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Development and Validation of a Near-Infrared Spectroscopy Method for the Prediction of Acrylamide Content in French-Fried Potato

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ABSTRACT: This study investigated the ability of near-infrared spectroscopy (NIRS) to predict acrylamide content in French-fried potato. Potato flour spiked with acrylamide (50−8000 μg/kg) was used to determine if acrylamide could be accurately predicted in a potato matrix. French fries produced with various pretreatments and cook times (n = 84) and obtained from quick-service restaurants (n = 64) were used for model development and validation. Acrylamide was quantified using gas chromatography−mass spectrometry, and reflectance spectra (400−2500 nm) of each freeze-dried sample were captured on a Foss XDS Rapid Content Analyzer-NIR spectrometer. Partial least-squares (PLS) discriminant analysis and PLS regression modeling demonstrated that NIRS could accurately detect acrylamide content as low as 50 μg/kg in the model potato matrix. Prediction errors of 135 μg/kg (R^2 = 0.98) and 255 μg/kg (R^2 = 0.93) were achieved with the best PLS models for acrylamide prediction in Russet Norkotah French-fried potato and multiple samples of unknown varieties, respectively. The findings indicate that NIRS can be used as a screening tool in potato breeding and potato processing research to reduce acrylamide in the food supply.

KEYWORDS: *acrylamide, near-infrared spectroscopy (NIRS), partial least-squares (PLS) regression, potato, Solanum tuberosum*

INTRODUCTION

Acrylamide is a known neurotoxin in humans, and low doses of acrylamide (0.5 mg/kg bw/day) have been shown to be genotoxic and carcinogenic in animal models.[1−5] Thus, the state of California has included acrylamide in the list of chemicals that can cause cancer (Proposition 65) and established 0.2 μg/day as a "no significant risk level".[6−8] It is well established that acrylamide is present in commonly consumed carbohydrate-rich foods processed at high temperatures during frying, baking, and roasting.[9−11] Therefore, the U.S. Food and Drug Administration recently recommended that food companies consider adopting approaches to reduce acrylamide formation and enable the monitoring of acrylamide content in finished food products.[12] The potentially negative effects of acrylamide on human health[13] and the potential for high chronic exposure to acrylamide through dietary consumption of foods such as processed potato products have stimulated the potato industry in the United States to search for new potato varieties that reduce acrylamide formation in processed products while retaining or exceeding the agronomic and consumer acceptance traits found in standard varieties.[14]

Analytical methods used to quantify acrylamide in foods are based on chromatography and mass spectrometry. These methods are expensive and time-consuming and are not suitable for quality control during the manufacturing process or for screening large numbers of samples for potato-breeding programs. As an alternative, several groups have investigated the feasibility of using finished product color as an indirect measure of acrylamide content.[15−20] In two studies related to fried potato products, acrylamide content correlated well with product lightness, measured as the luminosity component of color (L^*) and the chromatic color component (a^*), which is a measure of redness.[15,16] Vinci and colleagues evaluated several color scales commonly employed in the food industry for correlation to acrylamide formation potential of incoming raw potatoes. The selection of raw materials with <0.25 g/100 g reducing sugars or Agtron color values >61 after a three-step frying process was successful in correctly categorizing French fry samples with <500 μg/kg acrylamide 92 and 93% of the time, respectively.[17] The combination of reducing sugar content and the Agtron color value of fries from test batches was deemed a better predictor of acrylamide formation than the current quality control standards for incoming potatoes.[18] However, the use of a color measurement system on French-fried potato or chips as a global indicator of acrylamide content may not be a reliable surrogate because acrylamide is formed as an intermediate product during the Maillard reaction. Studies involving prolonged heating of green coffee, wheat flour, and potato chips indicated that changes in both acrylamide levels and the a^* parameter during heating at relatively high temperatures followed a typical kinetic pattern in which an initial increase to an apparent maximum was followed by a subsequent decrease.[19] This means that the changes in acrylamide level in foods with time during heating cannot be explained by means of simple linear regression models.

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Additionally, Taubert et al. concluded that the use of surface browning alone will not be a reliable predictor of acrylamide concentration in French fries because acrylamide content in fried potatoes depends on the surface-to-volume ratio: a linear correlation between browning and acrylamide concentration could be observed only for potato pieces with low surface-to-volume ratio (R^2 = 0.74, P < 0.001), with no correlation was observed for large-surface materials (R^2 = 0.06, P = 0.13).[20]

Near-infrared spectroscopy (NIRS) is a common technique for routine analyses because it allows fast and nondestructive analysis of samples. In potatoes, NIRS methods have been developed for dry matter,[21,22] sugars, and asparagine,[23] and as a quality control tool for potato chips.[24,25] A few recent studies have demonstrated the use of NIRS in quantification of acrylamide in potato chips. Segtnan et al.[26] reported that NIRS can be used as a screening tool for acrylamide in potato chips as a stand-alone method or in conjunction with process variable settings. NIRS could also be used online to separate samples with high and low acrylamide contents with an average prediction error of 266 μg/kg.[27] Ayvaz and Rodriguez[28] evaluated several hand-held and benchtop near-infrared and mid-infrared spectrometers for rapidly estimating acrylamide in potato chips, accomplishing acrylamide prediction in potato chips containing 169−2453 μg/kg with a standard error of prediction (SEP) of <100 μg/kg. Because of the low amounts of acrylamide in food, there is some question about the sensitivity of NIRS for measuring acrylamide in food. Usually, chemical components in the range of 100 μg/kg (0.00001%) are not detectable in NIR spectra.[26] The level of acrylamide in potato chips typically ranges from 117 to 4215 μg/kg, whereas that of French fries is 59−5200 μg/kg.[9,10,29] Moreover, the black box approach, which usually involves blind use of spectral data and regression methods in the development of NIR calibrations, makes it difficult to determine if NIR spectra were influenced solely by acrylamide in the studies cited.

In this study, we investigated the capability of NIRS as a tool in the prediction of acrylamide content in a potato flour model system and French-fried potato. Specifically, we (1) determined that NIRS can be used to accurately detect low concentrations of acrylamide in a potato flour model system, (2) identified the NIR wavelength bands associated with acrylamide content in a potato flour model and French-fried potato, and (3) validated NIRS calibration models for the prediction of acrylamide in French-fried potato.

MATERIALS AND METHODS

Chemicals and Materials. Acrylamide (99%) standard was obtained from the Sigma-Aldrich group (Milwaukee, WI, USA). Hexane of high-purity grade (95%) was obtained from Burdick & Jackson (Muskegon, MI, USA). Acetonitrile (MeCN), formic acid (98%), anhydrous magnesium sulfate ($MgSO_4$), American Chemical Society (ACS) grade sodium chloride (NaCl), and sorbent for dispersive-solid phase extraction (SPE) were purchased from Fisher Scientific (Fair Lawn, NJ, USA).

Standard Preparation. A stock solution of acrylamide standard (25 μg/mL) was prepared by dissolving acrylamide powder in acetonitrile (MeCN). From the stock acrylamide solution, acrylamide solutions of 50, 100, 200, 400, 1000, 2000, 6000, and 8000 μg/L were prepared by dilution. MeCN solution without acrylamide was used as zero level.

