Models for Predicting Quality of Solar-Dried Shrimp (Penaeus vannamei) during Storage Based on Protein Oxidation

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Abstract

The purpose of this study was to explore the correlation between protei oxidation and quality and to study the changes in various indexes of solar-dried shrimp (Penaeus vannamei) stored at 37°C and 20°C through vacuum packing and vacuum packaging with antipressure sterilization. The results showed that ΔE as well as TVB-N and carbonyl contents increased, whereas moisture and free thiol (SH) contents decreased with time. Furthermore, SDS-PAGE and scanning electron microscopy revealed protein degradation and damage of shrimp muscle microstructure during storage. A quality prediction model based on protein oxidation was established according to Arrhenius equation. Verification of shrimp quality prediction models revealed that the relative errors of the models based on SH and carbonyl contents were below 10%, indicating that these protein oxidation parameters can be used for reliable estimation of quality changes in dried shrimp during storage.

1. Introduction

Penaeus vannamei shrimp is one of the most popular seafood because of its attractive flavor and high nutritional value [1]. Fresh shrimps deteriorate easily and, therefore, must be preserved or processed soon after harvest to increase their shelf life [2]. Freshly processed dried Penaeus vannamei is delicious, and its flavor gradually becomes weaker with the prolonging of storage time. Drying predominates over other preservation and processing methods because it allows storage of dried shrimp at room temperature for a long time [3]. China is rich in solar energy resources, which not only prevent the invasion of mosquitoes and microorganisms but also provide effective solar energy utilization and high temperatures to perform solar drying, which, compared with natural drying, can significantly shorten the drying time and improve the quality of the final product [4].

During storage, many chemical reactions occur in seafood, the most important of which is protein oxidation leading to nutrient degradation and changes in food sensory properties [5]. In their study, M. Malgorzata et al. [6] found that high concentration of oxygen promoted the increase in disulfide bond cross-linking after myosin oxidation, which reduced the degradation of internyosin and the tenderness of meat. In their study, on the influence of high oxygen packaging on pork quality, R. M. Delles et al. [7] found that the high oxygen system causes protein oxidation, and myosin aggregates through disulfide cross-linking, which weakens the ability of protein to bind to water, leading to a decrease in water holding capacity. Many factors such as moisture, the packaging method, and storage temperature can influence the rate of protein oxidation [8]. Packaging and storage are important steps in the distribution and marketing of processed food, which critically affect the quality of the final product [9]. In particular, packaging methods and materials significantly influence the oxidation stability of dried seafood products; for example, vacuum packaging, including packing with antipressure sterilization, can delay protein oxidation and improve the overall quality of muscle foods through oxygen exclusion [10].

Constructing a quality prediction model of solar-dried shrimp based on protein oxidation can help to better understand the relationship between protein oxidation and quality formation. Artificial intelligence has a good application prospect in many fields. An image fusion based on multimodal medical images renders a considerable enhancement in the quality of fused images [11]. N. Zhu et al.
[12] used the artificial neural network to establish a multiple mass prediction model based on protein degradation. In order to study the relationship between quality change and protein oxidation of turbot during storage, changes in protein oxidation parameters and quality indicators during refrigeration (4°C) and ice (0°C) storage were determined, and the correlation among them was analyzed [13]. The classic Arrhenius model, which is commonly used to describe the relationship between chemical reaction rates and temperature, can potentially reduce experimental burden and provide a practical method for predicting and preventing quality deterioration of processed food during storage [14]. However, there are few reports on the relationship between protein and quality based on the Arrhenius model. Compared with previous studies, Arrhenius equation is often used to predict the shelf life of food. However, in this study, it was applied to analyze the correlation between protein oxidation and quality indicators, which is helpful to better regulate the storage quality of *Penaeus vannamei*.

In this study, solar-dried shrimp (*Penaeus vannamei*) was used as the research object to explore the change rule of protein and quality of shrimp in different packaging methods and storage temperatures. Using Arrhenius equation, a quality prediction model based on protein oxidation was established. Our results provide a theoretical basis and technical support for the quality assessment of dried products during storage.

