Kinetics and Nondestructive Measurement of Total Volatile Basic Nitrogen and Thiobarbituric Acid-Reactive Substances in Chilled Tabtim Fish Fillets Using Near Infrared Spectroscopy (NIRS)

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***Abstract*—Tabtim fish (*Oreochromis sp.*) fillets were prepared with a size 100-150g/piece, individual packed into zip lock polyethylene bag and kept at 0, 5, and 10°C up to 18 days. Total volatile basic nitrogen (TVB-N) and thiobarbituric acid-reactive substances (TBARS) were determined. The TVB-N and TBARS changes during storage were found to be adequately described by first-order reaction kinetics. The NIR spectra of TVB-N and TBARS in fish fillets (180 samples) were collected in the reflectance mode using NIRSystems 6500 at 25°C. Results showed that the TVB-N and TBARS were 9.40-99.7mg/100g and 0.13-2.06mg malondialdehyde/kg, respectively. The spectra were in range of short-wavelength region (700-1000nm) and long-wavelength region (1000-2500nm). Consequently, the partial regression model with cross validation was created and the optimized model was done with full NIR spectra, short wavelength and long wavelength. It was found that the acceptable model of TVB-N was obtained with 750-1000nm with determination coefficient of calibration (R^2_{cal}) = 0.78. A model of TBARS showed good data with short wavelength (750-1000nm). Considering the obtained results, it is suggested that the TVB-N model could be used as a screening step to determine the TVB-N of Tabtim fish fillets.**

***Index Terms*—near infrared spectroscopy, Tabtim fish (*Oreochromis sp.*), fish fillets, TVB-N, TBARS**

I. Introduction

The supply chain of fishery industries (fishers, producers, retailers and consumers) is great interest and attend in terms of fish freshness as essential for quality of final fishery products. It has always been recognized that fish freshness relates to the eating quality of the products for consumer consumption [1]. The quality of fresh fish is influenced by physical, microbiological and biochemical changes during handling and storage leading to rapidly deterioration and limited shelf life [2]. To preserve fish freshness, the raw materials or products should be maintained and stored in low temperature. Chilling is main process for keeping fish before further processing or consumption. Low temperature delays microbial growth, enzymatic, and biochemical reactions, resulting in long shelf life of fresh fish [3]. However, fish quality slowly changes during chilling process because of development of oxidative rancidity especially of its lipid content, autolysis by fish's own enzymes, and spoilage bacterial increasing [4].

The fish freshness or deterioration rate can be evaluated using either subjective methods (sensory test) or objective methods (physiochemical/biochemical and microbiological methods). The Chemical or biochemical methods including adenosine 5'-triphosphate (ATP)-breakdown compounds (*K* and related values), and related products and subsequently the action of proteolytic enzymes or other reactions e.g. total volatile basic nitrogen (TVB-N), trimethylamine (TMA), Thiobarbituric Acid-Reactive Substances (TBARS), and biogenic amines, etc., remain frequency used for fish freshness assessment [5], [6].

Near Infrared Spectroscopy (NIRS) analysis has become the alternative quality control in food industries due to its provide quick information and one of non-destructive methods that may requires small or no sample preparation [7], [8]. NIRS technique used Near Infrared (NIR) spectrum ranging about 700-2500nm that usually divides into short (700-1100nm) and long (1100-2500nm) wavelengths. The light of NIRS instrument works on principle of reflection, absorption, scattering and/or transmission in or through food materials, which simulates vibrations of outer electron of chemical bonds (C-H, N-H, O-H, and S-H) in foods [9], [10]. For the measurement of quality and chemical composition of various fishery products, NIRS technique has been successfully applied to predict chemical composition of Tilapia fillet [8], lipid characteristics and deterioration of frozen Saithe (Pollachius virens) and Hoki (Macruronus novaezelandiae) [7], chemical composition and classification of Sea bass fillets [11], discriminating between farmed and wild Sea bass [12], freshness

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estimation of Salmon fillets [13], chemical characteristics of minced raw fish [14].

