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Capsaicinoids are the main components of red pepper that determine the spiciness level of red–pepper powders. Current methods for pungency measurement are mostly dependent on HPLC measurement technique, which is a sample–destructive, labor–intensive, time–consuming, and expensive method. In this research, a nondestructive on–line pungency measurement method for red–pepper powder was developed using a Visible/Near–Infrared spectrometer with the wavelength range of 400–1000 nm. The system was constructed with a charge–couple device (CCD) spectrometer, a reference–measuring unit for calibration, and a sample transfer unit, which conveyed the red–pepper powder during measurements. Typically powder samples with eleven different spiciness levels were produced by controlling the mixture of predetermined non–spicy and spicy red–pepper powder. A total of 33 different samples, spanning 11 spiciness levels in three particle sizes (below 0.425 mm, 0.425 to 0.71 mm and 0.71 to 1.4 mm) were prepared for spectral measurements using the developed pungency measuring system. Partial Least Square Regression (PLSR) models were developed to predict the content of capsaicinoids in red–pepper powder from the visible/NIR spectra and reference values measured by HPLC. The R_v^2 and SEP of the PLSR model for red–pepper powders without regard to particle size were 0.973 and ±6.98 mg/100 g, respectively. The best result of the PLSR models (R^2=0.991, SEP=±4.33 mg/100 g) was achieved from the raw spectra of red–pepper powders for particle sizes below 0.425 mm. The obtained results demonstrate the potential of NIRS technique for non–destructive and on–line measurement of capsaicinoids content in red–pepper powder.

Key words: capsaicinoids, food quality, non–destructive measurement, red–pepper powder, spectroscopy

INTRODUCTION

Peppers are one of the most popular vegetables in South Korea. Red–pepper powder, a processed product made from raw peppers, is used as a raw material for variety of food preparations such as in seasonings, pepper paste, Kimchi, etc. The production process for making red–pepper powder consists of cleaning to removing dirt or pesticide residues, cutting the pepper to improve drying efficiency, drying for long–term which imparts quality preservation, effective grinding to produce appropriate particle size, blending for regulating the level of spiciness, and finally packing. The quality of red–pepper powder is assessed based on American Spice Trade Association (ASTA, 1997) with respect to color, surface color, pungency, microbiological analysis, impurity analysis, moisture content, and particle size.

Red–pepper powders of different particle sizes are used for manufacturing different kinds of spicy food products, such as Kimchi, red–pepper paste, etc.

The capsaicinoids content is the most significant quality factor for red–pepper powder. Non–uniformity of the capsaicinoids content in red–pepper powders commonly causes inconsistent quality control of spicy foods, which results in consumers' complaints. Red–pepper powders made from the same variety of red–peppers can vary with respect to the content of capsaicinoids. Capsaicinoids content is affected by various environmental factors during the growth of red peppers, such as rate of sunshine, precipitation amount, and distinctive soil features. For the production of foods of consistent spiciness, capsaicinoids content needs to be measured quantitatively. The current measurement method for capsaicinoids content is dependent on High Performance Liquid Chromatography (HPLC), but the application of this technique for the rapid measurement of capsaicinoids during the manufacturing process of red–pepper powder is hampered by the sample–destructive and time–consuming nature of the technique that also requires complicated sample pretreatments. Recently, there has been an increase in the demand for non–destructive, rapid, and low cost techniques for the measurement of capsaicinoids content (Barbero *et al.*, 2008; Ha *et al.*, 2008; Korel *et al.*, 2002; Kachoosnagi *et al.*, 2008).

In the past decades, visible (Vis) to near–infrared

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(NIR) spectroscopy technology has been explored as a rapid and non–destructive measuring tool for the quality of agricultural and food products (Suh *et al.*, 2011). NIR spectroscopy methods can estimate chemical components based on overtone and combination bands of specific functional groups. NIR spectroscopy was first used in agricultural applications to monitor moisture contents in grain (Norris, 1964). Since then the use of the technique has been expanded to predict protein and fat content, soluble solid content (SSC), and organic acids content of various agricultural materials (Norris *et al.*, 1984; Kawano *et al.*, 1993; Perris *et al.*, 1998; Park *et al.*, 2003; Lee *et al.*, 2004; Cho *et al.*, 2011, Suh *et al.*, 2012).

