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Determination of Capsaicinoids in Habanero Peppers by Chemometric Analysis of UV Spectral Data

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A novel spectrophotometric method for the determination of capsaicinoids in habanero pepper extracts is described that does not require prior analyte separation. The method uses partial-least-squares (PLS-1) multivariate regression modeling techniques in conjunction with ordinary UV absorption spectral data obtained on alcoholic extracts of habanero peppers (*Capsicum chinese*). The PLS-1 regression models were developed by correlating the known total concentration of the two major capsaicinoids (capsaicin and dihydrocapsaicin) in the extracts as determined by high-performance liquid chromatography with the spectral data. The regression models were subsequently validated with laboratory-prepared test sets. The validation studies revealed that the root-mean-square error of prediction varied from 4 to 8 ppm, based on the results obtained from models prepared from nine test sets. Once a regression model has been developed and validated, analyses of the extracts can be accomplished rapidly by ordinary spectrophotometric procedures without any prior separation steps.

KEYWORDS: Capsaicin; multivariate regression modeling; UV−vis spectrophotometry; pungency

INTRODUCTION

Capsaicin {*N*-[(4-hydroxy-3-methoxy-phenyl)methyl]-8-methyl-non-6-enamide} and dihydrocapsaicin {*N*-[(4-hydroxy-3-methoxy-phenyl)methyl]-8-methyl-nonanamide} are two members of a family of naturally occurring capsaicinoids (**Figure 1**), which make up the pungent components of *Capsicum* fruits. Capsaicinoids are all *N*-vanillylamides of fatty acids. When evaluating the pungency of a pepper, capsaicin and dihydrocapsaicin constitute approximately 80−90% of the total capsaicinoids present (*1*, *2*) and account for the majority of the "hotness". The concentrations of capsaicin and dihydrocapsaicin in a chile pepper vary, depending on the species of the pepper, its growing conditions, and the time when the pepper was harvested (*3*, *4*). The capsaicinoids are not evenly distributed throughout the pepper, and substantial amounts of the capsaicinoids exist in the pericarp and placenta of the individual fruits (*5*).

Capsicum fruits have long been utilized in food preparation (*6*), medicinal applications (*7*, *8*), and personal protection aerosols (*3*). Chile peppers and spices, such as paprika, with different capsaicin contents are used routinely in salsas and other food products (*5*). Pungent sauces in Mexican and Asian cuisine often contain pungent peppers such as the habanero (*9*). Capsaicin has been used in the treatment of rheumatoid arthritis, osteoarthritis, and other peripheral neuropathic disorders as a therapeutic pain reliever (*10*). Capsaicin is also used to gauge a patient's cough threshold after ingestion of an antitussive agent (*7*). Recent studies have used capsaicin in other applications, such as neurobiological research (*8*), weight management (*11*, *12*), local/topical analgesia (*13*), and antimicrobial defense (*14*, *15*). Personal self-defense aerosols rely on the lachrymator-like effects caused when capsaicin is introduced to the mucous membranes (*3*, *16*).

In the past, organoleptic tests, such as the Scoville heat test, introduced in 1912 by Wilbur Scoville, were used to determine pepper pungency (*17*). These methods have been replaced with modern instrumental analyses (*1*, *18*). Analytical methods employed for the determination of capsaicinoids have included colorimetry (*19*, *20*), gas chromatography (GC) (*21*−*24*), liquid chromatography (LC) (*9*, *16*, *25*−*35*), GC/LC-MS (*3*, *36*), NMR-flow probe analysis (*37*), spectrophotometry (*38*−*45*), amperometric titration (*46*), micellar electrokinetic capillary chromatography (*47*), and sensory methods such as an electronic nose (*48*).

Of these methods, the most widely used is high-performance liquid chromatography (HPLC), which offers sufficient accuracy and precision. The standard analytical method, as described by the American Spice Trade Association, employs HPLC for testing the pungency of capsicums and their oleoresins (*49*). In this method, a 4.6 mm × 250 mm C-18 column (10 μm packing) is used with a mobile phase consisting of a mixture of acetonitrile, dioxane, water, methanol, and perchloric acid depending on the sample concentration. For samples greater than 700 ppm, absorption UV detection at 280 nm is used. For samples less than 700 ppm, fluorescence detection is employed

