Prediction of Chemical Compositions for On-line Quality Measurement of Red Pepper Powder Using Near Infrared Reflectance Spectroscopy (NIRS)

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Abstract Applicability of near infrared reflectance spectroscopy (NIRS) was examined for quality control of red pepper powder in milling factories. Prediction of chemical composition was performed using modified partial least square (MPLS) techniques. Analysis of total 51 and 21 red pepper powder samples by conventional methods for calibration and validation, respectively, revealed standard error of prediction (SEP) and correlation coefficient (R^2) of moisture content, ASTA color value, capsaicinoid content, and total sugar content were 0.55 and 0.90, 8.58 and 0.96, 31.60 and 0.65, and 1.82 and 0.86, respectively; SEP and R^2 were low and high, respectively, except for capsaicinoid content. The results indicate, with slight improvement, on-line quality measurement of red pepper powder with NIRS could be applied in red pepper milling factories.

Keywords: red pepper powder, near infrared reflectance spectroscopy, capsaicinoids, ASTA color value, modified partial least squares

Introduction

The red pepper (*Capsicum annuum* L.), a commonly used vegetable, is widely utilized in processed foods, such as spice and paste, as well as consumed as a fresh fruit. The major factors involved in the international quality control of red pepper powder include pungency, color, moisture content, mixing ratio of seeds, and sanitation. The quality property of Korean red pepper is its unique sweet taste, as well as its pungency. However, the cost of quality assessment of red pepper powder is quite high in terms of time, money, and labor for chemical and microbiological analyses.

Near infrared reflectance spectroscopy (NIRS) is a simple, rapid, and non-destructive method, and a multivariate technique of large application in the quality assessments of foods and agricultural commodities (1). The NIR region spans the wavelength range of 400~2,500 nm, with the absorption bands corresponding, in general, to overtones and combinations of fundamental vibrations (1-2).

The lack of a representative and reproducible procedure for quantification has introduced problems with regard to quantitative analysis. Nevertheless, numerous researches have been performed aimed at determining the correlation between NIRS and chemical analysis for the application of NIRS in food industry, for example; starch analysis (3), authentication of the varieties (4), mold contents in tomato puree (5) and hay (6), among others. In addition, researches have also been conducted on the application of NIRS to the processings of red pepper and paprika, focusing on such aspects as moldiness of paprika powders (7), determination of ergosterol contents (8), mixing ratio

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of seeds (9), and determination of capsaicin content (10-11).

To develop a model equation, mathematical treatment of the spectral data is very important, and statistical techniques such as multiple linear regression (MLR) (5-6, 10, 12, 13), principal component regression (PCR) (12), and modified partial least square (MPLS) are generally used (4, 12, 15-17) . The MLR technique selects the wavelengths, which correlate most strongly with the reference data. The PCR technique is essentially a two-stage method, in which the data compression stage is carried out independent of the regression stage, and each principal component is set orthogonally to the previous one. In MPLS, these two stages are combined, not only because there are much spectral variations to be taken into account, but because these independent factors are correlated with the reference data (12).

The applicability of NIRS to the estimation of chemical components has been reported using MLR techniques (10-11). Recently, MPLS regression technique, which involves the treatment of optimum data to remove the spectral interference comparing the MLR and PCR techniques, has been predominantly applied (12).

The objectives of this study were to predict the chemical composition, including moisture, capsaicinoid, and total sugar contents, and color value using NIRS, and to investigate the applicability of NIRS to the on-line measurement of red pepper powder in milling factories.

Materials and Methods

Materials To develop a model equation for the red pepper powder, 72 dried red peppers were purchased from Joongang Seed Co. (Seoul, Korea) and Hungnong Seed Co. (Seoul, Korea). The samples were de-stemmed, and the seeds were separated out, after which 90% pericarp and 10% seeds were mixed. To homogenize the particle

size, all samples were powdered using a roll mill, and the particle size for each sample was controlled to below 1.0 mm using a Ro-Tap sieve shaker (Cheonggesa CG-213, Seoul, Korea). Fructose, glucose, sucrose, maltose, capsaicin, and dihydrocapsaicin were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA).