Potato Flour Model System. Twenty tubers free of defects were selected from two bags of Russet Norkotah potatoes purchased locally (Green Giant, Raleigh, NC, USA). The tubers were washed in water, peeled, and cut in half longitudinally. One half of each of the raw potato tubers was sliced, freeze-dried, and ground into a homogeneous flour. Potato flour (1 g) was weighed into 20 mL glass scintillation vials and spiked with each of the prepared acrylamide solutions of various concentrations. Eighteen independent replicates for each acrylamide level in the potato flour model system were prepared and scanned.

French Fries. Two sets of fried potato samples were used for development of the calibration models. The first set consisted of 84 French fry samples fried in canola oil in a 6 gal fryer (Vulcan, Baltimore, MD, USA) using the same potatoes (the other halves of the tubers previously mentioned) used for potato flour preparation. The raw tuber was sliced into strips (6.35 × 0.635 × 0.635 cm) and processed into fry samples using a protocol developed by the National Fry Processor Acrylamide trial for quick-service restaurant fry evaluation.[30] Sliced potato strips were blanched at a temperature of 77 °C for 7 min and dipped in a 0.5% sodium acid pyrophosphate solution (SAPP) at a temperature of about 66 °C for 15 s. The blanching procedure was to remove some of the reducing sugars, whereas the SAPP dip was to prevent darkening of the potato strips after cooking. The blanched potato strips were fried at a temperature of 177 °C for various times: 1, 2, 3, 4, 5, 6, 7, 8, and 10 min. Other batches of samples included blanched but not dipped in SAPP; dipped in SAPP but not blanched; and no blanching or SAPP pretreatment and fried for 2, 4, 6, and 8 min. After frying, the samples were freeze-dried to moisture content (MC) below 2% to reduce the influence of various moisture levels in the samples on NIRS spectra. Freeze-dried samples were homogenized using a food processor (KitchenAid model KFP7500B, Benton Habor, MI, USA). The second set of 64 commercial French fries was purchased from four different quick-service restaurant chains in Raleigh, NC, USA. To obtain these samples, four orders (replicates) of French fries were purchased from four different quick-service chain restaurants in four different locations. The 64 commercial samples were freeze-dried to MC below 2% prior to being homogenized using a food processor. After processing, all samples were placed in a sealed bag stored in a −24 °C cold room until they were analyzed for acrylamide content.

Quantification of Acrylamide by Gas Chromatography−Mass Spectrometry (GC-MS) Analysis. To extract the acrylamide from the samples, 1 g of homogenized potato sample was weighed into a 50 mL centrifuge tube and extracted using a combination of hexane, deionized water, and acetonitrile as described by Mastovska and Lehotay.[31] Quantification of acrylamide was accomplished using a GC-MS (models 7890A and 5975C, Agilent Technologies, Santa Clara, CA, USA) according to the procedure described in the Agilent Technology Application note on GC-MS approaches to the analysis of acrylamide.[32] Briefly, extracts were separated on a polyethylene glycol column and analyzed for acrylamide using MS detection with electron impact ionization in single ion monitoring mode for m/z 71, 55, and 44. Derivatization of acrylamide prior to analysis was not used in this study because the GC-MS detection limit for test samples was approximately 70 μg/kg, which is lower than the target goal of 100 μg/kg for the NIRS calibration. External standard calibration curves were obtained by spiking acrylamide into a freeze-dried French fry sample with low natural levels of acrylamide. Quantification of peak areas for m/z 71 was linear (regression coefficients > 0.995) for acrylamide concentrations in the range of 50−8000 μg/kg.

NIR Spectra Acquisition. Spectra were captured using a Foss XDS Rapid Content Analyzer-NIR spectrometer. The spectrometer is designed for the nondestructive rapid analysis of samples (solid, powder, slurries, and liquid), and it captured spectral data in reflectance mode from 400 to 2500 nm. Each potato flour model system sample or freeze-dried French fry sample was sealed in an XDS mini sample cup and scanned 32 times to obtain an average spectrum in ambient laboratory condition (20 °C, 65% relative humidity).

Data Analysis and NIRS Model Development. Multivariate data analysis of spectra was performed using The Unscrambler X software version 10.3 (Camo Smart, Woodbridge, NJ, USA). A total of 162 spectral samples were used for model development, generated from 18 replicates of each of 9 levels of acrylamide-spiked potato flour model solutions (0, 50, 100, 200, 400, 1000, 2000, 6000, and 8000 μg/L). Raw spectra and spectra preprocessed using Savitzky−Golay first-

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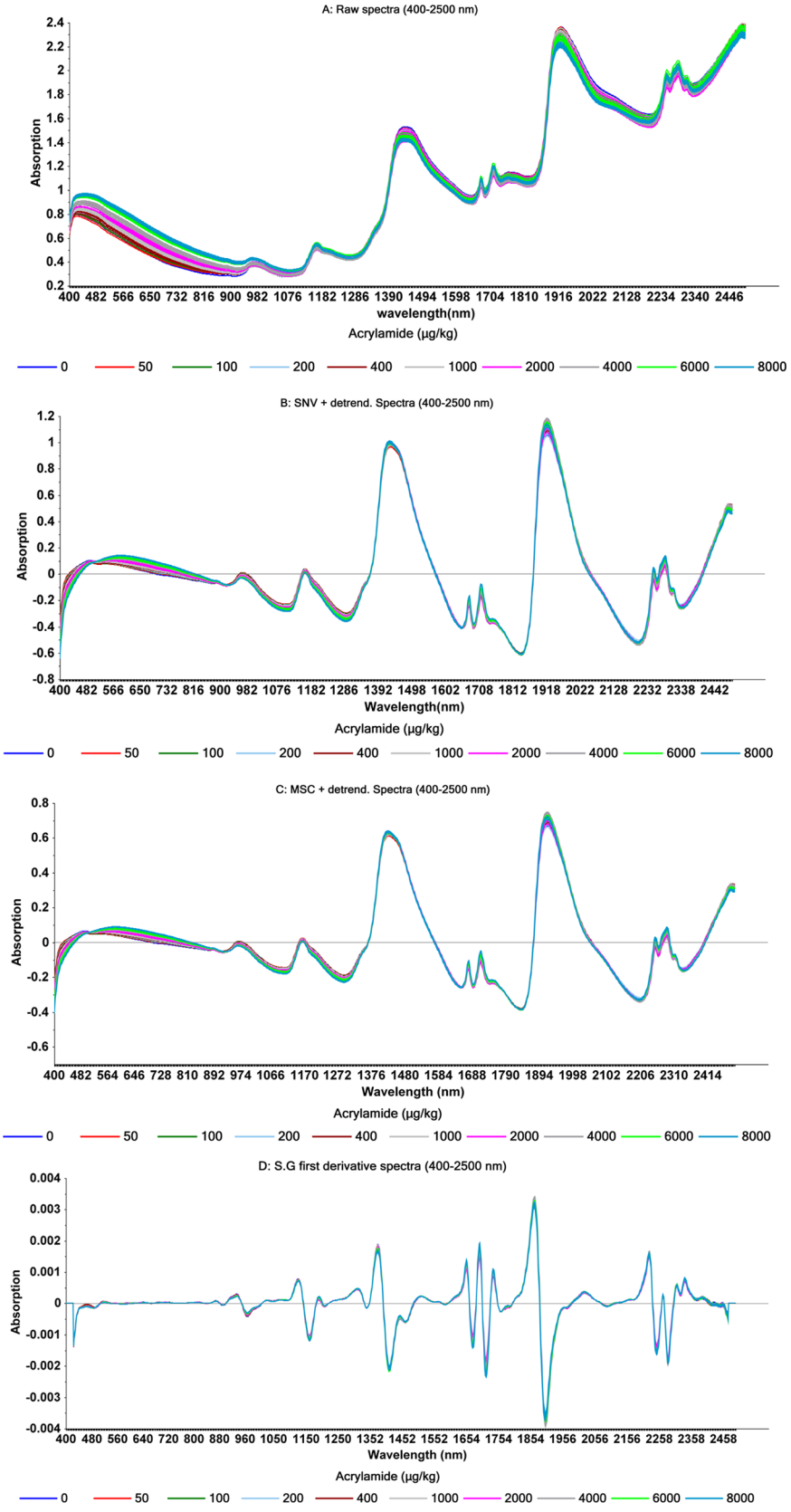