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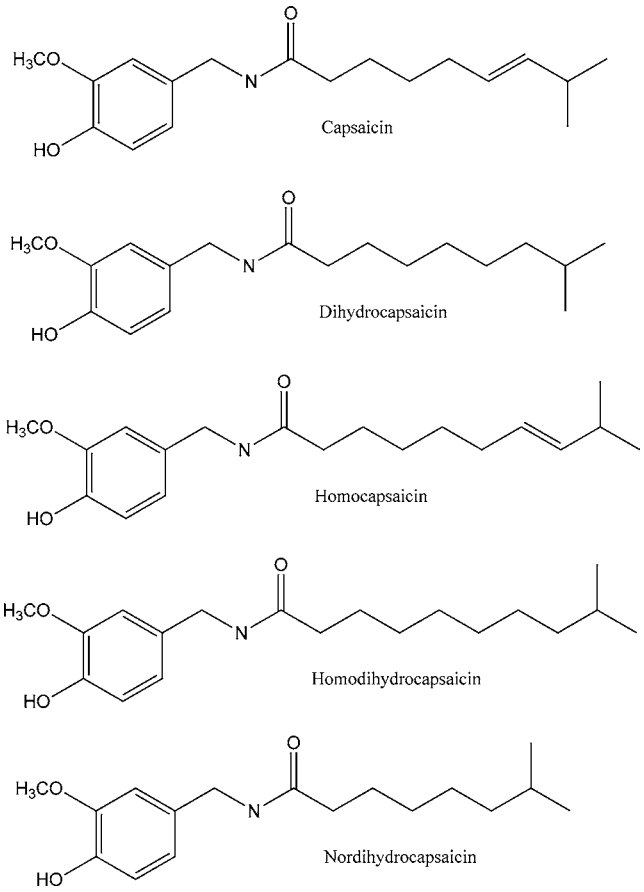

Figure 1. Chemical structures of some naturally occurring capsaicinoids.

with excitation at 288 nm and emission monitored at 320 nm. Drawbacks to chromatographic methods include the expense of columns and solvents, analysis time, and production of chemical wastes that require proper disposal.

While UV−visible spectrophotometry is often highly sensitive—particularly for analytes with high molar absorptivities—selectivity can frequently be a problem with complex samples because contaminants in the sample can produce absorption bands that overlap those of the analyte. As a result, ordinary UV−visible spectrophotometry at a single wavelength generally requires that the analyte be separated from the other absorbing constituents in the sample matrix before the absorbance measurements are made. This prior separation step can be problematic when the sample matrix is complex. In the case of chile pepper samples, capsaicinoids are generally extracted from the flesh of the pepper with solvents such as ethyl alcohol (*50*−*54*). While extracting capsaicinoids from a chile pepper is straightforward, other absorbing compounds—particularly carotenoids—are simultaneously coextracted from the pepper. Carotenoids are isoprene derivatives found in fruits, vegetables, and grains that impart a yellow or orange color (*50*). Carotenoids have certain common features, such as a polyisoprenoid structure, a long conjugated chain of double bonds, and near symmetry about the central double bond (*55*). The majority of carotenoids exhibit absorptions in the visible region of the spectrum between 400 and 500 nm. Several carotenoids, however, exhibit maximum absorbance in the UV region, such as phytoene with a λ_{max} at 285 nm (*56*). As a result, traditional spectrophotometric methods require sample purification before the absorbance spectrum is collected (*1*).

Although techniques for the chemical purification of pepper extracts have been developed for spectrophotometric procedures (*1*), sample cleanup can be expensive and time-consuming because it adds an extra step to the procedure and may generate chemical waste that requires proper disposal. In analytical situations such as this, chemometric techniques can be beneficial. Chemometric techniques have gained acceptance in the past several years as an effective tool in extracting information out of seemingly chaotic uncontrolled systems by use of different statistical algorithms. In chemistry, for example, multivariate regression modeling (MRM) has been used as a means of correlating spectral data with known compositional changes (*57*−*60*). Techniques such as partial-least-squares (PLS-1) regression modeling have been shown to be especially effective in removing the influence of unwanted variables that are impractical or impossible to control in the laboratory (*61*). The overall goal of this approach is to attempt to compensate for the interferences and other uncontrolled variables mathematically rather than resorting to chemical purification strategies.

The aim of PLS-1 is to develop a mathematical model that correlates two sets of data so that the independent *X*-variables (spectral data) can be used to predict the dependent *Y*-variables (total concentration of capsaicin and dihydrocapsaicin). PLS-1 regression modeling is a two-step multivariate process that makes use of actual real samples (rather than laboratory-prepared standards) in calibration, followed by validation. By using actual real samples for calibration, all possible interferences are ideally present in the spectral data used to develop the regression model. The procedure is multivariate because it makes use of spectral ranges rather than a single wavelength.

In the calibration phase of regression modeling, spectral data are acquired for a set of samples whose capsaicinoid concentrations are known independently from some reference analytical method (such as HPLC). In this phase of the process, the computer is trained to predict the capsaicinoid levels from spectral data obtained on real pepper samples that contain all of the possible interfering matrix components. In selecting the calibration samples, it is important to have as much inherent variability in the samples as possible so that all of the potential interferences can be included and accounted for by the model developed. The more samples in the calibration set, the more likely the regression model will be robust and provide acceptable prediction results.