Moisture content and ASTA color value Moisture content and ASTA (American Spice Trade Association) color value were determined by AOAC method (18) and ASTA-20.1 method (19), respectively. Red pepper powder (70-100 mg) was added to 100 mL acetone, and the mixture was stored at 0°C for 4 hr with intermittent stirring. An aliquot of the transparent extract was taken and measured at 460 nm for absorbance using a UV/Vis spectrophotometer (V-550, Jasco, Tokyo, Japan). The ASTA color value was then calculated using the following equation:

$$\text{ASTA color value} = \frac{\text{Absorbance of acetone extracts} \times 16.4 \times I_f}{\text{Sample weight (g)}}$$

(Instrument correction factor, I_f = Declared Absorbance of the Glass Reference Standard/Absorbance obtained at 465 nm on a Glass Reference Standard. A NIST SRM 2030 or 930, glass filter with an absorbance specified by NIST in the range of 0.4-0.6 at 465 nm, or equivalent.).

Capsaicinoids Capsaicinoids were extracted using the method of Vincent and Ken (20) with some modifications. Red pepper powder (4 g) was blended with 50 mL acetonitrile for 2 min. A 1.0-mL aliquot of the acetonitrile extract was taken for cleanup. A Sep-pak C_{18} (Waters, Milford, MA, USA) was conditioned with approximately 5 mL acetonitrile, followed by the addition of 5 mL distilled water. The extract (1.0 mL) was diluted with 9 mL water and injected into the conditioned Sep-pak. Capsaicinoids were then eluted with 4 mL acetonitrile, followed by 1.0 mL acetonitrile containing 1% acetic acid.

The capsaicinoids were analyzed by HPLC (Jasco, Tokyo, Japan), using a Model PU 980 pump, a Model UV 975 detector, and a Model 807-IT integrator under the following conditions: wavelength, 280 nm; mobile phase, MeOH/Water (70:30, v/v); μ-Bondapak C_{18} column (3.9 mm × 300 mm, 10 μm, Waters, Milford, MA, USA) connected to a guard-column (Waters Guard-Pak™, Milford, MA, USA); temperature, 25°C; and flow rate, 0.8 mL/min.

Total sugar Red pepper powder (2 g) was mixed with 50 mL of 80% ethanol and blended with a vortex mixer for 2 min. The extract was then filtered through a 0.45-μm filter (PVDF, Whatman, Clifton, NJ, USA). Total sugar contents corresponded to the sum of fructose, glucose, sucrose, and maltose contents analyzed by HPLC.

Total sugars were analyzed by HPLC (Jasco, Tokyo, Japan) using a Model PU 980 pump, a Model 830-RI detector, and a Model 807-IT integrator. The mobile phase consisted of acetonitrile/water (87:13, v/v), and a Carbohydrate analysis column (3.9 mm × 300 mm, 10 μm, Waters, Milford, MA, USA), with temperature and flow

rate set at 25 and 1.2 mL/min, was used.

Spectroscopic analysis by NIRS Reflection spectra of the red pepper powder were obtained using a visible/near-infrared scanning spectrophotometer (FOSS NIRSystems 6500, Silver Spring, MD, USA). Approximately 6 g each red pepper powder sample was scanned in triplicate in a small ring cup (NR-7073, i.d. 35 mm, depth 9 mm). All spectra were recorded over the wavelength range of 400~2500 nm. The spectroscopic procedures and data recording were performed using WinISI II version 1.50 software (FOSS NIRSystems, Silver Spring, MD, USA).

NIRS Calibration for the experimental contents of red pepper powder The prediction equations for capsaicinoids contents, moisture contents, ASTA color value, and total sugar contents were developed using the modified partial least square (MPLS) regression method. For correction of the scatter effect, standard normal variation and de-trend (SNVD) mathematical procedures were applied. The derivative treatment was "1, 4, 4, 1"; where the 1st digit is the number of the derivative, the 2nd is the gap over which the derivative is calculated, the 3rd is the number of data points in a running average or smoothing, and the 4th is the 2nd smoothing.

Statistics All chemical analysis data, with the exception of NIRS, were the averages of triplicate values obtained based on dry weights. Statistical analysis was performed by ANOVA and Duncan's multiple range tests (alpha = 0.05) using the SAS software (21).

