

การทำนายค่าปริมาณกรดทั้งหมดและค่าความเป็นกรด-เบสในส้มฟัก โดยใช้  
เทคนิคสเปกโทรสโกปีอินฟราเรดย่านใกล้แบบไม่ทำลาย

Prediction of Total Acidity and pH of Som-fug Using a Non-  
Destructive Near Infrared Spectroscopy

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## บทคัดย่อ

ส้มฟักเป็นอาหารหมักจากเนื้อสัตว์ที่ได้รับความนิยมในประเทศไทยโดยเฉพาะในภาคอีสาน มีวิธีการทำ  
โดยใช้เนื้อสัตว์บด เช่น เนื้อปลาหรือเนื้อหมูบดผสมกับเกลือ กระเทียมและข้าวสุก หมักทิ้งไว้ 2 - 3 วัน  
ที่อุณหภูมิห้อง (30 องศาเซลเซียส) ระหว่างการหมักปริมาณกรดจะถูกสร้างขึ้นทำให้เกิดรสเปรี้ยว  
ซึ่งเป็นที่ต้องการของผู้บริโภค แต่เนื่องจากกระบวนการหมักตามธรรมชาติขึ้นอยู่กับอุณหภูมิและการเจริญ  
เติบโตของแบคทีเรีย จึงเป็นการยากที่จะกำหนดเวลาการหมักที่มีปริมาณกรด (total acidity) และ  
ค่ากรด-เบส (pH) ในผลิตภัณฑ์ให้ตรงกับความต้องการของผู้บริโภค เทคนิคสเปกโทรสโกปีอินฟราเรด  
ย่านใกล้ (Near-infrared spectroscopy, NIR) เป็นวิธีที่มีประสิทธิภาพในการตรวจสอบปริมาณ  
สารเคมีในอาหารแบบไม่ทำลาย (a non-destructive analysis) โดยอาศัยคุณสมบัติการดูดกลืนแสง  
และการสั่นสะเทือนของโครงสร้างเคมีที่แตกต่างกัน การทดลองนี้ได้ศึกษาความเป็นไปได้ในการนำเทคนิค  
สเปกโทรสโกปีอินฟราเรดย่านใกล้มาใช้ทำนายปริมาณกรดทั้งหมดและค่า pH ในส้มฟัก 123 ตัวอย่าง  
ที่รวบรวมจากจังหวัดสกลนคร อุดรธานี และขอนแก่น และนำมาตัดให้มีขนาดกว้าง × ยาว × หนา  
เท่ากับ 1 × 1 × 1 เซนติเมตร วัดสเปกตรัมแถบการดูดกลืนแสงรังสีย่านใกล้ที่ 1000 - 2500 นาโนเมตร  
แล้วนำตัวอย่างไปวัดปริมาณกรดทั้งหมดและค่า pH โดยวิธีวิเคราะห์มาตรฐาน AOAC (2000) และ

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<sup>2</sup> คณะวิทยาศาสตร์และศิลปศาสตร์ มหาวิทยาลัยเทคโนโลยีราชมงคลอีสาน นครราชสีมา

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พีเอชมิเตอร์ (pH meter) จากนั้นนำค่าการดูดกลืนรังสีและค่าทางเคมีที่ได้มาสร้างสมการทางคณิตศาสตร์โดยใช้โปรแกรม Unscrambler ปรับแต่งสเปกตรัมรังสีโดยการปรับความแปรปรวนให้เป็นมาตรฐาน (standard normal variation) และการแปลงค่าเป็นอนุพันธ์อันดับสองในการสร้างสมการทางคณิตศาสตร์ ผลการทดลองพบว่าสมการที่ได้สามารถทำนายผลของปริมาณกรดทั้งหมดในส้มผักได้ค่อนข้างแม่นยำ โดยมีค่าสัมประสิทธิ์ของการตัดสินใจ ( $R^2$ ) เท่ากับ 0.72 ค่าความคลาดเคลื่อนมาตรฐานในการทำนายค่า (Standard error of cross-validation; SECV) ที่ 0.07% และมีค่าความเอนเอียง (bias) ที่ 0.005% แต่ไม่สามารถใช้ในการทำนายค่า pH ในส้มผักได้นัก

