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Prototype near-infrared (NIR) reflectance spectrometer for the analysis of maize flour

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ABSTRACT

Near-infrared spectroscopy has been employed for determination of biochemicals in agricultural products. In this study, a prototype near infrared instrument is reported capable of simultaneously determining primary and secondary biochemical compounds in agricultural products using supervised modeling method. Maize flour was used as the material for model development. Calibration models were developed for four primary components (moisture, protein, oil, carbohydrate) and eight secondary compounds (amylose, amylopectin, oleic acid, linoleic acid, tocopherol, carotenoid, lysine, and tryptophan). Support vector machine, which is a supervised modeling method, was utilized to develop models. The graphical user interface of the prototype was developed using the R platform, which provides the user with flexible options for analysis and data management. The results obtained from the models generated with the prototype were compared those obtained from a bench-top near infrared reflectance instrument and reference analyses. The developed prototype was able to make measurements between 900 and 2100 nm with 6 nm resolution and completed each measurement in 60 s. The prototype demonstrated adequate performance in terms of repeatability and yielded comparable results with the bench-top instrument. The best calibration model for the prototype was obtained for carotenoids. This prototype is suitable for the determination of biochemicals in additional products after appropriate prediction models are prepared.


KEYWORDS

Maize; near-infrared (NIR) spectroscopy; R language; support vector machine

Introduction

Near-infrared reflectance (NIR) spectroscopy is commonly used for agricultural products for compositional analysis. The history of NIR spectroscopy begins with studies conducted by William Hertchell in 1833 on the

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measurement of light that the human eye does not perceive.^[1] The use of NIR spectroscopy has increased considerably since Hertchell's day. The studies carried out by Norris et al. in the 1970s showed that the content of roughage samples may be performed in the NIR region.^[2] Since international standard organizations have accepted NIR as a reference,^[3] great effort has been made to develop devices with improved function.

Any NIR spectroscopy instrument can measure spectra, but the measurements must be treated to be meaningful to the user. Spectra are applied to prediction models, known as "calibration" which are developed using chemometric techniques. In most commercial devices, integrated calibration models have limitations and can only measure the primary biochemical compounds in the products. Most models are based on simple multiple linear regression or partial least squares regression in commercial instruments.^[4] Today, interest in supervised (learning-based) and non-parametric modeling approaches such as support vector machine and artificial neural network have increased. A trend has emerged for wavelength selection to be estimated by NIR^[5] because nonlinear relationships between light and matter are more effectively explained by these models. Support vector machine is commonly used in the literature and commercial instruments.

There are restrictions for calibration developers and users of NIR instruments. One is the calibration software of NIR devices are only compatible with classical modeling techniques. Hence, users develop the supervised calibration models but cannot utilize these models because old instruments are not capable of using modern chemometric methods. The other problem is the difference between the language used in modeling and control software in NIR instrumentation. Various problems may occur in the development and prediction steps when the language used in model development is different from that of the control software.

R software has a great potential for these problems^[6] and allows the development of prediction models using multivariate structured data. Suitable packages for spectral analysis in R software are mainly pls,^[7] e1071,^[8] mdatools,^[9] ChemometricsWithR^[10] and caret.^[11] These packages have algorithms for model development using classical and supervised-learning approaches. Furthermore, developers may connect to hardware using R serial package^[12] and command them using the interface developed within shiny framework.^[13] To our knowledge, there is not an exemplary study for an NIR spectrophotometer using R language, although examples exist for other software.

There are many studies on prototype NIR instruments and calibration models for different agricultural products.^[14–16] The use of NIR devices in agricultural analysis began with staples and shifted to other plants. Primary biochemical components are generally the focus. However, secondary biochemical components have not been carefully addressed in most studies.