Results and Discussion

To develop a model equation for the quality control of red pepper powder, chemical components of several varieties of red pepper powder were analyzed. The moisture contents, ASTA color values, total sugar, and capsaicinoid contents of 51 samples were analyzed for calibration in the ranges of 9.82~16.11%, 11.10~148.80, 4.97~9.82% d.w., and 22.68~471.10 mg/100 g d.w., respectively. For validation, 21 samples were analyzed in the ranges of 9.59 ~15.69%, 17.32~142.31, 4.62~20.92% d.w., and 14.87~179.75 mg /100 g d.w., respectively (Table 1).

Korean Industrial Standard for the quality of red pepper powder (KS standard Number: H 2157) requires moisture content to be below 13% (22), because moisture content directly affects the chemical components and causes sanitary problems in the red pepper industry. Higher initial

Table 1. Chemical compositions of red pepper powder in calibration and validation sets determined by conventional methods

	Calibration	Validation
Sample number	51	21
Moisture contents (%)	9.82-16.11	9.59-15.69
ASTA color value	11.10-148.80	17.32-142.31
Capsaicinoids (mg/100g d.w.)	22.68-471.10	14.87-179.75
Total sugar (%, d.w.)	4.97-9.82	4.62-20.92

moisture contents in paprika, less changes in color value, and moisture content increase of up to 14% enhanced the color stability and precluded pigment destruction (23-24). However, higher than 14% moisture content resulted in an acceleration of fungal growth. When moisture contents were in the range of 15~18%, ergosterol, used as a fungal marker for the red pepper powder, was detected in Korean commercial products at 1.48~4.96 mg/100 g d.w. (8, 25). Therefore, the regulation of moisture content is critical for the quality control of red pepper powder with regard to chemical deterioration and microbiological contamination.

Capsaicinoids contents and ASTA color values, which are directly related to the consumers' preference, were significantly different among KS standard commercial red pepper powder products. The level of capsaicinoids and ASTA color values were generally in the ranges of 10.54~250.87 mg /100 g and 64.55~124.07, respectively, in 47 varieties of Korean red pepper powder (26). The capsaicinoids contents of 'Chungyang' varieties were in the range of 250~400 mg/100 g, showing very strong pungency (27).

Figure 1 shows the NIRS obtained from triplicate results of 51 samples for calibration (n = 153) in a range of 400 to 2500 nm. Studies using MLR revealed wavelength were to the moisture content, capsanthin, total sugar, and capsaicin; with moisture to the wavelengths of 1460 and 1930 nm (28), capsanthin to the wavelengths of 1700,
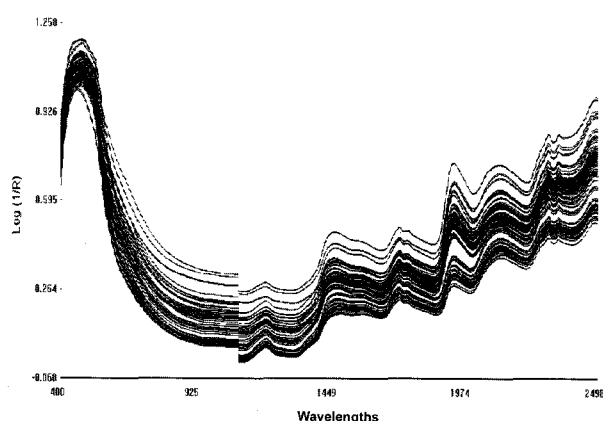

Fig. 1. Near infrared reflectance spectra of red pepper powder.

1768, 1164, and 2420 nm, and total sugar to the wavelengths of 1428 and 1432 nm (11). Capsaicin was related to the wavelengths of 2422, 2358, 1974, 1622, and 1820 nm (10); MPLS technique, an improved mathematical treatment technique, was not applied.

The optimum number of MPLS factors was determined by cross validation; the best calibration equation was developed based on the highest coefficient of determination (R^2) and the lowest standard error of cross-validation (SECV) to prevent over-fitting. SECV was calculated such that the number of cross-validation group was '4', and calibration by 3/4 samples in calibration set samples (n=153) and validation by others were also performed. After establishing the equations, samples in the validation set were used to assess the prediction accuracy of the calibration equation based on the standard error of prediction (SEP), coefficient of determination (R^2), and bias.

