association of Cereal Chemists (AACC) for crude protein measurement in whole grain. A collaborative study was performed to assess the accuracy, repeatability (within-laboratory precision), and reproducibility (between-laboratory precision) by NIRS measurements. NIRS is widely used for determination of protein in rapeseed [6, 7]. The American Oil Chemists’ Society (AOCS) has approved this method for the determination of oilseed quality components, including protein (Am 1–92). The use of near-infrared reflectance spectroscopy to evaluate soil properties has started to receive more attention in recent years. The method is being used for predicting organic C, total N, macro and micro elements [8–11].

The ability of NIRS to provide rapid analyses depends on prior preparation of mathematical cal-
librations used to predict analytes in unknown samples. A calibration is a statistical correlation model relating NIRS spectral data and laboratory data determined by conventional methods. Spectral pre-processing such as wavelength range selection, scatter correction method, mathematical treatment have been used to increase the robustness and predictability of the model [12–15].

This paper aims to examine and compare the effect of the scatter correction method, mathematical treatment, wavelength range and the number of the reflectance spectra selection on the strength of regression relationships between near-infrared reflectance spectra and the nitrogen concentration in agricultural objects differing in particle size, moisture or organic matter content.

EXPERIMENTAL

Sample preparation and chemical analysis. For the purpose of this experiment, six sample sets were prepared, including dried and ground grasses, undried grasses, maize silage with “as is” moisture, intact rapeseeds, whole wheat grains, and soil. Samples of perennial grass and herbage of meadows grown in various areas of Lithuania and different species and varieties from plant breeding plots were collected in 1998 through 2001. Fresh samples were chopped into pieces of 3 to 5 cm in length, dried at 105 °C for 15 min, then at 65 ± 5 °C for 16 h and ground by Cyclotec mill with a 1 mm sieve. Sample sets of undried grasses consisted of different species and varieties from plant breeding plots of Lithuanian Institute of Agriculture (LIA). The samples were collected at weekly intervals from the beginning of May to the first 10 days of June. For scanning, grasses of “as is” moisture were chopped into pieces not larger than 3–5 cm and mixed before wrapping by the cling film. Samples of wet undried maize silage produced in Lithuanian farms were well mixed. For NIRS calibration the database of intact rapeseed free of impurities was composed of samples of different varieties grown in various areas of Lithuania from 1994 to 2001. Most of them were grown in the experimental fields of the LIA, variety testing stations, individual farms or agricultural partnerships. Grain samples of different wheat varieties were included in the calibration database for this study. These samples were grown in different regions of Lithuania during the period 1997–2002. Cracked grains and impurities were removed before scanning. The calibration set of soil samples was composed of different soil samples from various areas of Lithuania, collected in 1996–2003. The sample group was composed of the major soil types of the country: from pasture soils rich in organic matter to mineral sandy and sandy loam soils taken from different depths ranging from 0 to 40 cm. For chemical analysis and NIRS scanning, visible roots and plant residues were removed. The air-dried soil was ground with an IKA micromill and sifted through a 0.25 mm sieve.

The total amount of N was measured colorimetrically using a Technicon Auto-Analyzer after wet digestion by the semi-micro Kjeldahl method. Nitrogen contents were expressed on a dry matter basis (Table 1).

Recording near-infrared spectra. All samples were scanned on a monochromator scanning spectrometer NIRS-6500 (FOSS NIRSystems Silver Spring, MD). Samples of dried grasses, air-dried soil and intact rapeseed were scanned with Spinning Module using a small ring cup (~ 4.7 cm). The number of complete scans to average for rapeseed was two, RMS – 100 (but no more than 7 scans were used), dried and ground grasses and soil were more uniform than intact rapeseed and were scanned once. Samples of undried forage and wheat grain were scanned using a Transport Module. The prepared undried grass and maize silage was wrapped in the cling film 235/0412/03 from Merck. The samples were scanned in triplicate using a high moisture / high fat cell. The samples of wheat grains were kept as whole grain kernels and scanned once in a large natural product cell with a removable back (Natural Product Cup, Cell & lid IH-0314). The reflectance spectral data as log 1/Reflectance values (log 1/R) collected covered the range 400 to 2498 nm and were recorded at 2-nm intervals. Calibrations were developed using 125–200 samples of each tested agricultural object selected from centred spectra files using the CENTER and SELECT procedures of ISI operation and calibration software (ISI-NIRS2 Ver-