Tabtim fish (*Oreochromis sp*.) is one of economic fish products for domestic consumption and exportation of Thailand. However, there are little information of non-destructive measurement using NIRS for determining the chemical qualities (TVB-N and TBARS) in Tabtim fish fillets during chilling storage. Therefore, the objective of this research was to study the feasibility of NIRS for TVB-N and TBARS determination of Tabtim fish fillets.

II. Metrials and Methods

A. Raw Materials and Sample Preparation

Live Tabtim fish (*Oreochromis sp*.) were purchased from a local farm in Chiang Mai Province, Thailand. Fish were transported to the Faculty of Agro-Industry laboratory, Chiang Mai University, Thailand with oxygen supply. The fish were stunned in ice, slaughtered using a knife, de-scaled, eviscerated, filleted, de-skinned by hand, and cleaned in cold water (10°C). Individual fillets size was in the range of 100-150g/piece. Then, the fillets were individual packed into zip lock polyethylene bags and subjected to storage at chilling temperature 0, 5, and 10°C up to 18 days.

B. Proximate Composition

Moisture, ash, crude protein, and fat contents (ether extraction) of Tabtim fish fillets were determined according the AOAC [15] standard methods 934.01, 942.05, 954.01, and 991.36, respectively. Crude protein of the fish fillets was expressed as 6.25 × nitrogen content. All analyses were done in triplicate.

C. TVB-N Determination

TVB-N in fish muscles was determined in triplicate using the Conway micro-diffusion method as described by Conway and Byrne [16]. The chopped fish fillets (2g) were homogenized with 8 ml of 4% Trichloroacetic Acid (TCA) and then the homogenate was filtered through a Whatman No. 1 filter paper. The filtrate was used for the analyses. A sealing agent (petroleum jelly, Vaseline, Unilever Thai Trading Ltd., Bangkok, Thailand) was applied to the edges of the Conway micro-diffusion units. Then 1ml of filtrate was placed into the outer ring of Conway micro-diffusion unit. A 1% boric acid containing the mixed indicator (bromocresol green and methyl red) was then pipetted into the inner ring. To initiate the reaction, 1ml of saturated K_2CO_3 was carefully pipetted into the outer ring and gently mixed with the sample extract. The unit was closed and incubated at 37℃ for 60 min. The inner ring solution with a green color was then titrated with 0.02N HCl until the green color became pink.

D. TBARS Determination

TBARS was determined in triplicate according to the method of Buege and Aust [17]. Chopped fish muscle (0.5g) was placed into a test tube and mixed with 5ml of a mixture containing 0.375g/100ml of Triobabuturi Acid (TBA), 15g/100 ml of Trichloroacetic Acid (TCA), and 0.875ml of concentrated HCl. The mixture was heated in boiled water for 10 min, followed by cooling with running tap water. The mixture was centrifuged at 3000×g for 15 min (model ZK 316, Hermle Labortechnik GmbH, Wehingenand, Germany). The absorbance of the supernatant was measured at 532nm using a UV/Vis spectrophotometer (Jasco 7800, Tokyo, Japan). The TBARS were calculated from the standard curve of using the 1, 1, 3, 3-tetraethoxy-propane (TEP) as a standard compound and expressed as mg malondialdehyde/kg muscle.

E. NIRS Analysis

NIRS analysis was performed using NIRSystems 6500 (Foss NIRSystems, Silver Spring, USA) equipped with spinning module at room temperature about 25°C. The measurement of the fish fillets spectra in the short and long wavelength region from 700nm to 2500nm. The spectra were obtained at 2nm intervals with the average scan of 32 times in reflectance mode. Fish fillets preparation, each fillet was cut with 10 mm of thickness, placed into zip lock polyethylene bag, and kept in an ice box to control temperature at 5-10°C throughout the measurements. Before scanning, flesh fish was packed into standard sample cup and subjected to spectra scanning. A total of 180 samples was carried out in this experiment.

F. Statistical Analysis

The collected spectra and value of TVB-N (mg/100g) and TBARS (mg malondialdihyde/kg muscle) were analyzed using Unscrambler software (version 9.3, Oslo, Norway) Calibration equations were calculated by Partial Least Square Regression (PLSR) to predict the TVB-N and TBARS using cross validation. The number of factors used as independent variables in the prediction equations was fixed at a maximum of 20 in order to avoid over fitting [8]. The accuracy of prediction equations were evaluated in terms of coefficient of determination in calibration (R^2_{cal}) and validation (R^2_{val}), Standard Error of Calibration (SEC) and Standard Error of Prediction (SEP). The optimization of NIR wavelength was done by step up in every 50nm wavelengths to fine the best results of R^2_{cal} and SEP.