Also, the prototypes are limited in the number of the analyzed parameters. Portable devices have limitations for the determination of secondary components. Therefore, there is a need to develop new instruments for staples such as maize.^[17] Various studies have been conducted to determine the major and minor biochemical components in maize by NIR spectroscopy. In addition to major components such as proteins, oils, and carbohydrates,^[18] minor components have also been determined by NIR.^[19,20] In most studies, classical modeling approaches were used. However, classical techniques have limitations explaining the non-linear relationships between light and sample.

This study reports a prototype NIR instrument that enables the determination of the primary and secondary biochemical components in maize flour. Machine learning based approaches were used in the design of the device with support vector machine models on the R platform. The graphical user interface has been prepared using the same programming language.

Materials and methods

Material

150 maize flour samples were analyzed that included 142 collected across Turkey and 8 commercial varieties. Approximately 100 g of sample from each genotype was conserved under suitable conditions until analysis.

Development of prototype

A schematic representation of the instrument is provided in [Figure 1](#). The details are provided in the [supplementary material](#). The spectra from the prototype was subjected to a reflectance calculation described by:

$$\text{Reflectance}(\%) = \frac{S-D}{R-D} \quad (1)$$

where D is the dark spectrum, R is the spectrum from the Spectralon reference, and S is the sample spectra. The prototype's graphical user interface was developed by RStudio utilizing 13 R packages ([Table S1](#)).^[8,9,13,14,21–29] The development of the instrument ([Figure S1](#)), the final prototype ([Figure S2](#)), sample measurements ([Figure S3](#)) and the graphical user interface ([Figure S4](#)) are available in the [supplementary material](#).

Spectra acquisition

In order to make spectral measurements on the prototype, the samples were ground in a laboratory mill and passed through 0.5 mm sieves. The

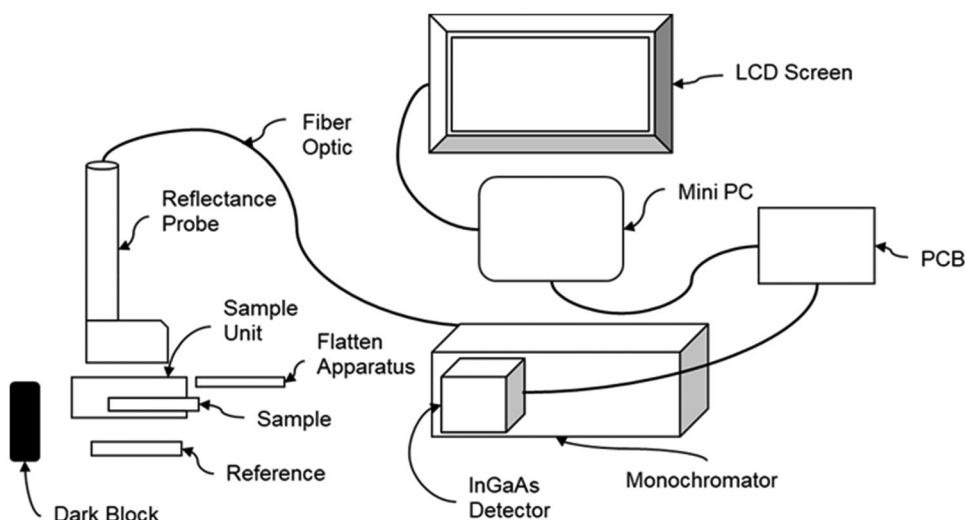


Figure 1. Schematic of the prototype near infrared reflectance instrument.

ground samples were placed in a sample tray that was manufactured for the probe and the spectra were collected from 900 to 2100 nm. The spectrum of each sample was saved as a comma separated value (csv) file through the interface. These data were combined into a single file for the calibration step.