<table>
<thead>
<tr>
<th>Table 1. Composition and characterisation of calibration sample sets</th>
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<tr>
<td>Parameter</td>
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<tr>
<td>n</td>
</tr>
<tr>
<td>Mean</td>
</tr>
<tr>
<td>Min</td>
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<tr>
<td>Max</td>
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<tr>
<td>CV %</td>
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</table>

n – a number of samples, Mean, Min and Max – nitrogen values, and CV – coefficient of variation of nitrogen content
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Calibration development. The selected spectra were matched with the reference data and the calibration models were developed using the MPLS regression (modified partial least squares) algorithm and cross-validation technique. Spectra were corrected for scatter and transformed into their different mathematical treatment models using the ISI-NIRS 2 Version 3 chemometric software. Mathematical treatment of spectra is described by four digits: the first means the derivative, the second is the gap over which the derivative is calculated, the third is the smooth, i.e. the number of data points in a running average, and the fourth is the second smooth. Four scatter correction methods and no correction were applied to spectra transformation. Spectra were corrected for scatter and transformed into their different mathematical treatment models using the ISI-NIRS 2 Version 3 chemometric software. Mathematical treatment of spectra is described by four digits: the first means the derivative, the second is the gap over which the derivative is calculated, the third is the smooth, i.e. the number of data points in a running average, and the fourth is the second smooth. Four scatter correction methods and no correction were applied to spectra transformation. Spectral data of wavelength intervals of 400–2500, 700–2500, 800–2500, 900–2500, 1000–2500 and 1100–2500 nm with different number spectral data points (with absorbance readings every 2, 4, 6, 8, 10 nm) were tested in order to optimise the accuracy of calibration. The CENTER algorithm was used for the calculation of principle components and Mahalanobis distance. This information was used for the description of spectral boundaries and detection of outliers. Following centering of the data, the SELECT algorithm was used for grouping the spectra into clusters with neighbourhood H distance.

In this study we confined ourselves to calibration performance expression by RSQ (determination coefficient in calibration) and analogous characteristic in cross-validation: 1-VR.

RESULTS AND DISCUSSION

Many factors affect the accuracy of near-infrared (NIR) reflectance spectroscopy for the analysis of agricultural products. Variations in particle size, shape, compaction, and other physical differences between samples can cause shifts in spectral baselines and hence interfere with quantitation. It is obvious that these differences are much larger than variation due to chemical composition. It is therefore necessary to find a method that can overcome these effects. Spectral pre-treatments are mathematical functions for handling such interferences in order to avoid their dominance over the chemical signal. Several pre-treatments are commonly used. The first of these pre-treatments are baseline correction, standard normal variate transformation (SNV), and multiple scatter correction (MSC) or other. These algorithms enhance the differences in spectra related to the chemical composition of samples by reducing differences in spectra related to physical characteristics of the sample (primarily particle size).

Effect of scatter correction method. Different scatter correction procedures were applied to the calibrating 1100–2500 nm wavelength range with no spectra derivatisation (“raw” spectra - math treatment 0, 0, 1, 1) and with mathematical treatment 1, 4, 4, 1 (Fig. 1). The present study shows that some methods of scatter correction enhance the relationship between spectral data and nitrogen content, i.e. the determination coefficients become higher. The application of various models had a different impact on the accuracy of the equations subject to the investigated object and spectra derivatisation. Especially important was the application of the scatter correction for “raw” spectra calibration, and this factor had the extralarge effect on the statistics of “raw” spectra calibration of maize silage.

