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Application of Near Infrared Spectroscopy to Predict Crude Protein in Shrimp Feed

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ABSTRACT

The preliminary study of using near infrared spectroscopy (NIRS) to predict crude protein in shrimp feed was carried out. The calibration set of 41 shrimp feed samples was laboratorily prepared against the validation set of 39 samples with similar distribution for the chemical content. The samples were scanned for the NIR spectra in the wavelength region of 1100 nm to 2500 nm. Both multiple linear regression (MLR) and partial least square regression (PLS) were performed to analyze data to create the best calibration equations. The results indicated that absorbances at wavelengths of 1686 nm, 1778 nm and 2486 nm were related with protein in the samples. At 1686 nm the absorbance was found to coincide with that of protein analytically found the most in the prepared shrimp feed. Following the use of wavelengths selected in MLR for constraining the wavelength region as input to PLS, the calibration equations were shown to improve in prediction accuracy.

Key words: near infrared spectroscopy (NIRS), protein, shrimp feed

INTRODUCTION

Aquatic animals are those of economic animals which earn a large amount of exporting incomes to Thailand. Thailand was the world number one in exporting aquatic animals particularly shrimp in 1992. Since 1988, shrimp farms have been expanded by 90% (Fishery Economics Division, 2002). In shrimp farming the feed accounts is the main cost accounting for 70-80% of the total investment. The feed as a result has been produced in an increasing amount every year. Since there are a large number of shrimp feed products available in the market, the quality control must be exercised to standardize the feed quality by the concerned authority.

In quality control of aquatic animal feed, the chemical analyses must be applied inevitably. The analyses have to be carried out in laboratory rooms using a number of instruments and both local and imported chemicals. The results obtained are accurate and internationally accepted but the analyses take time, need a lot of spending and employ several personnel. Besides the waste from the laboratory room is regarded as pollutants.

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Near Infrared Spectroscopy (NIRS) has been used efficiently in predicting the chemical contents in animal feed (De Boever *et al.*, 1994) and the Total Digestible Nutrients (TDN) in animal feed (Amari and Abe, 1997). De Boever *et al.* (1994) used partial least square regression (PLSR) to obtain the calibration model in the range of 1100 nm to 2500 nm for predicting the protein in feed for cow. The accuracy of the prediction was shown with correlation coefficient, R = 0.96 and root mean square error of prediction (RMSEP) = 1.4. Amari and Abe (1997) studied the use of NIRS technique to determine the content of TDN in silage animal feed and showed that the first term in the model developed using multiple linear regression (MLR) related to the absorbance at 2149 nm. Edney *et al.* (1994) investigated the quality of barley to use as animal feed using NIRS. They found that in the range 400 – 1800 nm the model with second derivative treated spectra could be used to predict the animal feed with R = 0.97 and SEP = 0.31. Iwamoto *et al.* (1984) reported the use of MLR model to predict the protein content in wheat flour. In the industry of the aquatic animal feed, the NIRS has been found to be used the most compared with other industries (Warunee *et al.*, 2001). The use of NIRS would reduce the chemical expense and shorten the analysis time. However the NIRS technique is not well-known and still new to Thai research community thus necessitating further study and development.

This study was aimed to preliminarily investigate the application of NIRS in prediction of protein content in the laboratorily prepared shrimp feed. The shrimp feed was prepared so as to have the protein content varying in similar range to that in the commercial feed. The prepared shrimp feed with known proportion of raw material was used as samples for this initial study. Provided the results were promising the extension to the commercial feed with large variation of raw material will be carried out in the future.

MATERIALS AND METHODS

Shrimp feed for the tests

Each individual raw material for formulating the shrimp feed such as wheat, ground fish, for instance was prepared and chemically analyzed for protein content. The obtained protein values were then used to calculate the proportion of each raw materials for preparing 80 samples of the shrimp feed with a range of protein between 12 to 50% i.e. covering the protein range of the feed in the market. The protein content in the feed was prepared in such a way that the content was increased from 12 to 50% in even increment. The feed was then dried in the oven at 50°C for 16 hours and kept at ambient temperature so that the moisture content was approximately not over 10%. Each sample of the shrimp feed was then ground by the mortar grinder to ensure the homogeneity of the sample. The sample was kept until the temperature was 25°C prior to being subjected to scanning by the spectrometer. After that the sample was chemically analysed for the protein content. Following the scanning and chemically analysing all 80 samples, the chemical data was used to divide the data into a calibration set of 41 samples and a validation set of 39 samples with both sets having similarity in even distribution of the protein content. The samples were sorted in descending order of the protein content and each sample was assigned alternatively to both calibration set and validation set. The calibration set contained the first and the last data in the sorted order. As a result the calibration set has the protein content in a larger range than the validation set.