Figure 1. Raw and transformed spectra of potato flour spiked with various acrylamide concentrations.

derivative (SG first deriv), a combination of standard normal variate and detrending (SNV + detrend), and a combination of multiplicative scatter correction and detrending (MSC + detrend) transformations were used to develop the calibration models. To determine which wavelength regions provided the most useful qualitative and quantitative information for acrylamide prediction in processed potato, models were developed using the visible−NIR region (400−2500 nm), the NIR region (780−2500 nm), the combination of second

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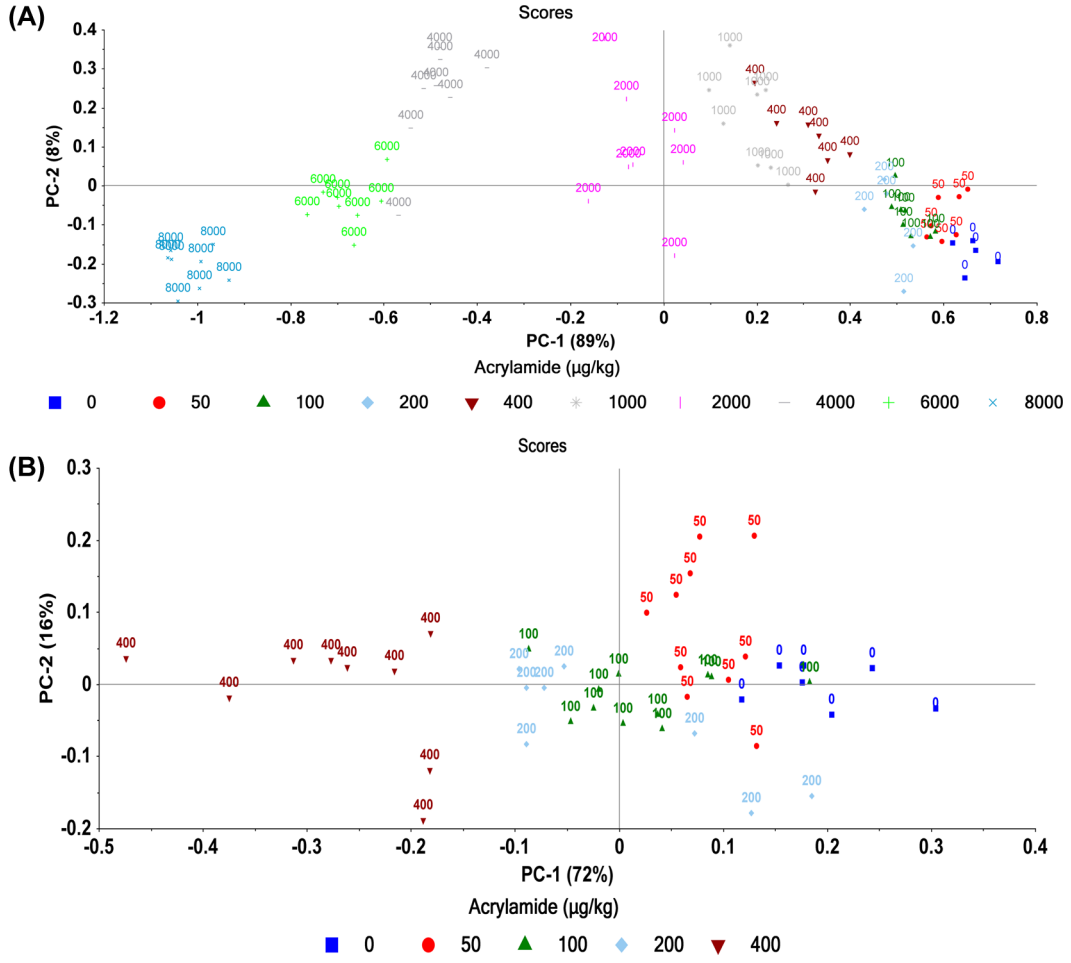

Figure 2. Score plots of PCA model developed using SNV + detrend transformed spectra (400−2500 nm): (A) acrylamide content of 0−8000 ppb; (B) model for samples spiked only with low amounts of acrylamide (0−400 ppb).

overtone and first overtone NIR regions (1100−2500 nm), and the 1800−2300 nm region. The broad wavelength regions and not individual wavelengths were selected to construct a model that gave the most efficient estimates of the amount of acrylamide. This is because NIR spectral data contain subtle information in wavelength absorption intensities, which are not visible as individual peaks.

Principal component analysis (PCA) was carried out to observe any clustering or separation in the data set and, subsequently, partial least-squares discriminant analysis (PLS-DA) was used to build prediction models and to test if NIRS can be used to differentiate and correctly classify samples with low amounts of acrylamide. PLS-DA, a linear classification method that combines the properties of partial least-squares regression with the discrimination power of a classification technique, was used to develop a model capable of assigning new samples to a classification group. PLS-DA is based on the PLS regression algorithm, which searches for latent variables with a maximum covariance with the *Y*-variables. The response variable (*Y*-variables) is a categorical one (replaced by a set of dummy variables describing the categories) expressing the class membership of the statistical units. Therefore, PLS-DA does not allow for other response variables than the ones that have been defined. As a consequence, all measured variables have the same role with respect to the class assignment. The main advantage of PLS-DA is that the relevant sources of data variability are modeled by the so-called latent variables (LVs), which are linear combinations of the original variables and, consequently, it allows graphical visualization and understanding of the different data patterns and relations by LV scores and loadings. Loadings are the coefficients of variables in the linear combinations that determine the LVs, and therefore they can be interpreted as the influence of each variable on each LV, whereas scores represent the coordinates of samples in the LV projection hyperspace.[33,34] PLS-DA models were developed and validated using 86 samples with acrylamide values ranging from 0 to 400 μg/kg. From the 86 spectral data, 66 samples were randomly selected and used as the calibration set, and the remaining 20 samples were used to validate the models.