Table 2 shows calibration statistics established using NIRS and MPLS techniques. The calibration set used the numbers of all samples (n = 153) without outliers in chemical components, with the exception of capsaicinoids. The calibration of capsaicinoids was calculated using 143 sample sets (sample removal ratio 6.5%). The increase of sample removal ratio renders the reduction of SEC and increase of R^2 possible. However, general ratios for the optimization of model equation were in the range of 15~20% in NIRS statistical analyses (8).

The numbers of terms and R^2 values were 9 and 0.97 for moisture content (Fig. 2a), 10 and 0.99 for ASTA color value (Fig. 3a), 10 and 0.89 for capsaicinoid content (Fig. 4a), and 10 and 0.96 for total sugar, respectively (Fig. 5a). Comparing SEC, SECV, and R^2 values, the model equations obtained by NIRS proved to be very accurate for all chemical components (Table 2, Fig 2a, 3a, 4a, and 5a). Validation process applied to the 21 red pepper powder samples (Table 3) revealed SEP and R^2 of moisture content, ASTA color value, and total sugar were 0.55 and 0.90 (Fig. 2b), 8.58 and 0.96 (Fig. 3b), and 1.82 and 0.86 (Fig. 4b), respectively, which indicate that the contents of the compounds analyzed by chemical analysis were strongly correlated with those predicted by NIRS, and that each model equation was applicable to a red pepper milling factory. However, the model equation did not work properly for capsaicinoid contents. The SEC and SECV values with regard to capsaicinoid contents were 33 and

Table 2. Calibration and cross validation statistics of red pepper powder in the calibration set (Math treatment: SNV+DT, 1,4,4,1)

Calibration statistics	Moisture contents (%)	ASTA color value	Capsaicinoids (mg/100g d.w.)	Total sugar (%)
N (Calibration set)	153	153	143	153
Number of Outliers	0	0	10	0
Term[a]	9	10	10	10
SEC[b]	0.27	3.15	33.00	0.87
R_1[2c]	0.97	0.99	0.89	0.96
SECV[d]	0.31	4.10	40.42	1.10
R_2[2e]	0.96	0.98	0.82	0.93

a: number of MPLS factor used in the regression equations
b: standard error of calibration
c: R^2 of SEC
d: standard error of cross validation
e: R^2 of SECV

Table 3. Validation statistics of red pepper powder by the calibration equation

Calibration statistics	Moisture contents (%)	ASTA color value	Capsaicinoids (mg/100g d.w.)	Total sugar (%)
N(validation set)	63	63	54	63
Number of Outliers	0	0	9	0
SEP[a]	0.55	8.58	31.60	1.82
Mean	12.10	68.57	6.72	12.17
SD	156.00	40.80	54.15	4.89
Bias	-0.07	-0.33	31.87	1.83
R^{2b}	0.90	0.96	0.65	0.86

a: standard error of prediction
b: R^2 of SEP
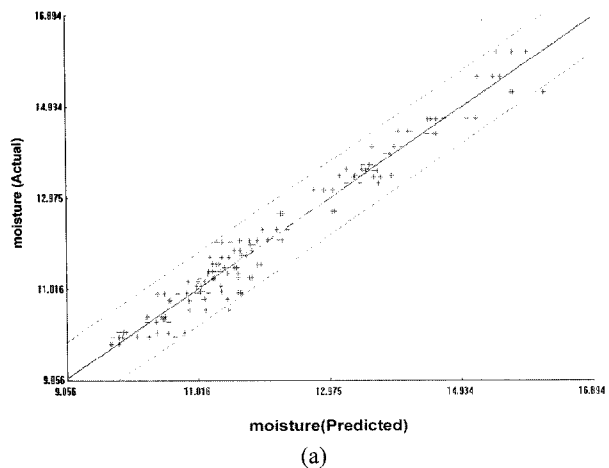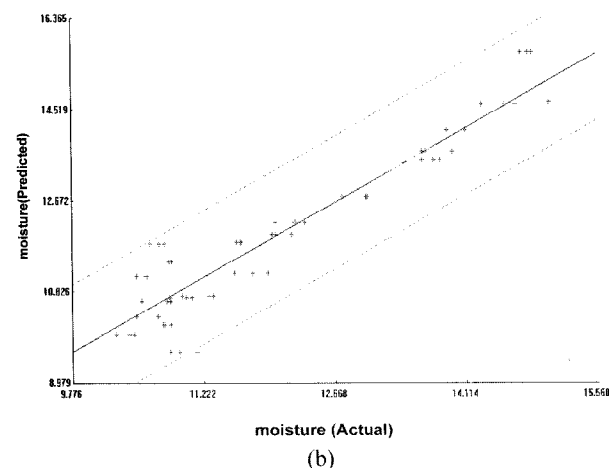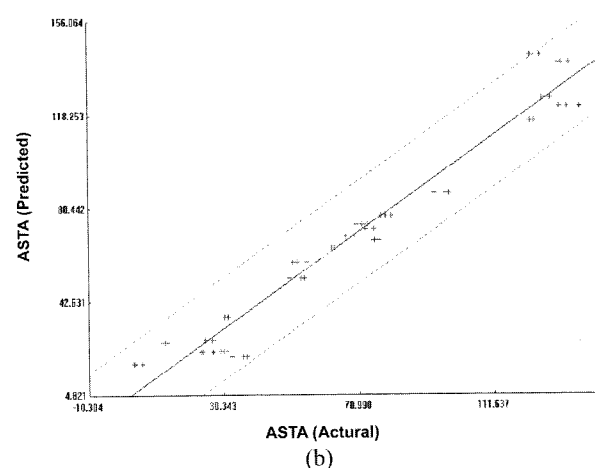