Measurement of the spectra

The samples of the shrimp feed were scanned in reflectance mode with NIR spectroscopy (Bran&Luebbe InfraAlyzer 500) in the near infrared region from 1100 nm to 2500 nm at 2 nm increment. The samples were packed in the standard close cup and each sample was

repacked and scanned three times for the average value. The NIR absorbance spectra were stored for subsequent development of the calibration equation with the reference i.e. the protein data.

Chemical analyses

The protein contents of the feed were determined by means of Kjeldahl method. The measurement was repeated three times for each sample and the measured values were averaged. The chemical values of the protein in the shrimp feed were shown in Table 1.

Data analyses

The data analyses were performed based on the assumption that other constituents in the shrimp feed would have the absorbance in other wavelength region than the protein content.

The samples from the calibration set were used to derive the calibration equation. The spectra were initially pretreated with second derivative in comparison with the multiplicative scattering correction (MSC) prior to analyses in order to compensate for the particle size and the scattering effect. The calibration equations or models were developed using multiple linear regression (MLR) analysis and partial least square regression (PLSR) for comparison. The models were validated with data in the validation set. The optimum model was the one that yielded the lowest value of standard error of prediction (SEP) and low value of bias.

For the multiple linear regression model, the software used was the Near Infrared Spectral Analysis Software (NSAS) which gave the calibration equation with four terms of the spectral variables at maximum as follows:

$\%Protein = K_0 + K_1F_1 + K_2F_2 + K_3F_3 + K_4F_4$

where K_i = coefficients of each term

F_i = function of absorbance at the wavelength i

Regarding the partial least square regression model, the UNSCRAMBLER software package was used to derive the PLS models relating the spectral bands to the protein content. The spectral bands were varied and analyzed for the PLS model with minimized errors in prediction. The software suggested the number of factors, which were optimum in describing all variance in the spectra.

RESULTS AND DISCUSSION

The NIR absorbance spectra of the shrimp feed in the calibration with protein content between 12.08 and 52.80% are illustrated in Figure 1. Clearly, the absorbance were affected by variation in particle size as the whole were shifted at all wavelengths. Additionally, scattering effect was apparent which tilted the spectra to have higher asorbance at longer wavelengths.

The particle size and the scattering effect imposed in the absorbance (Figure 1) were compensated for by second derivative or multiplicative scattering correction as shown in Figure 2 and Figure 3 respectively.

MLR model

The sample absorbance at each wavelength were correlated with the protein content and the resulted correlation coefficients were plotted against wavelength. The correlation plot in Figure 4, used as the tool in selection of

Table 1 Protein contents in the prepared shrimp feed.

	Protein content	
	Calibration set	Validation set
Number of samples	41	39
A range of protein content	12.80% – 52.80%	13.32% – 50.57%
Standard deviation	11.68%	11.19%

the first wavelength in the MLR model, showed high correlation between the treated absorbance and the protein content at various wavelengths at 1513, 1686, 1750, and 1840 nm for instance.

To assure the selection of the optimum absorbance band, the spectra of the protein type