คำสำคัญ: ส้มผัก; รังสีอินฟราเรดย่านใกล้; ปริมาณกรดทั้งหมด; ความเป็นกรด-เบส

## Abstract

*Som-fug* is a famous fermented food product in Thailand particularly in the Northeastern region. To prepare *som-fug*, freshly minced meats were used as materials such as minced fish and minced pork, mixing with a small amount of salt, garlic and steamed rice. After incubation at room temperature (30°C for 2-3 days), total acidity has been accumulated resulting in a sour taste related with consumer's preference. Due to the variation of fermentation temperature resulted in various growth conditions for bacteria, it is difficult to exactly determine total acidity levels and pH for the acceptable consumption. A non-destructive analysis, near infrared spectroscopy (NIR) was used to predict total acidity and pH in this study. One hundred twenty three samples of *som-fug* products were purchased from Sakon Nakhon, Udon Thani, and Khon Kaen provinces. The samples were kept in the ice box (4°C) during transportation to laboratory. The wavelength (1000-2500 nm) of reflectance mode was measured with 32 scans averaging. The total acidity and pH were then rapidly analyzed with a standard method. The obtained NIR spectra and chemical data were calculated by Unscrambler software. The spectra treatments (standard normal variation and second derivative) were employed. The results showed that NIR had high accuracy to predict total acidity with high determination coefficient ( $R^2$ , 0.72), low standard error of cross-validation (SECV, 0.07%) and low bias (0.005%). However, NIR could not provide a good model to predict pH in *som-fug*.

Keywords : *Som-fug*, NIR, Total acidity, pH

## Introduction

*Som-fug* is a famous fermented product from Thailand particularly in the Northeastern region. The materials, minced meat (100g), salt (2.5-5g), minced garlic (4 g) and steamed rice (2-15 g), were crushed, mixed together and kept in plastic bags for 2-5 days at 30°C for acid fermentation (Saisithi et al., 1986). Normally, fish meat is the popular material to make som-fug (Riebroy et al., 2008). Other fermented meat materials such as pork (Valyasevi et al., 2001) and beef (Hammes and Knauf, 1994) also are available in the market. By dominant growth of lactic acid bacteria (LAB) such as *Lactobacillus plantarum*, *Lactobacillus brevis*, *Lactobacillus fermentum*, and *Pediococcus pentosaceus* (Saisithi et al., 1986), promote an increasing amount of acid and pH drop. At low pH condition, it could inhibit growth of some spoilage bacteria and pathogens (Genigeorgis, 1976).

For *som-fug* products, sour taste produced from total acidity and pH are the two significant acceptances related with consumer's preference. Many customers prefer a strong taste while some do not. Considering the process of natural fermentation (about 30°C for 2-3 days), varying temperature and amount of salt used are the important factors for lactic acid bacteria growth. Therefore, it is difficult to exactly determine the fermentation time with appropriate total acidity levels and pH for the best consumption.

Near infrared spectroscopy (NIR), a non-destructive analysis, is considered as a promising technique for chemical analysis in various kinds of food sources; for instance determination of sugar in fruits (McGlone and Kawano, 1998; Saranwong et al., 2004), milk compositions (Melfsen et al., 2012) and quality of meats (Kapper et al., 2012; Gjerlaug-Enger et al., 2011). In this study, we used NIR to develop models for prediction of total acidity and pH in *som-fug*.

## Materials and Methods

### Chemicals and reagents

Distilled water (CO<sub>2</sub>-free) was obtained from Lab-Scan Asia (Thailand).