The PLS-1 algorithm is extremely powerful in dealing with interferences associated with the spectral data because it focuses on those spectral features that correlate with the parameter of interest while simultaneously minimizing the effect of spectral features that do not correlate with the parameter of interest (*62*−*64*). The mathematical model developed in calibration stage takes the form of a regression vector made up of regression coefficients that are determined by the PLS-1 algorithm. This regression vector can be represented by

$$\hat{y} = b_o + b_1x_1 + b_2x_2 + ... + b_nx_n \qquad (1)$$

where $\hat{y}$ is the concentration of capsaicinoids predicted by the model for a given sample, the *b* values are the regression coefficients determined by the PLS-1 algorithm during the calibration phase, and the *x* values are the measured absorbances at the different wavelengths that make up the spectral range from 1 to *n* (i.e., the spectrum of an unknown sample).

In the validation phase of the process, a second independent set of samples is collected and analyzed by the reference method to determine the analyte concentrations. The spectra of these samples are then input into eq 1, and the analyte concentrations, $\hat{y}_i$, are predicted. The predicted concentrations are then compared with those obtained by the reference analytical method. If the

predicted concentrations agree with the values obtained by the reference analytical method, then eq 1 can be used to determine the analyte levels of future samples simply from the absorption spectra of the samples (*57*−*60*).

In this paper, we report the application of multivariate statistical analyses to UV spectral data of habanero pepper extracts to develop regression models to predict the total capsaicin and dihydrocapsaicin content of pepper extracts from their UV−visible absorption spectra. The advantage of this approach is that it does not require prior separation of the analytes from the sample matrix.

MATERIALS AND METHODS

Reagents and Supplies. Natural capsaicin (∼65% capsaicin and 35% dihydrocapsaicin), ethanol, HPLC-grade acetonitrile, and HPLC-grade water were purchased from Aldrich Chemical. Glacial acetic acid was purchased from DuPont.

Preparation of Capsaicin Standard Solutions. Various standards (20, 40, 60, 80, 100, 120, 140, 160, 180, and 200 ppm) were prepared from a 1025 ppm stock solution of the natural capsaicin (∼65% capsaicin and 35% dihydrocapsaicin) in ethanol. These standards were subsequently used to prepare an HPLC calibration curve using the total of the peak areas for the capsaicin and dihydrocapsaicin peaks. The calibration curve was then used to determine the total amount of the two capsaicinoids in the pepper extracts.

Extraction of Fresh Peppers. Habanero peppers were purchased from local area supermarkets in the Waco metropolitan area and pureed in a Black & Decker Handy Chopper Plus blender. Approximately 4.0 g of the pepper mush was placed in a 150 mL beaker with 50 mL of 100% ethanol. The mixture was then stirred for 30 s. A watch glass was placed over the top of the beaker, and the contents were heated to approximately 78 °C. If the solvent level dropped below 10 mL, more solvent was added. After they were boiled for 30 min, the samples were removed from the heat and allowed to cool to room temperature. The liquid extract was then separated from the solids using vacuum filtration through a nylon filter mat into a 250 mL filter flask. The filtered extract was then transferred to a 50 mL volumetric flask and diluted to the calibration mark. Extracts were then stored in two 25 mL glass vials.

HPLC Analysis. The HPLC method used to separate the capsaicinoids and carotenoid pigments was adapted from established literature techniques (*53*, *54*, *65*−*67*). A Hewlett-Packard liquid chromatograph (model 1090) controlled by an HP-interface card (model 82341C) was employed for the HPLC analyses. Data analysis and instrument control were conducted with HP Chemstation software. Sample injections were performed with the HP-1090 sample injection valve, using a 5.0 μL sample loop. A Restek Corp. Pinnacle II (C-18) 250 mm × 4.6 mm column with 5 μm particle size, maintained at 40 °C, was used as the analytical column. A 1.0 cm × 4.6 mm Hypersil guard column with 5 μm particle size was attached to the column. The Hewlett-Packard UV−vis diode array detector (model 1050) was set to monitor at 284 nm. The solvents were degassed with helium. The binary gradient elution system used consisted of acetonitrile (A) and distilled water containing 1% acetic acid (v/v) (B). Separation was achieved using the following nonlinear gradient program: 0−2 min, 50% A; 2−5 min, 50−80% A; 5−7 min, 80% A; 7−8 min, 80−50% A; and 8−11 min, 50% A. The flow rate through the column was maintained at 2.0 mL min^{-1}, and the total run time was set for 11 min, which allowed for the late-eluting homocapsaicin (not measured) to come off the analytical column.