### 2. Materials and Methods

#### 2.1. Raw Material, Processing, Packaging, and Sampling

Fresh *P. vannamei* shrimp (average wet weight 19.52 ± 2.2 g, average body length 12.3 ± 0.5 cm) was obtained from Tangshan City, Hebei Province Caofeidian aquaculture area in October 2020. Ten kilograms of shrimp were frozen at −80°C and 80 kg was dried.

Fresh shrimp were graded, washed, and boiled at the following conditions: salt solution concentration, 3% (w/v); shrimp/salt solution ratio, 1:2 (w/w); and boiling time, 6 min. Weighed samples were placed in a single layer on plastic meshed trays and dried using solar drying equipment (Tangshan Luanfeng Breeding Co., Ltd.) for approximately 7 h. Dried shrimp were cooled, shelled, and subjected to vacuum packaging (VP) or vacuum packaging with antipressure sterilization (AP) at 110°C for 20 min.

For the storage experiment, the samples were divided into two groups stored at 37°C and 20°C, respectively. A total of 4 experimental treatments (the experimental design method is given in Table 1) were set; each treatment was a mutual control and stored for 30 days. Every 3 days, the samples were analyzed for color and moisture, TVB-N, SH, and carbonyl contents and every 6 days for protein composition by SDS-PAGE. At days 0 and 30, the samples were evaluated for the microstructure of muscle fibers under a scanning electron microscope (SEM).

#### 2.2. Color Analysis

The color of the shrimp surface was measured using a chroma meter (Konica Minolta Laboratory USA, Inc.) calibrated using a white board, and the CIE parameters *L*, *a*, and *b* (lightness from 0 to 100%, color from green to red, and color from yellow to blue) were obtained [15, 16]. The color results were expressed as the mean value of at least 10 samples, excluding the maximum and minimum values [17]. The total color difference ΔE was calculated as follows:

\[
\Delta E = \sqrt{\left( L^* - L_0^* \right)^2 + \left( a^* - a_0^* \right)^2 + \left( b^* - b_0^* \right)^2},
\]

where *L*₀, *a*₀, and *b*₀ are the shrimp color parameters before storage, and *L**, *a**, and *b* are the color parameters during storage. The difference between two colors was assessed according to a previously developed color scale: 6 < ΔE < 12 indicated strong difference and ΔE > 12 indicated distinct colors [18].

#### 2.3. Moisture Content Analysis

The moisture content was determined by drying the sample in an oven at 105°C until a constant weight was obtained [19]. Triplicate measurements were performed for each batch.

#### 2.4. TVB-N Measurement

The TVB-N content in shrimp samples was measured in a K1100 automatic Kjeldahl nitrogen analyzer according to GB 5009.228-2016 “National Food Safety Standard for the Determination of TVB-N in Foods.” Accurately weighed (3.0 ± 0.1 g) minced shrimp meat was placed into an Erlenmeyer flask with 50 mL of 20 g/L trichloroacetic acid (TCA), stirred every 5 min for 30 min, and filtered. Then, 10 mL of the filtrate was placed into a digestive tube, mixed with 5 mL of 10 g/L magnesium oxide solution, distilled for 5 min, and absorbed with 20 g/L boric acid; the absorption solution was titrated with 0.1 mol/L HCl. Blank control contained 10 mL of 20 g/L TCA and 5 mL of 10 g/L magnesium oxide without the shrimp sample. The TVB-N content was calculated as follows:

\[
X = \frac{(V_1 - V_2) \times C \times 14}{m \times (10/50)} \times 100,
\]

where *X* is the TVB-N content in the sample (mg/100 g), *V*₁ and *V*₂ are the volumes (mL) of the HCl titration solution used for the test sample and blank control, respectively, *C* is...
the HCl concentration (mol/L), and $m$ is the sample mass (g).

2.5. Determination of SH Content. The SH content was determined according to the modified Ellman method [20]. Dried shrimp (2.0 g) was homogenized in 10 mL of 50 mM phosphoric acid buffer (pH 8.0) and centrifuged to obtain the upper protein-containing fraction, which was diluted 10-fold and mixed with 0.02 ml reagent containing 2 mM/L 5, 5'-dithio (2-nitrobenzoic acid) (DTNB); the absorbance was measured at 412 nm after 1 h incubation at 25°C eddy dimming reaction. Protein content of the sample was determined using a BCA kit (Beijing Leagene Biotechnology Co., Ltd.), and the SH content was calculated as follows:

$$[	ext{SH}] = \frac{a \times d}{c \times b} \times \frac{1}{10 \times g \text{ protein}} \times \frac{10^5 \text{ g}}{c} \times 13,000 \text{ mol}$$(3)

where $a$ is the absorbance, $b$ is the protein concentration (mg/mL), $c$ is the molecular absorbance coefficient (13,600 mol⁻¹ cm⁻¹ L), and $d$ is the replacement coefficient of 10.