Fig. 2. Calibration curve of moisture contents in 51 red pepper powder (a) and scatter plot of the validation in 21 red pepper powder (b) determined by chemical analysis and NIRS.
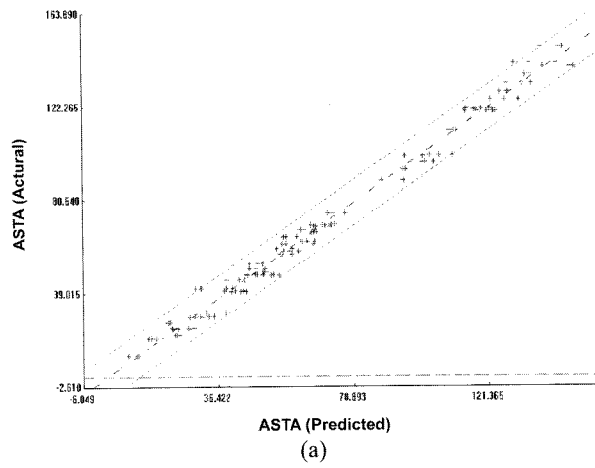
Fig. 3. Calibration curve of ASTA color values in 51 red pepper powder (a) and scatter plot of the validation in 21 red pepper powder (b) determined by chemical analysis and NIRS.

40.42, respectively, which were much higher than those of other components (Table 2).

In the validation process, the SEP and R^2 values for the capsaicinoids were 49.40 and 0.38 without outliers (n=63), and were poorly correlated. Removal of values exhibiting appreciable differences between data obtained by chemical analysis and those obtained by NIR improved the SEP (31.60) and R^2 (0.65) values (n=54, Fig 5), although the distribution of capsaicinoid contents became biased (Fig 5a).

A study on capsaicin contents reported that NIRS technique was applicable with a correlation coefficient and

a standard error of 0.993 and 0.0036%, respectively, in SMLR methods (10). However, Cho *et al.* reported that the standard error of the estimate was slightly higher, and thus model equation using NIRS was inapplicable to capsaicin (11).

