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Simultaneous detection of melamine and urea in gluten with a handheld NIR scanner

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Abstract The standard analytical methods for the determination of total protein content, which is an important measure of quality in many food products, can be easily miss leaded by adding nitrogen deriving from different source driving to serious adulteration of the various foods. Therefore, there is an immense need to develop rapid method to detect multiple adulterations with handheld instruments. The objective of the present work is to develop multivariate models for simultaneous prediction of melamine and urea in wheat gluten samples with a handheld NIR scanner. Wheat gluten samples from ten different manufacturers from different part of the world were mixed with melamine and urea in different ratios to provide a robust enough sample set for spectral data acquisition. In spite of the natural separation based on the geographical origin of the gluten samples it was possible to build accurate models for simultaneous quantification of common food adulterants, melamine and urea, in multiple mixtures of gluten. The results show Tellspec Enterprise Food Sensor as a rapid, cost effective and user friendly tool can be used for the determination of melamine and urea adulteration in wheat gluten down to 1 % concentration.

Keywords: Adulteration, near infrared spectroscopy, principal component analysis, partial least square regression.

1 Introduction

Protein content is very important in many food products due to its high impact on the sensory and rheological characteristics, moreover, due to its nutritional value [1]. Therefore, total protein content has been used as a measure of quality of many raw, intermediate and final products in the food industry. Standard analytical methods for total protein determination are based on measurement of nitrogen content (e.g. Kjeldahl, Dumas), thus, cannot distinguish if nitrogen derives from different source [2,3]. This fact leads to adulteration of the various foods with nitrogen reach compounds. Urea and melamine are known to be commonly used adulterants which can cause intoxication as the example showed in China in 2008. The addition of these chemicals cannot be detected by the standard methods. There are available analytical methods such as gas chromatography (GC), high-performance liquid chromatography (HPLC) and more [4–6] to quantify the specific adulterants but these methods are specific, expensive and laborious. Therefore, the development of cost-effective and rapid measurement techniques to identify and quantify melamine and urea as food adulterants are desired [7]. Mid- (MIR) and near-infrared (NIR) spectroscopy was found as an effective tool to detect melamine in dairy products, such as infant formula, milk powder, or liquid milk [8]. Surface enhanced Raman spectroscopy was also showed to be applicable to screen foods and detect melamine contamination in wheat gluten, chicken feed, cakes and noodle with lower than 1 % accuracy [9]. NIR spectroscopy combined with PLS-DA was also used to identify the presence of melamine in milk [10]. Yang et al. [11] showed mid-infrared spectroscopy can be used to quantify urea with linear model due to its molecular fingerprint in mid-infrared region. Near-infrared Raman spectroscopy was presented as applicable method for quantitative determination of urea adulteration in milk [12]. Several reports show NIR spectroscopy has been successfully applied for the detection and quantification of simultaneous detection of mixed adulterations, too [13]. However, there is an immense need to develop rapid method to detect multiple adulterations with handheld instruments. The objective of the present work was to develop multivariate models for simultaneous prediction of melamine and urea in wheat gluten samples based on data acquired with handheld NIR scanners and a user friendly mobile app.

2 Materials and Methods

Wheat gluten samples from ten different manufacturers were mixed with melamine (M) and urea (U) in different ratios to get total contamination from 0 to 18 % based on the following; M:U = 1:1 in 1, 2, 5, 10, 18 %; M:U = 2:1 in 2, 5, 10, 15 %; M:U = 1:2 in 2, 5, 10, 15 %; only U in 0.5, 1, 2, 4, 8 %; and only M in 0.5, 1, 2, 4, 8 % concentration. Total number of samples analyzed was 219. The samples were stored and scanned in plastic bags. The NIR spectra of the samples were collected with two Tellspec Enterprise Food Sensor g1 scanners (scanner 1 and scanner 2) (Tellspec Inc., Toronto, Ontario, Canada) in several sessions acquiring multiple spectra per sample in each session, with 2 nm spectral step in the 950–1630 nm spectral interval. Various sample pre-processing methods and multivariate data analyses techniques were used to process the spectral data. Principal component analysis (PCA) [14] was used to describe multidimensional patterns of the data and to discover outliers. Partial least squares regression (PLSR) was used for quantitative models [15] to evaluate the relationship between the melamine or urea concentration and NIR spectra. The PLSR models were optimized for both scanners separately by using cross-validation, where data of single samples with their repeats were left out of the calibration and were used for validation, iteratively. Finally, the trained models of scanner 1 were tested with data of scanner 2, and vice versa, to achieve independent prediction.