The feasibility of spectroscopic technique for non–destructive measurement of red–pepper components has been investigated recently. Cho *et al.* (1997) proposed that moisture and fat contents causing discoloration and rancidity of red–pepper powder could be predicted by using NIR spectroscopy. Lee *et al.* (2005) predicted moisture content, ASTA color, capsaicinoids content, and total sugar content in red pepper powder using Vis/NIR in the range of 400 to 2500 nm. In the published work, the correlation coefficient of capsaicinoids content prediction was 0.65 and the standard error of prediction was 31.60%. Pungency of red–pepper powder is mostly influenced by the placenta, which accounts for 2 to 3% of a red pepper's mass but can contain ten times or even hundreds of times more capsaicinoids than a red pepper's pericarp or seeds (Shin *et al.*, 1991). For accurate prediction of the capsaicinoids content in red–pepper powder, spectroscopy measurements must encompass relatively large sample areas of a powder sample to avoid the bias found in point–source measurements of the non–uniform, heterogeneous sample material.

In this study, an on–line pungency measuring system was developed for the prediction of capsaicinoids content in red–pepper powder using a visible/near–infrared (VNIR) spectrometer from approximately 400 to 1000 nm. Partial least squares regression (PLSR) models were investigated to predict the capsaicinoids content in red–pepper powders with different spicy level and particle size. The prediction results were compared with results from HPLC measurements.

MATERIALS AND METHODS

Sample preparation

Red–pepper powders made of two different red–pepper cultivars of two notably different levels of spiciness and harvested in Younggwang–Gun, Jeonnam, South Korea, were purchased and used for measurements. The less spicy powder and the more spicy powder, as determined by human experts, were mixed to obtain powder mixtures spanning 11 spiciness levels of samples by 10% mixture increments (i.e., mass–based ratio mixtures of the less spicy and more spicy powders: 0:100, 10:90, 20:80, … , 90:10, and 100:0). Each of these powder mixtures were separated into three groups

based on their particle size, (I) below 0.425 mm, (II) 0.425 to 0.71 mm, and (III) 0.71 to 1.4 mm, using a standard sieve (Chunggye Industrial MFG, Gunpo, South Korea). In this study, a total of 33 samples of red–pepper powder, of different capsaicinoids content (spiciness levels) and particle size, were used for VNIR spectral and HPLC measurements.

On–line System Design

Figure 1 shows a schematic diagram and a photo of the on–line pungency measurement system. The VNIR spectroscopy system is based on a charge–couple device (CCD) based spectrometer (SM442, Toshiba, Japan) that operates with a spectral range of 200 to 1050 nm. Sample illumination is provided by a 100 W Tungsten–Halogen lamp (ASTN–D1–W100–L, Spectral Products, Seoul, South Korea). A bifurcated fiber optic probe (with 12.7° divergence) consists of 7 fibers where one fiber in the center is connected to the entrance slit of the spectrometer for spectral measurements and six outer fiber bundles (surrounding the center one) are coupled with the light source for sample illumination. The field of view (FOV) of the probe at the sample surface is approximately 17.2 mm diameter. The system includes an automated reference measuring unit consisting of a reference material and a motorized positioning system for reference measurements. The automated reference measur-
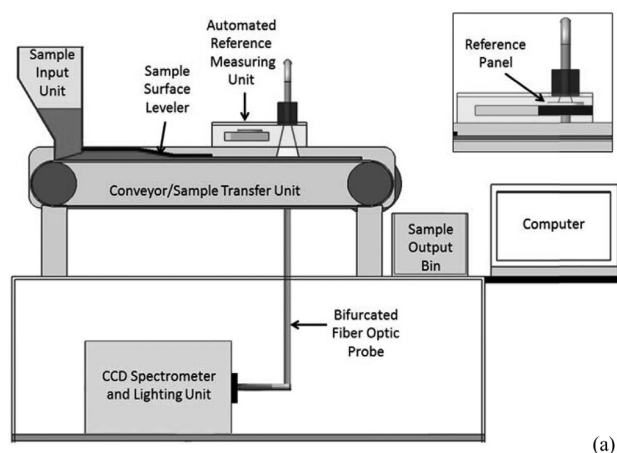
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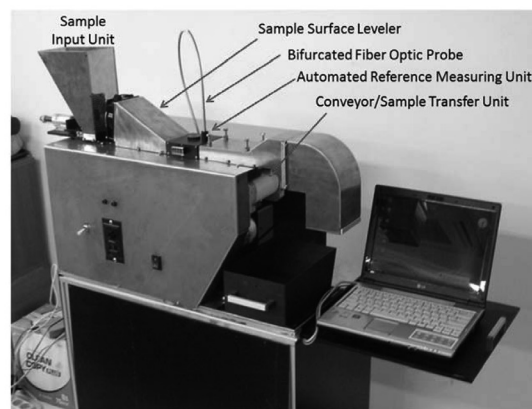
(b)