UV−vis Analysis. Absorbance spectra were collected with a Hewlett-Packard photodiode array UV−vis spectrometer (model 8455) using a 2.0 mm path length quartz cell over the wavelength range from 190 to 1100 nm. The wavelength region selected for the regression modeling was from 215 to 300 nm. The spectral data were written to a Microsoft Excel spreadsheet for subsequent data analysis.

Regression Analysis. The mean-centered spectral data were subjected to multivariate analysis using The Unscrambler 9.1 (CAMO, Inc., Woodbridge, NJ). PLS-1 was performed on the spectral data using test set validation.

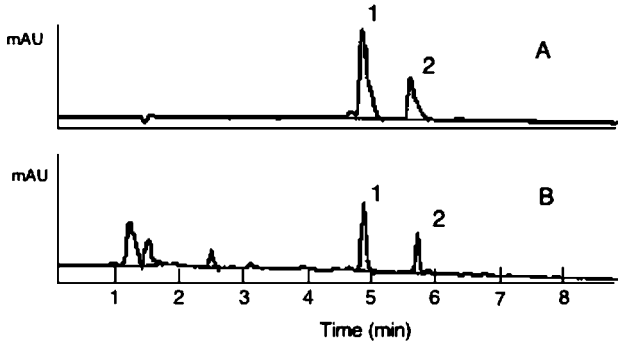

Figure 2. (**A**) HPLC chromatogram of natural capsaicin in ethanol at 284 nm. The total concentration of capsaicin and dihydrocapsaicin for this sample was 160.0 ppm. Compounds: **1**, capsaicin; **2**, dihydrocapsaicin. (**B**) HPLC chromatogram of habanero pepper extract in ethanol at 284 nm. Compounds: **1**, capsaicin; **2**, dihydrocapsaicin.

RESULTS AND DISCUSSION

Chromatographic Analysis of the Pepper Extracts. The HPLC chromatogram obtained with a 160 ppm sample of commercially available natural capsaicin using absorbance detection at 284 nm is shown in **Figure 2A**. The sample used to obtain the chromatogram in **Figure 2A** contained ∼65% capsaicin and ∼35% dihydrocapsaicin along with minor amounts of the other capsaicinoids. Standard solutions of this commercially available capsaicin were prepared and subsequently used to prepare an HPLC calibration curve of total peak area (the sum of the peak areas for capsaicin and dihydrocapsaicin) vs the total concentration of the two major capsaicinoids (i.e., the sum of capsaicin and dihydrocapsaicin). This HPLC calibration curve was used subsequently to determine the reference concentrations for the samples used to prepare the multivariate regression models relating the absorption spectral data with the total concentration of the two major capsaicinoids in the pepper extract samples.

Figure 2B shows a typical HPLC chromatogram obtained with a habanero pepper extract using absorbance detection at 284 nm. The peaks eluting prior to 4 min on this chromatogram are carotenoids and other plant pigment materials in the sample matrix. Under these chromatographic conditions, both capsaicin and dihydrocapsaicin give strong, well-resolved peaks. The sum of the peak areas for these two peaks was used, in conjuction with the previously prepared HPLC calibration curve to determine the total concentration of the two major capsaicinoids in the pepper extracts (i.e., the sum of capsaicin and dihydrocapsaicin). A total of 31 habanero pepper extracts were analyzed by HPLC to determine their capsaicin levels.

Absorption Spectral Data. The UV absorption spectra obtained from 31 habanero pepper extracts from 215 to 300 nm can be seen in **Figure 3A**. These spectra show two broad peaks at 230 and 280 nm, characteristic of capsaicinoids (*5*) and other absorbing components in the pepper extracts. **Figure 3B** shows a plot of the mean-centered spectral data over the wavelength range from 215 to 300 nm. The mean-centered plot was obtained by averaging the 31 individual spectra on a wavelength-by-wavelength basis (i.e., adding the absorbances of each spectrum on a wavelength-by-wavelength basis and dividing each sum by 31). The average spectrum was then subtracted from each individual spectrum on a wavelength-by-wavelength basis to give the mean-centered spectra. The mean-centered spectra show the regions of the pepper extract spectra that vary the most among the different pepper samples. These