PLS regression calibration models capable of predicting concentrations of acrylamide in French fry samples were developed in a two-stage process. French fry samples from Russet Norkotah potatoes fried with NFTP protocols in the pilot plant at different fry times (1−10 min) and different pretreatments (blanched, SAPP dip, no treatment) were used to develop calibration models. These models were subsequently referred to as single-variety calibration (SVC) models. The total sample set for the SVC models consisted of 84 samples, of which 50 samples were randomly selected as a calibration set, and the remaining 34 samples were used as a testing set to validate the calibration. The second stage of calibration model development involved using 148 samples, comprising all 64 samples acquired from four commercial quick-service restaurants plus the 84 Russet Norkotah potato (SVC samples fried in the pilot plant). These models were subsequently called multiple-variety calibration (MVC) models. Prior to development of calibration models, all spectra were standardized using autoscaling. This involved the combination of mean centering and scaling by the inverse of the standard deviation so that the resulting data and model may be interpreted in terms of the variation around the mean. To obtain an initial overview of data, PCA was carried out on the specimens to observe any clustering or separation and outliers in the specimen set. Spectral data of 12 samples fried between 1 and 3 min in the pilot plant were identified as outliers and removed from the models because of high spectral distance to the center within the space described by the PCA model and high residual

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and leverage; hence, a total of 136 samples were used for the final calibration. Samples (n = 80) were randomly selected for the calibration set, and the remaining 56 samples were used as test data to validate the models. The calibration models were constructed with an X-matrix of broad wavelength ranges of 400−2500, 780−2500, 1100−2500, and 1800−2300 nm and the GC-MS quantified acrylamide contents of samples as the Y-matrix. Different calibration models were developed using raw spectra and spectra preprocessed using various pretreatment methods to determine which spectral pretreatment method improved the interpretation of the spectra, integrity, and applicability of the calibration models. The limit of detection (LOD) and limit of quantification (LOQ) for the models were determined from the PLS regression curves. LOD was calculated as 3 times the standard deviation of the Y-residuals/slope, and LOQ was calculated as 3 times the LOD.

RESULTS AND DISCUSSION

Potato Flour Model System. The potato flour model system was used to determine if NIRS can detect acrylamide present in a potato matrix at levels relevant to processed potatoes and to determine the limit of detection in such a matrix. Figure 1 shows the raw spectra from 400 to 2500 nm (A), the SNV + detrend transformed spectra (B), the MSC + detrend transformed spectra (C), and the SG first deriv transformed spectra (D) for the potato flour spiked with various amounts of acrylamide (0−8000 μg/kg). Because of the low intensity of NIR, the spectral bands (peaks) in the NIR region are broad and are not as sharp as absorption peaks from the mid-infrared region; therefore, assignment of peaks to individual vibrations in NIR region is almost impossible. The frequency range of the NIR (800−2500 nm) covers mainly overtones and combinations of lower energy fundamental vibrations associated with C−H, O−H, and N−H bonds. The overtones and combination bands are types of vibrations related to the fundamental vibrations of the mid-infrared region. These are significantly weaker in absorption compared with the fundamental vibrational bands from which they originate. The weak signals from these vibrations are similar to resulting spectra consisting of many broad and overlapping bands as opposed to the mid-IR (2500−25000 nm). The raw spectra and the spectra preprocessed with MSC transformation were similar in appearance and showed two large broad peaks centered around 1440 and 1930 nm in the NIR region. Absorption in the visible region (400−780 nm) increased with acrylamide concentration in the potato flour matrix as shown in Figure 1A. Absorption in the area of the two major broad peaks found in the wavelength regions of 1400−1700 and 1900−2200 nm showed inverse relationships with increasing concentrations of acrylamide. This observation was consistent for most of the spectra of samples spiked with acrylamide ranging from 200 to 8000 μg/kg. However, there was no clear visual separation of spectra for samples spiked with acrylamide concentrations below 200 μg/kg. PCA of spectra was carried out to obtain an overview of the data and check for underlying patterns or relationships. PCA score plots were generated for all models developed with the raw and preprocessed spectral data and for each of the broad wavelength regions. PCA score plots revealed clustering of replicate samples and clear separation of samples with differing amounts of acrylamide (Figure 2A). The ability to differentiate NIR spectra of potato matrix samples spiked with various levels of acrylamide provided a strong indication that predictive models could be developed. However, visual separation of samples spiked with acrylamide concentrations below 200 μg/kg was less evident as shown in the PCA plot (Figure 2B) of samples spiked with low amounts of acrylamide (0−400 μg/kg). There was no clear distinction between samples spiked with 200 or 100 μg/kg of acrylamide, which suggests the need for further model development to differentiate samples with low concentrations of acrylamide.

Various transformation algorithms were evaluated to facilitate more accurate acrylamide concentration prediction in the lower ranges (0−400 μg/kg), because the target for potato-breeding efforts is to develop cultivars that consistently have acrylamide content below 200 μg/kg after processing into French fries. Table 1 summarizes the results of PLS-DA model cross-validation statistics for all models developed with raw spectral data and spectra preprocessed with different processing methods using various spectral wavelength regions. The plot of weighted regression coefficients (Figure 3) for all spectral data (400−2500 nm) revealed that multiple spectral wavelength regions play an important role in the PLS-DA models. The magnitude of the regression coefficients, whether positive or negative, is an indication of the importance of the variable in modeling for the response. The performance of all PLS-DA models was evaluated by using each of the PLS-DA models in the prediction of 20 samples not used in model development. The response of the validation samples as predicted with models developed with spectral data preprocessed using a combination of SNV + detrend is presented in Figure 4 as a plot of prediction with deviation as a function of the true values of 400, 200, 100, 50, and 0 μg/kg. The predicted response of each sample is shown as a thick horizontal line, and the box

Table 1. Statistics for PLS-DA Classification Model Development To Predict Low Acrylamide Concentrations (0−400 ppb) in a Potato Flour Model System

spectral treatment[a] (wavelength range)	N	LVs[b]	cal R^2[c]	RMSEC[d]	SEC[e]
raw (400−700 nm)	66	3	0.96	0.27	0.27
raw (400−2500 nm)	66	5	0.98	0.19	0.19
raw (780−2500 nm)	66	6	0.94	0.32	0.32
raw (1100−2500 nm)	66	7	0.92	0.38	0.39
raw (1800−2300 nm)	66	7	0.73	0.68	0.69
SG (400−700 nm)	66	2	0.95	0.29	0.30
SG (400−2500 nm)	66	5	0.97	0.23	0.23
SG (780−2500 nm)	66	4	0.93	0.35	0.36
SG (1100−2500 nm)	66	5	0.92	0.38	0.38
SG (1800−2300 nm)	66	6	0.94	0.34	0.34
SNV(400−700 nm)	66	3	0.95	0.32	0.32
SNV(400−2500 nm)	66	4	0.99	0.15	0.15
SNV(780−2500 nm)	66	4	0.99	0.15	0.15
SNV(1100−2500 nm)	66	4	0.97	0.22	0.22
SNV(1800−2300 nm)	66	7	0.94	0.33	0.33
MSC (400−700 nm)	66	3	0.96	0.27	0.28
MSC (400−2500 nm)	66	4	0.99	0.15	0.15
MSC (780−2500 nm)	66	4	0.98	0.18	0.18
MSC (1100−2500 nm)	66	4	0.97	0.23	0.24
MSC (1800−2300 nm)	66	7	0.96	0.29	0.29

[a]Raw = untreated spectra; SG = Sovitsky−Golay first derivative; SNV = single normal variate; MSC = multiplicative scattering correction. [b]Optimum number of latent variables. [c]Calibration R-square. [d]Root mean square error of calibration. [e]Standard error of calibration.