2.6. Determination of Carbonyl Content. The carbonyl content was estimated using the method of M. Morzel et al. [20] with slight modifications. The method is based on the formation of protein hydrazones with 2, 4-dinitrophenylhydrazine (DNPH). Dried shrimp (2 g) was homogenized in 10 mL of 0.6 M NaCl (pH 6.5) in a blender, centrifuged at 5,000 rpm for 10 min, and the supernatant (400 μL) was mixed in a 1.5 mL Eppendorf tube with 200 μL of 2 M HCl containing 10 mM DNPH. The samples were incubated for 1 h in the dark at 37°C in a water bath under agitation, and proteins were precipitated with 1 mL of 40% TCA. After 20 min, the samples were centrifuged at 13,000 rpm for 15 min, and the resultant pellets were washed with 1 mL of ethanol:ethyl acetate (1:1) and centrifuged at 13,000 rpm for 10 min to remove free DNPH; washing was repeated until the supernatant became colorless. The pellets were dissolved in 3 mL of 6 M guanidine-HCl and left overnight; then, 200 μL was placed in triplicate into a 96-well plate, and the absorbance was measured at 370 nm in a microplate reader (1510, Jingqiao Export Processing Zone, Pudong New Area, Shanghai). Protein concentration was determined using the BCA method, and the results were expressed as nmol of carbonyl/mg protein.

2.7. SDS-PAGE. Salt-soluble proteins were extracted from shrimp samples (0.1 g) using 10 mL of PIPA lysis buffer (strong) (50 mM Tris, pH 7.4, 150 mM NaCl, 1% Triton X-100, 1% sodium deoxycholate, and 0.1% SDS) supplemented with protease inhibitors [21]. After sufficient homogenization in a high-throughput tissue grinder (Ningbo Xinzhi Biotechnology Co., Ltd.), the samples were incubated for 20 min on ice to achieve complete cell lysis, centrifuged at 14,000 × g for 10 min, and the supernatant was transferred to a new tube and used for further analysis as the total sample protein. Protein concentration was determined using the BCA method, adjusted to 1,500 μg/mL for all samples, and 40 μL of each lysate was mixed with 5× reducing SDS-PAGE loading buffer for 40 s and heated in a water bath at 100°C for 3–5 min. If the sample was not used immediately, it was stored at −20°C. Samples were loaded into SDS-polyacrylamide gels (5% stacking and 10% separating) and subjected to electrophoresis in a JY600C Electrophoresis Cell (Beijing Junyi Dongfang Electrophoresis Equipment Co., Ltd.) at 130 V constant voltage for approximately 100 min. The gels were stained for 15 min in SDS-PAGE staining buffer, followed by decolorization in distilled water, and the molecular weights of individual proteins were determined by comparison with standard protein markers (10–250 kD) (Shanghai Saiao Biotechnology Co., Ltd.) [22].

2.8. Muscle Microstructure Analysis. Shrimp muscle microstructure was analyzed by SEM as previously described with slight modifications [23, 24]. The second shrimp segment was cut into 5 × 5 × 3 mm pieces, fixed in 2.5% (w/v) glutaraldehyde solution at 4°C overnight, washed three times with distilled water for 20 min, and dehydrated in gradient ethanol solutions (25%, 50%, 75%, and 95%, w/v) for 20 min, then in 100% (w/v) ethanol for 20 min three times, and finally in isooamyl acetate for 20 min three times. Afterward, the sample was gold-coated at 20 mA and 10 Pa vacuum for 5 min in a vacuum ion sputtering instrument (Beijing Zhongke Instrument Co., Ltd,) and observed at 1000× magnification under a 4863-P SEM (Amitech Trading (Shanghai) Co., Ltd.).

