

Development of prediction models for pH and total acidity of *pla-a-som* using reflectance near infrared spectroscopy

Juthamat Ratha *

Amporn Sae-Eaw **

Sirinda Yunchalard ***

Abstract

For the development of pH and total acidity (TA) prediction models, *pla-a-som* samples (N=188) were randomly drawn from production processes with a wide range of fermentation times and recipes. The samples were divided into 2 groups for calibration (N=141) and validation (N=47). The prediction models of both pH and TA values were developed using partial least squares (PLS) regression with Savitzky-Golay and N-point smoothing pretreatment methods. The best calibration models for pH and TA had R^2 (0.89, 0.72), SEC (0.16, 0.50), and R^2_{max} (1.0), respectively. Hence, both pH and TA calibration models gave a reasonable fit and were able to estimate pH and TA values in the validation step. The prediction capability of the pH model showed a good correlation ($R^2=0.89$) with a standard error of prediction (SEP) and bias of 0.19 and 0.30, respectively, while the prediction capability for the TA model showed only a moderate correlation ($R^2=0.72$), SEP (0.50), and bias (0.32), respectively. Although the TA model displayed a lower correlation value for prediction, pH and TA models were still able to give reasonable predictions for all tested *pla-a-som* samples. Thus the two models developed in this study have revealed potential applications for large scale *pla-a-som* manufacture since they can predict two important acid parameters that directly correlate with sourness of the product.

Keywords: Near infrared spectroscopy (NIRS), *pla-a-som*, acid parameter, prediction model

* Graduate School, Khon Kaen University, Khon Kaen 40002, Thailand

** Department of Food Technology, Faculty of Technology, Khon Kaen University, Khon Kaen 40002, Thailand

*** Department of Biotechnology, Faculty of Technology, Khon Kaen University, Khon Kaen 40002, Thailand

Corresponding author: sirinda@kku.ac.th

Introduction

Plaa-som, a traditional home style acid fermented fish product of Thailand, is expected to have a pronounced sour taste that stands out among other tastes. The sour taste of *plaa-som* is considered a natural development from acid fermentation and its distinguishing characteristic (Valyasevi & Rolle, 2002). Both pH and total acidity (TA) are therefore two fundamental acid parameters which should be recognized and monitored for the product's flavor specification. The sour taste of this fermented food results from organic acids, mainly lactic and acetic acids, released during lactic acid fermentation by predominant lactic acid bacteria (LAB) at an ambient temperature not exceeding 35°C for 3-5 days (Phithakpol *et al.*, 1995; Kopermsub & Yunchalard, 2010). The increasing organic acid content detected in fermentation samples directly related with a decrease in pH and an increase in TA levels. Both pH and TA therefore serve as key acid indicating parameters that can contribute to the specification of the finished *plaa-som* product.

TA and pH of *plaa-som* serve as indicators of the success or failure of the *plaa-som* fermentation (Paludan-Müller *et al.*, 1999, Kopermsub & Yunchalard, 2008). In addition to indicating acid properties of *plaa-som*, these parameters also directly relate to the level of sourness given by sensory evaluations due to the relationship between total acidity and pH. These acid properties can be quantified and determined using standard physicochemical analytical methods. However, these methods are time consuming and laborious procedure. As a consequence, where there is a limitation on sample numbers and sizes, especially in cases where the sample is to be kept and maintained after analysis, near infrared spectroscopic (NIRS) is an advantageous technique to use. When using NIRS, one sample can be used to simultaneously determine the values of many parameters since it is non-destructive to the sample, it has relatively high accuracy, and it is also a rapid analytical technique (Prieto *et al.*, 2009; Galvez-Sola *et al.*, 2010; Xie *et al.*, 2011; Liu *et al.*, 2015). NIRS is recognized as a non-destructive technique since it is not required to have sample treated before analysis. As a result, the sample is not destroyed by various chemical reactions during any standard physicochemical analytical technique. Moreover, it can be used to monitor not only the final stage of the product but also the various stages of the production process. Additionally, NIRS requires relatively little or no sample pretreatment and no sample preparation prior to analysis, therefore it is also considered a fast and environmentally friendly technique since it involves no or relatively less chemical (Misal, 2016). NIRS uses light of wavelengths in the near infrared region between 700 and 2500 nm. This light generates signals indicating vibrational energies of organic bonds in the sample (Reich, 2005). The emitted spectral signals are translated into absorption

