



## Research article

# Microbiological, chemical and physical attributes and mathematical models for total volatile basic nitrogen formation of Asian seabass (*Lates calcarifer*) fillets stored under refrigerated and temperature-abuse conditions

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## Abstract

**Importance of the work:** Understanding the quality features and applying mathematical models to the total volatile basic nitrogen (TVB-N) content in refrigerated Asian seabass fillets can be beneficial in predicting their freshness and shelf life, as well as for developing fish spoilage indicators.

**Objectives:** To examine the quality attributes of Asian seabass fillets under refrigerated and temperature-abuse conditions and identify the best-fitting mathematical models for the TVB-N content.

**Materials & Methods:** The investigation covered the microbiological (total viable count [TVC]) and *Pseudomonas* spp.), chemical (TVB-N content and pH), physical (color and texture) properties of Asian seabass fillets at 4°C and 10°C and the best-fitting mathematical models of the TVB-N content and activation energy ( $E_a$ ).

**Results:** Asian seabass fillets were noticeably mushy and reached 6 log colony forming units/g on day 3 at 10°C and on day 6 at 4°C, while the TVB-N content was below 25 mg N/100 g and reached 25.59 mg N/100g on day 4 at 10°C and 25.67 mg N/100g on day 7 at 4°C. The pH, color and TPA of the seabass fillets did not provide good indications of quality. TVB-N correlated well with both TVC and specific spoilage organisms (SSOs, *Pseudomonas* spp.) at 4°C and 10°C. The best-fitting mathematical models for TVB-N changes in fillets was a 2<sup>nd</sup>-order kinetics, yielding an  $E_a$  value of 58.55 kJ/mol.

**Main finding:** The good correlation between TVB-N and SSOs might be useful as a chemical spoilage index to determine fish quality during refrigerated storage. The best-fitting mathematical models predicted  $E_a$ , suggesting that future research should investigate intelligent labeling based on TVB-N to monitor fish and food spoilage.

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## Introduction

Aquatic products, such as fish, are perishable and spoil faster than other foodstuffs because after the fish dies, major fish molecules are degraded by enzymatic activity inside muscle tissues, resulting in chemical and biological changes (Food and Agriculture Organization, 2005). Typically, protein decomposition leads to organoleptically detectable decay and microbial growth produces metabolites such as amines, sulfides, alcohols, aldehydes, ketone and organic acids that lead to unacceptable off-flavors and sensory rejection (Gram and Dalgaard, 2002; Erkan and Özden, 2006). With the proper storage conditions, specific spoilage organisms (SSOs) can dominate the remaining microorganisms and produce metabolites (volatile organic compounds, VOCs). The number of SSOs at rejection can be considered as the minimum spoilage level (MSL) and the concentration of VOCs associated with spoilage can be used as a chemical spoilage index (CSI; Gram and Huss, 1996; Dalgaard, 2003; Parlapani et al., 2015). *Pseudomonas* spp. are mostly psychrotrophic bacteria responsible for the deterioration of temperate freshwater fish (Lunestad and Rosnes, 2008) and cold-stored fish and crustaceans (Fung, 2009). The microbial load of good-quality fish should be less than 5 log colony-forming units per gram (log CFU/g) (Pal et al., 2016) and a microbial load higher than 6 log CFU/g in fish is not favorable for long-term storage and is unsuitable for consumption (Olafsdóttir et al., 1997; Erkan and Özden, 2006). Fresh fish with total viable count (TVC) and *Pseudomonas* levels of 7 log CFU/g reached the cutoff index for spoilage (Pacquit et al., 2007; Fung, 2009). According to the Microbiological Quality Criteria for Food and Food Contact Containers in Thailand, the number of microorganisms in refrigerated and frozen seafood products, such as shrimp, fish and crab, must be lower than 6 log CFU/g and the seafood must be cooked prior to consumption (Department of Medical Sciences, 2017).

Total volatile basic nitrogen (TVB-N), comprising ammonia, dimethylamine (DMA) and trimethylamine (TMA), is a chemical index of increased toxicity and deterioration. The generation of volatile species is dependent on the species and variety of fish (Whittle and Howgate, 2002). In the literature, different TVB-N levels have been established as acceptable limits for various fish species. For example, the TVB-N levels of newly captured fish were in the range 5–20 mg N/100 g, whereas freshwater and marine fish were refused at 20 mg N/100 g and 30 mg N/100 g, respectively (National Health

and Family Planning Commission of the People's Republic of China, 2015). As specified by the European Commission (95/149/EC), TVB-N levels of 25–35 mg N/100 g are defined as the maximum permitted levels for different species of fish (European Union, 1995). However, fish with TVB-N levels above 25 mg N/100 g have been reported as unfit for consumption in rainbow trout fillets (Giménez et al., 2002), Asian seabass (Masniyom et al., 2002), Nile perch fillets (Amegovu et al., 2012) and Chinese seabass (Ma et al., 2021).

The Asian seabass (*Lates calcarifer*, Bloch 1790) is an indigenous species of Thailand and a commercially important fish across Asia and the Pacific (Food and Agriculture Organization, 2022). The Asian seabass has high economic value in Thai aquaculture due to its rapid growth rate, exquisite flavor and nutritional value, especially its polyunsaturated fatty acids and omega 3 fatty acids (Pechsiri et al., 2020). Asian seabass fillets are in high demand for packaged goods and are traded globally due to their flavor, nutritional value and easy of storage, with the consumption of Asian seabass recently increasing in emerging nations and sales growing at a compounded annual growth rate (CAGR) of 4.1% between 2016 and 2020 and predicted to grow at a CAGR of 5.5% through 2031 (Asian Seabass Market, 2021).