The best results are obtained with WMSC for dried grasses, SNVD for maize silage, rapeseed and soil, SNV for wheat grains and none for undried...
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Grasses “raw” spectra calibration. When the spectra of the first order derivatisation were calibrated (except for soil), determination coefficients in most cases were higher than the coefficients in calibration and cross-validation of “raw” spectra. Calibrations were improved more by derivatisation than by scatter correction. It should be noted that the best algorithm of scatter correction for “raw” spectra calibration was not the best for calibration of derivatised spectra for all test objects.

Mathematical pre-treatment. The next spectra pre-processing is mathematical treatments, such as using derivatives (first, second, or more) on the spectra, averaging over spectral ranges (smoothing). Different mathematical treatments of NIR spectra were tested before correlating the NIR and laboratory values. Equations were developed using the raw log1/R, first-, second- and third-order derivatised data with gap, smooth, smooth2 combination 4, 4, 1.

For that we calibrated spectra of the wavelength interval 1100-2500 nm with the absorbance readings of every eighth nanometer. The variation of correlation coefficients under the effect of this factor was obvious, but different for each of the test objects (Fig. 2).

The first derivative seemed to be the most efficient for the accuracy of calibration. The determination coefficients in cross validation of equations were highest with application of both none and SNVD algorithms, except for wheat grains, also for rapeseed and soil with SNVD scatter correction. Application of the second and third derivative for spectra strengthened the correlation between optical and laboratory data only in the first chain of equation accuracy estimation, i.e. in calibration (RSQ). The choice of the scatter correction method as well as of spectra derivatisation level were ultimate on silage calibration statistics, while these factors least influenced the accuracy of the equation for the prediction of nitrogen content in ground dried grasses.

The mathematical treatment also allows for selection of the gap over which the derivative will be calculated and the degree of smoothing for the calibration. The effect of the gap over which the first derivative on non-scatter corrected spectra on calibration efficiency is presented in Fig. 3. The choice of gap for the calculation of the derivative has no lesser effect on the accuracy of calibration than spectra derivatisation. For the optimisation of the correlation in calibration of optical data and nitrogen content in dried and undried grasses, maize silage and wheat, most suitable is the size of gap 3, with data point 1 in a running average (smoothing), i.e. mathematical treatment 1, 3, 1, 1, when the scatter correction algorithm is not to be used. For rapeseed and soil the optimal gap was 2, i.e. mathematical treatment 1, 2, 1, 1. The impact of gap with smo-
othing data point 2 on the improvement of calibration was less significant than with smoothing 1 for calibration of spectra of the first order derivative.

Smoothing is used to reduce instrument and/or sample noise and is performed by multiplying points along the NIR spectrum with a weighting function, increasing values at low frequency points and decreasing values at high frequency points [13].

The impact of the smoothing on the calibration statistics was differently effective for the test objects (Fig. 4). The smoothing interval of 2 nm (data point 1) was favourable for the statistics of calibration for dried and ground grasses, 4 nm (data points 2) - for wheat grains and soil. For the rest of the objects the optimal smoothing value depended on the other spectra pretreatment models included in this study, i.e. scatter correction and derivative.

Number of spectral points. The number of spectral points associated with each sample was gradually increased from 139 or 140 (1100–2500 nm, absorbance readings of every tenth nanometer) to 693 or 700 (every second nanometer) before further spectral pre-processing. The following data points for raw optical data with and without scatter correction were tested: 700, 350, 234, 175, 140, and for first order derivatised data: 693, 346, 231, 173,139. The number of calibrated wavelengths did not have any significant effect on the statistics of the equations for the prediction of nitrogen in all investigated objects, irrespective of the pre-treatment of optical data (Table 2).

Wavelength range. Finally, the effect of wavelength range on the correlation of nitrogen content and optical data was studied. The ranges of two detectors (Si and PbS) chosen were 400–2500 nm, 700–2500 nm, 800–2500 nm, 900–2500 nm, 1000–2500 nm and 1100–2500 nm with absorbance readings every eighth nanometer. Absorbance readings of every second nm only for the interval of 1000–1100 nm were tested (Fig. 5).