Reference analyses

In reference analyses, the moisture, protein and oil content were measured according to methods defined by the Corn Refiners Association,^[30–32] the carbohydrate content according to Gerhardt et al.,^[33] the amylose and amylopectin content per Kaufman et al.,^[34] lysine and tryptophan content according to Galicia et al.,^[35] total tocopherol content analysis by Baker and Frank,^[36] total carotenoids according to Rodriguez-Amaya and Kimura,^[37] and oleic and linoleic acid by David et al.^[38]

Development of calibration models and evaluation of the prototype

The calibration models used in the prototype were developed in RStudio using e107 package which is based on SVM regression.^[8] Before the spectra are utilized in the model development, irregularities were eliminated with spectral pretreatments. In this context, standard normal variate transformation (SNV) was applied to the spectra. Spectra collected with the prototype for each target were used as independent variables to develop calibration models. Outlier elimination was performed based on T and H values of calibration samples. To eliminate outliers, critical values of T and H were

selected to be 2.5 and 3, respectively. SVM models were independently developed in RStudio platform for each parameter using the remaining samples after outlier elimination and combined into a single file with the RDS extension. In the SVM models, eps-regression was applied, and radial basis function was used as the kernel function. The grid search was performed for the determination of the most suitable hyper-parameters for the gamma, cost, and epsilon values. Grid search was performed for hyperparameter tuning and the determined values for gamma, cost and epsilon were used to create best SVM models for each trait using the SVM relationships:

$$RBF = \exp\{-\gamma|u-v|^2\} \quad (2)$$

$$SVM = \omega \times x + b \quad (3)$$

where γ is the parameter with the highest gamma value, u and v are support vectors in kernel function, x is the input vector, ω is the distance to the hyper plane, and b is the bias.

The variation within the sample set was evaluated by the mean, minimum, maximum, and standard error. The reliability of the models developed with the support vector machine was evaluated according to the root mean square error of calibration (RMSEC), standard error of calibration (SEC), R^2 (regression coefficient), and RPD (ratio of performance to deviation). Also, developed calibrations were tested with leave-one-out cross validation due to the limited number of the samples in the study. The root mean square error of prediction (RMSEP) and standard error of prediction (SEP) were calculated for the validation set and evaluated. Finally, the robust calibration models were transferred to the device using the graphical user interface. The performance of the prototype NIR device was compared with a bench-top NIR instrument (Spectrastar 2400 D, Unity Scientific, USA) using previous calibration models. The prototype output and reference analysis results were also evaluated using Pearson moment correlation.

Results and discussion

Variation in the samples for targets for calibration development

The results of the reference analyses are presented in Table S2. Studies have been carried out on the variation of major and minor biochemical components in maize flour and their analysis by NIR spectroscopy. In this work, the variation in the measured compounds was similar to the previous results for maize genotypes. In addition, there are limits in the literature for major and minor components to specify maize genotypes. These limits indicate that maize genotypes may be classified into groups. For example, maize genotypes containing more than 6–7% oil are called high-oil maize,^[39] while those with carotenoids above 50 micrograms per gram are

high carotenoids^[40] and genotypes are quality protein maize when they have specified values of essential amino acids^[41] (Table S2). The variation in the sample set has importance for NIR calibration in two ways. Firstly, the presence of high and low values in the set has a positive effect on the calibration statistics, especially R^2 values. Secondly, these genotypes positively affect the potential of the developed calibration and allow the base to expand.

Evaluation of prototype instrument

The developed prototype collects spectra that are incorporated to the models and the results are displayed on the screen in approximately 60 seconds. The measurement time is significant for NIR analysis.

The portability of the instrumentation with an internal processor is important. In addition, it is also desirable to analyze samples away from the device using reflectance or transmittance probes.^[42] The prototype in this study is portable and provides internal data analysis after measurement with the integrated fiber optic probe. However, some portable NIR devices require an external computer. In addition, an external light source must be connected to these devices.

Although miniature spectrophotometers are advantageous in terms of portability, they have disadvantages compared to the bench-top devices in terms of scanning range.^[4] The majority of portable NIR devices have a 900 to 1700 nm scanning range. The developed prototype, on the other hand, is able to scan between 900 and 2100 nm.