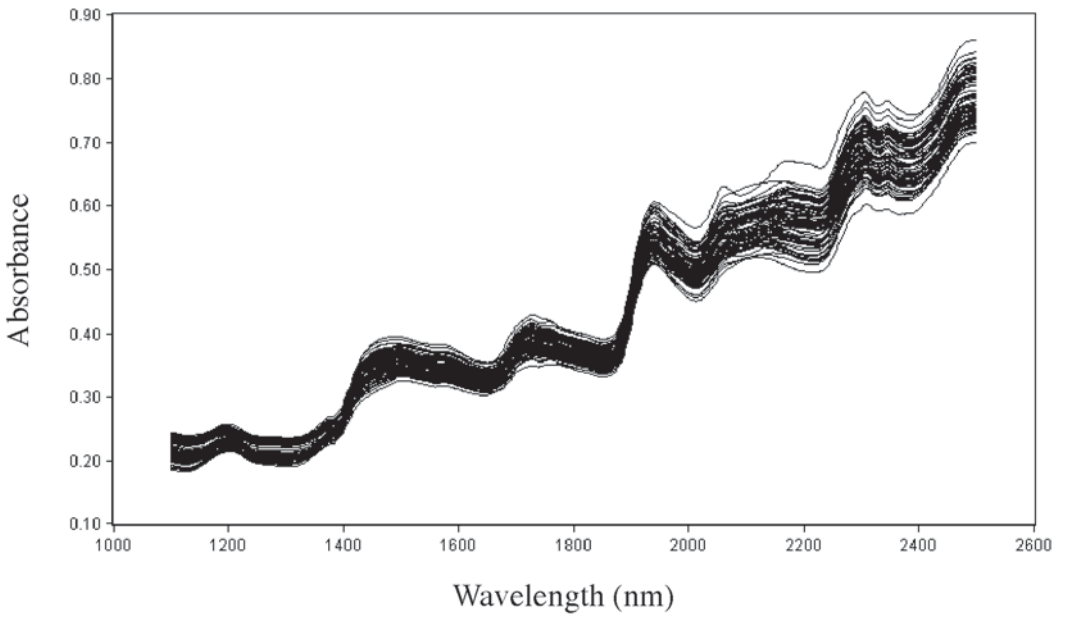

Figure 1 Original spectra of shrimp feed for the calibration set having protein content ranging from 12.08 to 52.80%.

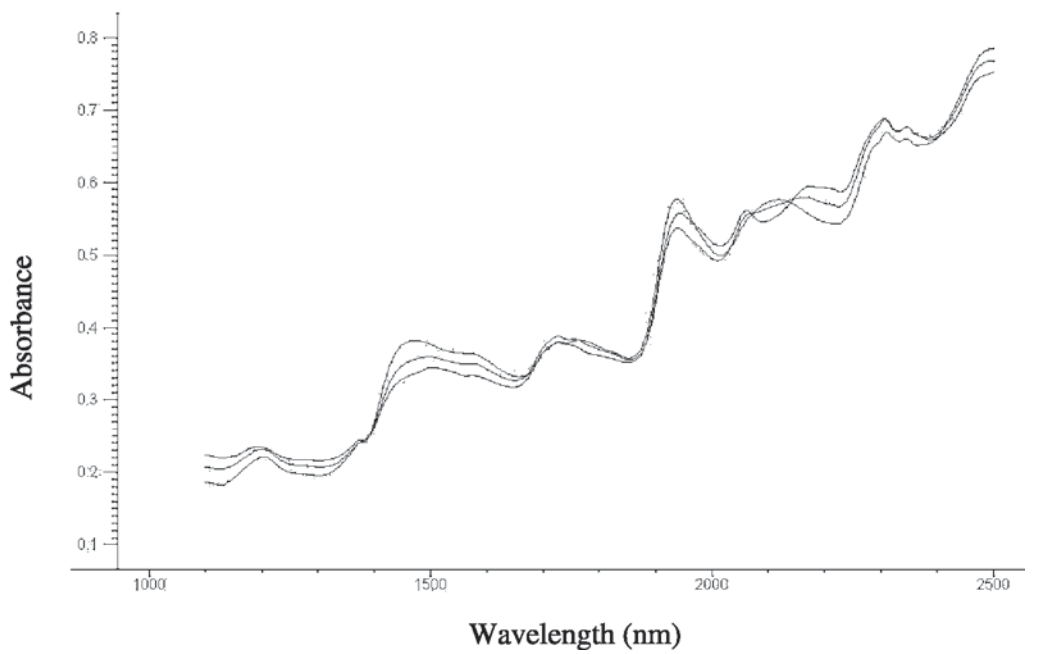

Figure 2 MSC pretreated spectra of shrimp feed showing reduction of particle size and scattering effect.

found the most in the shrimp feed was also examined. Representatively the shrimp feed samples with the lowest value and the highest value of the protein contents (i.e. 19 and 45% respectively) were analyzed by the high performance chromatography (HPLC). The analysis results would indicate the type of protein contributing to the maximum proportion.