Asian seabass, like other fresh fish, has a limited shelf life even when chilled or refrigerated. Specifically, temperature and time have a major influence on the quality attributes of refrigerated Asian seabass, especially regarding microbial growth and metabolite formation, with these quality attributes during storage being described using mathematical models (Heising et al., 2014; García et al., 2022). The temperature dependence may be simulated in a variety of ways, including the Arrhenius model, which is commonly used to describe the temperature dependence of microbiological and quality deterioration in the food system (Gudmundsson and Kristbergsson, 2009). Mathematical models have been developed to predict changes in fish quality during storage for Japanese seabass (Chang et al., 1998), gilthead seabream fillets (Tsironi et al., 2010), crucian carp (Yao et al., 2011), cod fish (Heising et al., 2014), stored fresh fish fillets (Peleg, 2016), rohu fish (Prabhakar et al., 2021) and rainbow trout (Yin et al., 2022).

The simplest method for evaluating the deterioration process in a food product as a function of time and the reaction order is linear regression analysis (van Boekel, 2008; Phimolsiripol and Suppakul, 2016). When the effects of temperature on reactions in foods have been established, the activation energy ( $E_a$ ) obtained from the food quality index would be beneficial for indicating temperature dependence

(van Boekel, 2008). In addition, if the food quality index and food spoilage indicator have a similar temperature dependence, a difference in  $E_a$  values of less than 5 kJ/mol indicates that the indicator can be used accurately to monitor the freshness of food products, as suggested by Nopwinyuwong et al. (2010) and Suppakul (2011). However, no information has been published on mathematical models of the quality of Asian seabass fillets during refrigerated storage. Consequently, the current study aimed to examine the changes during storage of microbiological, chemical and physical attributes of Asian seabass fillets under refrigerated (4°C) and temperature abuse (10°C) conditions. A quality function with time associated with mathematical models and the Arrhenius equation was established to determine the activation energy for TVB-N formation in seabass fillets for the future development of fish spoilage indicator.

## Materials and Methods

### Materials

Potassium dihydrogen phosphate 99.5% (Q RēC; New Zealand), plate count agar (PCA; Merck; Germany) and *Pseudomonas* agar base CM559 (Oxoid; UK) with *Pseudomonas* CFC (cetrimide fucidin cephaloridine) selective agar supplement SR0103 (Oxoid; UK) were used to examine total viable count and *Pseudomonas* count, respectively. Bromocresol green (BCG), potassium carbonate ( $K_2CO_3$ ) and potassium hydroxide (Ajax Finechem; New Zealand), ethyl alcohol, boric acid, trichloroacetic acid (TCA) and hydrochloric acid 37% (HCl; Merck; Germany) and methyl red (MR; Panreac Química; Spain) were used to determine the TVB-N.

### Experimental setup

Asian seabass (average weight of 0.9–1.0 kg) were purchased from a local market near Kasetsart University, Bangkok, Thailand. The samples were immediately washed with cold tap water, descaled, filleted, individually packed in a low-density polyethylene pouch and kept in separate controlled refrigerators at 4°C and 10°C. Filleted samples for microbiological, chemical and physical analysis were randomly taken on days 0, 2, 4, 6, 7, 8 and 9 for 4°C (refrigerated) storage and on days 0, 1, 2, 3 and 4 for 10°C (temperature abuse). All tests were conducted in triplicate.

### Microbiological analysis

The seabass fillets were aseptically cut and chopped. Each chopped sample (50 g) was mixed with 450 mL Butterfield's phosphate buffer (pH 7.2) and homogenized for 2 min using a Stomacher 400 (Seward; Germany) and the homogenate was decimal diluted in a series. Each dilution (1 mL) was pipetted into a polystyrene Petri dish (90 mm diameter, 14 mm depth), poured with PCA and incubated at 35°C for total viable count (TVC) determination (Maturin and Peeler, 2001). Each dilution (0.1 mL) was spread on a *Pseudomonas* agar base with *Pseudomonas* selective agar supplement and then incubated at 30°C for the *Pseudomonas* count (Pacquit et al., 2007; Kuswandi et al., 2012). After 48 hr, both colonies were counted. A feasible range for counting colonies was 25–250. The data were presented as the logarithm of CFUs per gram.

### Total volatile basic nitrogen analysis

The TVB-N content was determined using Conway's assay (Conway and Byrne, 1933; Pathanasriwong et al., 2020). A dorsal fish meat sample (2 g) was extracted with 4% w/v of TCA (8 mL), finely ground, left for 30 min and passed through Whatman's filter paper no.41. A solution (1 mL) containing 1% boric acid and a mixed indicator (0.01 g of BCG and 0.02 g of MR in 10 mL of ethyl alcohol) was added to the inner ring of the Conway plate. Then, sample extract (1 mL) was transferred to the outer ring, followed by saturated  $K_2CO_3$  solution (1 mL) to initiate the reaction. As a reference color, TCA (1 mL) was transferred to the outer ring instead of a sample. The Conway unit was sealed and incubated at 35°C for 60 min. The green solution in the inner ring was titrated with 0.02 N HCl until it became pink. The amount of TVB-N (measured as milligrams of N per 100 g of sample) was calculated using Equation 1:

$$\text{TVB-N content} = [(V_S - V_B) \times (N_{HCl} \times A_N) \times (W_S \times (M/100)) + V_E] \times 100 / W_S \quad (1)$$

where  $V_S$  and  $V_B$  are the volumes of HCl used to titrate the sample and blank, respectively,  $N_{HCl}$  is the concentration of HCl,  $A_N$  is the atomic mass of nitrogen,  $W_S$  is the sample weight,  $M$  is the percentage of moisture in the muscle sample and  $V_E$  is the sample volume.