Fig. 1. Schematic diagram (a) and photo (b) of the pungency measuring system for red–pepper powder.

ing unit is incorporated in between the fiber optic probe and sample to provide reflectance reference measurements. The positioning of the reference material is controlled by a motorized arm. The upper right diagram in Figure 1a exhibits the position of the reference panel in reference–measuring mode. Reference material is positioned 7.7 mm below the fiber optic probe tip, but 33.9 mm above the red–pepper powder, to prevent surface contamination from red–pepper powder. A grey reference panel (reflectance factor=0.781) is used to avoid detector saturation and to better utilize the dynamic response range of the spectrometer. Note that the use of the reference is not to determine absolute reflectance or absorbance values of the samples, but to correct for any system drifts such as lighting conditions. The sample transfer unit is equipped with an adjustable speed conveyer belt to move the sample powder. A surface leveler is devised to provide uniform surface height across the conveyor width for the VNIR measurements.

Data acquisition and analysis

Spectrum acquisition and recording were performed by MS–Windows–based in–house software developed using Labview (version 8.5, National Instruments Corp., USA) in conjunction with the software development kit provided by the CCD spectrometer manufacturer. When red–pepper powder is transferred by the conveyer belt, spectra of the entire length of red–pepper powder surface were continuously obtained. Typically, fifty spectra measured during each 5–second period were averaged and the respective average spectrum was stored for further analyses. The conveyer belt ran at a speed of 14.3 mm/s and the reflectance spectra from the sample surface were measured from 200 to 1050 nm at a spectral interval of approximately 0.3 nm. The acquired spectra were smoothed using the Savitzky–Golay smoothing method, with 6.5 nm interval, to minimize the system noise. A total of 297 spectra were obtained for the set of 33 samples, with every sample subjected to a measurement 3 times a day on 3 different days. Note that spectral data shorter than 400 nm and longer than 1000 nm were excluded from further analyses due to poor signal to noise of the spectrometer. After excluding the noisy spectral region and following the pretreatments, each spectrum contained 450 spectral data points between 400 and 1000 nm, with a spectral interval of approximately 1.5 nm.

Analysis of Capsaicinoid Contents

The standard ASTA analysis method for measurement of capsaicinoids content was used. Extracts were obtained from 1 g samples of red–pepper powder by heating with 25 mL of HPLC grade ethanol at 60°C in a pressurized container for 2 hours and 30 minutes. Extracts were filtered through a GHP Acrodisc Syringe filter (PALL, P/N 6054563–1, USA) and subsequently 20 μL of the sample extract were injected into the HPLC system (Waters Associates model ALC/GPC equipped with a M.6000A pump, a U6K injection) equipped with a column of Unison UK–C18 (Imtakt, USA). Measurements were

accomplished via a variable wavelength UV detector set at 280 nm. The isocratic mobile phase was methanol/ Phosphoric acid (65:35 v/v) with a flow rate of 1.0 mL/ min. Capsaicin (Sigma, M–2028, USA) and dihydrocapsaicin (Sigma, M–1022, USA) were identified using standards compounds.