2.9. Establishment of a Kinetic Model. Changes in the quality characteristics of dried shrimp during storage at different temperatures could be expressed based on the rate constant ($k$) and its dependence on temperature. The rate of changes in the selected quality indices can be represented by the following kinetic equation:

$$\frac{dB}{dt} = kB^n$$

(4)

where $B$ is the value of quality index after storage for time $t$ (days), $k$ is the rate constant, and $n$ is the kinetic order of reaction [25]. Rate constant $k$ is temperature dependent and often described by the following Arrhenius equation [26]:

$$k = k_0 \exp\left(-\frac{E_A}{R}\right)$$

(5)

where $k_0$ is the preexponential constant, $E_A$ is the activation energy in kJ/mol, $R$ is the universal gas constant (8.3144 J/K·mol), and $T$ is the absolute temperature. Fitting equation (5) into equation (4) yields

$$\int_{t_0}^{t} \frac{dB}{B^n} = k_0 \exp\left(-\frac{E_A}{RT}\right) dt$$

(6)

Intergrating equation (6) results in

$$\int_{B_0}^{B} \frac{dB}{B^n} = \int_{t_0}^{t} k_0 \exp\left(-\frac{E_A}{RT}\right) dt$$

(7)

At $n = 0$, a zero-order kinetic equation is obtained:
3. Results and Discussion

3.1. Changes of Indexes during Storage. The appearance of the product is one of the most important parameters in consumer acceptance, and the color is a characteristic indicator of dried shrimp quality. \( \Delta E \) is used to quantitatively assess the total difference between two colors; the larger the \( \Delta E \), the greater the difference. Changes in \( \Delta E \) of VP and AP shrimp samples during storage at 37°C and 20°C are shown in Figure 1(a). All samples showed a significant increase in \( \Delta E \) with the time of storage; however, at the same temperature, \( \Delta E \) was higher for AP than for VP shrimp, which is consistent with previous reports [27]. In average, the \( \Delta E \) value of all samples increased by 19.74% at the end (day 30) compared with the start (day 0) of the storage period, which could be due to water loss and subsequent browning of dried shrimp. In order to avoid great changes in total color difference of dried shrimp during storage, VP-20°C treatment should be selected to maintain the stability of color and luster for up to 21 days.

Moisture content of dried shrimp decreased during 30 days of storage at different temperatures (Figure 1(b)). Under the same packing method, the moisture content was significantly lower in samples stored at 37°C than in those stored at 20°C, indicating that higher temperature accelerated the loss of moisture, which is consistent with the results of a previous study [28]. At the same time, the packaging method also affected the rate of moisture loss, which was higher in AP than in VP shrimp. In order to reduce the water loss of dried shrimp during storage, the storage condition of VP-20°C should be adopted, and the best storage time is 6 days to ensure that the water content does not change greatly.

TVB-N value, as the main hygienic index, indicates the freshness of meat products and reflects the spoilage degree of meat products. Protein degradation due to enzymes and microorganisms generates alkaline ammonia, amines, and nitrogen-containing substances, such as volatile basic nitrogen compounds, which are small molecular substances and toxic nonprotein nitrogen-containing compounds, such as metabolism products of amino acids and nucleotides [29]. Figure 1(c) shows that the TVB-N content in all samples increased with storage time \( (p < 0.05) \); however, the increase was slower for AP shrimp than for VP shrimp, indicating that vacuum packaging after antipressure sterilization could delay the spoilage of dried shrimp. Therefore, in order to maximize the freshness of dried shrimp, the AP-20°C treatment group should be selected for storage.

SH groups in cysteine residues are particularly vulnerable to attack by reactive oxygen species and other radicals, resulting in a wide array of oxidative changes [30]. Figure 1(d) shows a significant storage time-dependent decrease of SH content in all samples \( (p < 0.05) \), which was more pronounced in VP than in AP shrimp, indicating that antipressure sterilization packaging was better in inhibiting protein oxidation. Similar results were reported in previous studies [31, 32]. In order to delay the process of protein oxidation, the best treatment group was AP-20°C, and the original quality could be best maintained after 6 days of storage.