### pH measurement

The pH of seabass fillets was determined using the procedure described by Qiu et al. (2014). Fillet meat (10 g) was homogeneously blended with distilled water (90 mL) for 2 min and the pH of the mixture was measured using a pH meter (Metrohm 827; Mettler Toledo; Germany).

### Color measurement

The color intensity—based on the L\*, a\* and b\* parameters (CIE, 1986)—was scaled using a Minolta CM-3500d (Konica Minolta; Japan). The colorimeter was calibrated with a standard white plate and measured under D65 illumination. The total color differences (TCD,  $\Delta E$ ) of the seabass fillets at 4°C and 10°C were recorded and calculated using Equation 2:

$$\Delta E = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \quad (2)$$

where L\* is lightness (100 = brightest white, 0 = darkest black), a\* is redness/greenness (+a\* = redder, -a\* = greener) and b\* is yellowness/blueness (+b\* = yellower, -b\* = bluer) values as defined by CIE (1986) and  $\Delta L^*$ ,  $\Delta a^*$  and  $\Delta b^*$  are the differences between the color parameters of fresh fish on day 0 and at sampling time.

### Texture profile analysis

The texture profile analysis (TPA) was modified from Zakhariya et al. (2015). Samples of the middle (loin) of each seabass fillet were collected and cut using a sharp knife into 3 cm  $\times$  2 cm  $\times$  1.5 cm pieces. The hardness, gumminess, chewiness, springiness and cohesiveness were evaluated. Prior to analysis, the fillets were equilibrated at 25°C for 1 hr. All measurements were performed using a texture analyzer (TA HD/50; Stable Micro Systems; UK) and a 500 N load cell. The measurements were obtained using a cylinder probe with 50 mm diameter and a crosshead that ran at a deformation rate of 2 mm/s to a depth of 7.5 mm. For all measurements, the fillets were punctured with a trigger force of 1 N.

### Comparison of mathematical models for estimating the activation energy for total volatile basic nitrogen

Generally, mathematical models for changes in food quality during storage are described by the order of reaction (n) in the kinetics equation (Equation 3). Changes in the TVB-N content

of seabass fillets at 4°C and 10°C can be predicted theoretically. Under isothermal conditions, the linear regression analysis was depicted on a graph of quality indicator (TVB-N content) versus time (t) for zero-, 1<sup>st</sup>- and 2<sup>nd</sup>-order kinetics, as shown in Equations 4–6, respectively:

$$\frac{dC}{dt} = kC^n \quad (3)$$

For zero-order reactions ( $n = 0$ ),

$$C = C_0 + kt \quad (4)$$

For 1<sup>st</sup>-order reactions ( $n = 1$ ),

$$C = C_0 \exp(kt) \quad (5)$$

For 2<sup>nd</sup>-order reactions ( $n = 2$ ),

$$C = 1 / (1 + C_0 kt) \quad (6)$$

where  $C_0$  is the initial concentration, C is the actual concentration at time (t) and k is the reaction kinetics constant. In the above models, the Arrhenius equation is usually applied to explain the temperature-dependent rate constant (k), as showed in Equation 7:

$$k = k_0 \exp(-E_a/RT) \quad (7)$$

where  $k_0$  is a pre-exponential constant,  $E_a$  is the activation energy (kJ/mol), R is the universal gas constant (8.314 J/K mol) and T is the absolute temperature (Kelvin, K).

The  $Q_{10}$  value is another parameter that is frequently adopted to describe the relationship between temperature and the reaction rate constant, as shown in Equations 8–9:

$$Q_{10} = [(dC_2)/dt] / [(dC_1)/dt] = [\exp(E_a / (R(T+10))] / [\exp(E_a / RT)] \quad (8)$$

$$Q_{10} = k_2 / k_1 = \exp[10 E_a / RT(T + 10)] \quad (9)$$

where T is the absolute temperature (Kelvin, K),  $k_0$  is a pre-exponential constant,  $E_a$  is the activation energy (kJ/mol) and R is the universal gas constant (8.314 J/K mol).

The Arrhenius equation and  $Q_{10}$  can be used to demonstrate the effect of temperature on the reaction rate. The linearized form was applied to estimate  $E_a$ . Given that the rate of loss of fillet quality (k) at 4°C was  $k_1$  and at 10°C was  $k_2$ ,  $E_a$

can be solved by modifying Equation 9 to Equation 10 where the temperature difference is 6°C:

$$Q_6 = k_2 / k_1 = \exp [6 E_a / RT(T + 6)] \quad (10)$$

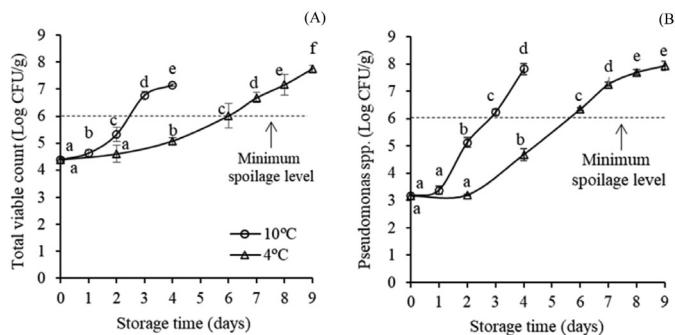
### Statistical analysis

All data were obtained from experiments with three replications and the results were expressed as mean  $\pm$  SD. Statistical analysis was conducted using the Statistical Package for the Social Sciences (SPSS) software (SPSS Inc.; USA). One-way analysis of variance was used and a multiple-comparison procedure was also performed using Duncan's new multiple range test at  $p < 0.05$  for a significant difference and ( $p < 0.01$ ) for a highly significant difference.