Development of Prediction Model

Prediction models of capsaicinoids content were developed for red–pepper powders of three different particle sizes: below 0.425 mm (case I), between 0.425 and 0.71 mm (case II), and between 0.71 and 1.4 mm (case III). Each case included 99 samples of red–pepper powder; a total of 297 spectra from 400 to 1000 nm were used for analysis. The partial least squares regressions (PLSR) model were developed using Unscrambler software (v9.2, Camo., Norway), to predict capsaicinoids content in the red pepper powders based on the spectral measurements. Several preprocessing techniques, such as smoothing, mean normalization, maximum normalization, range normalization, 1st order derivative, 2nd order derivative, standard normal variate (SNV), and multiplicative scattering correction (MSC) techniques, were applied to the measured spectra to reduce systematic noise and variation emanating from light sources and the effect of light scattering, which were all dependent on the powder particle size. For each particle size (Cases I, II, and III), a PLSR prediction model for capsaicinoids content in the red–pepper powder was developed by using the preprocessed spectra, and the performance of each model was evaluated using a cross–validation method. The coefficient of calibration (R_c^2), standard error of calibration (SEC), determination coefficient of validation (R_v^2), standard error of prediction (SEP), and the number of factors (F) of the three prediction models were compared. Standard error of calibration and prediction were calculated by the following equations.

$$SEC = \sqrt{\frac{\sum_{i=1}^{n}(Y_i - \overline{Y}_i)^2}{n - f - 1}}$$

$$SEP = \sqrt{\frac{\sum_{i=1}^{n}(Y_i - \overline{Y}_i)^2}{n}}$$

where, n is the number of samples, Y_i is the actual concentration, $\overline{Y}_i$ is the predicted concentration and f is the number of factors.
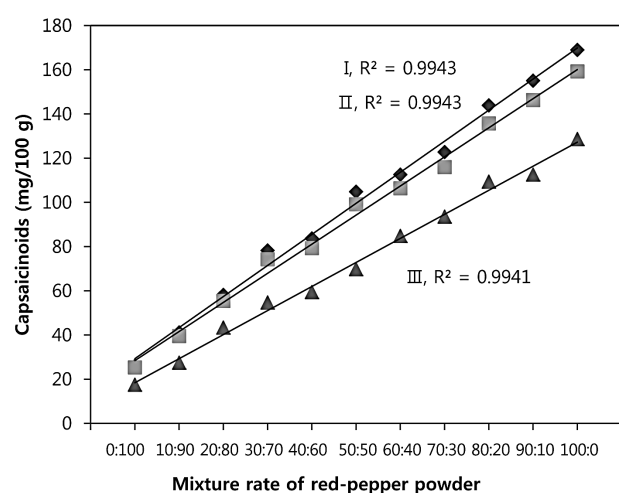
RESULTS AND DISCUSSIONS

Analysis of capsaicinoids content

Table 1 shows the results for capsaicin content in red–pepper powder as measured by HPLC. In case of mixtures of red–pepper powder, which were coarser and of larger particle size (Case III) consistently showed the lowest level of capsaicinoids, ranging from 17.50 to 128.66 mg/100 g, while the same mixtures with the finer particle size (Case I) consistently showed the highest level of capsaicinoids, ranging from 26.02 to 168.97 mg/ 100 g. Figure 2 shows the correlation between the pre-

Table 1. Capsaicinoids content in the mixture of red–pepper powder as measured by HPLC

Sample	Mixture rate of red–pepper powder (non–spicy:spicy)	Capsaicinoids (mg/100 g)		
		Case I*	Case II*	Case III*
1	0:100	26.02	25.34	17.50
2	10:90	41.15	39.52	27.47
3	20:80	58.12	55.40	43.34
4	30:70	78.25	74.27	54.66
5	40:60	83.67	79.31	59.41
6	50:50	104.80	99.14	69.77
7	60:40	112.58	106.42	84.80
8	70:30	122.77	115.96	93.55
9	80:20	143.88	135.72	109.38
10	90:10	155.08	146.29	112.57
11	100:0	168.97	159.24	128.66