Carbonyl content is one of the most reliable indices of the extent of protein oxidation [33]. Amino acids with NH or NH₂ groups on their side chains are highly sensitive to hydroxyl radicals and are transformed into carbonyl groups during protein oxidation [34, 35]. The carbonyl content of dried shrimp showed an increasing trend over the storage period (Figure 1(e)). Under the same packaging method, the level of carbonyls was higher at 37°C than at 20°C; at the same time, it was lower in AP shrimp than in VP shrimp. Similar results were reported by [36]. In order to ensure that a large degree of protein oxidation does not occur in the storage process of dried shrimp, AP-20°C should be used, and the storage time can reach 18 days.

Electrophoresis analysis of shrimp proteins showed that the main protein components of dried shrimp were myosin heavy chain (MHC, 220 kDa), actin (43 kDa), and troponins T (37 kDa), I (23 kDa), and C (18 kDa) Figure 2. After storage, the MHC band disappeared in all treatment groups, which is in agreement with previous findings [37, 38]. MHC degradation was probably due to oxidation, which may also lead to degradation, cross-linking, and aggregation of actin as indicated in an earlier report [39]. Indeed, the actin band in VP and AP shrimp samples decreased but did not completely disappear during storage. Similarly, the troponin T band gradually decreased with storage at 37°C; however, it did not significantly change during storage at 20°C. The same trend was observed for troponin I, which showed a less significant decrease at 20°C than at 37°C; still, the corresponding band was reduced or even disappeared in all storage at 20°C.

\[
B(t) = B_0 + k_d t \exp\left(\frac{-E_A}{RT}\right),
\]

where \( B_0 \) is the initial value of the selected quality indices.

2.10. Validation of Predictive Models. Protein oxidation indices (SH and carbonyl content) of samples stored at 20°C were used to validate models predicting shrimp quality changes, and the calculated predicted values were compared with the observed values.

2.11. Statistical Analysis. All experiments were repeated three times, and the results are shown as the mean ± standard deviation. Statistical analysis and correlation analysis were performed using SPSS 23 (SPSS Inc., Chicago, IL, USA). Differential significance analysis was performed using the Duncan method, and \( p < 0.05 \) indicated statistically significant differences. A data chart was created using Origin 2018 software.
Figure 1: Variation in $\Delta E$ (a), moisture content (b), TVB-N (c), SH (d), and carbonyl content (e) of dried shrimps during storage.
samples. The troponin C band also decreased or almost disappeared in AP shrimp at the end of the storage period, but did not significantly change in VP shrimp. Therefore, in order to delay the occurrence of protein degradation in the storage process of dried shrimp, the optimal treatment group should choose VP20°C.

Alterations in muscle microstructure are an important indicator of the quality of dried shrimp. Analysis of shrimp muscle structure by SEM indicated that before storage (day 0), muscle fibers were well maintained; they were relatively thin and evenly spaced, and there was no aggregation (Figure 3). However, at the end of the storage period (day 30), muscle fibers became coarser and appeared to aggregate, the gap between fiber bundles increased, and the uniform and delicate muscle microstructure was destroyed. AP shrimp showed larger crevices in muscle fibers and coarser muscle tissue than VP shrimp both at 37°C and 20°C. Similar results were obtained by Yingying et al. [40] who studied the influence of long-term storage on shrimp muscle microstructure. In order to better maintain the muscle microstructure of dried shrimp, VP packaging should be used during storage, and it should be placed in a room temperature environment of 20°C.

3.2. Establishment of the Kinetic Model. Next, we developed kinetic models for predicting quality of VP shrimp during storage. The results of the kinetics order of reactions determined by a graphical method are given in Table 2. Moisture, TVB-N, SH, and carbonyl contents were important indicators of quality changes in dried shrimp during storage at different temperatures as evidenced by regression coefficients ($R^2$), which were all above 0.89. Changes of moisture and SH contents were well-fitted into the second-
order kinetics model, whereas those of TVB-N and carbonyl contents obeyed the zero-order kinetics model.

The activation energy \( (E_A) \) values of moisture, TVB-N, SH, and carbonyl contents were 37.64, 16.47, 8.10, and 12.22 kJ/mol, and the corresponding preexponential constant \( (k_0) \) values were \( 3.08 \times 10^3 \), \( 3.08 \times 10^2 \), 0.08, and 5.99, respectively, at 37°C and 20°C. Protein degradation and quality prediction models for dried shrimp based on moisture, TVB-N, SH, and carbonyl contents were obtained by inserting the \( E_A \) and \( k_0 \) values into equation (7).