3 Results and Discussion

Smoothed (Savitzky-Golay filter) and normalized average spectra of pure melamine, urea and wheat gluten samples were calculated and plotted to test the performance of the handheld NIR scanners for the detection of the absorption peaks of the tested main chemicals (Fig. 2.1).

Average absorbance spectrum of melamine shows peaks at 1021, 1473, 1494 and 1522 nm, while that of urea at 1031, 1231, 1305, 1413, 1470, 1499 and 1529 nm and of wheat gluten at 1029, 1205 and 1506 nm which is in a good harmony with the results available in the corresponding references [16–18].

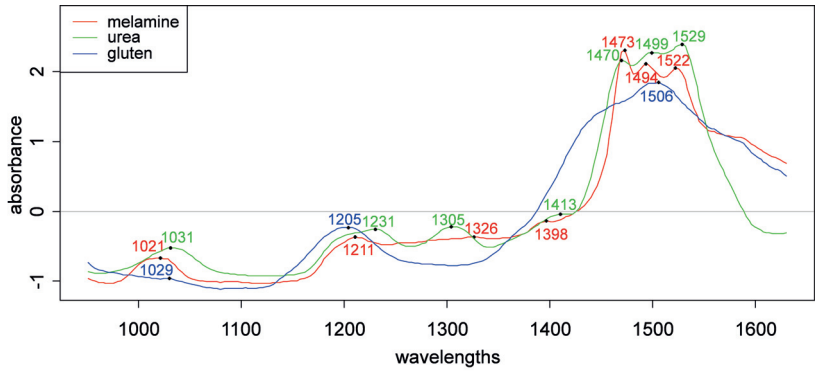

Figure 2.1: Smoothed (Savitzky-Golay filter) and normalized average spectra of pure melamine ($n_{melamine}$ = 50), urea (n_{urea} = 50) and wheat gluten (n_{gluten} = 1675) samples acquired with scanner 1 with their assigned absorption peaks.

The calculation of PCA models were performed both merging the data of scanner 1 and scanner 2 and separately to discover the multidimensional patterns of the spectral datasets. Results of PCA calculated on the averaged, smoothed (Savitzky-Golay filter) and pretreated spectra of pure and adulterated wheat gluten samples using the range between 950 and 1630 nm acquired with scanner 1 is shown in Fig. 2.1. Score plot of PC1 and PC2 presenting more than 60 % of the spectral variation presents good tendency of separation based on the total gluten content of the pure and adulterated wheat gluten samples (Fig. 2.2 a) mainly based on PC1. Separation of two main groups was observed in the PCA score plots in the case of data of both scanners (Fig. 2.2 b, for scanner 1). The grouping of samples was caused by the spectral differences of the wheat gluten samples originated from Europe and Asia.

Further analysis were performed coloring the PCA scores based on melamine or urea concentration and results showed separation based on the concentration of the adulterants in PC1 and PC2 plain similar to the result of separation of the different concentration wheat gluten. The wavelengths having highest importance of the separation of the samples containing different concentration of adulteration i.e. melamine and/or urea, can be discovered based on the loadings of the PCA mod-

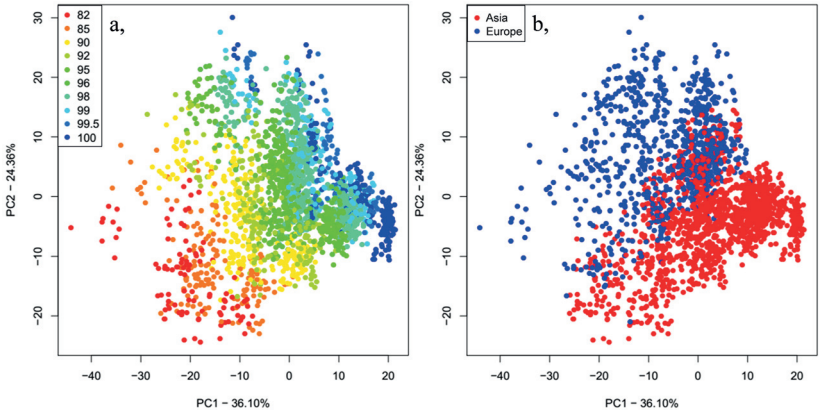

Figure 2.2: PCA score plots (PC1-PC2) of pure and adulterated wheat gluten samples calculated by the averaged, smoothed (Savitzky-Golay filter) and pretreated spectra of the 950–1630 nm range (n = 2314) acquired with scanner 1. a) Represents the score plot colored by wheat gluten concentration. b) Represents the score plot colored by origin of wheat gluten samples.

els (Fig. 2.3 for scanner 1). Loadings of PC1 and PC2 beside others highlights the importance of 1030, 1218, 1302, 1326, 1468, 1490 and 1532 nm which are in the ranges of the absorption peaks of the main components of the mixtures i.e. wheat gluten, melamine and urea.