In the present study, the effect of altering the spectral range on the accuracy of equations for prediction of nitrogen content was examined by developing equations with different spectral pre-treatment. The spectra pre-treatment model had some...
effect on the efficiency of correlation subject to wavelength range. In many cases, a greater or lesser improvement in the precision of equations was obtained when pre-treated by first derivatisation spectral data of entire spectra were calibrated for dried and undried grasses, silage and rapeseed. The use of scatter correction procedure for soil spectra highlighted the advantage of using spectral information of wavelength range of 1000–2500 nm with absorbance readings of every eighth nanometer in both segments of spectra, for non-scatter corrected entire spectrum or interval of 700–2500 nm. Absorbance readings of every second nm for the wavelength interval 1000–1100 nm and every eighth nm for the interval 1100–2500 nm were most suitable for the development of equation for nitrogen prediction in wheat grain.

**DISCUSSION**

Chemometric analysis in the application of NIR spectroscopy involves the use of numeric factor analysis such as MPLS regression to extract information from spectral data that relates to an analyte measured within a population. The goodness of calibration for the property of interest can depend on the degree to which this property can be modelled from spectral information of samples within the property domain of the calibration set. The present study has over again demonstrated that the accuracy of an NIRS prediction depends on the successful completion of several factors of calibration. The discussed spectra pre-treatment affected the accuracy of equations (in calibration, cross-validation) by different intensity subject to object. Dardenne et al. [16] have reported that the optimal scatter correction algorithm for wheat and whole maize silage is SNVD, however, for fresh grass silage no pre-treatment for scatter correction is recommended. Our findings do not contradict, but also do not completely coincide with the above-mentioned results, since the scatter correction effect was investigated for different combinations of derivative, gap and smoothing. Development of an accurate equation for nitrogen determination in maize silage was more complex and was more strongly affected by more factors compared with other agricultural objects. The first reason why this sensitivity in the calibration model was very high could be that undried maize silage of whole plant was the least homogeneous object of all the objects tested. Literature sources point out that the analytical accuracy of calibration was higher for ground than for coarse sample sets [17]. Scatter correction together with mathematical treatment subdues the effect of particle size [3, 13, 14]. Gap size is important in calibration sensitivity to system noise [13]. Although a large gap may render the calibration inefficient due to a lower sensitivity to sample variation, a small gap could produce the opposite effect and make the calibration sensitive to variations resulting from sample noise. It should be noted that in all investigated cases the combination of all math treatment parameters (derivative, gap and smooth) is relevant. Mathematical treatment of the calibrated spectra affects the accuracy of equations for prediction of the dried grasses quality in all levels of their robustness test and subdue the effect not only of particle size but also of other sample preparation inaccuracies [18]. Trial and error is the only way to fix the best spectra pre-treatments [14, 18].

Increasing the number of calibrated wavelengths by changing absorbance reading points, in the range 1100–2500 nm did not have any significant effect on the accuracy of the equations for the prediction of nitrogen in all investigated objects regardless of pre-treatment of optical data. There is little evidence on this issue in the literature, however, some researchers [19] confirmed that the use of 693 data point spectra in calibration development did not improve the predictive abilities of individual glucosinolate content.

Nitrogen in plant tissues associates with proteins. Bands for NH indicating protein are found at 1040, 1210, 1496, 2050, and 2140 through 2180 nm [3]. Consequently, the best results of calibration of nitrogen content in wheat grains evidently demonstrated the advantage of using spectral information of the wavelength range 1000–2500 nm. The positive

**Table 2. The range of the coefficient of determination in calibration (RSQ) as affected by the number of wavelength of spectral range 1100-2500 nm**

