Prediction of Freshness Quality and Phosphate Residue of White Shrimp Products Using Near-Infrared Spectroscopy

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HIGHLIGHTS

- Near-Infrared (NIR) spectroscopy is proposed as a rapid technique for quality control of shrimp.
- NIR spectroscopy can be effectively used for monitoring the phosphate content of fresh and frozen shrimp.
- Prediction results of total volatile basic nitrogen and phosphate by Partial Least Square regression were highly accurate.

ABSTRACT

Background: The manufacturing of frozen shrimp is an important industry for the economy of Thailand. The objective of this study was to use Near-Infrared (NIR) spectroscopy to determine the freshness quality, includingTotal Volatile Basic Nitrogen (TVB-N) and Water Holding Capacity (WHC) of white shrimp (whole and chopped shrimp) and phosphate residues of shrimp.

Methods: Sixty white shrimp samples of a size of 70-80 shrimp/kg were stored at 4 ˚C. The sample was divided into two groups by soaking in two kinds of phosphate solutions, including Sodium Tripolyphosphate (STPP) and Mixed Phosphate (NAN101). The samples were evaluated using NIR which was performed before freezing and seven days after freezing. Calibration models of the freshness and phosphate residues of fresh and frozen shrimp products were built by Partial Least Square (PLS) regression between the spectral data and the reference methods.

Results: Satisfactory PLS results were obtained from the calibration model of TVB-N of chopped shrimp with a correlation coefficient (R) of 0.94 and Ratio of Prediction to Deviation (RPD) of 3.07. However, the NIR data indicated an unreliable prediction for the WHC (R<0.5). For the determination of phosphate residuals from STPP and NAN 101, the best calibration results were R>0.94 and RPD>3.00.

Conclusion: The NIR spectroscopy was feasible for monitoring the TVB-N as well as phosphate residues of shrimp products.

Introduction

Frozen and processed shrimp are the most important exports among the fishery commodities of Thailand. In 2019, Thailand exported more than 400,000 tons of shrimp products, worth approximately 100 million baht

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and accounting for 8.15% of the global market. Pacific white shrimp (*Litopenaeus vannamei*) is an important commercial species primarily cultured in Thailand. Shrimp quality includes many attributes, such as texture, colour, pH, freshness, and residual phosphate (Nirmal and Benjakul, 2011; Zhang et al., 2015).

Freshness is considered a fundamental indicator of the quality and safety of shrimp (Huang et al., 2015). During storage, shrimp are usually perishable because of bacterial action, autolysis, and, accordingly, changes in shrimp lipid oxidation, protein denaturation, and chemical composition, such as trimethylamine content, Total Volatile Basic Nitrogen (TVB-N), and Water Holding Capacity (WHC) (Huang et al., 2016). The TVB-N scales of acceptability previously reported for raw shrimp are <12 mg N/100 g for fresh shrimp, 12-20 mg N/100 g for edible but a little decomposed shrimp, 20-25 mg N/100 g for borderline shrimp, and >25 mg N/100 g for decomposed and inedible shrimp (Okpala et al., 2014). In the food industry, the freshness of shrimp is an important factor in determining the quality of raw material, which is related to the price and grade of shrimp. However, the current method used in the industry is sensory evaluation (Baka et al., 2018; Nirmal and Benjakul, 2011), which depends on the experience of the staff.

Processing of frozen shrimp involves various steps that affect the protein structure of the muscle by chemical-physical factors that affect the characteristics of the product. One of the crucial steps for frozen shrimp production is soaking the shrimp in a phosphate solution before freezing to increase the WHC of the shrimp. Soaking is an important step in the preparation of frozen raw shrimp products. Shrimp are soaked in a phosphate compound to improve their functional properties (Baka et al., 2018). Phosphates have been widely used to improve the yield and WHC during processing, to make peeling easier and minimize yield loss during freezing. Phosphates affect pH and anionic strength, which enables interaction with divalent cations and myofibrillar proteins (Guðjónsdóttir, 2011). According to the Ministry of Health (Thailand), phosphate in a frozen shrimp product must not exceed 5,000 mg/kg (Panseri et al., 2020).