The pattern encoded in the spectral dataset revealed by the exploratory data evaluation gives the rise to build regression models for simultaneous quantification of melamine and urea concentration in wheat gluten samples.

Results of the PLSR model built to predict melamine concentration based on data of scanner 1 is shown in Fig. 2.4 a,. High coefficient of determination (R^2) was found in model training (R^2tr = 0.9877) and as well as in cross-validation (R^2cv = 0.9858). The error of training (RMSEC) and cross-validation (RMSECV) were 0.3182 and 0.3429 %, respectively. The independent prediction performed with the data of scanner 2 (Fig. 2.4 b,) also proved the high accuracy and robustness of the model (R^2_{pr} = 0.9818 and RMSEP = 0.39 %). Similar results could be achieved with the model built on the data of scanner 2; parameters of the model, validation and prediction are R^2tr = 0.9815, RMSEC =

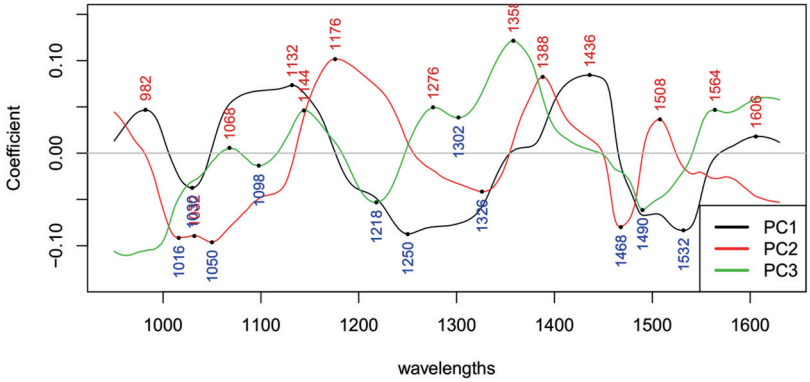

Figure 2.3: PCA loadings plot (PC1-PC3) of pure and adulterated wheat gluten samples calculated by the averaged, smoothed (Savitzky-Golay filter) and pretreated spectra of the 950–1630 nm range (n = 2314) acquired with scanner 1.

0.3927 % and R^2cv = 0.9798, RMSECV = 0.104 % and R^2_{pr} = 0.9775 and RMSEP = 0.43 %, respectively.

Models built to predict urea concentration were also found to be reliable. PLSR model built on the data of scanner 2 showed slightly better accuracy than that of scanner 1 (Fig. 2.5).

The coefficient of determination for the training (R^2tr) and for the cross-validation (R^2cv) were found 0.9163 and 8773, respectively (Fig. 2.5 a,), while for the independent prediction based on the data collected with scanner 1 (R^2_{pr}) was 0.9034 (Fig. 2.5 b,). The average prediction error of training (RMSEC = 0.8944 %) and cross-validation (RMSECV = 1.0597 %) as well as of independent prediction (RMSEP = 0.9607 %) confirmed that the determination of urea concentration in wheat gluten powder is also possible with 1 % average prediction error beside the changing concentration of melamine with the handheld food scanner.

Regression coefficient vectors of PLSR models provide information about the wavelengths of highest importance in the quantitative regression models (Fig. 2.6).

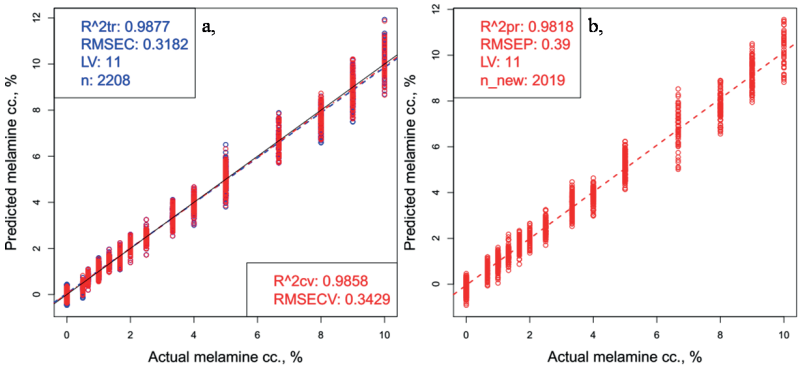

Figure 2.4: Calibration, cross- and independent validation of PLSR on melamine concentration in wheat gluten samples. a) Results of calibration model (blue color) and cross-validation (red color) built on data of scanner 1. b) Results of independent prediction based on data of scanner 2.