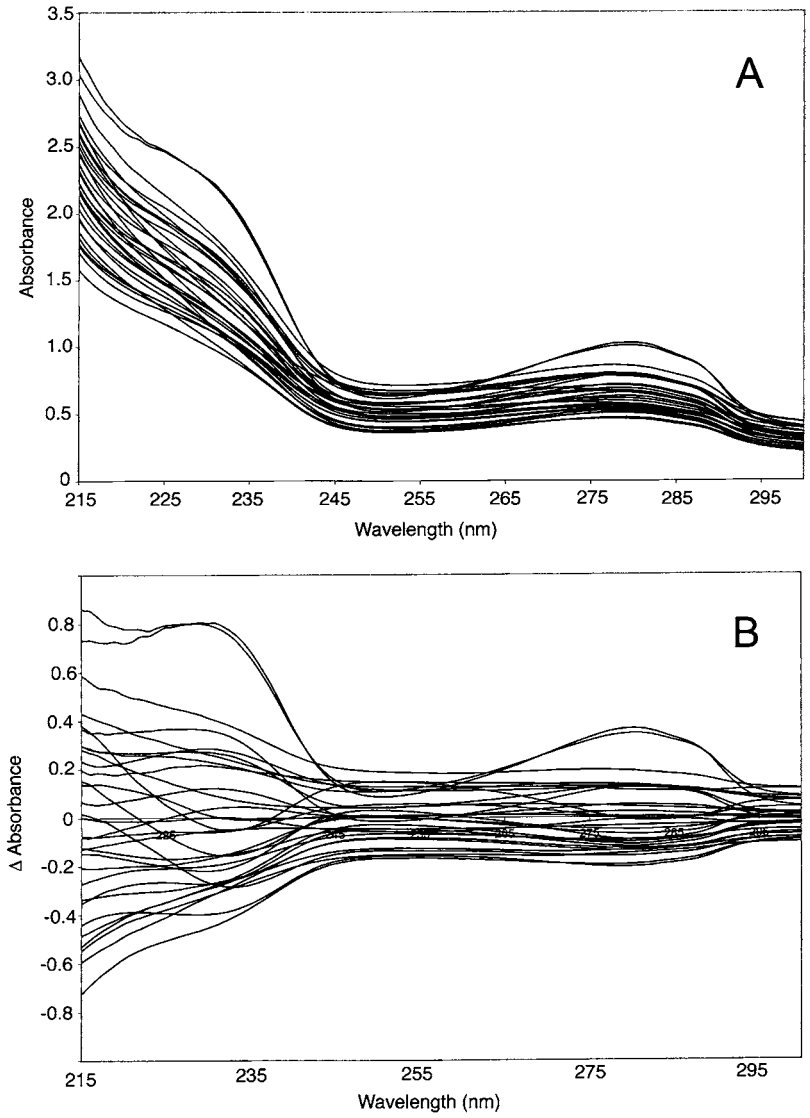

Figure 3. UV spectra of 31 habanero peppers extracted with ethanol. (**A**) Raw spectra from 215 to 300 nm. (**B**) Mean-centered spectra from 215 to 300 nm.

spectral ranges where variation occurs are likely candidates for MRM studies.

MRM Studies. In this study, a total of nine regression models were prepared by randomly selecting 21 pepper extracts from the 31 samples that were analyzed by HPLC. These 21 randomly selected samples were then used to prepare a particular regression model, and the remaining 10 samples were used to validate that particular model. This process was repeated nine times with different samples selected randomly to prepare the model and validate it.

PLS-1 regression modeling was the algorithm selected to develop the regression models. Because UV spectral data are relatively noise-free, no data pretreatment was employed, and the raw spectral data were input directly into the multivariate software. **Figure 4** shows the summary of results for the first regression model prepared using 21 samples. **Figure 4A** shows a scores plot for the first two PLS components in model 1. From the scores plot, which shows the samples plotted on the new coordinate system made up of PLS components, there are no apparent groupings in the sample set, and the first PLS component appears to be related primarily to the total concentration of the two major capsaicinoids (it accounts for 76% of the variance in the concentration data).

Figure 4B shows a plot of the unexplained variance as a function of the number of PLS components in the model. The plot seems to indicate that, while most of the variance in the data can be explained with just two PLS components, additional prediction ability can be gained by using four PLS components.

Figure 4C shows a plot of the regression coefficients that make up the model as a function of wavelength. This plot shows the values for the different b_i coefficients in eq 1. The plot shows that over the wavelength range from 215 to 300 nm the coefficients vary sinusoidally with some being positive and others being negative. The maximum positive values in the plot occur at 230 and 280 nm, which correspond to regions in the pepper extract spectra that show the most variability for the different samples (**Figure 3A**,**B**). Wavelengths in these regions contribute significantly to the regression model in a positive direction. By contrast, the region around 250 nm contributes significantly to the regression model in a negative direction.