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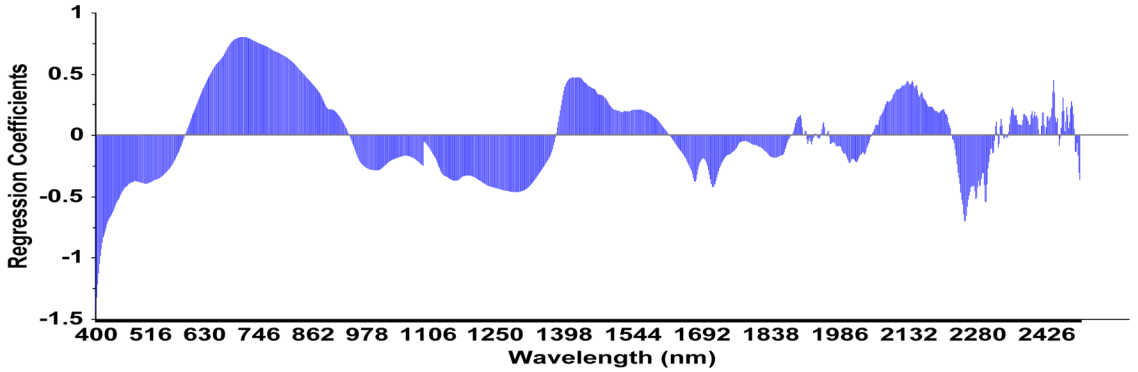

Figure 3. Weighted regression plot showing important variables in modeling acrylamide concentration with SNV transformed spectra (400−2500 nm) of potato flour spiked with various amounts of acrylamide.

around the predicted value spans the deviation in both directions. This deviation of the predicted sample from the measured one is an estimate of the prediction uncertainty. For a prediction to be trusted, the predicted sample must not be too far from a calibration sample true value. Large deviations (uncertainties) associated with prediction of new samples with the PLS-DA models developed using only the visible region (400−780 nm) or with the combination band of the longer wavelength region (1800−2300 nm) indicated that the predictions of acrylamide in the model system based on these spectral regions alone cannot be trusted (Figure 4D,E). Models developed with either the full spectra (400−2500 nm) or the full NIR spectra (780−2500 nm) pretreated with SNV + detrend transformation performed best (Table 1 and Figure 4) for prediction of new samples in the model system. These models had lower prediction error with fewer latent variables required to explain the variance while maintaining a high correlation coefficient. However, it should also be noted that MSC + detrend pretreated spectra (400−2500 or 780−2500 nm) could also be used to achieve a similar result as they had comparable predictive statistics as shown in Table 1.

French-Fried Potatoes. The acrylamide content of the 84 potato samples fried in the pilot plant and acrylamide contents in the commercial French fry samples ranged from 211 to 3381 μg/kg, within the range of 59−5200 μg/kg reported in previous studies.[9,10,29] Respective calibration and predictive statistics for calibrations developed using the potato samples fried in the pilot plant (SVC) and for the 60 restaurant samples plus the 84 Russet samples fried in the pilot plant (MVC) are presented in Table 2.

Spectral Features and Modeling. Full spectra (400−2500 nm) data treated with MSC transformation for all potato fry samples are presented in Figure 5. In the NIR spectra region (780−2500 nm), there were six wavelength ranges of 942−1084 nm (third overtone region), 1140−1250 nm (second overtone region), 1400−1600 nm, 1650−1850 nm (first overtone region), 1900−2200 nm, and 2300−2400 nm (combination band region) that showed some relationship with levels of acrylamide. Spectral features at wavelength ranges of 1400−1600, 1650−1850, and 1900−2200 nm all had a positive correlation with acrylamide as absorption at these wavelength regions increased with increases in acrylamide content (Figure 5). In contrast, the wavelength regions of 942−1084, 1140−1250, and 2300−2400 nm showed a negative correlation with acrylamide concentration where the absorption intensity of the spectra was reduced with increases in acrylamide. The wavelength regions that showed the most prominent differences related to acrylamide levels in the fried samples were found in the 942−1084 nm range, with a peak centered at 994 nm, and in the 1900−2200 nm range, with a peak centered around 1930 nm (Figure 5). Spectral features with pronounced differences were also observed in the visible range (400−700 nm). The spectra absorption in the visible region of 400−700 nm increased with fry time and amount of acrylamide for all fried samples as shown in Figure 5. This observed relationship between spectra of fried samples, acrylamide content, and fry time may be related to color changes as a result of the browning process, or it could also be attributed to the fact that VIS-NIR spectra captured chemical changes in the fried samples that may or may not be relevant in terms of acrylamide.[35] Because of the observed relationship between both browning and acrylamide content with spectral changes in the visible region, one may be tempted to think that the French fry model is measuring color changes as a result of the browning process rather than acrylamide. However, as exemplified by the potato flour model system, the plot of weighted regression coefficients (Figure 6) for the VIS-NIR spectra (400−2500 nm) revealed that multiple spectral wavelength regions, including a large part of the visible region (400−780 nm), played an important role in the PLS model for prediction of acrylamide. However, all models developed to predict the concentrations of acrylamide in a model system and fried potato samples with only the restricted visible spectral wavelength range of 400−780 nm were less accurate when compared to models developed with the full VIS-NIR spectra, NIR region, or restricted wavelength region of 1100−2500 nm (Table 2). These results are consistent with previous studies that have shown that the use of a color measurement system on potato fries or chips is not a reliable indicator of acrylamide content.[19,20]

NIRS Prediction Models. The performances of all the PLS models developed for this study were evaluated using the coefficient of determination (R^2), standard error of calibration/standard error of prediction (SEC/SEP), root-mean-square errors of calibration/prediction (RMSEC, RMSEP), and the ratio of SEP of prediction samples to standard deviation of validation samples, known as the relative prediction deviation (RPD). For an accurate prediction model, it is always desirable to have low prediction error, with fewer latent variables to explain the variance while at the same time maintaining a high correlation. A high coefficient of determination is an indication that the model fits the calibration samples with minimal deviation. The SEC/SEP is an expression of the amount of error to expect when using the calibration models in the