## Results and Discussion

### Microbiological changes in seabass fillets

The initial TVC and *Pseudomonas* spp. counts of seabass fillets were 4.38 log CFU/g and 3.16 log CFU/g, respectively. At the minimum spoilage levels (MSL) of 6 log CFU/g (Erkan and Özden, 2006; Department of Medical Sciences, 2017), the TVC and *Pseudomonas* spp. counts of seabass fillets reached 6.02 and 6.35 log CFU/g, respectively, on day 6 at 4°C, whereas at 10°C, these counts were 6.77 and 6.23 log CFU/g on day 3, respectively (Fig. 1). At the spoilage index of 7 log CFU/g suggested by Fung (2009), the *Pseudomonas*

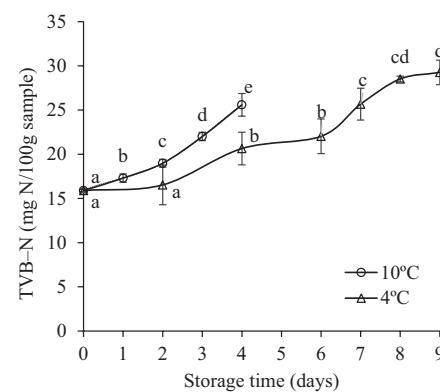


**Fig. 1** Microbial counts of seabass fillets during storage at 4°C and 10°C: (A) total viable counts; (B) *Pseudomonas* spp. counts, where minimum spoilage level is the cell concentration of specific microorganism at rejection, different lowercase letters indicate significant ( $p < 0.05$ ) difference between means in same treatment, error bars indicate  $\pm$  SD and CFU = colony forming units

spp. count reached 7.26 log CFU/g on day 7 and TVC reached 7.17 log CFU/g on day 8 at 4°C, while the *Pseudomonas* spp. count and TVC reached 7.82 log CFU/g and 7.16 log CFU/g, respectively, on day 4 at 10°C. Although the storage time required to reach the MSL was similar for the TVC and *Pseudomonas* spp. counts at each temperature, there were notable variations in *Pseudomonas* spp. counts at the index of spoilage, with *Pseudomonas* spp. displaying higher levels of spoilage than TVC.

### Total volatile basic nitrogen changes in seabass fillets

Changes in the TVB-N content of the seabass fillets are shown in Fig. 2. The initial TVB-N contents in the seabass fillets were 15.9 mg N/100 g and gradually rose during the storage period. At the MSL, the TVB-N contents continuously increased to 22.03 mg N/100g on day 6 at 4°C and to 22.01 mg N/100g on day 3 at 10°C. In this study, the TVB-N content was below 25 mg N/100 g sample, indicating that all the seabass fillets remained fresh even after reaching the MSL. At the index of spoilage, all seabass fillets were deemed unsuitable for consumption due to the presence of an off-odor and the TVB-N content being above the acceptable limit on day 7 at 4°C (25.67 mg N/100g) and on day 4 at 10°C (25.59 mg N/100g). A TVB-N level of 25 mg N/100 g was used to identify Asian seabass (Masniyom et al., 2002) and Chinese seabass (Ma et al., 2021) as unfit for human consumption.

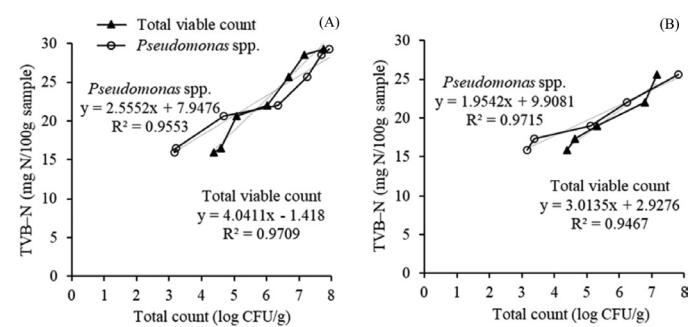


**Fig. 2** Total volatile basic nitrogen (TVB-N) content of seabass fillets at 4°C and 10°C, where different lowercase letters indicate significant ( $p < 0.05$ ) difference between means in same treatment and error bars indicate  $\pm$  SD