Figure 4D shows a plot of the predicted total concentration of the two major capsaicinoids vs the values obtained by HPLC. For the calibration set, this linear plot has a slope of 0.994, an offset of 0.665, and correlation coefficient of 0.997. A perfect

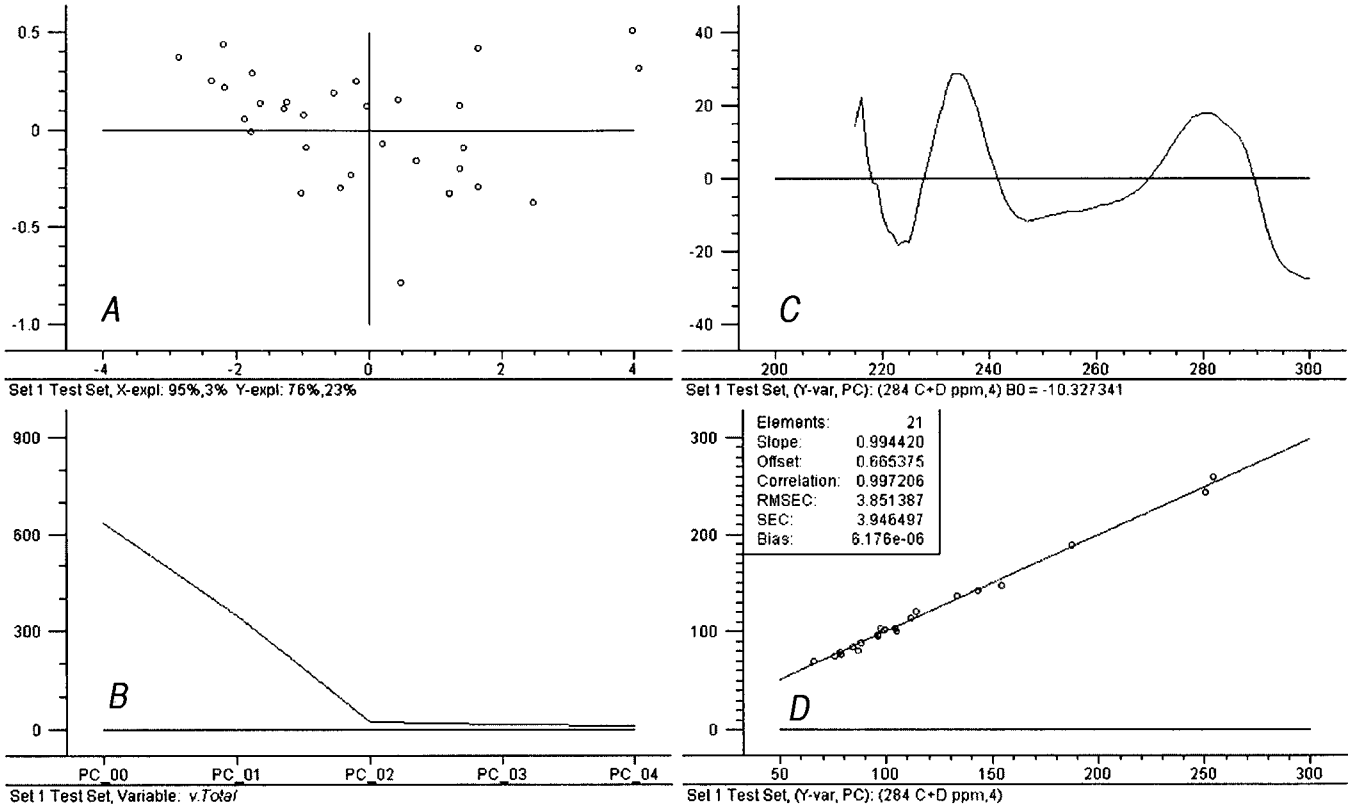

Figure 4. Unscrambler plots for test set 1 (**A**, scores plot; **B**, unexplained variance vs number of PLS components in the model; **C**, regression coefficients vs wavelength; and **D**, predicted vs measured plot).

Table 1. Prediction Results for the 10 Samples in Test Set 1[a]

total (ppm)[b]	predicted (ppm)[c]	error (ppm)
144	138	–6
83	79	–4
118	124	6
124	121	–3
135	132	–3
135	135	0
92	88	–4
92	91	–1
142	144	2
163	167	4
		RMSEP = 4

[a] Four PLS components were used in this model. [b] Total concentration of capsaicin and dihydrocapsaicin as determined by HPLC at 284 nm. [c] Total concentration of capsaicin and dihydrocapsaicin as predicted by the regression model.

model would have a slope of 1, an offset of 0, and a correlation coefficient of 1. It should be stressed that **Figure 4D** is not a calibration curve.

While the above model parameters look quite good, the real test of any regression model is its ability to predict future samples correctly. This ability is evaluated in the validation phase of regression modeling. In this part of the study, the spectra of the 10 samples that were not used to develop a given model were input into the model and the total of the capsaicin and dihydrocapsaicin levels were predicted using the model and the spectral data. **Table 1** gives the results obtained for this phase of the study for model number 1. In this table, the total capsaicin concentration (capsaicin plus dihydrocapsaicin) predicted by the model is given along with the corresponding value determined by HPLC. From this data, the root-mean-square error of prediction (RMSEP), given by

$$\mathrm{RMSEP} = \sqrt{\frac{\sum_{i=1}^{n} (\hat{y} - y_i)^2}{n}} \quad (2)$$

was calculated for the 10 samples. In eq 2, $\hat{y}$ is the predicted total concentration, y_i is the concentration determined for the i-th validation sample by HPLC, and n is the number of validation samples in the set. The RMSEP produces direct estimates of the prediction error of a given model. In other words, RMSEP expresses the average error to be expected associated with future predictions. The root-mean-square error of calibration (RMSEC) indicates the modeling error in the Y (concentration) data. RMSEC is also described by eq 2; however, y_i is the concentration determined for the i-th calibration sample by HPLC.