Currently, Near-Infrared (NIR) spectroscopy is popularly applied in the food processing industry due to its benefits of nondestructive analytical techniques, rapid methods capable of online application, minimal or no sample preparation, and environmentally friendly methods (Porep et al., 2015). This technique has been used for quantitative analyses of chemical constituents and qualitative determination of multicomponent systems. NIR spectroscopy has multivariate chemometrics for freshness determination in food products, and it has been applied to determine the TVB-N values in duck meat (Qiao et al.,

2017). NIR spectroscopy has been used for the quantitative determination of the four major food constituents of seafood: water, protein, fat, and carbohydrates (GuÐjónsdóttir et al., 2011; Sringarm et al., 2022). Therefore, consumer safety and quality control are important considerations. The standard procedure for determining the freshness quality and phosphate quantification in factories has the disadvantages of being time-consuming, destructive, chemically wasteful, and consequently unsuitable for online application in modern industry (GuÐjónsdóttir et al., 2011; Okpala et al., 2014). Therefore, the objective of the current work was to determine the feasibility of using NIR spectroscopy to determine the freshness quality and phosphate residues of white shrimp.

Materials and methods

Materials

Pacific white shrimp (*L. vannamei*) with a size of 70-80 shrimp/kg in this study were purchased from a local market in Phitsanulok province, Thailand from July to December in 2018. Shrimp were kept on ice during transportation to the laboratory at Naresuan University, Phitsanulok, Thailand, and then peeled and deveined tail-off before performing the experiments.

Determining TVB-N and WHC using NIR spectroscopy

-Sample preparation

Sixty shrimp samples were packed in sealed bags and then stored at 4 °C different storage times up to 9 days was determined in terms of TVB-N and WHC to ensure the quality of white shrimp (whole and chopped shrimp). During storage, samples were scanned using NIR spectroscopy, and then the TVB-N values and WHC value were analysed to obtain various levels of these chemical values.

-NIR spectral acquisition

A Multi Purpose Analyser (MPA) Fourier transform NIR spectrometer (Bruker Optics, Ettlingen, Germany) equipped with an integrating sphere and an indium gallium arsenide (InGaAs) detector was used. The NIR spectrum of the shrimp sample was acquired in rotating reflectance mode at 8 cm^{-1} spectral resolution with a background of 32 scans. A total of 150 g of shrimp sample was filled in a quartz cup [100(Ø)×20(H) mm] and covered using an aluminium reflector. In this study, each sample was NIR-measured twice, first on a whole shrimp and then on a chopped shrimp.

-NIR measurement and data analysis

NIR spectral data, ranging from 4,000-12,000 cm^{-1}, were analysed using open-source software package (OPUS) version 7.8 software (Bruker Optics). Spectral pre-treatment through Standard Normal Variate (SNV) method was performed to reduce multiplicative interferences of scatter and particle size in the spectral scan (Da Costa Filho, 2009; Numthuam et al., 2017). The procedure of selecting samples for modelling and the test was performed. All analytical samples were sorted according to their respective reference values, and one of every four samples entered the validation set. Therefore, the calibration set contained 45 samples, and the prediction set contained 15 samples. Calibration models were established by Partial Least Squares (PLS) regression to obtain the fundamental relation between the NIR spectral data and the reference TVB-N and WHC values. Latent variables (factors) of the PLS models were determined based on a leave-one-out cross-validation method (Pu et al., 2020). The performances and predictive ability of the PLS models were evaluated in terms of the correlation coefficient (R), Root-Mean-Square Error of Prediction (RMSEP) and the Ratio of Prediction to Deviation (RPD).

-Chemical analyses

TVB-N value of shrimp was determined using the Conway microdiffusion method. Nitrogen compounds in shrimp were extracted with trichloroacetic acid (Fisher, England) and then mixed with boric acid salt and a mixed indicator (bromocresol green and methyl red). The resulting solution was incubated at 37 °C for 1 h and titrated with Hydrochloric Acid (HCl) to determine TVB-N.