**Table 1** and **Fig. 3** show the correlation between TVB-N and total count (TVC and *Pseudomonas* spp.) of the seabass fillets stored at 4°C and 10°C. The TVB-N contents were highly significantly correlated with TVC ( $r = 0.9853$  at 4°C,  $r = 0.9730$  at 10°C) and *Pseudomonas* spp. (Pearson correlation coefficient,  $r = 0.9774$  at 4°C,  $r = 0.9856$  at 10°C). *Pseudomonas* spp., which are psychrotrophic bacteria and SSOs, that tended to grow more rapidly than the TVC which was based on aerobic mesophilic bacteria, after 6 d at 4°C and 3 d at 10°C (**Fig. 1**). These results indicated that the volatile components (TVB-N) were generated by spoilage organisms (*Pseudomonas* spp.) and might be adopted as a CSI to ascertain fish quality during refrigerated storage. According to Ólafsdóttir et al. (1997), Erkan and Özden (2006) and Department of Medical Sciences (2017), seabass fillets were considered unacceptable on day 6 at 4°C and day 3 at 10°C due to the presence of microorganisms exceeding the safety limit of 6 log CFU/g, despite having somewhat reasonable TVB-N levels for fresh fish (TVB-N < 25 mg N/100g). These results revealed they were unsafe for consumption and should be rejected.

### pH changes in seabass fillets

pH changes in seabass fillets at 4°C and 10°C are presented in **Table 2** and **Table 3**. Fresh seabass had an initial pH of 6.37. At 4°C, the pH of the seabass fillets was not significantly on days 8, 6, 4 and 2 (**Table 2**). At 10°C, the pH decreased considerably on days 1 and 3, while there were no significant differences on days 2 and 4 of storage (**Table 3**). In general,



**Fig. 3** Correlation between total volatile basic nitrogen (TVB-N) content and total counts (TVC and *Pseudomonas* spp.) of seabass fillets at: (A) 4°C; (B) 10°C, where CFU = colony forming units and  $R^2$  = coefficient of determination

**Table 1** Pearson correlation coefficients ( $r$ ) for total volatile basic nitrogen (TVB-N) content and total viable count (TVC) and *Pseudomonas* spp. in Asian seabass fillets at 4°C and 10°C

Correlation	4°C			10°C		
	TVB-N	TVC	<i>Pseudomonas</i> spp.	TVB-N	TVC	<i>Pseudomonas</i> spp.
TVB-N	1	0.9853**	0.9774**	1	0.9730**	0.9856**
TVC	0.9853**	1	0.9786**	0.9730**	1	0.9730**
<i>Pseudomonas</i> spp.	0.9774**	0.9786**	1	0.9856**	0.9730**	1

\*\* = correlation is highly significant ( $p < 0.01$ ; 2-tailed)

**Table 2** Changes in pH and color of Asian seabass fillets at 4°C

Parameter	Storage time (days)						
	0	2	4	6	7	8	9
pH	6.37±0.06 <sup>ab</sup>	6.47±0.02 <sup>bc</sup>	6.38±0.08 <sup>abc</sup>	6.38±0.05 <sup>abc</sup>	6.33±0.05 <sup>a</sup>	6.48±0.06 <sup>c</sup>	6.49±0.06 <sup>c</sup>
$L^*$	36.29±0.14 <sup>d</sup>	35.18±0.39 <sup>c</sup>	34.87±0.76 <sup>c</sup>	34.64±0.27 <sup>c</sup>	32.61±0.19 <sup>a</sup>	33.52±0.77 <sup>b</sup>	33.69±0.83 <sup>b</sup>
$a^*$	-2.89±0.30 <sup>a</sup>	-2.70±0.12 <sup>a</sup>	-2.11±0.19 <sup>b</sup>	-2.15±0.33 <sup>b</sup>	-1.91±0.26 <sup>b</sup>	-2.10±0.17 <sup>b</sup>	-2.58±0.47 <sup>a</sup>
$b^*$	-2.33±0.47 <sup>ab</sup>	-2.43±0.74 <sup>ab</sup>	-2.75±0.48 <sup>a</sup>	-2.52±0.78 <sup>a</sup>	-2.43±0.46 <sup>ab</sup>	-1.34±0.37 <sup>c</sup>	-1.59±0.77 <sup>bc</sup>
$\Delta E$	-	1.32±0.33 <sup>a</sup>	1.78±0.65 <sup>a</sup>	1.96±0.37 <sup>a</sup>	3.84±0.19 <sup>c</sup>	3.07±0.76 <sup>b</sup>	2.89±0.62 <sup>b</sup>

Values (mean ± SD) in same row with different lowercase superscript are significantly ( $p < 0.05$ ) different.

**Table 3** Changes in pH and color of Asian seabass fillets at 10°C

Parameter	Storage time (days)				
	0	1	2	3	4
pH	6.37±0.06 <sup>b</sup>	6.19±0.09 <sup>a</sup>	6.36±0.04 <sup>b</sup>	6.24±0.01 <sup>a</sup>	6.39±0.05 <sup>b</sup>
$L^*$	36.29±0.14 <sup>b</sup>	35.08±0.86 <sup>a</sup>	35.46±0.95 <sup>ab</sup>	34.76±0.74 <sup>a</sup>	35.05±0.32 <sup>a</sup>
$a^*$	-2.89±0.30 <sup>ab</sup>	-3.11±0.15 <sup>a</sup>	-2.51±0.15 <sup>cd</sup>	-2.62±0.19 <sup>bc</sup>	-2.27±0.36 <sup>d</sup>
$b^*$	-2.33±0.47 <sup>b</sup>	-1.04±0.13 <sup>c</sup>	-3.27±0.43 <sup>a</sup>	-2.58±0.32 <sup>b</sup>	-2.45±0.69 <sup>b</sup>
$\Delta E$	-	1.92±0.41 <sup>a</sup>	1.66±0.33 <sup>a</sup>	1.64±0.62 <sup>a</sup>	1.54±0.35 <sup>a</sup>