The software spectrometers are expected to display the spectra, input data to the internal model, and transfer the results by Bluetooth or physical recording. The separation of the spectrum acquisition and analysis process is an important feature for calibration development. The software of the developed prototype has all basic functions.

Evaluation of calibration models and comparison of performance

The results for the calibration models developed for the biochemical parameters are shown in Table S3. The R^2 values of the models related to the targets were between 69.90% and 94.16%, and the ratio of performance to deviation values were from 1.73 to 4.18. For the NIR prediction models to be successful, the model should have low RMSEC and SEE values, and high R^2 and ratio of performance to deviation (RPD) results. Models with RPD exceeding 2.25 are suitable for NIR and the success increases with the RPD value.^[43]

9 of the 12 developed models in this study are suitable for measurements in the prototypes. Correlation graphs showing the relationship between the reference analysis in the calibration set of the models and the prototype predictions are shown in Figure S5. The correlation plots support the results based on evaluation statistics. A number of studies have determined biochemical components in maize samples. Some literature results were similar to the findings from the prototype,^[18,22,44,45] while others gave conflicting results.^[46–49] The scanning properties, the modeling for calibration, the approach in the biochemical measurements and the variations in samples influence the results.

The samples analyzed on the prototype were also characterized by a bench-top NIR instrument. The results obtained by local calibration models on the bench-top for some targets were compared with the prototype measurements^[19,20,44] as shown in Table S4. High similarities in moisture, protein, oil, oleic, linoleic, lysine, and tryptophan contents were observed. Correlations for amylose, amylopectin, carotenoid and tocopherol content were poor because different methods were used in the model development.

The models for carotenoid and tocopherol contents in the bench-top NIR instrument are based on chromatographic measurements.^[11] These features in prototype were done by ultraviolet absorption spectroscopy. Similarly, the models for the bench-top NIR device for amylose and amylopectin content were based on an enzymatic procedure,^[20] while these parameters were analyzed by colorimetry in the prototype. These findings show that the method used in calibration influences the results. Since the reliability of the comparison between methods was not a study goal, detailed analyses were not performed. However, this issue is worthy of consideration in future studies.

There are no NIR instruments that can be exactly compared with the developed prototype. However, some comparisons are possible. Ayalew and Ward^[14] developed a prototype which has the ability of measuring moisture content in millet peat. This instrument analyzes only one compound using two wavelengths. Spielbauer et al.^[50] developed a benchtop prototype for protein, oil, starch, and weight of corn kernels using PLS calibration. This instrument made measurements from 900 to 1700 nm with 1 nm resolution and provided good results for targets. The developed prototype has significant advantages in the scanning interval and the measurement of multiple compounds. The reported device also has limitations compared to other instruments. Its main limitations are the resolution and sample validation. The prototype has a 6 nm resolution that should be improved for scanning studies. Also, this device has only been characterized for maize flour based on a specific calibration set that is low in number compared to commercial calibration sets. More samples

should be analyzed with appropriate calibration models with a larger number of samples.

Conclusion

12 biochemical components were determined simultaneously in maize flour using the reported prototype. The models for proteins, oils, carotenoids, and tocopherol content were successful compared to other analytes. Since modeling based on machine learning was used, the relationship between the spectra and the analytes were determined accurately. The developed instrument has a wide scanning range from 900 to 2100 nm, uses learning-based algorithms, and is more convenient than current portable near infrared instrumentation.

High similarity was obtained for samples on a bench-top instrument and the prototype for some biochemical compounds. The similarity of measurements by the prototype and the bench-top instrument is lower for those components for which different reference methods were used in calibration development. The interface uses the R language and the integration of models with the same software provides high compatibility. Further studies should evaluate the biochemical content of additional agricultural products. Also, the software should include cloud-based service for use over the internet. The limitations of resolution and few sample applications have been identified in the prototype. The amelioration of these issues will improve its functionality.

Disclosure statement

No potential conflict of interest was reported by the authors.

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