### Samples

One hundred twenty three samples of *som-fug* were purchased from Sakon Nakhon, Udon Thani, and Khon Kaen provinces. *Som-fug* samples produced from different fish species; BLANC'S STRIPED FEATHERBACK FISH (*Chitara blanci*), GIANT SNAKE-HEAD FISH (*Channa micropeltes*) and pork were used

in this study. The samples were kept in the ice box (4°C) during transportation to the NIR laboratory (Rajamangala University of Technology Isan, Khon Kaen campus). Consequently, the samples were placed in a temperature controlled room at 25°C with an average relative humidity of 67% for 30 minutes and then they were sliced into a cubical shape of 1 × 1 cm<sup>2</sup> with 1 cm thickness. The samples were covered with polyethylene sheet (0.25 mm thickness) before NIR spectra scanning (Figure 1). After spectral acquisition, the total acidity and pH level of samples were then rapidly analyzed with a standard conventional method.

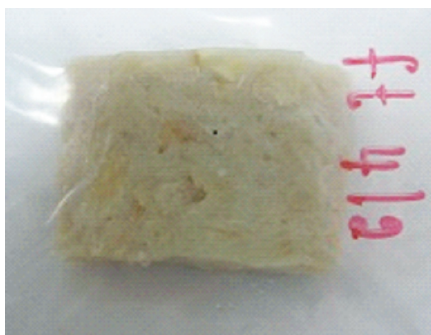


Figure 1. A display of *som-fug* sample with dimension of 1 cm × 1 cm × 1 cm thickness covered with polyethylene sheet for NIR acquisition.

#### Determinations of total acidity and pH

The total acidity and pH of the samples were measured following the method of AOAC (AOAC, 2000). In brief, *som-fug* (5 g) was suspended in 40 ml of CO<sub>2</sub>-free distilled water and homogenized at 11,000 rpm for 60 s (model T25, Selangor, Malaysia). A pH meter (pHep®3, Hanna Instruments, USA) was used to measure pH of *som-fug* solution at room temperature. The supernatant was filtered through a filter paper (Whatman No. 4). The filtrate was titrated against 0.1 N NaOH until it reached the end point (the appearance of pink color solution) using phenolphthalein as an indicator. The total acidity (%) in *som-fug* was calculated with following equation; Total acidity (%) =  $N \times V \times 90 \times 100 / W \times 1000$  where N is normality of NaOH, V is titrated volume of NaOH (ml), and W is weight of samples (g).

#### NIR conditions and spectral acquisition

The BUCHI NIRFlex N-500 (Buchi, Switzerland) equipped with N510-000 NIRFlex Solids was used to obtain spectra of the samples. The wavelength of 1000-2500 nm with reflectance mode was used in this study. The NIR parameters were optimized as 32 scans averaging (using time of 500 ms).

The obtained NIR spectra and chemical values (total acidity and pH) were imported to Unscrambler software (Ver. 9.8: CAMO AS, Trondheim, Norway). The spectra treatments (standard normal variation (SNV) and second derivative) were applied for the best partial least square (PLS) model to predict total acidity and pH.

The statistical results were investigated. The determination coefficient ( $R^2$ ) indicates the percentage of total variability explained by the PLS model while the standard error of calibration (SEC) is a parameter to indicate the quality of the NIR calibration fitting of the model. The standard error of cross-validation (SECV), calculated with all PLS predictions, expresses the standard deviation of the differences between the predicted and measured validation data. Ratio of prediction to deviation (RPD) is the ratio of standard deviation of reference data in the validation set to SECV.

## Results and discussion

### Total acidity and pH of *som-fug*

The total acidity of *som-fug* was in range of 0.9 - 1.5 % (w/w) while pH was varied between 5.0 and 5.9. As the results, pH of *som-fug* was relatively high when compared to other reports as about 4.5 - 4.6 (Riebroy et al., 2004). This is possibly due to difference in raw materials, recipe, and fermentation time. *Som-fug* with shorter fermentation time is commonly available in the market for longer distribution. The total acidity range found in this study (0.9 - 1.5% w/w) was in the same range with the previous reports (Visessanguan et al., 2006; Riebroy et al., 2004).