It was found from Table 2 that L-Glutamic acid was accounted for the maximum percentage in both samples. The shrimp feed either with low protein content (19%) or high protein content (45%) contained L-Glutamic acid as a protein type with the maximum percentage. The

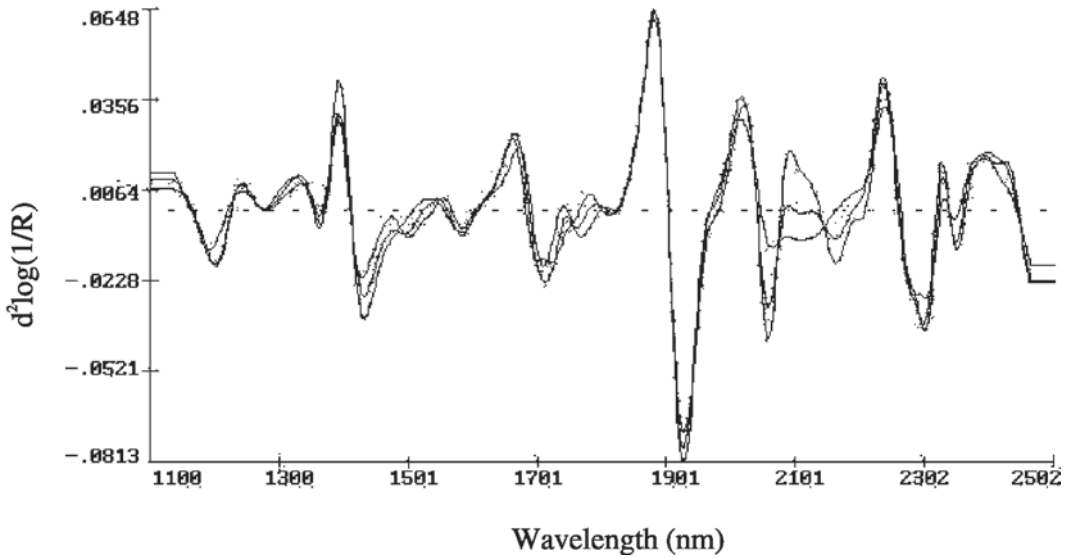

Figure 3 Second derivative of the absorbance of shrimp feed.

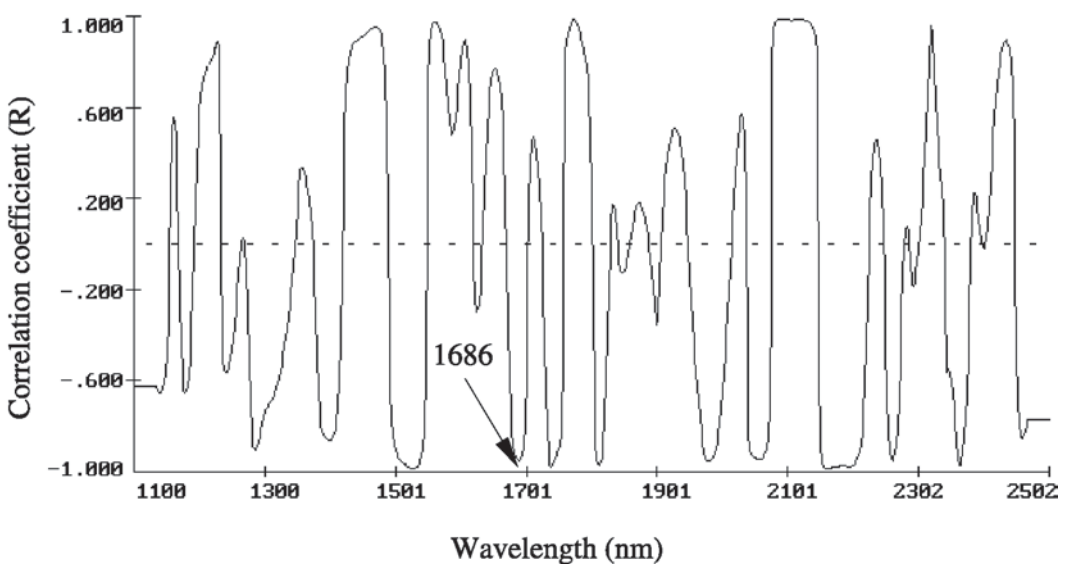

Figure 4 Second derivative correlation plot for wavelength selection of the first term in the MLR calibration model.

absorbance region of L-Glutamic acid would be useful in selection of the wavelength at which the absorbance was prominent.