The residual predictive deviation (RPD) is a metric that reflects the predictive capability of the model and is defined as (*68*):

$$\mathrm{RPD} = \frac{\mathrm{SD}_y}{\mathrm{SEP}} \quad (3)$$

where SD_y is the standard deviation of the sample values in the validation set and SEP is the standard error of prediction for the model. The standard error of prediction is given by:

$$\mathrm{SEP} = \sqrt{\mathrm{RMSEP}^2 - \mathrm{bias}^2} \quad (4)$$

where RMSEP is the root-mean-square error of prediction and the bias is given by:

$$\text{bias} = \frac{\sum_{i=1}^{n} (\hat{y} - y_i)}{n} \quad (5)$$

Models with RPD values of 6.5−8.0 are considered to have good predictive capabilities (*69*).

In developing a regression model, selecting the proper number of PLS components to use is an important consideration. Using too few PLS components will result in suboptimal predictive capability, while using too many PLS components will result in overfitting the model. Overfitting a model leads to incorporation of noise into the model, which has no predictive capability. The maximum number of PLS components possible in a model is given by the smaller of (i) the number of variables in the model or (ii) one less than the number of samples in the model. In this study, there were 86 variables (wavelengths) and 21 samples. As a result, the maximum number of PLS components would be 20 with this set of data.

In examining **Figure 4A**, it can be seen that two PLS components account for 98% of the variance in the spectral data and 99% of the variance in the concentration data. **Figure 4B** also indicates that two PLS components account for essentially all of the variance in the data. This means that two PLS components should be all that are needed in the model.

To determine the optimum number of PLS components to use in the model, the predictive abilities of models with two and four PLS components were studied with the nine models developed as described above. **Figure 5** shows the results of this study in terms of a bar graph, and it is clear that in almost all cases, models with four PLS components had better predictive capabilities (lower RMSEP values) than models with only two PLS components. Only for models 7 and 9 was the RMSEP value for a model with two PLS components less than the corresponding RMSEP value for a model with four PLS components. As a result, four PLS components were used for the models in this study.

The statistics in **Table 2** summarize the results obtained from the calibration of the nine regression models prepared in this study. The average correlation and slope values, 0.997 and 0.995, respectively, demonstrated a linear relationship between the measured concentration of capsaicinoids determined by HPLC and the predicted concentrations generated by the multivariate models. The RMSEC values for the models range from 3 to 4 ppm. This is quite good considering that only 21 samples were used in the calibration phase of the regression modeling. Smaller error values would be expected if a larger calibration set were employed.

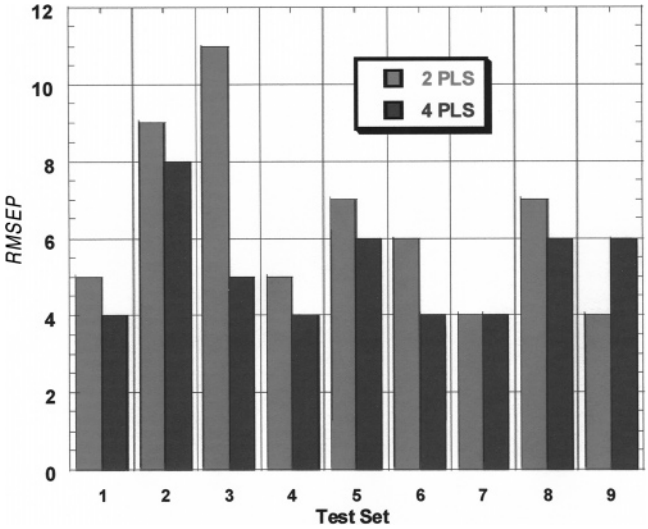

Figure 5. Comparison of RMSEP from nine test sets with two and four principal components.

The predictive abilities of the models were investigated, and the results are shown in **Table 3**. As was seen during calibration, the average correlation and slope values, 0.991 and 1.02, respectively, were both approximately one. The RMSEP for the individual models ranged from 4 to 8 ppm.