spectra within the NIR spectrophotometer. The radiation in the NIR region is absorbed by various related functional groups that contain a bond with one hydrogen atom which are commonly found in all organic compounds, for instance, carbon-hydrogen (C-H), nitrogen-hydrogen (N-H), and oxygen-hydrogen (O-H) bonds. When such a bond absorbs light in the near infrared region, it vibrates in stretching and bending vibration patterns (Ozaki *et al.*, 2007). These absorbance and consequent vibrations between organic bonds in the sample cause overtones and/or combinations of other types of responses. An overtone occurs when vibration appears while the molecule is excited from its background at levels up to 2, 3, or higher, resulting in wider based spectra often found in wavelengths between 800 to 1800 nm. Combinations occur when vibration of the organic bonds happen in various patterns at the same time and are found at wavelengths between 1800 to 2500 nm. Spectral pretreatment is commonly used to reduce or to eliminate spectral fluctuations or to standardize their impacts on the spectra (Cen & He, 2007; Ntsame Affane *et al.*, 2011). Particularly, Savitzky-Golay and N-point smoothing methods are two popular mathematical pretreatment methods used to remove undesirable effects from either noise or baseline shift and to enhance peak separation by removal of scattering effects due to non-homogenous distribution in the spectra (Pi *et al.*, 2009).

NIRS can be used for organic compound identification and quantification of some properties of fermented food or organic products, at various production stages or in the final product (Reich, 2005; Xie *et al.*, 2010). NIRS has been applied in a number of areas including food industry in which NIRS can be used to monitor and to create product fingerprints (Collell *et al.*, 2010; Melfsen *et al.*, 2012; Ji-yong *et al.*, 2013). However, scant information is available for fermented foods, particularly fermented fish products such as *pla-a-som*. The aim of this study is to evaluate the feasibility of using near infrared reflectance spectroscopy to develop prediction models for both pH and TA. Since these two acid parameters directly relate to the sourness levels obtained from sensory evaluations of *pla-a-som* makes this study offered potential applications for large scale *pla-a-som* production process.

Materials and Methods

1. *Pla-a-som* samples

A total of 188 randomly selected *pla-a-som* samples were sourced from local markets available from several provinces of Thailand with different recipes at various fermentation times. Although all *pla-a-som* samples were produced using different recipes, they were all prepared to fit the specification in accordance with the Thai industrial standard for *pla-a-som* (26/2548) (TISI, 2005) by mixing cleaned, scaled, and gutted whole silver barb fish

(*Barbomyrus gonionotus*) with either boiled white rice or steamed sticky rice, freshly crushed garlic bulbs, and salt. The samples were kept at -20 °C until analyzed.

2. Sample preparation

Thirty grams of each *pla-a-som* sample was homogenized for 5 minutes using a homogenizer (Moulinex Multi Moulinette, Moulinex® Tefal® KRUPS®, France) at the highest speed for 3 minutes. Approximately 10 grams of homogenized sample was made into a slab of 1 cm thickness using a 55 mm glass petri dish with a lid covered by a piece of aluminum foil according to Tsuchikawa (2007) (Figure 1). Duplicate samples were made. Subsequently, these prepared homogenized samples were subjected to NIR spectroscopy.



Figure 1. A glass dish containing 1 cm thick homogenized *pla-a-som* sample with a lid covered by a piece of aluminum foil.

3. NIR Spectroscopy

The spectral data for *pla-a-som* samples were obtained using a NIR spectrometer (XDS Masterlab Tablet Analyser, FOSS, USA) in reflectance mode at full wavelength setting ranging from 400-2500 nm. Scanning was performed twice per sample. Each spectrum used an average of 32 scans at a wavelength spectral resolution of 2 nm. All spectra were recorded at a constant temperature (25±1°C). The spectral data were expressed as absorbance values for wavelengths in the near infrared region ranging from 1100 to 2500 nm. This was done to develop calibration and validation equations. All spectra were analyzed using VISION® software Version 4.0.1 (Metrohm AG, Herisau, Switzerland).