Subsequently, 99% L-Glutamic acid was measured to obtain the spectrum and its corresponding second derivative NIR spectra was derived as shown in Figure 5 and Figure 6 respectively. From Figure 5 the band of the spectrum was apparently broad and made it difficult to choose for the significant bands of the spectrum.

With the second derivative NIR spectrum, the bands were markedly resolved. It could be seen in Figure 6 that the important bands of the L-Glutamic acid were at 1686, 2150, 2284 and 2390 nm and these wavelengths closely coincided with those proposed by William *et al.* (1990).

The band at 1686 nm was therefore chosen as the first term in the MLR model. Analysis results suggested that the optimum MLR model included spectra at wavelengths 1686, 1778, and 2486 nm with R=0.99, Standard error of calibration (SEC) = 1.30 and SEP=0.99. The model used the MSC treated spectra that were found giving lower errors than the second derivative treated ones. In Figure 7 is shown the NIR calculated protein contents plotted against the actual values of the validation set.

The absorbance at 1686 nm given by the correlation plot in the MLR model was also the wavelength of the significant band in the L-Glutamic acid. Consequently the best MLR model is as follows:

%Crude protein = 581.39 + 744.02 F1686 – 1172.06F1778 – 485.33F2486

where Fi = MSC treated log(1/R) or absorbance at wavelength i

R = Reflectance

Table 2 The first three of protein types accounting for the maximum percentage in shrimp feed samples.

Type of protein (w/w)	Feed with 19% protein	Feed with 45% protein
L-Glutamic acid (%)	2.88	6.50
L-Aspatic acid (%)	1.75	3.97
L-Leucine (%)	1.39	2.82

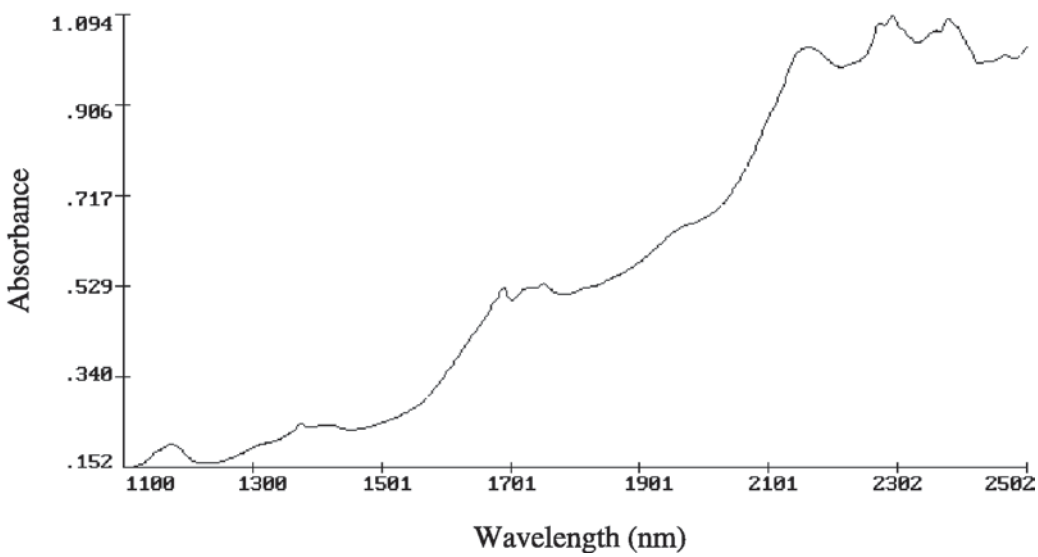

Figure 5 Absorbance spectrum of 99% L-Glutamic acid.