III. Results and Discussions

A. TVB-N and TBARS Changes during Storage

Chemical composition of Tabtim fish fillets were 77.94±0.60% of moisture, 18.17±0.35% of crude protein, 2.37±0.18% of fat, and 1.17±0.11% of ash contents. The TVB-N content and TBARS values of fillets stored at 0, 5, and 10°C up to 18 days are shown in Fig. 1A and Fig. 1B, respectively. At day 0, the TVB-N content of fish fillets was about 10mg/100g. As the storage time increased, a continuous increase in TVB-N content was observed in all storage temperatures. The samples stored at 10°C was rapidly increased after 6 days of storage. At 5°C, fish fillets showed unacceptable quality for consumption with more than 25mg/100g of TVB-N value [18] after day 12 but the TVB-N content of fillet stored at 0°C was value

lower than the upper limit during 18 days. The initial value of TBARS value of fillets was 0.20mg malondialdihyde/kg muscle. The TBARS values of all the Tabtim fish fillets slowly increased throughout the storage period, especially for the values of fillets stored at 10°C, and the increasing rate became slower as lower storage temperature due to the oxidation of lipid is slowly reacted at low temperature [19].

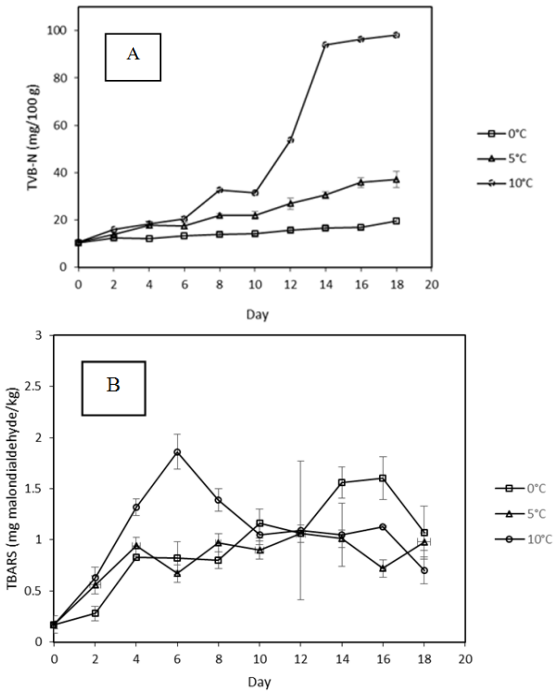

Figure 1. (A) TVB-N content (mg/100g) and (B) TBARS values (mg malondialdehyde/kg) of Tabtim fish fillets during chilling storage.

The kinetic reaction of quality deterioration was determined as reaction rate constant (k) and regression coefficient (R^2) using data based on experiments (Table I). The k of zero or first-order of TVB-N reactions were obtained from slope of regression of TVB-N or ln [TVB-N] versus time, respectively as well as the k of zero or first-order of TBARS reactions were obtained from slope of regression of TBARS or ln [TBARS] versus time, respectively. The TVB-N and TBARS changes were found to be adequately described by first-order reaction kinetics. In previous study Zhang *et al.* [19] reported that fish freshness correlated to storage time and temperature by the 1st-order reaction with high regression correlations.