Values (mean ± SD) in same row with different lowercase superscript are significantly ( $p < 0.05$ ) different.

during early storage, post-mortem glycolysis could cause lactic acid accumulation and pH reduction (Qiu et al., 2014). Over longer storage, the pH increased due to the accumulation of alkaline components, such as ammonia and TMA, which were mostly caused by bacterial activity during fish muscle breakdown (Jeon et al., 2002). Simeonidou et al. (1997) reported that the pH of fresh fish was in the range 6.0–6.5 and increased with the storage period. The pH post-mortem could be in the range 6.0–7.1 depending on the season, species and other factors, with the acceptable limit usually in the range 6.8–7.0. According to the literature, under refrigerated storage (4°C), the pH in Asian seabass fillets was in the range 6.1–6.6 for 15 d (Ahmad et al., 2012), the pH of scale-less filleted European seabass was in the range 6.43–6.77 for 12 d (Alparslan et al., 2012) and the pH in Japanese seabass was in the range 6.5–6.8 for 12 d (Cai et al., 2014). In the current study, the pH changes in the Asian seabass fillets were in the ranges 6.33–6.49 at 4°C for 9 d and 6.19–6.39 at 10°C for 4 d. These pH fluctuations remained within the acceptable range, even if the microorganism and TVB-N contents exceeded the safety limit and an off-odor was detectable. These findings suggested that pH measurement may not be effective for assessing the quality of seabass fillets, which was in agreement with the conclusions made by Simeonidou et al. (1997).

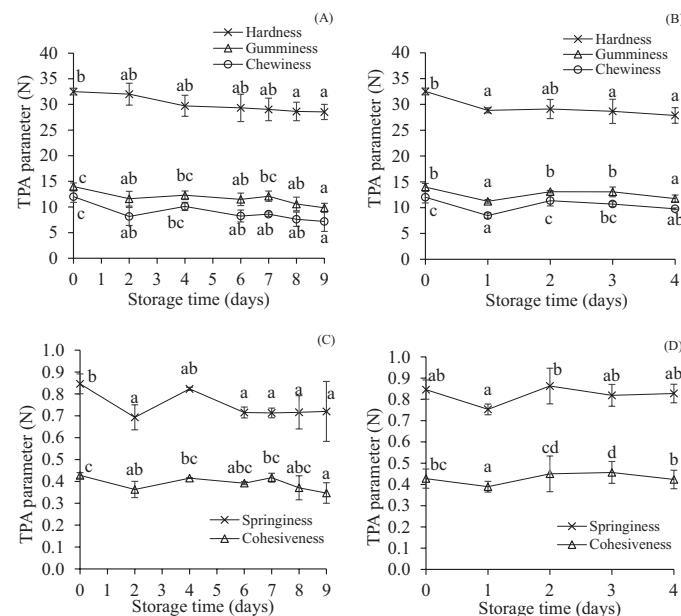
#### Color changes in seabass fillets

Color changes in seabass fillets at 4°C and 10°C are presented in Table 2 and Table 3, respectively. Initially the seabass fillets had a greenish-hue with values for  $L^*$ ,  $a^*$  and  $b^*$  of 36.29, -2.89 and -2.33, respectively. At 4°C, the  $L^*$  value decreased on day 2, the  $a^*$  value increased between days 4 and 6 and then decreased on days 8 and 9, while the  $b^*$  value increased on day 4 and then decreased noticeably on day 8. At 10°C, the  $L^*$  value decreased on days 1 and 3, the  $a^*$  value decreased on days 2 and 4, while the  $b^*$  value decreased sharply on day 2 before increasing rapidly on day 3. The TCD was calculated using delta  $L^*$ , delta  $a^*$  and delta  $b^*$  color values, which collectively provide a comprehensive numerical description of the color in a rectangular coordinate system. The TCD of the seabass fillets changed significantly on day 7 at 4°C (TCD = 3.84), while there was no significant difference in the TCD during 4 d at 10°C. In general, TCD values greater than 5 can be easily detected by the naked eye, while TCD values greater than 12 indicate a completely distinct color space (Francis, 1983). In the current study, all the seabass

fillets turned from a greenish-hue to dark a greenish-hue and released a strong off-odor on day 8 at 4°C and on day 4 at 10°C, indicating complete deterioration. However, the color changes were below the values that the naked eye could detect (TCD  $\geq 5$ ), as shown in Tables 2 and 3. Therefore, color change was not a good indicator for quality.

#### Texture profile analysis

The hardness, gumminess, chewiness, springiness and cohesiveness of the seabass fillets were determined and are reported in Fig. 4. The seabass fillets became noticeably mushy and soft in texture on day 6 at 4°C and on day 3 at 10°C. At 4°C, there was a significant decrease in gumminess and chewiness on day 6, followed by a decrease in hardness and springiness on day 8 and a decrease in cohesiveness on day 9. At 10°C, there was a considerable significant decrease in hardness on day 3, as well as a decrease in gumminess and chewiness on day 4. However, neither springiness nor cohesiveness decreased significantly. Fish muscle gets softer and loses its rigidity after death due to the enzymatic degradation of muscle protein; these changes occur before and independently of microbial breakdown (Ocaño-Higuera et al., 2011). In the current study, the reduction in hardness, gumminess and chewiness of the



**Fig. 4** Changes in texture profile analysis (TPA) of seabass fillets: (A) and (C) at 4°C and (B) and (D) at 10°C, where different lowercase letters indicate significant ( $p < 0.05$ ) difference between means in same treatment and error bars indicate  $\pm$  SD.