### Data acquisition

The original spectra of total 123 samples are shown in Figure 2. The regression coefficient of models treated with SNV and second derivative are shown in Figure 3. It was found that there was the high correlation at the wavelength of 1170, 1720, 1910 and 2290 nm that was the significant wavelengths of lactic acid bands according to previous report by Furukawa et al. (Furukawa et al., 2007). This could imply that most of acid found in *som-fug* is lactic acid. For lactic acid, the NIR bands have been reported to be dependent on adjacent compounds. Riley et al. (Riley et al., 2000) observed the lactic acid bands at 2262 and 2288 nm in the presence of glucose. Macedo et al. (Macedo et al., 2002) observed the lactic acid bands at 2236, 2278, and 2322 nm in nutrient broth. For the pH spectra, the narrow segment size and combination derivative with complex spectra could be found. Jiang et al. (Jiang et al., 2012) had reported that there was a wide combination of wavelength band used for prediction of pH.

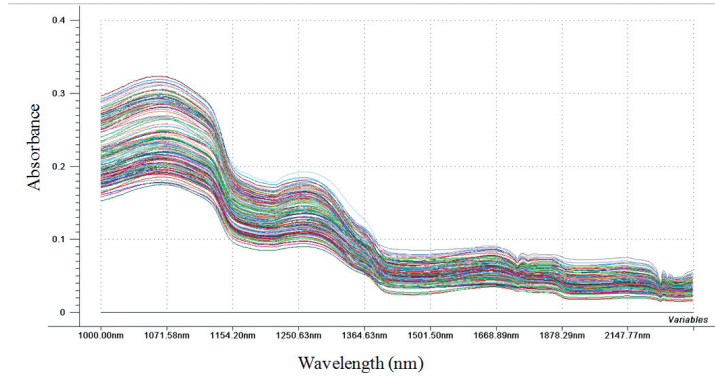


Figure 2. The original spectra of total 123 samples of som-fug.

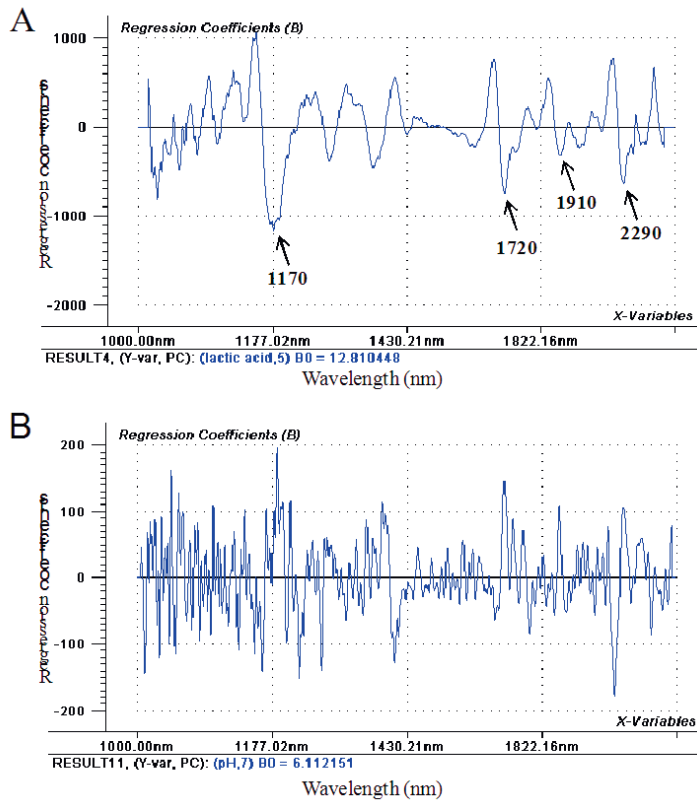


Figure 3. The regression coefficient of second derivative model of total acidity (A) and pH (B).