<table>
<thead>
<tr>
<th>Object</th>
<th>None*: 0,0,1,1</th>
<th>None*: 1,4,4,1</th>
<th>SNVD**: 0,0,1,1</th>
<th>SNVD**: 1,4,4,1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dried grasses</td>
<td>0.936–0.940</td>
<td>0.942–0.951</td>
<td>0.941</td>
<td>0.944–0.956</td>
</tr>
<tr>
<td>Undried grasses</td>
<td>0.873–0.879</td>
<td>0.891–0.898</td>
<td>0.866–0.867</td>
<td>0.891–0.898</td>
</tr>
<tr>
<td>Maize silage</td>
<td>0.473–0.585</td>
<td>0.743–0.770</td>
<td>0.625–0.639</td>
<td>0.751–0.764</td>
</tr>
<tr>
<td>Wheat grains</td>
<td>0.898–0.899</td>
<td>0.921–0.936</td>
<td>0.903–0.906</td>
<td>0.940–0.954</td>
</tr>
<tr>
<td>Rapeseed</td>
<td>0.906–0.914</td>
<td>0.911–0.918</td>
<td>0.913–0.915</td>
<td>0.918–0.920</td>
</tr>
<tr>
<td>Soil</td>
<td>0.929</td>
<td>0.926–0.940</td>
<td>0.954–0.955</td>
<td>0.940–0.960</td>
</tr>
</tbody>
</table>

Scatter correction: *None – non-scatter correction, **SNVD – standard normal variate and detrend. The 700, 350, 234, 175, 140 data points for raw optical data with and without scatter correction and for first order derivatised data 693, 346, 231, 173, 139 were tested.
effect of absorbance readings of every second nm for the segment 1000–1100 nm may be associated with the fact that during the process of spectral segmentation, some information essential to quantification of NH bands was left behind when absorbance readings of every eighth nm were used. In addition to protein, other nitrogen-containing plant constituents potentially have absorption features in the near infrared. Chlorophylls are other major nitrogen-containing components of green plants. The chemical structures of chlorophylls are markedly different from proteins. Chlorophyll a and b in plants do not contain amide bonds and N-H absorption features do not appear in their infrared spectra [20]. Chlorophylls exhibit strong absorption in the visible region, arising from conjugated carbon-carbon single and double bonds of the porphyrin ring and the Mg ion. The differential absorption properties of protein and chlorophyll might lead to the development of algorithms which use both visible and near-infrared wavelength regions to estimate total nitrogen content in forage material and rapeseed, whose immature seed contains some chlorophyll. In this way, elimination of 400–1100 nm segment, which is most frequently suggested in the literature, did not give any positive results for the improvement of the equation. Park et al. [14] confirmed also that little improvement in accuracy was obtained by extending the wavelength range beyond 1100–2500 nm.

CONCLUSIONS

1. Spectral pre-processing such as scatter correction method, mathematical treatment, wavelength range and absorbance readings interval selection affect the strength of the correlation between spectral data and total nitrogen content in various agricultural objects differing in particle size, moisture or organic matter content. The investigated factors had a greater effect on the relationship between spectral data and nitrogen content in undried silage of whole plant maize, wheat grains and undried grasses. In most cases spectra derivatisation effect was more important than scatter correction or wavelength range.

2. The number of calibrated wavelengths in the range 1100–2500 nm did not have any significant effect on the accuracy of the equations for the prediction of nitrogen in all investigated objects irrespective of pre-treatment of optical data.

3. Improvement in the accuracy of equations was obtained when spectral data of entire spectra (400–2500 nm) pre-treated by derivatisation were calibrated for dried and undried grasses, silage and rapeseed.

4. Absorbance readings of every second nm for the segment 1000–1100 nm and every eighth nm for the segment 1100–2500 nm were most suitable for the development of equation for nitrogen prediction in wheat grain.

Received 17 December 2004
Accepted 28 January 2005

References

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KALIBRACIJOS MODELIO ĖTAKA SPECTRINIO DUOMENŲ IR AZOTO KIEKIO ĖAIRIUOSE ŢEMĖS ŪKIO OBJEKTUOSE KORELIACINIO RYÐIO STIPRUMUI

Santrauka