The WHC values of shrimp were measured with the centrifugal method described by GuÐjónsdóttir et al. (2011). Samples were weighed approximately 5 g and then centrifuged at 6,300×g at 4 °C for 10 min and then the shrimp samples were dried at 105 °C for 24 h using a hot air oven and expressed as %WHC.

Determining phosphate residues using NIR spectroscopy

-Sample preparation

White shrimp of a size of 70-80 shrimp/kg with TVB-N values ranging from 6.0 to 11.0 mg N/100 g, as the standard freshness value of edible shrimp, were used as samples in this experiment. The shrimp samples were divided into two groups by soaking in two kinds of phosphate solutions, which were 2.5% Sodium Tripolyphosphate (STPP) and 2.5% Mixed Phosphate (NAN 101) at 4 °C. The ratio of shrimp in soaking solution was 1:1.2 (w/w). Then, the shrimps were left on the sieve for 2 min to remove the phosphate solution. This preparation was

performed at 2-4 °C. Then, the soaked shrimp were packed in sealed bags and then stored at -18 °C to obtain frozen shrimp for further experiments. The chemical analysis and NIR measurement were performed before freezing and seven days after freezing.

-NIR spectral acquisition

A Matrix-F Fourier transform NIR spectrometer (Bruker Fibre Optics, Ettlingen, Germany) equipped with a 1.0 m fibreoptic diffuse reflectance probe and an extended thermoelectrically-cooled InGaAs (indium gallium arsenide) detector was used. The scattered light is collected and guided via a fibre optic cable to the spectrometer. The NIR spectra of shrimp samples were acquired in rotating reflectance mode at 8 cm^{-1} spectral resolution with a background with 32 scans. A total of 150 g of shrimp sample was filled in a quartz cup [100(Ø)×20(H) mm]. Two types of soaked shrimp, fresh and frozen shrimp, were scanned, and NIR absorption was recorded for data analysis.

-NIR measurement and data analysis

Software and spectral pre-processing methods identical to those in Experiment 1 were used. The total of 135 shrimp soaked in STPP solutions were divided as follows: 90 samples, which represented the calibration set and 45 samples for validation set. Similarly, of the total 99 samples of the shrimp soaked in NAN 101 solution were divided into calibration set (n=66) and validation set (n=33). Reference phosphate residue of shrimp soaked in STPP and NAN 101 were ranged from 1,830 to 5,344 mg/kg and 2,247 to 4,433 mg/kg, respectively.

Phosphate analysis

The phosphate content was determined using a spectrophotometer (Thermo Spectronic Genesys 20, USA) at a wavelength of 823 nm (AOAC, 1995). The shrimp samples were converted into dry ash with zinc oxide (Kemaus, Australia). Then, the samples were mixed with ammonium molybdate (Kemaus, Australia) in ascorbic acid, which reacted with phosphate, producing a molybdenum blue complex that was detected at 823 nm using a spectrophotometer. The phosphate content, expressed as P$_2$O$_5$, was calculated using a standard curve.

Results

Determining TVB-N and WHC using NIR spectroscopy

The reference values of TVB-N for developing PLS calibration ranged from 6.0 to 28.9 mg N/100 g, and the range of the reference values resulted in a prediction set

that was covered by the range from 8.4 to 23.8 mg N/100 g in the calibration set, as shown in Table 1. The accuracy of TVB-N prediction was obtained from spectral data of chopped shrimp, which was superior to those of whole shrimp (R>0.9, RPD>3) as shown in Table 3. Figure 1 (a) illustrates the relation between the measured and NIR spectroscopy-predicted values of TVB-N. A good linear correlation was demonstrated between the reference values and NIR spectroscopy-predicted TVB-N values of chopped shrimp. The WHC of shrimp was determined using NIR spectroscopy, and 60 shrimp with different freshness levels had WHCs ranging from 93.7 to 99.9%. The PLS results for the WHC of whole and chopped shrimp based on NIR spectroscopy are shown in Table 3. Dissatisfied results were obtained from the calibration models of both whole and chopped shrimps, with R<0.50, and RPD<2. Figure 1 (b) illustrates a poor relationship between the actual and NIR spectroscopy-predicted WHC values of chopped shrimp.