* Particle size–Case I: <0.425 mm, Case II: 0.425~0.71 mm, Case III: 0.71~1.4 mm

Fig. 3. Representative absorbance spectra of red–pepper powder for medium particle size samples for 100:0, 50:50, and 0:100 mixtures (25.34, 99.14, and 159.24 mg/100 g capsaicinoids contents) with the pretreatment of maximum normalization and baseline correction. Relative absorbance differences from the non–spicy sample (100:0 mixture, 25.34 mg/100 g) are shown in the upper right graph.

Fig. 2. Correlation between mixture ratio of red–pepper powder and capsaicinoids content as measured by HPLC (◆: case I, ■: case II, and ▲: case III).

pared red–pepper mixture ratios and their capsaicinoid contents as measured by HPLC. The results exhibit strong linearity in case of all the three particle sizes, with determination coefficients above 0.994. The powders of small particle size contain more capsaicinoids because they contain more placental material that crumbles more easily into a fine powder than other parts of the pepper, such as pericarp and seed.

Representative VNIR spectra of red–pepper powder

Figure 3 shows representative absorbance spectra of the medium particle size (0.425 to 0.71 mm) red–pepper powders with three different capsaicinoids content (25.34, 99.14, and 159.24 mg/100 g) acquired with the on–line pungency measurement system. The spectra were subjected to the pretreatment of maximum normalization and baseline correction. The dominant absorbance peak at approximately 500 nm was observed in all the sample spectra. Relative absorbance differences from the least spicy powder spectrum are shown in the upper right graph. The normalized absorbance difference plot reveals the presence of minute variations in photosynthetic pigment contents such as chlorophylls (e.g., 670 nm) and carotenoids. In addition, spectral variations in the NIR portion from approximately 700 nm to 950 nm were also observed.

Prediction model for capsaicinoid content in red–pepper powder

The spectra with the pretreatments were used in developing PLSR models for the prediction of the capsaicinoids content for each of the three powder particle sizes (Cases I, II, and III) and also for the case in which all the particle sizes were included together (Case IV). Optimal pretreatments of the spectra to enhance the performance of PLSR models for the prediction of capsaicinoids content were also investigated. The determination coefficients and standard error of calibration and validation of each PLSR model with its respective pretreatments are shown in Table 2.

Case I (particle size<0.425 mm)

The results of calibration and validation of the PLSR prediction models with various pretreatments for the prediction of capsaicinoids content in the red–pepper powder for Case I (particle size below 0.425 mm) are listed in the upper part of Table 2. PLSR models with pretreatments showed slightly better performance than the model using only the raw spectra. The best PLSR model was the one which included 1st derivative pretreatment with a 43 nm spectral interval, wherein the optimal factor number was 4. The determination coefficient of calibration (R_c^2) was 0.994 and the standard error of calibration (SEC) was ±3.39 mg/100 g. The determination coefficient of validation (R_v^2) was 0.991 and the standard error