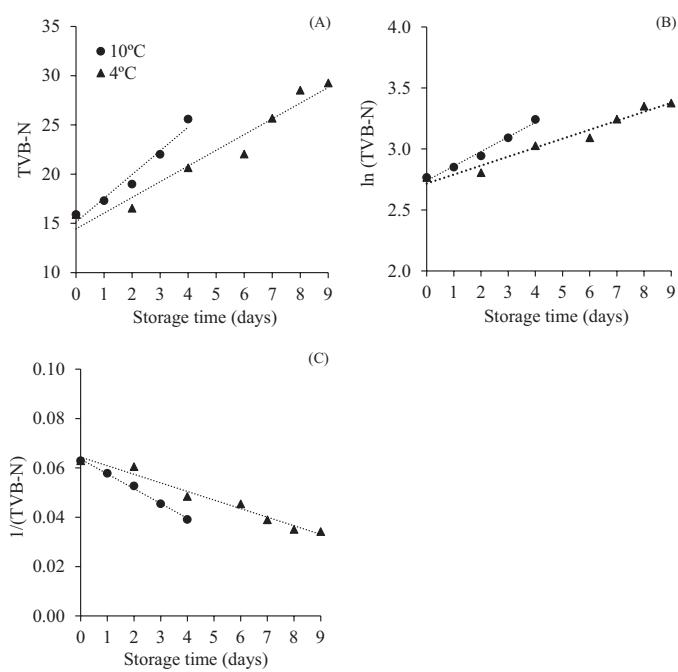
seabass fillets at 4°C followed a similar trend to that reported for Japanese seabass by Cai et al. (2014) and for pompano fillets reported by Gao et al. (2014). Despite reaching the spoilage threshold, TPA was not a reliable indication of the freshness of the seabass fillets. However, texture may be assessed using less intrusive methods. Unlike other food matrices, such as beef, the texture of fresh fish is not often regarded as a good predictor of its freshness (García et al., 2022).

#### Comparison of mathematical models for estimating the activation energy for total volatile basic nitrogen

Changes in the TVB-N content of the seabass fillets under isothermal conditions may be theoretically predicted using mathematical models (see Equations 4–6) by plotting the quality index (TVB-N) versus time and rearranging it into a linearized form (Fig. 5). Table 4 presents the quality function in linearized form, along with the response rate constant (k), coefficient of determination ( $R^2$ ), root mean square error (RMSE) and activation energy for the TVB-N of seabass fillets at 4°C and 10°C. Under isothermal conditions, 1<sup>st</sup>-order and 2<sup>nd</sup>-order kinetics models could be chosen for predicting TVB-N formation in seabass fillets during storage at 4°C and 10°C. The optimal models in the current study for TVB-N formation in seabass fillets kept at two temperatures were those with  $R^2$  values greater than 0.97 and the lowest RMSE. The best fit models for seabass fillet stored at 4°C ( $R^2 = 0.9707$ , RMSE = 0.0018) and 10°C ( $R^2 = 0.9945$ , RMSE = 0.0006) were 2<sup>nd</sup>-order kinetics models with an  $E_a$  value of 58.55 kJ/mol.

During the initial stage of storage, the TVB-N content of the seabass fillets remained relatively low; however, a substantial increase in the TVB-N content at high microbial counts (6–7 log CFU/g) indicated the beginning of major spoilage. Similar to the microbial growth curve, the correlation between microbial count and TVB-N content indicated that the kinetics of TVB-N under refrigerated storage at 4°C and

10°C did not follow a zero-order. The TVB-N formation is a function of enzymatic and bacterial actions influenced by the storage conditions. TVB-N is an indicator of protein degradation and bacterial activity, frequently applied to determine the freshness and quality of fish (Prabhakar et al., 2021). In chemical kinetics, the reaction order refers to the exponent to which the concentration of a reactant is raised in the rate equation. It determines how the concentration of reactants influences the rate of a chemical reaction. For zero-order reactions, the rate of the reaction is independent of the concentration of the reactant. The 1<sup>st</sup>-order reactions depend on a single reactant and the exponential value is one. For 2<sup>nd</sup>-order reactions, the reaction rate is proportional



**Fig. 5** Plot of total volatile basic nitrogen (TVB-N) content in seabass fillets as function of time and temperature for fillets at 4°C and 10°C: (A) zero-order reaction; (B) 1<sup>st</sup>-order reaction; (C) 2<sup>nd</sup>-order reaction

**Table 4** Quality function, response rate constant (k), coefficient of determination ( $R^2$ ), root mean square error (RMSE) and activation energy ( $E_a$ ) for total volatile basic nitrogen (TVB-N) content of Asian seabass fillets at 4°C and 10°C

Model	Temperature (°C)	Quality function F(X)	TVB-N			$E_a$ (kJ/mol)
			k	$R^2$	RMSE	
Zero-order	4	$y = 1.5969x + 14.437$	1.5969	0.9472	1.1471	44.68
	10	$y = 2.4094x + 15.137$	2.4094	0.9607	0.6893	
1 <sup>st</sup> -order	4	$y = 0.0735x + 2.7165$	0.0735	0.9652	0.0425	52.61
	10	$y = 0.1193x + 2.7402$	0.1193	0.9817	0.0230	
2 <sup>nd</sup> -order	4	$y = -0.0035x + 0.0644$	0.0035	0.9707	0.0018	58.55
	10	$y = -0.006x + 0.0636$	0.0060	0.9945	0.0006	

to the square of the concentration of the reactant or the product of the concentrations of two reactants (Labuza and Riboh, 1982).