Determining phosphate residues using NIR spectroscopy

The total number of shrimps in the STPP and NAN 101 samples in the calibration and prediction sets is summarized in Table 2. The statistical results of the PLS models for the phosphate residues of fresh shrimp and frozen shrimp soaked in STPP and NAN 101 are shown in Table 4. PLS regression models for prediction results of frozen shrimp soaked in STPP had a higher prediction error than those of fresh shrimp soaked in STPP solution. The best PLS results for fresh and frozen shrimp in STPP samples had R values of 0.95 and 0.96, RMSEP of 225 and 219 mg/kg and RPD of 3.14 and 3.44, respectively.

Latent variables (factors) were estimated by an external set of samples to evaluate and validate the model performance. A model with nine factors maximized the R at 0.95, RMSEP at 130 mg/kg and RPD at 3.14 for fresh samples. A model with eight factors was selected for frozen shrimp in NAN 101 with R, RMSEP, and RPD values of 0.96, 165 mg/kg, and 3.39, respectively. The scatter plot of phosphate residues of fresh and frozen shrimp obtained from the reference methods and NIR predictions are shown in Figure 3 (a,b). Significant linearity was observed for predictions of phosphate residues.

For the prediction of the phosphate residues obtained from STPP combined with NAN 101, the reference values used for this PLS calibration ranged from 1,830 to 5,595 mg/kg both fresh and frozen shrimp. The best PLS results of shrimp soaked in STPP combined with NAN 101 were for an R of 0.94 and 0.95, RMSEP of 252 and 295 mg/kg, and RPD of 3.01 and 3.10, respectively, as shown in Table 4. Figure 3 (a,b) illustrate the relationship between the reference phosphate values and the phosphate predicted by NIR regression for the total fresh and frozen shrimp, respectively.

Figure 3 (c) shows the regression coefficients obtained by the PLS models. The spectral regions from 4,250-5,450 cm^{-1} corresponded to phosphate concentrations for shrimp (*Pandalus borealis*) and the absorption band of shrimp at 5,241 and 5,258 cm^{-1} is related to the phosphate functional groups.

Discussion

The raw NIR spectra of the whole and chopped shrimp with different freshness are not shown, and PLS regression was used to select the optimum spectral interval of 9,403-4,242 cm^{-1}, which corresponds to 8,921-8,655 and 9,191-8,924 cm^{-1} in the spectral regions (Cai et al., 2011). Clearly, the overall absorption intensity of the spectra of chopped shrimp was stronger than that of the whole sample because the effects of particle size, distribution, surface texture, and compaction are expressed in a sample's scattering constant (Ely et al., 2008; Pasikatan et al., 2001). The absorbance units of reflectance spectra of chopped shrimp samples were lower than the spectra of whole shrimp samples, this result agreed with to Manley et al. (1994), and Tamburini et al. (2017) which reported that the absorbance value of spectra of ground grain sample is lower than the absorbance value of coarser samples. The absorbance peak at approximately 6,896 cm^{-1} and 5,235 cm^{-1} were clearly observed in the spectral data of whole and chopped shrimp which might be related to first overtone of O-H stretching in water as water is the major composition in shrimp (Simeone et al., 2017). The absorption bands of other shrimp constituents, such as WHC, pH (Brodersen and Bremner, 2001), and TVB-N (Dai et al., 2016), were relatively weak in comparison with the water band and were thus difficult to discern in the spectra.