As a final step in validating this spectrophotometric procedure, a series of 12 fresh pepper samples was prepared and analyzed with a regression model that had been prepared over 5 months prior to this validation study. The analysis results obtained for these pepper samples can be seen in **Table 4**. The RMSEP value obtained for these samples was 4 ppm, suggesting that the regression model prepared from the UV spectral data is quite stable over time. The 12 pepper samples were then stored in the dark at room temperature for a period of 25 weeks. During this time, the UV lamp in the spectrophotometer failed and was replaced with a new one. The 12 samples were then taken out of storage, and their spectra were acquired with the spectrophotometer using the replacement bulb. **Table 5** shows the prediction results obtained for these samples. The RMSEP obtained was 5 ppm, which demonstrates that the model is stable over time, as well as when instrument adjustments occur.

Table 2. Statistics for Calibration Phase of Capsaicinoid Determinations

test set	1	2	3	4	5	6	7	8	9
correlation	0.997	0.998	0.997	0.997	0.997	0.997	0.997	0.998	0.997
slope	0.994	0.997	0.994	0.995	0.995	0.994	0.994	0.996	0.994
offset	0.665	0.390	0.733	0.632	0.636	0.997	0.725	0.458	0.661
SEC	3.95	2.80	3.87	3.62	3.13	3.89	3.86	3.05	3.21
RMSEC	3.85	2.73	3.77	3.53	3.05	3.79	3.77	2.97	3.14
bias	6.18×10^{-6}	-3.36×10^{-7}	-1.09×10^{-6}	-2.54×10^{-6}	2.54×10^{-6}	-1.09×10^{-6}	4.72×10^{-6}	5.45×10^{-6}	1.09×10^{-6}

Table 3. Statistics for Prediction Phase of Capsaicinoid Determinations

test set	1	2	3	4	5	6	7	8	9
correlation	0.992	0.981	0.991	0.990	0.995	0.994	0.994	0.984	0.995
slope	1.05	1.07	1.03	1.04	0.930	1.03	0.991	0.987	1.06
offset	–7.50	–4.73	–3.51	–4.56	6.16	–3.48	2.75	0.845	–7.44
SEP	3.73	7.41	5.11	4.61	6.09	4.18	3.92	6.62	6.13
RMSEP	3.65	7.52	4.85	4.38	6.24	3.96	4.08	6.32	5.82
bias	–0.894	2.67	0.188	–0.152	–2.36	–0.0891	1.68	–0.647	0.280
RPD	7.06	4.55	6.84	6.57	8.68	8.27	9.50	5.60	8.47

Table 4. Prediction Results for 12 New Samples with Model Prepared 5 Months Prior to Validation[a]

total (ppm)[b]	predicted (ppm)[c]	error (ppm)
114	116	2
112	119	7
144	147	3
229	223	–6
130	131	1
115	116	1
89	90	1
152	156	4
119	123	4
90	92	2
146	138	–8
113	111	–2
		RMSEP = 4

[a] Four PLS components were used. The calibration set used all 31 samples. [b] Total concentration of capsaicin and dihydrocapsaicin as determined by HPLC at 284 nm. [c] Total concentration of capsaicin and dihydrocapsaicin as predicted by the regression model.

Table 5. Prediction Results for 12 New Samples after Light Source Replacement[a]

total (ppm)[b]	predicted (ppm)[c]	error (ppm)
118	115	–3
115	117	2
142	145	3
230	220	–10
138	129	–9
121	115	–6
88.2	88	0
154	154	0
123	120	–3
93	90	–3
137	136	–1
111	108	–3
		RMSEP = 5

[a] Four PLS components were used. The calibration set used all 31 samples. [b] Total concentration of capsaicin and dihydrocapsaicin as determined by HPLC at 284 nm. [c] Total concentration of capsaicin and dihydrocapsaicin as predicted by the regression model.

Our motivation in conducting this study was to show that MRM of spectral data could be used as a means of developing regression models that could be used to predict pepper pungency from ordinary absorption spectrophotometric data. We chose to predict the sum of the capsaicin and dihydrocapsaicin levels because they are the major capsaicinoids present that primarily determine the pepper pungency. If one wanted, however, similar models could be made individually for capsaicin and dihydrocapsaicin separately. We selected habanero peppers for this study because they are one of the hotter commonly available peppers with relatively high levels of capsaicinoids. Because they have high capsaicinoid concentrations, these pepper extracts were more compatible with our diode array absorption HPLC detection systems than less pungent peppers that would require fluorescence HPLC detection.

This study has shown that reasonable multivariate regression models can be prepared from ordinary absorption spectrophotometric data obtained on pepper extract samples using HPLC as a reference method to determine the actual capsaicinoid content of the pepper extracts. For the habanero peppers used in this study, the predictive relative error of approximately ±5 ppm was observed for regression models prepared with 21 samples. It should be realized that regression modeling is an ongoing process and models can be updated periodically with new samples. As this is done, the predictive error will decrease.

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