Table 2. Comparison of PLSR Models with respect to pretreatment

Case		x^1	Normalization			1st D	2nd D	SNV	MSC	Smoothing	Baseline
			Mean	Max	Range						
I	R_c^2	0.977	0.990	0.992	0.992	**0.994**	0.984	0.991	0.991	0.994	0.977
	SEC	6.706	4.388	4.076	4.076	**3.385**	5.603	4.130	4.216	3.596	6.705
	R_v^2	0.969	0.982	0.984	0.984	**0.991**	0.980	0.982	0.982	0.990	0.969
	SEP	7.916	6.121	5.779	5.779	**4.331**	6.304	6.025	6.112	4.531	7.916
	PC	5	6	6	6	**4**	2	5	5	7	5
II	R_c^2	0.985	0.989	**0.992**	0.992	0.988	0.985	0.987	0.986	0.984	0.985
	SEC	5.060	4.302	**3.639**	3.642	4.516	5.148	4.692	4.894	5.261	5.063
	R_v^2	0.976	0.983	**0.987**	0.986	0.985	0.981	0.980	0.979	0.978	0.976
	SEP	6.522	5.585	**4.900**	4.910	5.151	5.786	5.964	6.135	6.300	6.531
	PC	5	6	**6**	6	4	2	5	5	5	5
III	R_c^2	0.968	0.972	0.975	0.975	**0.982**	0.982	0.976	0.976	0.981	0.968
	SEC	6.177	5.786	5.416	5.457	**4.696**	4.611	5.368	5.390	4.100	6.251
	R_v^2	0.959	0.965	0.967	0.967	**0.977**	0.974	0.969	0.969	0.975	0.960
	SEP	7.032	6.566	6.297	6.339	**5.326**	5.648	6.120	6.146	5.508	7.005
	PC	5	5	5	5	**4**	3	5	5	7	5
IV	R_c^2	**0.977**	0.960	0.974	0.973	0.967	0.968	0.968	0.965	0.975	0.975
	SEC	**6.364**	8.461	6.835	6.870	7.622	7.482	7.541	7.868	6.692	6.605
	R_v^2	**0.973**	0.957	0.968	0.967	0.965	0.966	0.961	0.960	0.971	0.971
	SEP	**6.983**	8.755	7.600	7.626	7.921	7.833	8.311	8.487	7.171	7.171
	PC	**7**	5	6	6	3	3	6	5	7	7

[1] X: Non–Pretreatment, 2) 1st D: 1st Derivative, 3) 2nd D: 2nd Derivative

of validation (SEP) was ±4.33 mg/100 g with a bias of 0.0541 mg/100 g. The predicted and measured values are displayed in figure 4 which shows the strong linear correlation between the values.

Case II (particle size 0.425 to 0.71 mm)

The PLSR models to predict capsaicinoids content in red pepper powder of particle size between 0.425 mm and 0.71 mm were developed using pretreated spectra. The optimized pretreatment for the PLSR model was the maximum normalization method. The number of PLS factor was 6. The predicted and measured values are shown in Figure 5. The R_c^2 and SEC of this model were 0.992 and ±3.64 mg/100 g for calibration, respectively; the R_v^2, SEP, and bias were 0.987, ±4.90 mg/100 g, and –0.0549 mg/100 g, respectively, for validation.

Case III (particle size 0.71 to 1.4 mm)

The best PLSR results were achieved for the prediction of capsaicinoids content of the Case III red–pepper powder with particle size between 0.71 mm and 1.4 mm, using 1st derivative spectral pretreatment with a 34 nm spectral interval and a 4–factor model. The R_c^2 and SEC were 0.982 and ±4.70 mg/100 g, respectively; R_v^2, SEP, and bias were 0.977, ±5.33 mg/100 g, and 0.0438 mg/ 100 g, respectively. The predicted values correlated well with the measured values as shown in Figure 6.

Case IV (mixed particle sizes up to 1.4 mm)

A mixture of red–pepper powder of the three different particle sizes (Case I, Case II, Case III) were used to develop a PLSR model for the prediction of capasicinoids content. The best result was obtained with the non–pretreated spectra. The model predicted capasicinoids content with R_c^2 of 0.977, SEC of ±6.36 mg/100 g, R_v^2 of 0.973, and SEP of ±6.98 mg/100 g (Fig. 7). Although the prediction error of this PLSR model was higher than the ones of the models developed for powders of more limited particle sizes, its performance demonstrated feasibility for use in the on–line pundency measurement system.