Normally, the TVB-N contents gradually accumulated at a relatively slow rate as the metabolism of protein was effectively restrained (Ely et al., 2008; Huang et al., 2014) which were comparable to the TVB-N levels specified in the raw shrimp system. Food samples containing the TVB-N content over than 25 mg N/100 g should be rejected for human consumption (Sriket et al., 2007). The PLS model obtained from shopped shrimp showed a high ability to predict the content of TVB-N, as the R, RMSEP, and RPD of 0.94, 1.58 mg N/100 g, and 3.07, respectively. This result demonstrated considerable progress compared with Dai et al. (2016) who reported that unsatisfactory predicting accuracies (R <0.5, RPD<2.0) of TVB-N level predicted from NIR spectra
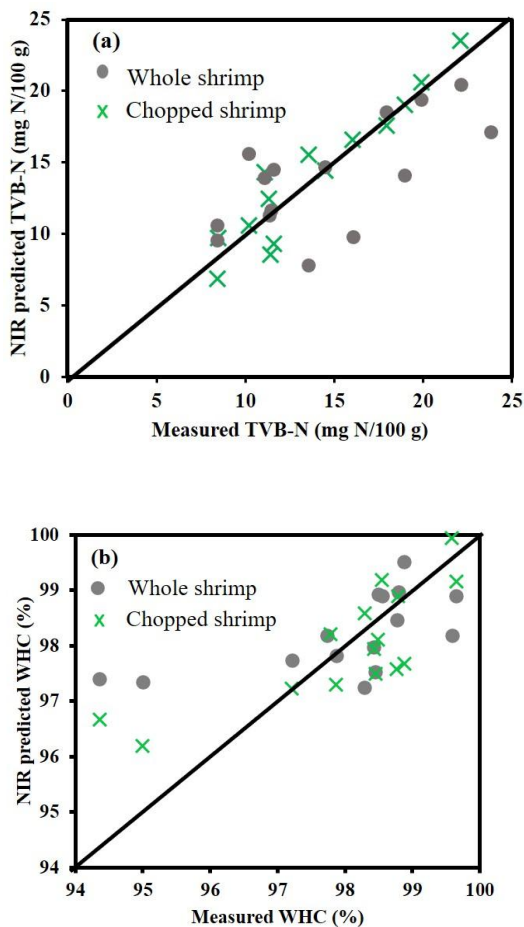
Figure 1: The relation between actual and Near-Infrared (NIR) predicted values of Total Volatile Basic Nitrogen (TVB-N) (a) and Water Holding Capacity (WHC) (b) in validation set of whole shrimps (gray points) and chopped shrimp (green point) samples
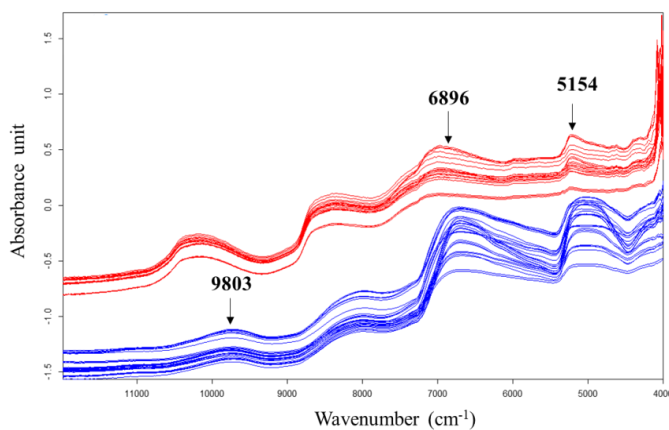

Figure 2: Near-Infrared (NIR) spectral of fresh (red line) and frozen (blue line) soaked shrimps

(a)

(b)

(C)