B. Original NIR Spectra and Data Acquisition

Collected NIR spectra with reflectance mode had a trembling line that caused by the scattering of fillet due to different size and type of compounds affected by NIR in different ways (Fig. 2). Therefore, Tabtim fish fillets had light and dark muscle that might some reflected to absorbance as discussions of previously reports [7]-[13]. The pretreated spectra techniques (smoothing, 2nd derivative transformer and multiplication scattering correction; MSC) were approached to original spectra. The results showed that smoothing at 10 points with all wavelengths (700-2500nm) provided the good models compared to other techniques (Table II). Considering to NIRS wavelength regions, a model created by short NIR wavelength (700-1000nm) and long NIR wavelength were compared. It was found that the good model obtained with short NIR wavelength for TVB-N (R^2 = 0.58, SEP = 16.22mg/100g) better that long wavelength (R^2 = 0.57, SEP = 16.44mg/100g) as well as results of TBARS; short NIR wavelength R^2 = 0.40, SEP = 0.33mg malondialdihyde/kg and long wavelength (R^2 = 0.09, SEP = 0.41mg malondialdihyde/kg), respectively (data not shown). This is probably due to greater penetration of short NIR wavelength to fish muscles. Although long wavelength have more sensitivity but it is lower penetration. To find the finest model, wavelength optimization with short wave was done with 50 wavelengths stepwise (Table III).

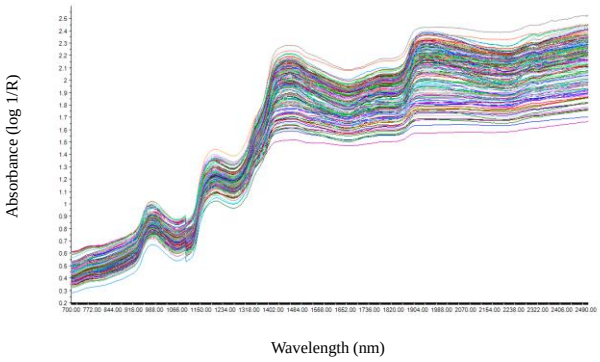

Figure 2. Original spectra of chilled Tabtim fish fillets by using short and long NIR wavelength.

TABLE I. REACTION RATE CONSTANTS AND CORRELATION COEFFICIENTS OF TVB-N CONTENT AND TBARS INCREASING IN TABTIM FISH FILLETS DURING CHILLING STORAGE

Temperature (°C)	TVB-N				TBARS			
	Zero-order reaction		First-order reaction		Zero-order reaction		First-order reaction	
	k_0 (mg /100g. day)	R^2_0	k_1 (day^{-1})	R^2_1	k_0 (mg malondialdehyde /kg. day)	R^2_0	k_1 (day^{-1})	R^2_1
0	0.879	0.973	0.061	0.974	0.162	0.972	0.199	0.974
5	2.922	0.985	0.131	0.984	0.076	0.730	0.133	0.984
10	9.113	0.972	0.266	0.980	0.026	0.169	0.830	0.381

Note: k_0 and k_1 are zero-order and first-order reaction rate constants, respectively; R^2_0 and R^2_1 are zero-order and first-order regression coefficients, respectively.

The TVB-N and TBARS are the quality index refer to spoilages of fish by microbiological and biochemical reactions [5], [6]. The chemical component of TVB-N value including NH_3, dimethylamine, and TMA, while malonaldehyde contents related to TBARS value [18]. Those were unstable and a small amount in the samples (the range of 9.40-99.7mg/100g for TVB-N and 0.13-2.06mg malondialdihyde/kg for TBARS). Other compounds could show higher effects in the model such as bands of water (760nm) and protein (910nm) [20].

TABLE II. COEFFICIENT OF CALIBRATION (R^2_{VAL}) AND STANDARD ERROR OF PREDICTION (SEP) OF DIFFERENT SPECTRA TREATMENT'S MODEL

Chemical value	Smoothing (10 points)		MSC		2nd derivative transform	
	R^2	SEP	R^2	SEP	R^2	*SEP*
TVB-N	0.58	16.2	0.57	16.4	0.55	16.8
TBARS	0.40	0.33	0.22	0.38	0.24	0.37

TABLE III. COEFFICIENT OF CALIBRATION (R^2_{VAL}) AND STANDARD ERROR OF PREDICTION (SEP) FOR OPTIMIZATION OF SHORT NIR WAVELENGTH

Chemical value	700-1000nm		750-1000nm		800-1000nm		850-1000nm	
	R^2	SEP	R^2	SEP	R^2	SEP	R^2	SEP
TVB-N	0.68	14.2	0.75	12.2	0.64	15.1	0.65	14.8
TBARS	0.20	0.38	0.21	0.23	0.20	0.38	0.19	0.39