4. Physicochemical analyses for pH and TA

Homogenized *pla-a-som* samples used for NIR scanning were then analyzed for pH and TA by standard AOAC methods (AOAC, 2005). Samples were analyzed in duplicate. Five grams of *pla-a-som* sample was placed in 40 ml of fresh carbon dioxide free distilled water and homogenized in a stomacher (Masticator Basic, IUL S.A., Barcelona, Spain) three cycles (1500 rpm for 90 sec/cycle). Filtrate was obtained by passing the homogenized sample through a filter paper (WhatmanTM No.1) and was used for both pH measurement and TA determination. The pH was measured using a pH meter (UB-5, Denver Instruments, NY, USA). The total acidity was determined using standard titration against 0.1 N NaOH and calculated as a lactic acid equivalent expressed as % total acidity (w/w).

5. Spectral data processing

Model development was divided into two parts; calibration and validation. In this study, the NIR spectra were in the wavelength range of 1100-2500 nm. Using the default values of the VISION[®] program, 141 samples (75%) were randomly selected to check their correlation with reference data obtained from standard physicochemical analyses. After repeated spectral scanning, the precision of agreement between the near infrared spectra and reference data were determined. Standard deviation (SD) of the differences between duplicates reflected the repeatability of reference data obtained from the 141 samples. The maximum coefficient of determination (R^2_{max}) was calculated according to Dardenne (2009).

6. Near infrared spectroscopy analysis

Spectral pretreatment was performed using VISION[®] version 4.0.1 software. In this study, regression models were generated by partial least squares (PLS) with mathematical pretreatment using Savitzky-Golay and N-point smoothing methods. The PLS regression method used the whole range of spectral data (wavelengths of 1100-2500 nm) and calibration sets of pH and TA values. The optimum model in this study was selected based on the model's correlation coefficient, coefficient of determination (R^2), standard error of calibration (SEC), and standard error of cross validation (SECV). The most predictive model was obtained by performing a cross-validation based on the correlation and the standard error of prediction (SEP) that related the SECV and residual prediction deviation (RPD) from validation samples and bias. The RPD usually shows the ratio between the standard error of the reference data set and the SEP.

Results and Discussion

A composite of the NIR spectra of 188 available *pla-a-som* samples is shown in Figure 2. The NIR region contained many bands due to some overtones and combination modes that overlapped among the samples. NIR radiation is predominantly absorbed by C-H, O-H, and N-H bonds that existed in the primary constituents of a sample under analysis. Additionally, each of these functional groups has its own specific vibration pattern at each wavelength, which is indicated by its absorbance. The homogenized sample used in this study changed the signal intensity due to reflectance, scattering, and absorption of the sample (Pasquini, 2013).

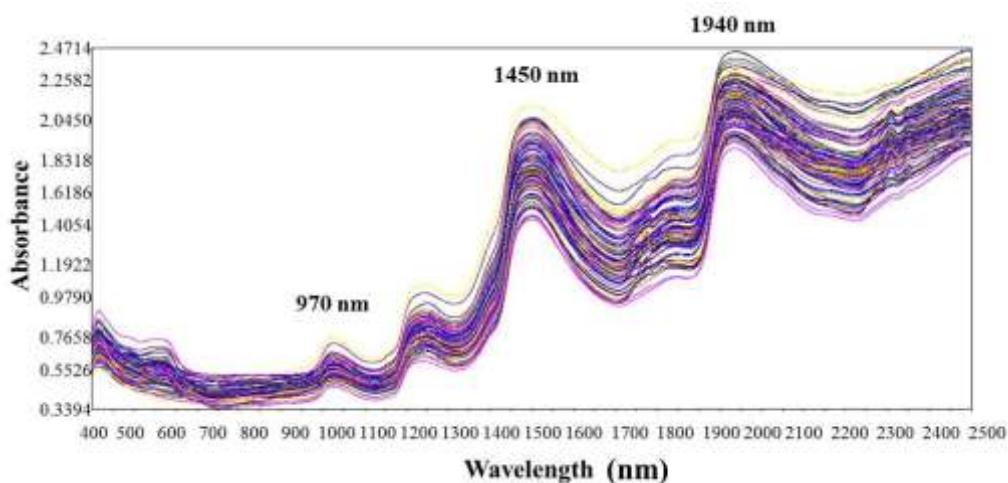


Figure 2. NIR reflectance mode spectra from 400 to 2500 nm for *pla-a-som* samples.