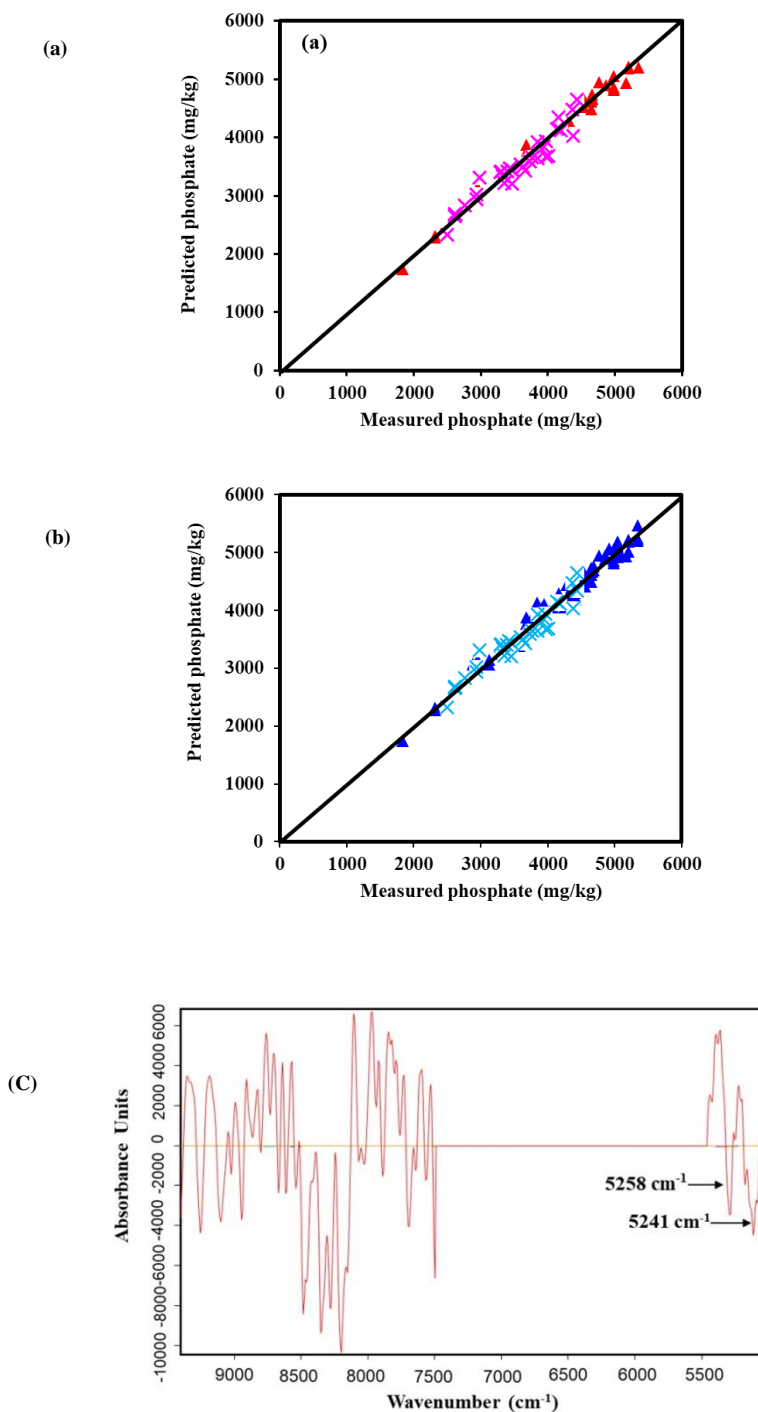**Figure 3:** The relation between actual and Near-Infrared (NIR)-predicted values of phosphate in validation set of totals of fresh soaked shrimps. Sodium Tripolyphosphate (STPP) red triangle and Mixed Phosphate (NAN 101) pink cross (a) and total of frozen soaked shrimps (STPP and NAN 101 light blue cross) (b). The regression coefficient plots of calibration models for phosphate using spectral data of shrimp (c)

Table 1: Characteristic of calibration and validation samples used for establishment of calibration model for TVB-N and WHC

Parameters	*Calibration set*					*Validation set*				
	n	Mean	Min	Max	SD	n	Mean	Min	Max	SD
TVB-N (mg N/100 g)	45	13.6	6.0	28.9	6.4	15	14.6	8.4	23.8	4.9
WHC (%)	45	97.8	93.7	99.9	1.4	15	98.0	94.4	99.6	1.5

Min=Minimum value of data; Max=Maximum value of data; SD=Standard Deviation of data; TVB-N=Total Volatile Basic Nitrogen; WHC=Water Holding Capacity.