The performance of the PLSR models for the prediction of capsaicinoids content was better for red–pepper powders of smaller particle size. This may be the result of the smaller particle size providing a uniform sample presentation with less scattering than that of larger particle size. The performance of the PLSR models for the prediction of capsaicinoids content in the red–pepper powders of restricted particle size was found to be better than that of the model for powder that included the entire range of particle sizes. Thus, it is recommended that the use of a PLSR model developed using powder of a narrowly specified particle size range would be desirable to optimize the precise prediction of the capsaicinoids content in red pepper powder.
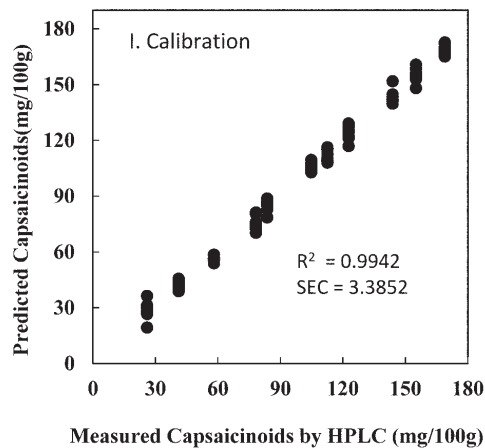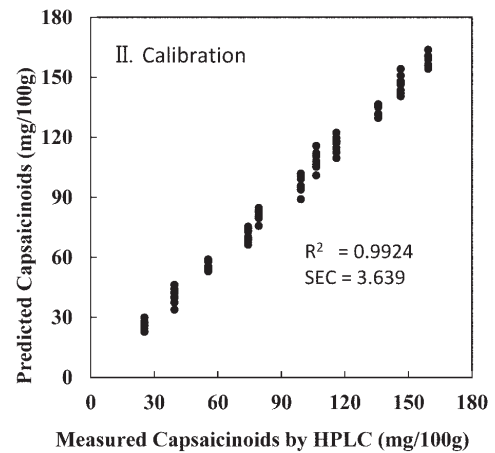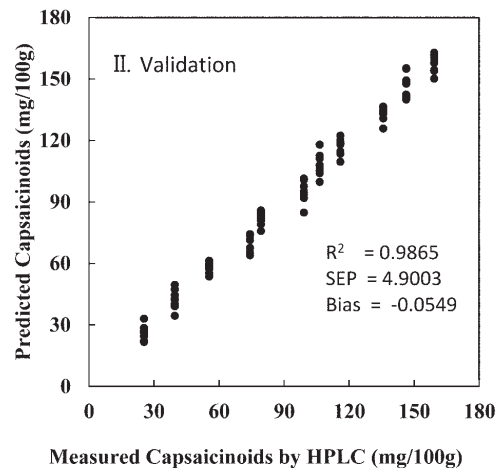
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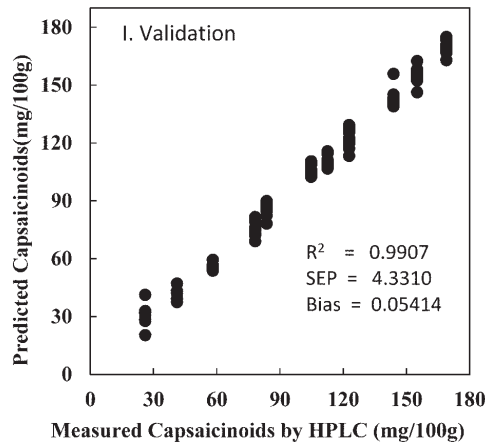
(b) Validation

Fig. 4. Calibration and Validation results using PLSR model for Case I red–pepper powder (particle size below 0.425 mm).
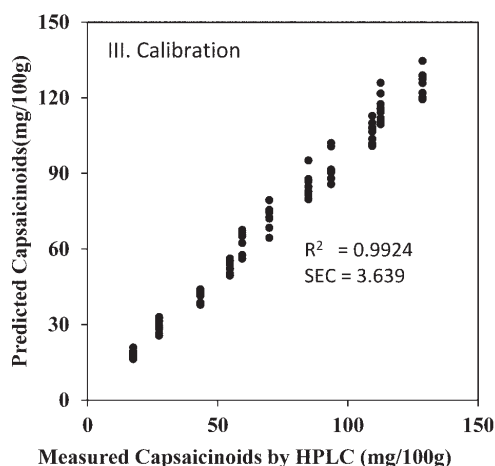

(a) Calibration

(b) Validation

Fig. 5. Calibration and Validation results using PLSR model for Case II red–pepper powder (particle size between 0.425 and 0.71 mm).