Figure 2 shows the absorbance peaks associated with the primary chemical components of *pla-a-som*. For example, the bands at 970, 1450, and 1940 nm are due to moisture contained in each sample. According to Büning-Pfaue (2003), strong NIR water absorption bands close to 1400-1440 nm and 1900 to 1950 nm have often been used for quantification analysis of the moisture content in samples. These bands relate to OH bonds, with an OH second overtone and OH stretch first overtone, and combination overtones at these wavelengths, respectively (Fernández-Cabanás *et al.*, 2011; Zhao *et al.*, 2015). Correlation bands at 1150-1250 and 1730-1760 nm are due to lactic acid (Sivakesava *et al.*, 2001).

The raw NIR spectra of all homogenized *pla-a-som* samples obtained from reflectance NIR included some noises in addition to sample information. Therefore, the spectral data was preprocessed to remove or to reduce these undesirable variations before developing models and calibration. To further ensure better correlation between the spectral and reference data, the spectral mathematical pretreatment methods of Savitzky-Golay and N-point smoothing were applied. PLS regressions with these commonly used mathematical

pretreatments were conducted to produce spectra of the sample. Savitzky-Golay is a well-known smoothing method thru derivative calculation which relies on the least squares fit of a polynomial to spectral segments based on a data smoothing algorithm. N-point smoothing is another method for spectral smoothing based on spectral values placed in the middle of the spectral segment (Wang & Zhou, 2011).

PLS regression is a conventional method used to develop prediction models for any food product, and to eliminate spectral fluctuation. This generally results in a good correlation obtained between absorbance and reference data. PLS regression with full cross validation was performed on the spectra at 1100-2500 nm to analyze pH and TA values of the *pla-a-som* samples. The wavelength region used was a default setting of the VISION® software. This software was used to select samples during the development of the models for both calibration and validation. The performance of the final model was evaluated according to values of three parameters; R^2 , SEP, and SECV. Statistical results obtained from descriptive analysis (maximum, minimum, mean, range, and standard deviation) of pH and TA in *pla-a-som* samples used in this study from calibration (141 samples) and validation (47 samples) sets are summarized in Table 1. The pH of *pla-a-som* samples used in this study was determined according to the Thai Industrial Standard for *pla-a-som* (26/2548) (TISI, 2005). Samples ranged from pH 4.0 to 6.0. TA of *pla-a-som* samples from both sets ranged from 0.84 to 5.09 % (w/w). The correlation coefficient between pH and TA was -0.73 indicating a strongly negative correlation. The pH was found to have linearly decreased as TA increased. Repeatability (R^2_{max}) of pH values was 1, indicating that the reference test was accurate, and the model was reasonable.

Table 1. Statistical data for acidity values in calibration and validation sets used in model development.

Constituents	Data set	No.	Min.	Max.	Mean	Range	SD
pH (unit)	Total sample	188	4.05	6.08	4.76	2.03	0.45
	Calibration set	141	4.05	6.08	4.69	2.03	0.49
	Validation set	47	4.20	5.92	4.56	1.72	0.22
TA (% w/w)	Total sample	188	0.84	5.09	2.53	2.56	0.98
	Calibration set	141	0.84	5.09	2.50	2.56	0.97
	Validation set	47	1.12	4.94	2.24	3.82	1.01

Note: No. = Number of samples

Min. = Minimum value of data

Max. = Maximum value of data

SD = Standard deviation of data

From the various pretreatment methods used with the pH and TA evaluations, the best calibration model was obtained using the mathematical pretreatment methods of Savitzky-Golay and N-point smoothing (Ntsame Affane *et al.*, 2011; Ritthiruangdej *et al.*, 2011; Porep *et al.*, 2015). The R^2_{Cal} , the coefficient of determination of calibration is used to determine the efficiency of the calibration model, the higher value of R^2_{Cal} indicates better calibration model. The R^2_{Pred} is the value obtained from the prediction model developed from calibration model and has been used to verify the prediction accuracy. For the best pH calibration model, R^2_{Cal} and SEC were found to be 0.89 and 0.16, respectively (Table 2 and Figure 3A). The best TA calibration model yielded R^2_{Cal} and SEC values of 0.72 and 0.50, respectively (Table 2 and Figure 3B).