The coefficient vector of the regression model built for the determination of the melamine concentration in wheat gluten samples (Fig. 2.6 a,) proves the high importance of the wavelengths 1472, 1492 and 1522 nm which are the characteristic absorption peaks of melamine. The wavelengths found in the regression vector of the urea model (Fig. 2.6 b,) at 1042, 1224, 1304 nm and in the range between 1468 and 1538 nm also obviously present that the spectra of the adulterated wheat gluten samples acquired with the handheld food scanner hold the information of the absorption of urea.

4 Conclusions

Spectra of pure melamine, urea and wheat gluten samples showed that the handheld NIR scanner is applicable to accurately measure the characteristic absorption peaks of the tested chemicals. Results of principal component analysis presented good tendency of separation based on the total gluten content of the pure and adulterated gluten samples based on their NIR spectra. Separation of two main groups was ob-

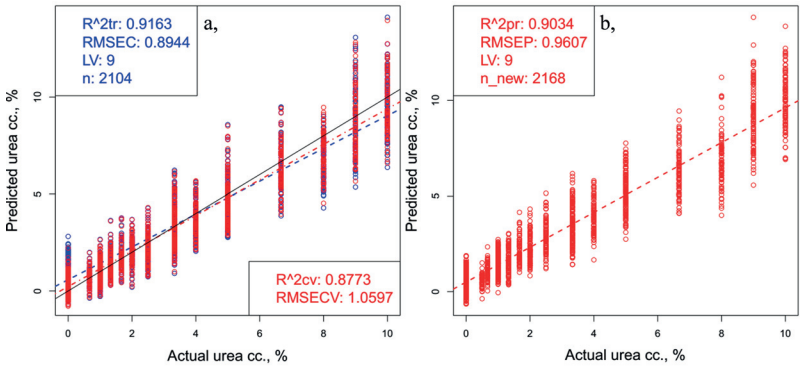

Figure 2.5: Calibration, cross- and independent validation of PLSR on urea concentration in wheat gluten samples. a) Results of calibration model (blue color) and cross-validation (red color) built on data of scanner 2. b) Results of independent prediction based on data of scanner 1.

served in the PCA score plots, which was caused by the spectral differences of the gluten samples originated from Europe and Asia. In spite of this natural separation it was possible to gain robust models to predict melamine and/or urea concentration accurately. Accurate models were built for simultaneous quantification of melamine and urea, in multiple mixtures of gluten. The achieved results prove that the Tellspec Enterprise Food Sensor as a rapid, cost effective and user friendly tool can be used for simultaneous quantification of the most common food adulterants in wheat gluten powders at or lower than 1 % adulteration level.

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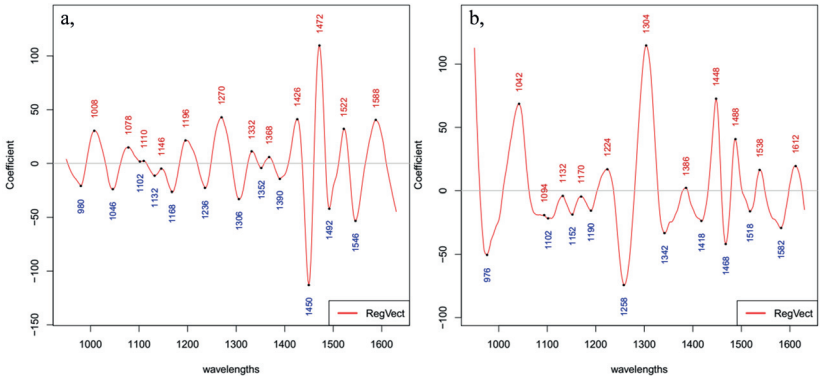

Figure 2.6: Regression coefficient vectors of PLSR models with the assigned wavelengths having the highest weight in the regression models. a) PLSR on melamine concentration in wheat gluten samples (Fig. 2.4). b) PLSR on urea concentration in wheat gluten samples (Fig. 2.5).

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