The whole spectra (1000-2500 nm) were used to create the PLS model with cross validation. The statistical results were shown in Table 1. The results of total acidity showed slightly high  $R^2$  of calibration and validation set (0.77 and 0.72), low SECV (0.07%), low bias (0.005%) and high ratio of prediction to deviation

(RPD) of 1.87 using 5 factor numbers for model calculation. Considering this results, it can be concluded that NIR could provide accuracy prediction of total acidity in *Som-fug* (Figure 4a). For pH prediction, the statistic parameters showed low of  $R^2$ -Val (0.54), low RPD (1.43) and high SECV (0.16) with using 7 factors numbers. Considering low  $R^2$ , it indicates the low percentage of total variability explained by the PLS model. In addition, the model showed a high pH error (0.16) comparing to the narrow pH distribution (pH 5.0-5.9) of *som-fug* samples. This result suggested that NIR could not provide a good prediction for pH in *som-fug* (Figure 4b). This is possibly due to the fact that pH is an indirect measurement. Thus, there are no specific bands recognized by NIR (Karouia et al., 2006). In addition, since the samples in this study come from various materials (BLANC'S STRIPED FEATHERBACK FISH, GIANT SNAKE-HEAD FISH and pork), there was the difference in viscosity and homogeneity that effect to NIR absorption. Therefore, it was difficult to create an accuracy pH model in this study.

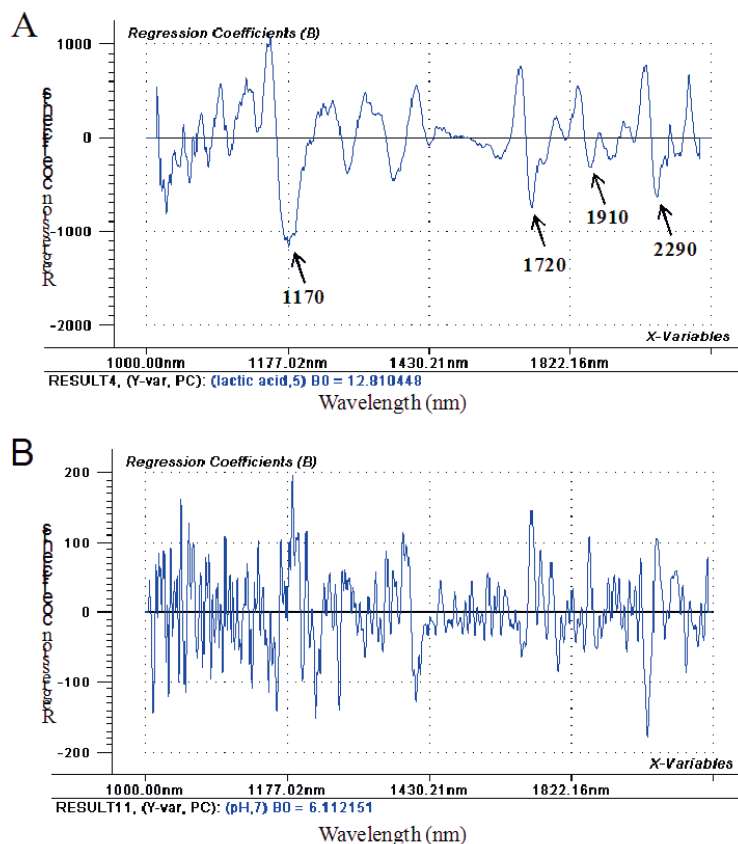


Figure 4. Scatter plot of actual total acidity and predicted total acidity (A) and scatter plot of actual pH and predicted pH (B).

Table 1 Statistical results of partial least square (PLS) regression models.

Item	F	R <sup>2</sup> -Cal	R <sup>2</sup> -Val	SEC	SECV	Bias	RPD
Total acidity (%)	5	0.73	0.72	0.70	0.07	0.005	1.87
pH	7	0.88	0.54	0.08	0.16	0.005	1.43

## Conclusions

The total acidity of *som-fug* was in the range of 0.9 - 1.5% and pH was varied in the range of 5.0 - 5.9. By using wavelength as from 1000-2500 nm with reflectance mode, NIR has potency to predict total acidity with high  $R^2$  of calibration and validation set (0.73 and 0.72), low SECV (0.07%) and low bias (0.005%). For pH, the results showed low  $R^2$  of validation (0.54), high SECV (0.16) and low RPD (1.43). It can be concluded that NIR could not provide a good model to predict pH in *som-fug*.

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