Table 2. Calibration and cross-validation results for pH and TA in *plaa-som* using PLS with Savitzky-Golay and N-point smoothing pretreatment methods.

Parameters	R^2_{Cal}	SEC	R^2_{Pred}	SECV	SEP	Bias	RPD
pH	0.89	0.16	0.93	0.31	0.19	0.30	2.37
TA	0.72	0.50	0.89	0.69	0.50	0.32	1.96

Note: R^2_{Cal} = The coefficient of determination of calibration

R^2_{Pred} = The coefficient of determination of prediction

SEC = The standard error of calibration

SECV = The standard error of cross-validation

SEP = The standard error of prediction

RPD = The residual prediction deviation

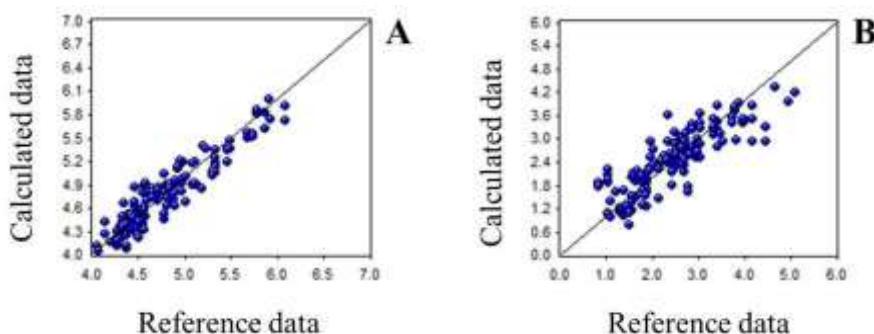


Figure 3. Calibration plots of calculated and reference data for *plaa-som* samples. A: pH model and B: TA model.

The R^2_{Cal} values of the pH (0.89) and TA (0.72) prediction models were higher than 0.70, indicating good capability in predicting both pH and TA values (Fernández-Cabanás *et al.*, 2011). According to Gholizadeh *et al.* (2015), the R^2_{Cal} value of the pH model was very good while the R^2_{Cal} value of the TA model was moderately good. The R^2_{pred} value attained from pH and TA prediction models were 0.93 and 0.89, respectively indicates the relatively good accuracy of these two models since they were reasonable high in their values. The values of R^2_{Pred} , SEP, SECV, and bias for the pH model were 0.93, 0.19, 0.31, and 0.30; respectively, while for the TA model they were 0.89, 0.50, 0.69, and 0.32, respectively, as collectively listed in Table 2. The RPD values for the pH and TA models were 2.37 and 1.96, respectively. These values indicate the validation precision of the calibration model. RPD value of about 3 indicates good model for screening, value in the range of 3-4 indicate good model for quality control, and value of 4 or more indicate excellent model (Malley *et al.*, 2005). The pH calibration model constructed in this study was therefore considered to be moderately successful with an RPD of 2.37. However, the TA calibration model, with its lower RPD at 1.96, was considered only moderately useful. Therefore, the TA model could only be expected to make approximately predictions. Even though the pH and TA models were found to be less than ideally accurate, according to their RPD values and other statistical data like SEP, SECV, and bias values nonetheless indicated that they were acceptable as they were in agreement with those of Ritthiruangdej *et al.* (2011). They reported that models for ash composition in Thai steamed pork sausages had low RPD value (RPD<3.0), low errors of prediction (SEP) value (0.15), and high R^2_{Pred} value (0.96). The high R^2_{Pred} and the low SEP values indicated that the models were good to predict pH and TA values (Williams & Sobering, 1996). Shenk and Westerhaus (1996) stated that a prediction model with an R^2_{Pred} of more than 0.9 has excellent precision, while the R^2_{Pred} between 0.5 and 0.9 reflect good precision. The SEP value of 0.5 (Table 2) was lower than the SD value of 0.9 obtained from the TA model in the current study, (Table 1) indicating that the TA model was not as good as the pH model. A validation plot between calculated and reference data for pH and TA in *plaa-som* samples is shown in Figure 4., where good accuracy can be observed with the high R^2_{Pred} values of pH and TA at 0.93 and 0.89, respectively. The R^2_{Pred} values of both pH and TA models indicate that more than 90% of the analysis of variance is explained by each of the prediction models shown in Figure 4.