PLS model

The comparison between the PLS models established from variably different bands of spectra was made. The full band of the spectra was firstly used to make the PLS model. Secondly the minimum and the maximum wavelengths in the obtained MLR model were used to define a range of wavelengths for the PLS model analysis as

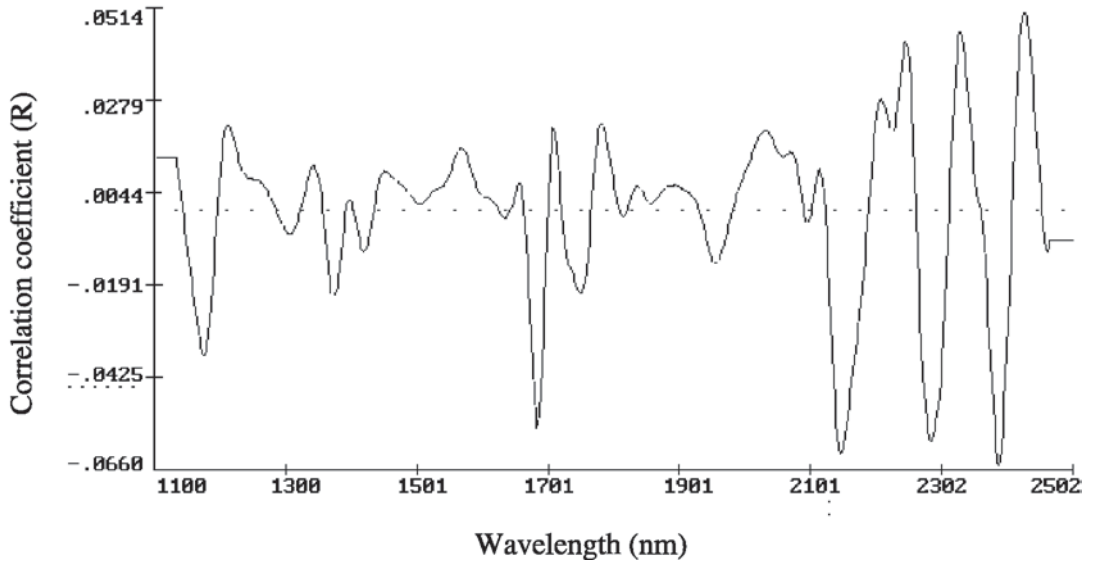

Figure 6 Second derivative of NIR spectrum of 99% L-Glutamic acid.