Table 2: Characteristic of calibration and validation samples used for establishment of calibration model for phosphate residues in shrimp

Parameters	Calibration set					Validation set				
	n	Mean	Min	Max	SD	n	Mean	Min	Max	SD
Phosphate residue (mg/kg) STPP	90	4,165	1,830	5,344	794	45	4,199	1,830	5,205	827
Phosphate residue (mg/kg) NAN 101	66	3,520	2,247	4,433	705	33	3,532	2,499	4,433	509

Min=Minimum value of data; Max=Maximum value of data; SD=Standard Deviation of data; STPP=Sodium Tripolyphosphate; NAN 101=Mixed Phosphate.

Table 3: The prediction results of TVB-N content and WHC in whole shrimps and chopped shrimps with PLS model

Parameters	Type	Factor	R	RMSEC*	RMSEP*	RPD	Region (cm^{-1})
TVB-N (mg N/100 g)	Whole shrimp	4	0.66	2.14	3.61	1.34	9,403-4,242 4,605-6,096
	Chopped shrimp	6	0.94	2.21	1.58	3.07	9,403-6,094 4,605-4,242
WHC (%)	Whole shrimp	1	0.22	1.27	1.37	1.08	4,605-4,242
	Chopped shrimp	3	0.33	0.92	1.18	1.61	9,403-6,094 5,453-4,242

Remark * Units; R=Correlation Coefficient in validation; RMSEC=Root Mean Square Error of Calibration; RMSEP=Root Mean Square Error of Prediction; RPD=Residual Predictive Deviation (RPD=SD/RMSEP); TVB-N=Total Volatile Basic Nitrogen; WHC=Water Holding Capacity.

Table 4: The prediction results of phosphate content in raw and frozen shrimps with Partial Least Square (PLS) model

Sample		Factor	R	RMSEC*	RMSEP*	RPD	Region (cm^{-1})
Fresh shrimp	Phosphate residue (mg/kg) STPP	7	0.95	210	225	3.14	9,403-6,094 5,454-4,597
	Phosphate residue (mg/kg) NAN 101	9	0.95	123	130	3.14	6,102-4,242
	Phosphate residue (mg/kg) STPP+NAN 101	7	0.94	251	252	3.01	9,403-6,094 5,454-4,597
Frozen shrimp	Phosphate residue (mg/kg) STPP	9	0.96	150	219	3.44	9,403-7,498 6,102-5,446
	Phosphate residue (mg/kg) NAN 101	8	0.96	156	165	3.39	9,403-7,498 4,605-4,420
	Phosphate residue (mg/kg) STPP+NAN 101	10	0.95	246	295	3.10	9,403-7,498 6,102-5,446

Remark * Units; R=Correlation Coefficient in validation; RMSEC=Root Mean Square Error of Calibration; RMSEP=Root Mean Square Error of Prediction; RPD=Residual Predictive Deviation (RPD=SD/RMSEP); STPP=Sodium Tripolyphosphate; NAN 101=Mixed Phosphate.

of whole prawns during cold storage. This might be because of a higher light scattering within the layers of the whole shrimp than the chopped shrimp, resulting in more interference (Tamburini et al., 2017).

The PLS regression model of WHC in whole and chopped shrimps with NIR spectroscopy are shown in Table 2. Dissatisfactory results were obtained from the calibration models of both whole and chopped shrimp with the low R and RPD values. These results were similar to Huff-Lonergan et al. (2002) and Prevolnik et al. (2010) that reported a low R between NIR absorbance data and reference methods (R <0.50). This low prediction accuracy of WHC might come from a poor precision of the reference method (Prevolnik et al., 2010) and it might be due to the association with the other constituents such as liquid content (Huff-Lonergan et al., 2002).