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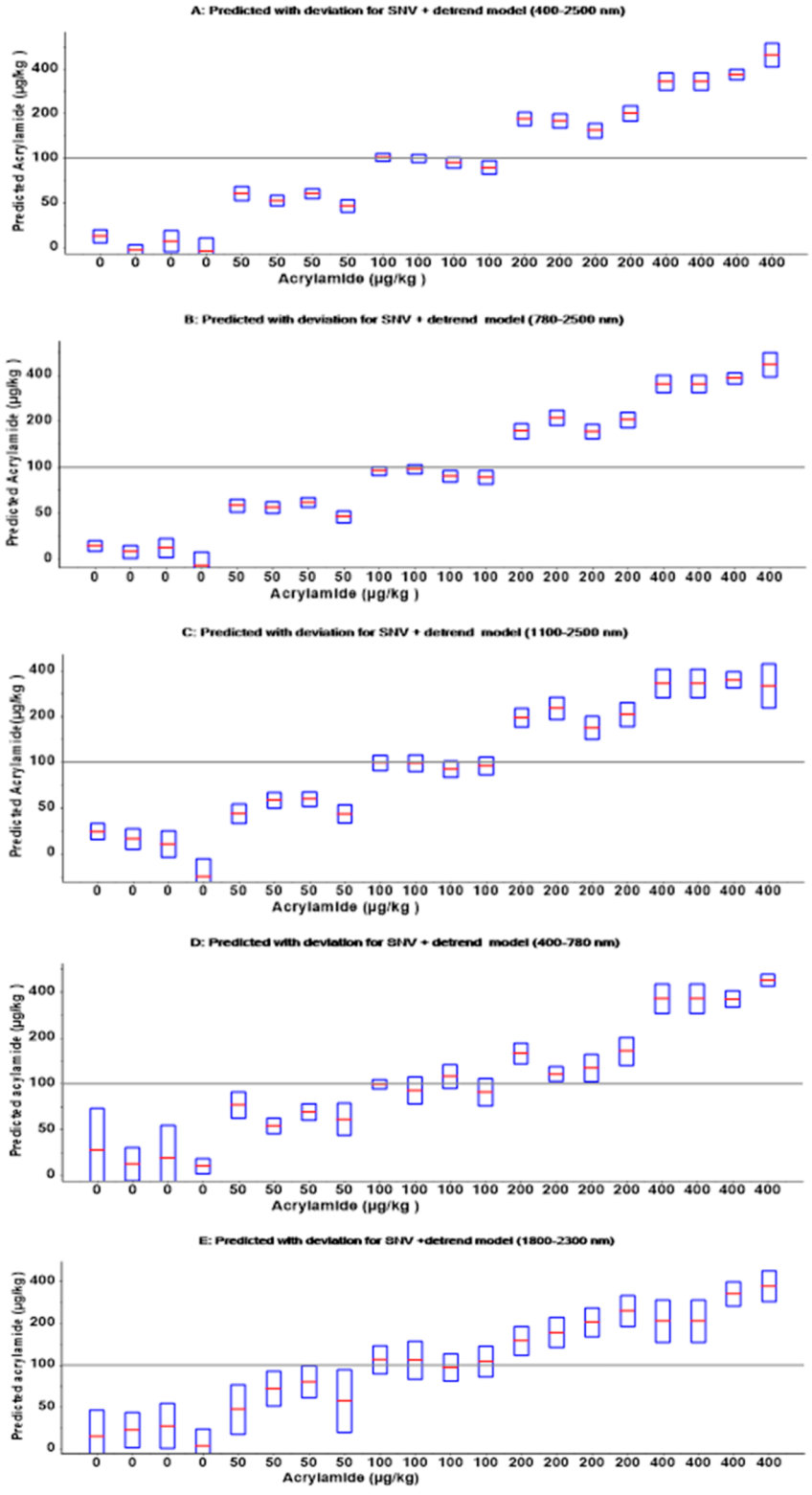

Figure 4. Prediction with deviation for potato flour spiked with acrylamide (μg/kg) for models developed using SNV transformed spectra and different spectral wavelength regions.

prediction of new samples, whereas the RMSEC and RMSEP are direct estimates of the prediction error and the modeling error in the *Y*-matrix, respectively. RMSEP is also useful in comparing different models regardless of how the models were developed with regard to weighting, preprocessing of the *X*-variables, or the number of components used. RPD is a simple statistic that enables the evaluation of the SEP in terms of the standard deviation of the validation data and is calculated as the ratio of the standard deviation (SD) of the validation set to SEP.

For the SVC models, calibration samples (n = 50) and a validation set (n = 34) were used for model development and

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Table 2. Calibration and Validation Statistics for PLS Models Developed To Predict Acrylamide Concentration (μg/kg) in French Fries

		single-variety (Russet Norkotah) calibration model (N cal = 50; N val = 34; SD = 907)								multiple-variety calibration model (N cal = 90; N val = 54; SD = 881)							
spectral treatment	wavelength (nm)	no. LVs[a]	cal[b]	LOD[c]	LOQ[d]	pred[e]	RMSEP[f]	SEP[g]	RPD[h]	no. LVs	cal	LOD	LOQ	pred	RMSEP	SEP	RPD
none	400−780	3	0.94	48	143	0.93	222	224	4.0	3	0.89	60	180	0.79	318	321	2.7
none	400−2500	3	0.95	46	138	0.94	211	214	4.2	5	0.91	59	177	0.80	319	323	2.7
none	780−2500	3	0.90	58	174	0.87	303	307	3.0	6	0.79	79	236	0.69	429	456	1.9
none	1100−2500	11	0.98	44	133	0.94	209	208	4.4	16	0.91	63	188	0.77	360	385	2.3
none	1800−2300	11	0.98	47	140	0.93	231	220	4.1	13	0.88	69	208	0.69	414	453	1.9
SG first deriv	400−780	2	0.94	48	145	0.93	228	230	3.9	2	0.88	64	191	0.76	350	358	2.5
SG first deriv	400−2500	4	0.96	43	128	0.95	186	189	4.8	5	0.92	55	166	0.80	289	307	2.9
SG first deriv	780−2500	4	0.92	53	159	0.9	265	268	3.4	11	0.94	59	178	0.80	347	365	2.4
SG first deriv	1100−2500	7	0.95	53	159	0.89	282	281	3.2	11	0.91	65	195	0.74	389	409	2.2
SG first deriv	1800−2300	7	0.91	61	184	0.83	347	321	2.8	12	0.92	71	213	0.66	475	508	1.7
SNV	400−780	1	0.93	49	148	0.92	234	238	3.8	3	0.90	58	173	0.80	298	300	2.9
SNV	400−2500	2	0.95	46	137	0.94	208	211	4.3	4	0.91	54	162	0.84	267	270	3.3
SNV	780−2500	7	0.97	42	127	0.95	188	187	4.9	6	0.93	53	160	0.85	273	278	3.2
SNV	1100−2500	9	0.98	36	109	0.97	140	135	6.7	7	0.93	54	162	0.85	281	288	3.1
SNV	1800−2300	7	0.96	47	142	0.92	231	220	4.1	4	0.90	59	178	0.82	317	322	2.7
MSC	400−780	2	0.95	45	136	0.94	206	208	4.4	3	0.9	58	173	0.80	298	300	2.9
MSC	400−2500	3	0.96	46	137	0.94	214	220	4.1	4	0.92	53	158	0.84	262	265	3.3
MSC	780−2500	4	0.95	47	140	0.93	219	223	4.1	5	0.92	52	157	0.86	258	261	3.4
MSC	1100−2500	3	0.94	49	147	0.92	235	243	3.7	5	0.93	51	154	0.86	252	255	3.5
MSC	1800−2300	3	0.95	48	143	0.93	228	236	3.8	4	0.91	56	169	0.83	291	295	3.0

[a]Number of latent variables. [b]Calibration *R*-square. [c]Limit of detection. [d]Limit of quantification. [e]Prediction *R*-square. [f]Root mean square error of prediction. [g]Standard error of prediction. [h]Relative prediction deviation of validations to SEP.