In the current study, TVB-N formation during fish spoilage was either 1<sup>st</sup>-order or 2<sup>nd</sup>-order (no zero-order kinetics). There are several reasons why the rate of TVB-N accumulation in fish tissue should be proportional to the concentration of various reactants, such as proteins and microorganisms. In the context of fish decomposition described by 1<sup>st</sup>-order kinetics, the rate of TVB-N formation increases proportionally with the concentration of reactive species (such as proteins) in the fish tissue. This is the result of enzymatic or microbial processes that break down proteins into volatile basic nitrogen compounds. For 2<sup>nd</sup>-order kinetics, the rate of TVB-N formation may depend on the interaction between two reactive species, such as proteins and bacteria. The presence of bacteria can increase the rate of TVB-N formation by contributing to the degradation of proteins. Therefore, 2<sup>nd</sup>-order kinetics may be applicable when both protein degradation and bacterial activity contribute substantially to the TVB-N formation process. Based on these considerations and empirical observations, it was concluded that the TVB-N kinetics during fish spoilage likely followed 1<sup>st</sup>-order or 2<sup>nd</sup>-order kinetics, rather than zero-order kinetics. Further research and modeling may help to determine the exact order of the reaction and to estimate the activation energy more accurately.

Different models are carried out to understand the quality loss of different types of seafood stored at different temperatures. Comparatively, the best-fitting models for TVB-N production in Rohu fish stored at -5°C, 0°C and 5°C were 1<sup>st</sup>-order kinetics ( $R^2 = 0.98$ ;  $E_a$  of 58.16 kJ/mol) as reported by Prabhakar et al. (2019). Due to various complex biochemical activities at -5°C, it was determined that the exponential model ( $R^2 = 0.98$ ) provided the best fit for TVB-N prediction for Rohu stored at 0°C and 5°C (Prabhakar et al., 2021). Srivastava et al. (2020) examined the shelf life of chilled white shrimp in the range 2–10°C. Their findings revealed that the 1<sup>st</sup>-order kinetics model ( $R^2 = 0.9792$ ) was comparable with TVB-N with an  $E_a$  of 57.78 kJ/mol. However, the  $E_a$  value obtained using the TVB-N content changed in Asian seabass fillets stored at 4°C and 10°C, with it being comparable to that obtained from Japanese seabass stored at 5°C and 10°C ( $E_a = 52.72$  kJ/mol) (Chang et al., 1998). Even under refrigeration, enzymatic activity and microbial growth can cause the deterioration of fresh fish and seafood (Peleg, 2016). Typical  $E_a$  values for food quality losses resulting from enzymatic activity and microbial growth have been reported as 41.84–62.76 and 83.68–251.04

kJ/mol, respectively (Suppakul, 2012). The current experiment yielded moderate  $E_a$  values, revealing that minor temperature fluctuations had negligible impact on seabass quality. A higher range in  $E_a$  values of 118–156 kJ/mol for TVB-N formation in frozen shrimp was reported by Tsironi et al. (2009) and an  $E_a$  value of 132.82 kJ/mol for TVB-N formation in Rohu fish was reported by Prabhakar et al. (2019), indicating that this product has a strong temperature dependence and should be stored at a low temperature to prevent spoilage reactions.

In the current study, it was necessary to determine the  $E_a$  of food systems (seabass fillets stored at 4°C and 10°C) using an appropriate mathematical model. The findings of our study indicated that TVB-N content, as a CSI, followed either 1<sup>st</sup>-order or 2<sup>nd</sup>-order kinetics. However, the best-fitting models for TVB-N formation in the seabass fillets along with the Arrhenius equation could be used to estimate  $E_a$  via the  $Q_6$  equation. This would benefit future research on fish spoilage indicators. This information would also be useful for predicting the shelf life of fish (Heising et al., 2014; Prabhakar et al., 2019), designing and developing an efficient supply chain for fishery products (Prabhakar et al., 2021), developing a food spoilage indicator (Nopwinyuwong et al., 2010) and a time-temperature indicator for monitoring packed fish quality (Katsouli et al., 2022).

## Conclusions

An increase in storage temperature for Asian seabass fillets from 4°C to 10°C decreased their product shelf life from 6 d to 3 d. The TVB-N content correlated well with the TVC and *Pseudomonas* spp. count. These findings indicated that the TVB-N content, produced by specific spoilage microorganisms, could be applied as a CSI to evaluate fish deterioration under refrigerated storage. The amount of TVB-N in Asian seabass fillets stayed within an acceptable range (< 25 mg N/100g sample) for fresh fish throughout 6 d at 4°C and 3 d at 10°C. However, Asian seabass fillets with TVB-N contents of 22 mg N/100g sample were considered unfit for consumption and should be rejected due to the presence of microorganisms exceeding the safety limit of 6 log CFU/g. The determination of  $E_a$  in this study would be useful for further research in the area of intelligent packaging by applying the TVB-N content as a chemical metabolite for developing a food spoilage indicator label for real-time monitoring of protein-based food deterioration during refrigerated storage.

## Conflict of Interest

The authors declare that there are no conflicts of interest.

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