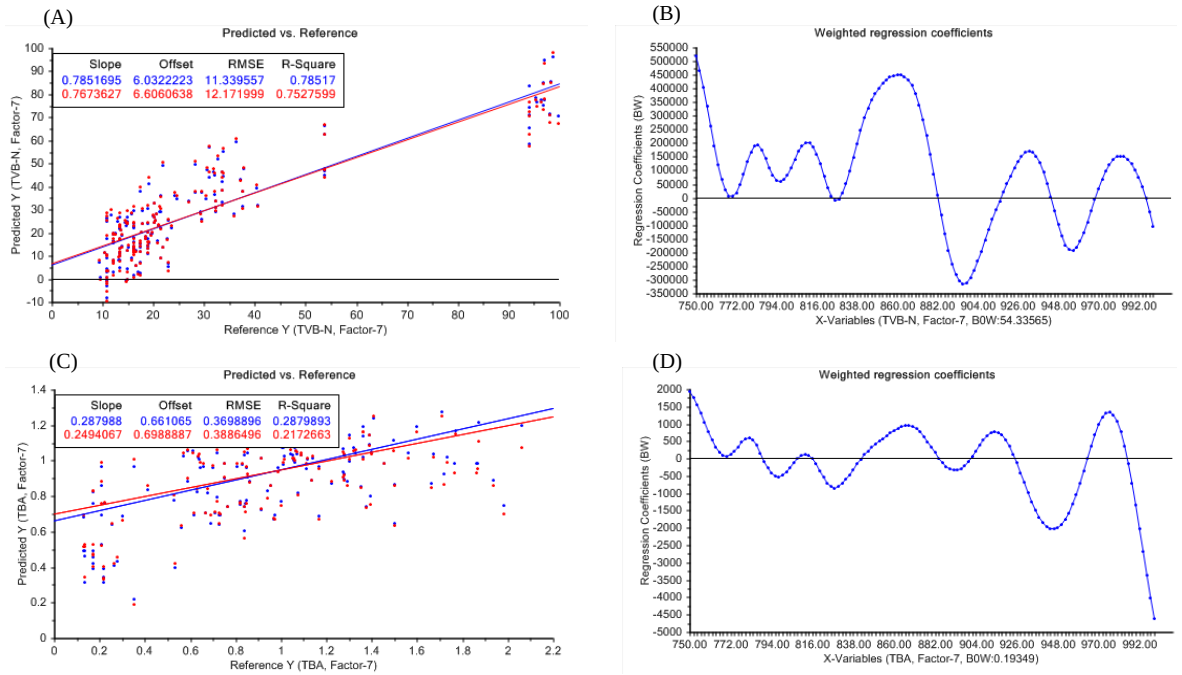

Figure 3. Scatter plots and regression coefficients of TVB-N (A, B) and TBARS (C, D).

The scatter plots and regression coefficients were shown in Fig. 3A and Fig. 3B for TVB-N and Fig. 3C and Fig. 3D for TBARS values. For scatter plot, it shows the accuracy of predicted values from the model and real values from conventional method. The higher prediction could be observed with that scatter point stands by the trend line whiles the regression coefficient plots showed the level of each wavelength effected in predicted model. Considering regression coefficients plots (Fig. 3B and Fig. 3D), it could be not specific wavelength and it could might showed as a noise in the model. However, model created by full wavelength (750-1000nm) gave high coefficient of calibration (R^2_{cal}) of 0.78, determination coefficient of validation (R^2_{val}) = 0.75, standard error of prediction (SEP) = 12.20mg/100g and bias of 0.01 and this could be used for screening test for TVB-N but the model showed low accuracy for TBARS.

IV. CONCLUSIONS

TVB-N and TBARS was investigated by near infrared spectroscopy in chilled Tabtim fish fillets. It was found that short-wavelength region (750-1000nm) gave higher accuracy with determination coefficient of calibration (R^2_{cal}) = 0.78, determination coefficient of validation (R^2_{val}) = 0.75, standard error of prediction (SEP) = 12.20mg/100g and bias of 0.01. This model could be used for screening for TVB-N determination. However, TBARS model was not accuracy model for prediction of fish quality. Future work is required to specify targeted main compounds for TVB-N and TBARS to obtain the higher accuracy for NIRS prediction.

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