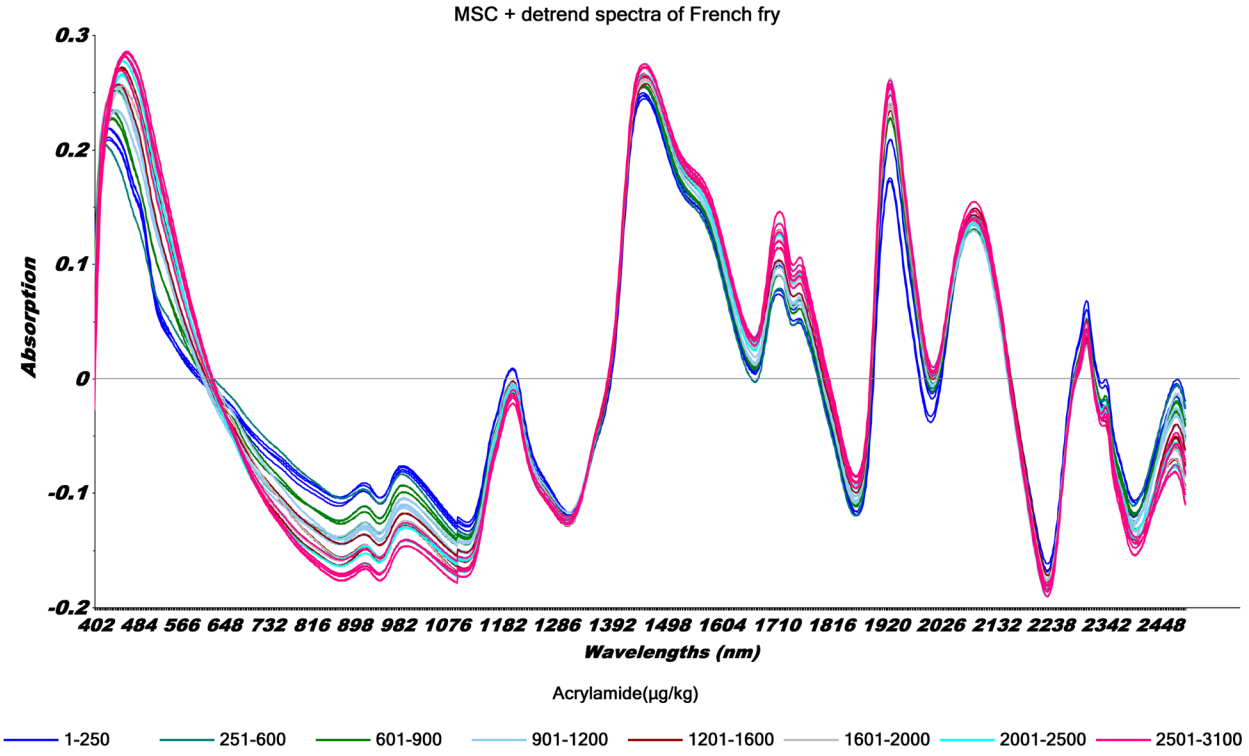

Figure 5. MSC + detrend preprocessed spectra of French fry samples prepared with different processing methods (representative spectra from several French fry samples in each range of acrylamide content are plotted with colors indicating various ranges of acrylamide concentration that were observed).

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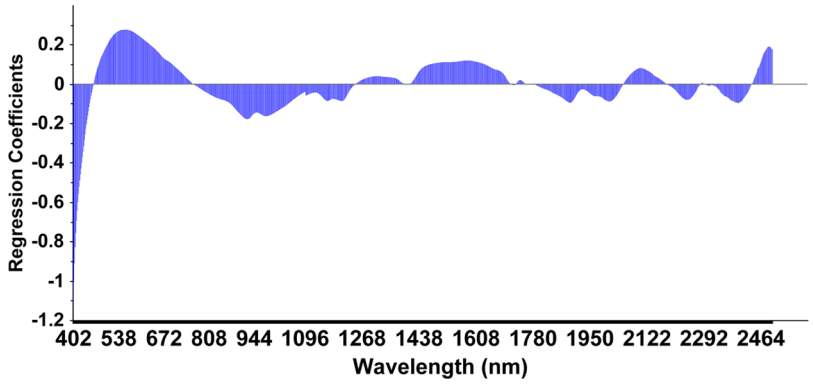

Figure 6. Plots of regression coefficients showing important wavelength regions for acrylamide prediction using MSC + detrend preprocessed spectra of French-fried potato (400−2500 nm).

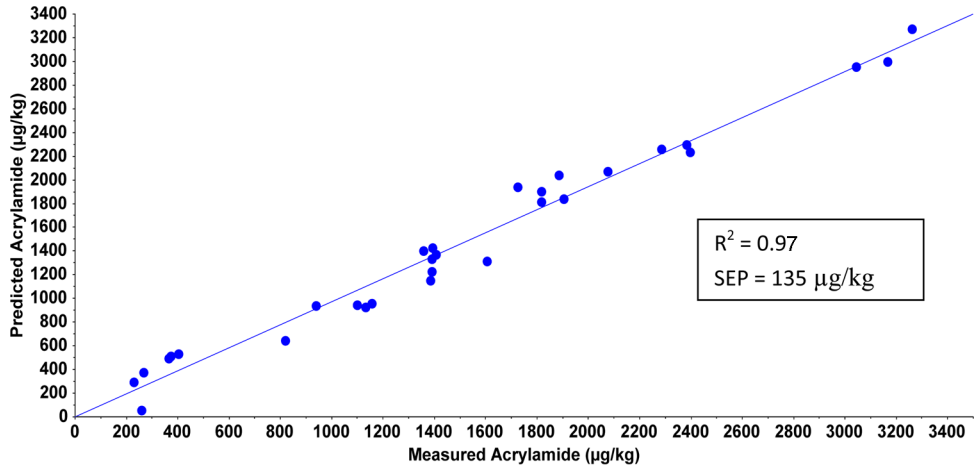

Figure 7. PLS regression plot of predicted versus measured acrylamide content (μg/kg) for a single-variety calibration (SVC) model using NIR spectral wavelength region 1100−2500 nm preprocessed with SNV + detrend.

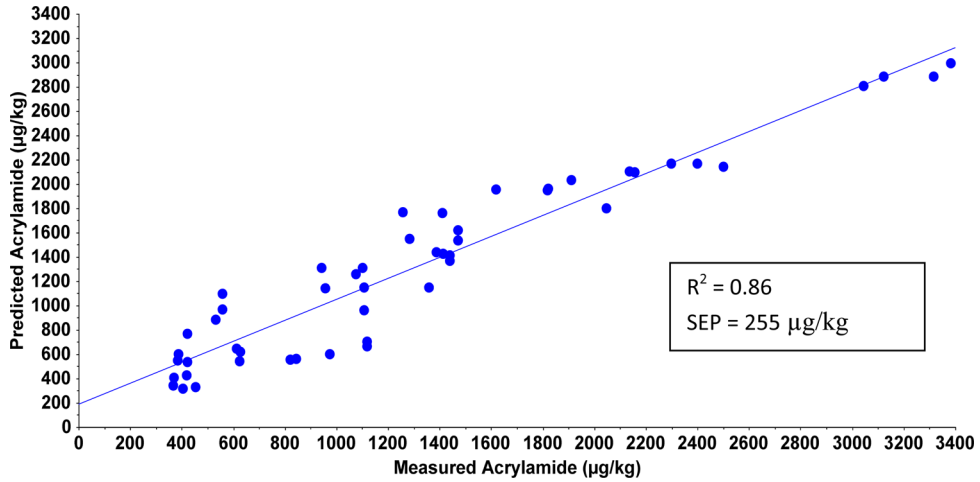

Figure 8. PLS regression plot of predicted versus measured acrylamide content (μg/kg) for a multiple-variety calibration (MVC) model using NIR spectral wavelength region 1100−2500 nm preprocessed with MSC + detrend.