The prediction of the model equation poses several problems when applied to capsaicinoids. First, capsaicinoid content of red pepper powder was lower than those of other components, such as moisture content and total sugar, and could be disturbed by other components when measuring the wavelength, in spite of their reduction via
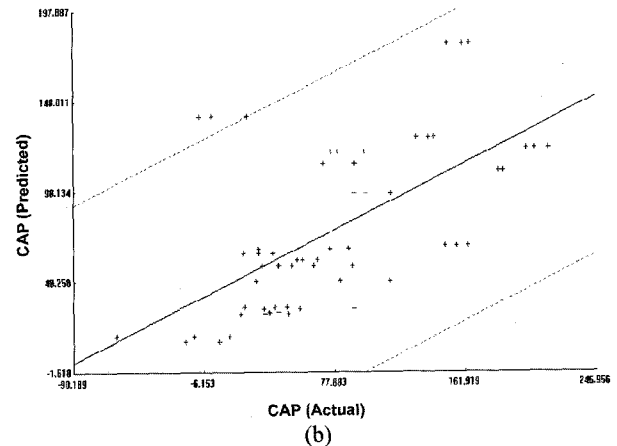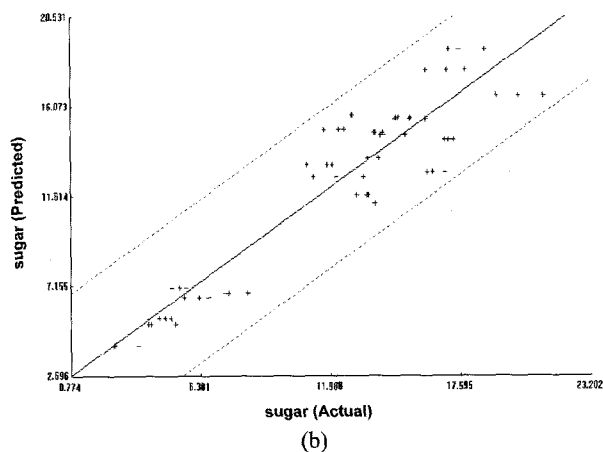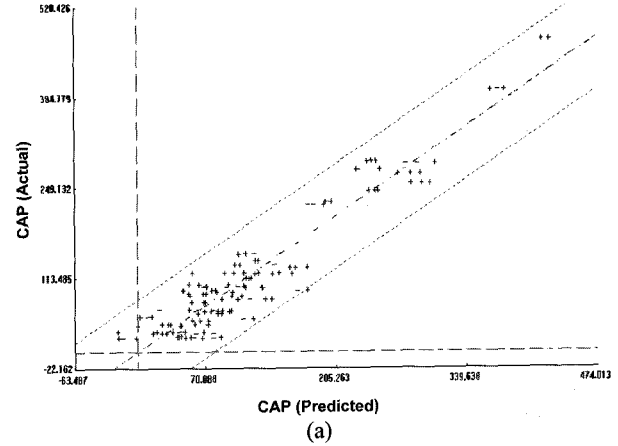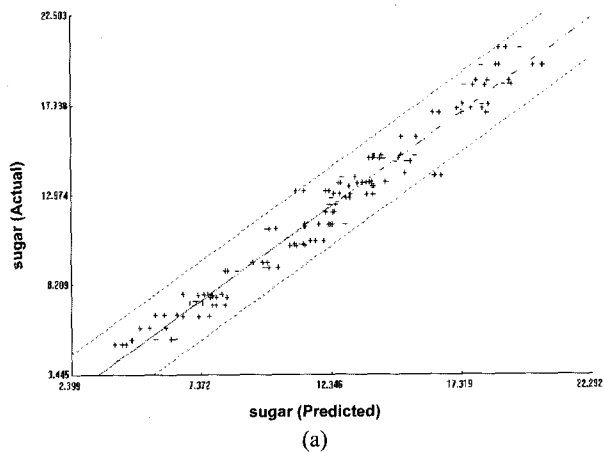
Fig. 4. Calibration curve of total sugar contents in 51 red pepper powder (a) and scatter plot of the validation in 21 red pepper powder (b) determined by chemical analysis and NIRS.

Fig. 5. Calibration curve of capsaicinoids contents in 51 red pepper powder (a) and scatter plot of the validation in 21 red pepper powder (b) determined by chemical analysis and NIRS.

mathematical techniques. Secondly, homogeneous samples must be prepared to prevent disturbance effects of particle size, moisture content, and other components. Thirdly, the preparation of evenly distributed and widely ranged samples is critical for proper measurement of capsaicinoid content. The red pepper harvested in Korea originated from a single genus, *Capsicum annuum*, L., and the range of capsaicinoid content was mostly below 80 mg/100g d.w., in addition to the biased distribution (26). Fourthly, this technique requires the preparation of a large number of samples. In this experiment, although 72 samples had been collected for several years, they proved to be insufficient.

In this study, the chemical compositions of red pepper powder were predicted by NIRS, and the results confirmed that the NIRS technique is applicable to the estimation of moisture content, ASTA color value, and total sugar content, with the exception of capsaicinoids. Furthermore, preparation of a large number of evenly distributed samples and development of mathematical treatment techniques could effectively improve the estimation of capsaicinoid contents for application to an on-line quality measuring system in red pepper milling factories.

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