CONCLUSION

The on–line pungency measurement system was constructed to measure capsaicinoids content, which is the main ingredient influencing the pungency of red–pepper powder, in an industrial processing environment. The visible and near–infrared spectra, spanning from approximately 400 to 1000 nm, were acquired and used for the development of prediction models of capsaicinoids content in red–pepper powder. Partial least square regression (PLSR) models were developed to predict capsaicinoids content in red–pepper powders of three different particle sizes. The respective R_v^2 and SEP of the prediction models were 0.991 and ±4.33 mg/100 g, respectively, for the samples of particle size below 0.425 mm; 0.987 and ±4.90 mg/100 g, respectively, for particle sizes between 0.425 mm and 0.71 mm; and 0.977 and ±5.33 mg/100 g, respectively, for particle sizes between 0.71–1.4 mm. The R_v^2 and SEP of the PLSR model for the red–pepper powder of varying particle sizes below 1.4 mm were 0.973 and ±6.98 mg/100 g, respectively. The predictions of the model for the smallest particle size group of red–pepper powders showed better performance for predicting the capsaicinoids content. These results demonstrate the potential of the presented NIRS–based on–line pungency measurement method for grading the spiciness level of red–pepper powders.
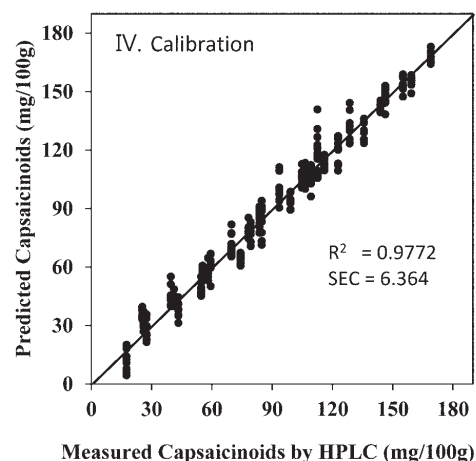
(a) Calibration

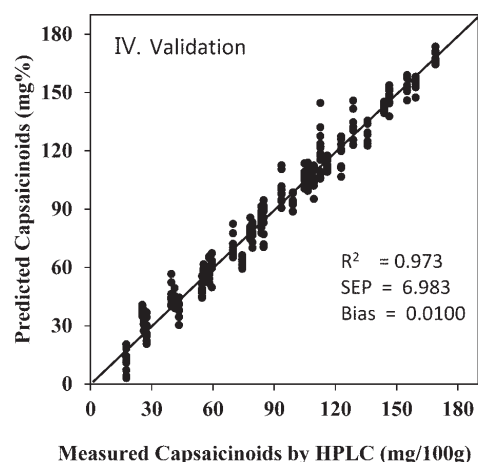(a)Calibration
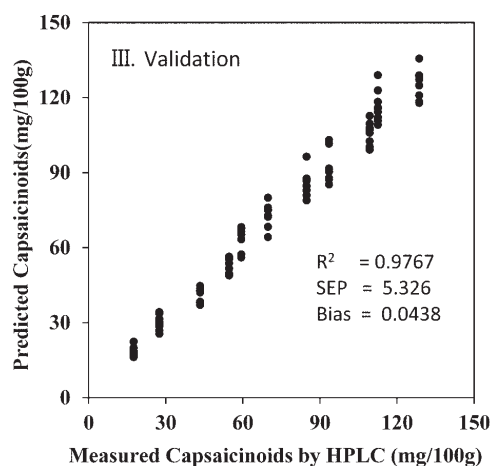
(b) Validation

(b) Validation

Fig. 6. Calibration and Validation results using PLSR model for Case Ⅲ red–pepper powder (particle size between 0.71 and 1.4 mm).

Fig. 7. Calibration and Validation results using PLSR model for the red–pepper powders of mixed particle sizes below 1.4 mm (Case Ⅳ).

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