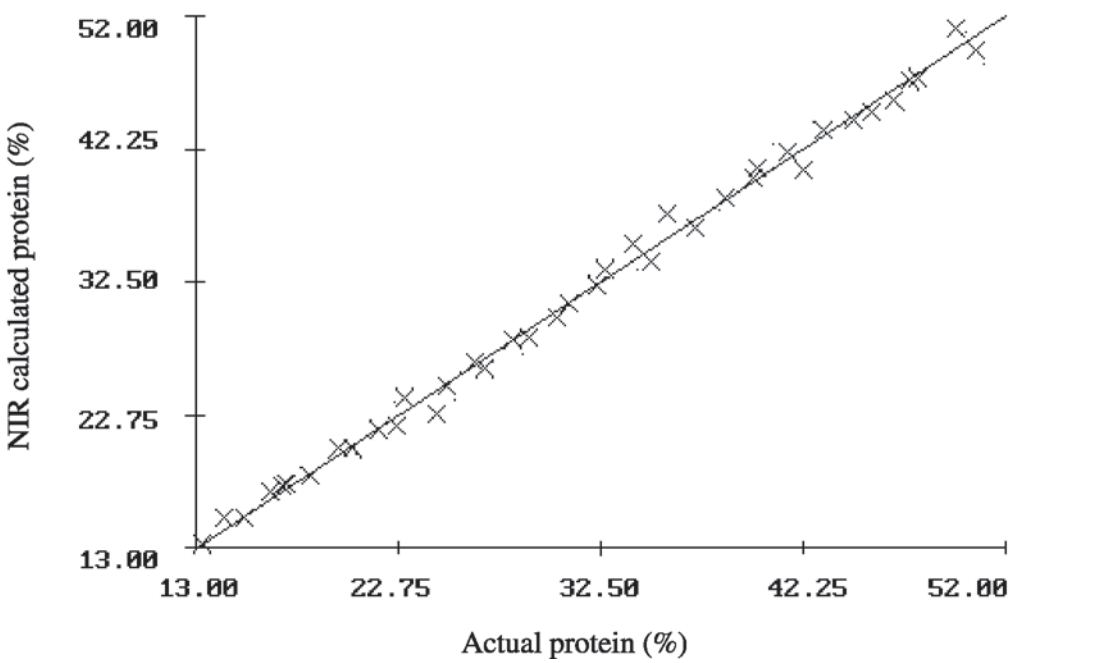

Figure 7 The plot of NIR calculated protein contents against the actual values of the validation set with SEP of 0.99 and bias of –0.31.

Table 3 The analysis results of PLS calibrations in comparison with MLR calibration for prediction of protein contents.

Model	Range of wavelengths (nm)	F[a]	R	SEC	SEP	Bias
PLS	1100 - 2500	2	0.99	1.29	1.05	-0.19
PLS	1600 – 2500	2	0.99	1.32	1.00	-0.34
MLR	1686, 1778 and 2486	3	0.98	1.30	0.99	-0.31

[a] Number of factors used in the PLS calibration or the number of terms in the MLR calibration.

suggested by Saranwong *et al.*, 2001). The PLS models developed from the above two selections of the band were compared.

The PLS analyses in Table 3 showed that using the whole spectrum (1100 to 2500 nm) for establishing the PLS model gave the highest error in prediction (SEP=1.05) but the bias was low. This was probably due to the inclusion of interference or unwanted absorbance in the model. The second PLS model took the starting wavelenth at 1600 nm and the ending wavelength in the range at 2500 nm. Both terminal wavelengths were empirically taken from the first (1686 nm) and the last wavelengths (2486 nm) of the MLR model. The accuracy in prediction of the second PLS model was slightly improved (SEP = 1.00) over the first model. However the bias was poorer at –0.34.

CONCLUSION

Prediction of protein content in laboratorily prepared shrimp feed was achieved using NIR spectroscopy. The MLR model was optimal when pretreated with MSC and the MSC treated spectra at 1686 nm was involved in the model. The spectra band at 1686 nm was coincident with the dominant band of L-Glutamic acid which was the protein type accounting to the maximum proportion in the shrimp feed. Similarly, MSC treatment resulted in the optimum PLS model. Comparative investigation for the optimum spectral range for PLS analysis was conducted. The results indicated that the range between 1600 nm and 2500 nm was the optimum range in establishing the PLS model with the lowest error of prediction.

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LITERATURE CITED

Fishery Economics Division. 2002. **Fishery Statistics of Thailand**, Department of Fisheries, Bangkok.

Amari, M. and A. Abe. 1997. Application of near infrared reflectance spectroscopy to forage analysis and prediction of TDN contents. **J. Jpn. Grassl. Sci.** 31: 55 – 63.

De Boever, J.L., B.G. Cottyn, J.M. Vanacker and Ch.V. Boucque'. 1994. The use of nirs to predict the chemical composition and the energy value of compound feeds for cattle. **Anim. Feed Sci. Technol.** 51: 243-253.

Osborne, B.G., T. Fearn and P.H. Hindle. 1993**. Practice NIR Spectroscopy with Applications in Food and Beverage Analysis**. Longman Scientific & Technology. Essex, 227 p.

Sirinapa, S., J. Sornsrivichai and S. Kawano. 2001. Improvement of PLS Calibration for

brix value and dry matter of mango using information from MLR calibration. **J. Near Infrared Spectrosc.** 9: 287-295.

Warunee, T., A. Terdwongworakul and V. Haruthaithanasan. 2001. Present conditions of R & D for nondestructive techniques including NIR spectroscopy in Thailand. pp. 36. *In* **Proc. of the Symposium on Nondestructive Techniques the 17 th**. Held at Tsukuba University, Japan.

Williams, P. and K. Norris. 1990**. Near – infrared Technology in the Agricultural and Food Industries.** American Association of Cereal Chemists, Inc. Minnesota, 330 p.