The complexity of the TVB-N structures, characterized by the presence of many strong peaks, may be due to the changes in metabolite sources of microorganisms (Huang et al., 2014). The region from 5,050-4,830 cm^{-1} was dominated by N-H absorption bands of proteins (Don et al., 2018), which may be related to the high protein source of TVB-N. N-H bonds at 5,897 and 5,870 cm^{-1} were clearly found, which suggested the presence of TVB-N (Osborne, 2006). Furthermore, the protein absorption peak was considered to be centred at approximately 4,545-4,655 cm^{-1} (Manley, 2014).

For the detection of phosphate residues, NIR spectra of fresh and frozen shrimp with various levels of phosphate concentrations were collected using NIR spectroscopy in the reflectance mode and are shown in Figure 2. We found all the prominent spectral features that were expected for fresh and frozen shrimp in the covered wavelength region. Strong absorption bands of water were noticed at 5,154 cm^{-1} and 6,896 cm^{-1} from both fresh and frozen shrimp spectra. Our results were consistent with those of Büning-Pfaue (2003) and Cruz-Tirado et al. (2022) that reported the two dominant and broad peaks of water near 5,154 and 6,896 cm^{-1}. Interestingly, the dominant absorption band at 9,803 cm^{-1} was observed only from the frozen shrimp spectra. This result was in accordance with the spectra of frozen salmon that clarified the band at 9,803 cm^{-1} as O-H stretching band of ice (Ottestad et al., 2009; Wu and Sun, 2013).

The models of phosphate residues of fresh and frozen shrimps were quite robust and accurate for predicting new samples. The validation sets presented excellent correlation (R>0.94, RPD>3.0) between phosphate reference values and NIR predicted phosphate values. These results were consistent with GuÐjónsdóttir et al. (2011), that reported a satisfied calibration model with a high value of R and RPD values (R>0.90 and RPD>2.4). With regard to the RPD values, the RPD ratio relates the SEP to variance in the original reference data, considering that

RPD should ideally be at least 2.4 (Kennard and Stone, 1969). All models in Table 4 presented RPDs above 3 that suggest the possibility of NIR for screening of phosphate content in shrimp. However, it should be underscored that the accuracy of models depended on its application and the errors of prediction (RMSEP) (Simeone et al., 2017). NIR models may be of great help to evaluate phosphate residues in frozen shrimp before expertise from industry so that the product is in accordance with the Ministry of Health.

For the regression coefficients corresponding to phosphate, these wavenumbers could be ascribed to a combination of C-H stretching and O-H stretching of water and P-OH which is consistence to Todd (2011) who report that the absorbance at 5,241 and 5,258 cm^{-1} is related to the P-OH functional groups.

Conclusion

This study showed that NIR spectroscopy could predict the TVB-N content in shrimp. The performance of NIR spectroscopy as a non-destructive measurement can support its use as a rapid rough screening tool. However, NIR spectroscopy had low feasibility for the determination of WHC values. In this study, NIR spectroscopy was effectively used to monitor the phosphate content of fresh and frozen shrimp after soaking in STPP and NAN 101 solutions. This study provided valuable information and theoretical foundations for the industrial application of NIR spectroscopy techniques to monitor the freshness quality and phosphate residues of shrimp products in the industry. The proposed technique can save time, reduce the analysis steps and be suitable for many samples at the industrial scale. This technique can be used to assist in production planning and control product quality to confirm consumer safety and ensure the timely delivery of growing market demand.

Author contributions

C.S. did the conceptualization, methodology, software, formal analysis, data curation, and writing original draft**;** S.S. did the methodology and formal analysis; S.N., S.D., and T.K. did the conceptualization, methodology, writing review and editing; S.R. did the conceptualization, methodology, software, writing, and editing, data curation, supervision. All authors read and approved the final manuscript.

Conflicts of interest

There is no conflict of interest regarding the present research.

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