validation. The latent variables required for models varied from 2 to 11. LOD and LOQ values ranged from 36 to 61 μg/kg and from 109 to 184 μg/kg, respectively. Most of the models performed very well in predicting samples not included in the calibration set (columns 3−10, Table 2). The R^2 for measured and predicted samples ranged from 0.90 to 0.98 with SEP ranging from 135 to 321 μg/kg. This means that the prediction models explain between 90 and 98% of the variation in acrylamide concentration, with the best model shown in Figure 7. Predictive statistics for the MVC models (columns 11−18, Table 2) showed that for most of the models, there were slight increases in the number of latent variables required for prediction, ranging from 2 to 16, and LOD and LOQ values ranged from 51 to 79 μg/kg and from 154 to 213 μg/kg,

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respectively, whereas prediction R^2 values ranged from 0.66 to 0.86 with SEP ranging from 255 to 508 (Figure 8). The RPD values ranged from 2.8 to 6.7 and from 1.7 to 3.5, respectively, for SVC and MVC models. A model for use as quality control should have SEP values much lower than the SD value and a RPD value higher than 5.[35] Most of the models developed in the study had RPD values between 2.5 and 4, indicating that most of the calibration models developed would be adequate for screening in potato-breeding research. SVC models developed using the spectral regions of 780−2500 and 1100−2500 nm and pretreated with SNV + detrend had the highest RPD values of 4.9 and 6.7, indicating that these NIRS models would be most applicable for development of a quality control tool in the industrial production of French fries.

In general, most of the models in the SVC category had better predictive statistics in terms of higher R^2, lower RMSEP/SEP, fewer latent variables, and higher RPD values when compared to the MVC models (Table 2). This may be attributed to the use of a single potato variety and a strict fry protocol that minimized natural variability among samples. In the case of the MVC, 60 samples from the commercial quick-service restaurants were fried by several vendors and may have been subjected to various sample pretreatments, sourced from multiple potato varieties, and/or produced with different frying protocols. These factors are likely to increase variation within the sample set. However, the addition of these fry samples contributed to the development of a more robust model that is more representative of reality. Calibration and prediction statistics for most of the models developed using only the 1800−2300 nm spectral region performed poorly when compared with corresponding models in the SVC and MVC categories. The 1800−2300 nm region is the reflectance part of NIR spectral region where the first 1 mm contributes as much as 99% of the variation of the spectrum, and hence uneven distribution of components, such as drying at the surface or separation of a water or oil layer in the glass scanning cup, may result in sample spectra that do not represent the entire sample.[36]

The findings of this study showed that the VIS-NIR (400−2500 nm), NIR (780−2500 nm), and restricted wavelength (1100−2500 nm) regions could all be used to develop calibration models with accurate prediction ability that will perform better than the models developed using wavelength regions of 400−780 or 1800−2300 nm alone (Table 2). This may be attributed to the fact that the full VIS-NIR spectra (400−2500 nm), the full NIR (780−2500 nm), and the wavelength region of 1100−2500 nm contain features or variables that are subtle and unknown, but important in the prediction of acrylamide in the fried potato samples.

There were significant improvements in the predictive ability of models with spectra preprocessed with SNV+ detrend and MSC + detrend methods when compared to models developed with raw (unprocessed) spectra. The use of the SG first deriv preprocessing method did not show any significant improvement compared to the models developed with the raw unprocessed spectra. Overall, the best model for the SVC category in terms of prediction performance was developed using the restricted spectral wavelength regions of 1100−2500 nm and preprocessed using the SNV + detrend method (Figure 7). The model had an optimum number of nine latent variables, the highest prediction R^2 of 0.97, the lowest SEP value of 135 μg/kg, and a RPD value of 6.7. The model developed using the restricted spectral wavelength regions of 1100−2500 nm and preprocessed using the MSC + detrend method was judged to be the best in the MVC category with an optimum number of five latent variables, prediction R^2 of 0.86, lowest SEP value of 255 μg/kg, and RPD value of 3.5. The difference in preprocessing methods to achieve the best models for SVC and MVC may be attributed to variations in fried samples used in the MVC category.

The results obtained in this study are comparable to those of similar studies on potato chips. Segtnan et al.[26] concluded that VIS-NIR spectroscopy could be used for rough screening of acrylamide in processed potato crisps and developed a model (R^2 = 0.952, RMSECV = 245.4 μg/kg) using the whole spectral region (400−2500 nm) with spectra preprocessed using extended MSC transformation, standardization, and jackknifing for removal of nonsignificant variables. Models developed in this study have slightly better predictive statistics with R^2 of 0.98 and SEP of 135 μg/kg for the SVC model. This difference could be attributed to larger sample size, range and distribution of acrylamide in samples used for this study, different frying protocols, or sample preparation, and it could also be a result of slightly different mathematical preprocessing methods. In another study conducted by Pedreschi et al.,[27] an average prediction error of 266 μg/kg was achieved when using NIR interactance and visible reflectance signal in online prediction of acrylamide content in potato chips. In a recent study by Ayvaz and Rodriguez,[28] acrylamide prediction in potato chips with SEP < 100 μg/kg and RPD values of 2.0, 4.7, and 4.8 was achieved respectively for NIR fiber optic, benchtop NIR, and MicroPHAZIR hand-held NIR. However, it should be noted that for this study, the authors treated replicate samples from each of the 42 bags of potato chips as independent samples, which may result in lower prediction errors.

The findings of this study showed that NIR spectroscopy methods can detect and differentiate various concentrations of acrylamide in a model system and in fried potato samples. VIS-NIR region, NIR region (780−2500 nm), and the restricted wavelength region of 1100−2500 nm could all be used in developing NIRS models. A singe-variety (Russet Norkotah) model developed using the spectral wavelength range of 1100−2500 nm and preprocessed using SNV + detrend was able to achieve prediction of acrylamide with SEP value of 135 μg/kg and RPD value of 6.7. The best model (SEP = 255 μg/kg, RPD = 3.74) developed for the prediction of acrylamide for the multiple variety calibration was achieved using NIR spectra from 11000−2500 nm preprocessed using MSC + detrend. The prediction capabilities for both the single-variety and multiple-variety models are accurate enough to conclude that NIR or VIS-NIR spectroscopy could be used for quality control in an industrial system for a single-variety line or as a screening tool for potato breeding. However, it should be pointed out that the results presented here demonstrate the feasibility of NIR for prediction of acrylamide in French-fried potatoes. To be useful in an industrial production environment, calibration models should include many factors such as instrument and process variable variations, environmental changes, and raw material variations.

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Notes

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