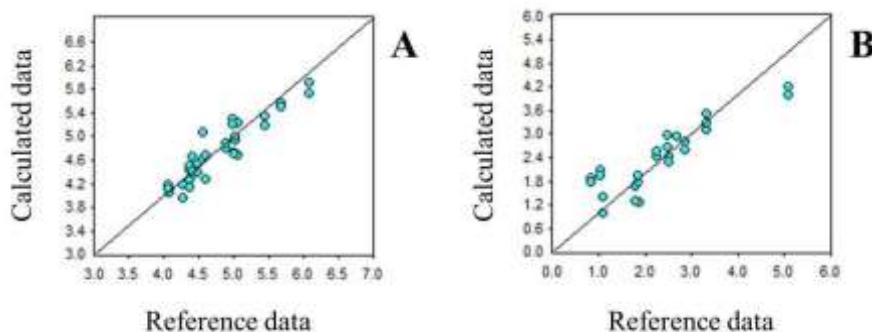


Figure 4. Validation plots of calculated and reference data for *plaa-som* samples. A: pH model and B: TA model.

These two models were well validated, since the SEP of 0.19 for the pH model is considered low when compared with a SEP of 0.5 obtained from the TA model. SEP represents how well the prediction model predicts values of the validation sample set, with low SEP values being desirable (Porep *et al.*, 2015). A good validation was obtained from the pH model with R^2_{Pred} (0.93), and SECV (0.31). The TA validation model was not as good, even though the R^2_{Pred} (0.89) and SECV (0.69) values were high as illustrated in Table 2 and Figure 4. SECV is an index that indicates how well the prediction model can predict the reference sample set when the samples are not used in the calibration step. The SECV is the value used to signify the error gotten from cross-validation. The lower in SECV value is being considered a better validation because it will indicate more errors in the value attained from the standard analytical technique (Fernández-Cabanás *et al.*, 2011; Escuredo *et al.*, 2013; Porep *et al.*, 2015). Hence, the desired SECV value is expected to be low in order to validate the accuracy of prediction model. Problems with the TA validation model may have been due to inaccuracies arising from the standard analytical method used to determine TA together with its susceptibility to human error, particularly in the titration step, as reflected by the relatively high SECV value. The pH is simply measured using a pH meter, thereby minimizing human error.

The two models are suitable to predict the two acid parameters, pH and TA in the samples. In order to test the validation models, 47 *plaa-som* samples were used to predict pH and TA values. The capability of the PLS model to predict the pH values obtained was shown to be very good with its high correlation ($R^2_{\text{Pred}}=0.93$, $\text{SEP}=0.19$, and $\text{bias}=0.30$), while the capability of the PLS model to predict the TA values obtained was shown to be only good with its moderate correlation ($R^2_{\text{Pred}}=0.89$, $\text{SEP}=0.50$, and $\text{bias}=0.32$). The best calibration and validation for both pH and TA models were found to yield low RPD values. The current study

presents a first in its field to model pH and TA values in *plaa-som* samples. However, further study is needed to improve the calibration of the TA model. For instance, using High Performance Liquid Chromatography, HPLC, to analyze the acidity offers more accuracy than the titration technique. However, in the practical aspect, the titration technique is more popular as it is a simpler and faster technique.

Conclusions

The results of this study verify that NIR spectroscopy offers an alternative analytical method to predict acid parameters in fermented foods, particularly *plaa-som*. The best calibration and validation models obtained for pH and TA values yielded robust predictions. However, further work is still needed to improve calibration models to provide better regression values. It is noted that these two models cannot be used to determine pH and TA of *plaa-som* sample. Finally, this is the first study to provide predictions for both pH and TA in *plaa-som* based on their acid profiles determined using NIRS requiring minimal sample preparation and avoiding sample destruction. Moreover, the rapid and accurate analysis using NIRS, especially for quantification, could make it the technique of choice. The developed NIRS model might also be used to predict shelf-life and quality of *plaa-som*, such as the sourness.

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