The quality prediction model for dried shrimp is based on moisture content:

\[
\frac{1}{y_1(t)} = 0.02 + 3.08 \times 10^3 t \exp\left(\frac{-37640.12}{RT}\right).
\]  

(11)

Figure 3: Influence of different packing methods and storage temperatures on the microstructure of dried shrimp muscle (magnification: 1000). (a) 0 day. (b) 30-day VP 37°C. (c) 30-day AP 37°C. (d) 30-day VP 20°C. (e) 30-day AP 20°C.
The quality prediction model for dried shrimp is based on TVB-N content:

\[ y_{3(t)} = 10.48 + 3.08 \times 10^2 t \exp\left(\frac{-16469.16}{RT}\right). \] (12)

The protein oxidation prediction model for dried shrimp is based on SH content:

\[ \frac{1}{x_{1(t)}} = 7.09 + 0.08t \exp\left(\frac{-8099.06}{RT}\right). \] (13)

The protein oxidation prediction model for dried shrimp is based on carbonyl content:

\[ x_{2(t)} = 2.40 + 5.99t \exp\left(\frac{-12218.01}{RT}\right), \] (14)

where \( y_1(t) \), \( y_3(t) \), \( x_1(t) \), and \( x_2(t) \) are the predicted values of moisture, TVB-N, SH, and carbonyl contents, respectively, in dried shrimp stored for a certain time at 37°C and 20°C.

### 3.3. Correlation Analysis of Protein Oxidation and Quality and Establishment of the Prediction Model

Table 3 provides the correlation between protein oxidation indexes and the quality of dried shrimp during storage under VP conditions at 37°C and 20°C. The results indicated that protein oxidation indexes were significantly associated with moisture and TVB-N content, some showing positive and the other negative correlations. Thus, there was a significant correlation between SH and TVB-N contents with high Pearson coefficient and \( p < 0.01 \) in a two-tailed test. Carbonyl and moisture contents also showed a significant correlation with high Pearson coefficient and \( p < 0.01 \) in a two-tailed test. Therefore, the changes in moisture and TVB-N contents could be predicted by carbonyl and SH contents, respectively.

According to equations (11) and (14), the model predicting moisture content based on carbonyl content can be calculated as

\[ \frac{1}{y_1} = 0.024 + 513.167(x_2 - 2.402)\exp\left(\frac{-25422.09}{RT}\right). \] (15)

According to equations (12) and (13), the model predicting TVB-N content based on SH content can be calculated as

\[ y_3 = 10.48 + 3691.185\left(\frac{1}{x_1} - 0.141\right)\exp\left(\frac{-8370.14}{RT}\right). \] (16)

### 3.4. Validation of the Predictive Models

The validity of quality prediction models based on protein oxidation in dried shrimp during storage at 20°C was verified by comparing the predicted and observed values of each protein index. Table 4 provides the moisture content measured in real samples and predicted based on carbonyl content in dried shrimp at 293 K. Table 5 provides TVB-N content measured and predicted based on SH content in dried shrimp.
shrimp at 293 K. The relative differences between the predicted and observed values of each selected quality index were used to assess the performance of quality prediction models based on protein oxidation [41]; if the relative errors were below 10%, the model was considered to be acceptable [42]. In the present study, the relative errors for moisture and TVB-N contents were within 10%, indicating that the two models were sufficiently reliable.

4. Conclusions
In this study, we analyzed the changes in the quality and protein oxidation of solar-dried P. vannamei shrimp during storage. According to the variations in \( \Delta E \), protein composition, muscle microstructure, and moisture content, VP shrimp was preserved better than AP shrimp. The Arrhenius equation used to analyze the kinetics of various indexes in VP shrimp during storage indicated that moisture and SH contents fitted well into the second-order kinetics model, whereas TVB-N and carbonyl contents obeyed the zero-order kinetics model. There was a significant correlation between moisture and carbonyl contents and between TVB-N and SH contents. The models of predicting moisture content based on carbonyl content and TVB-N content based on SH content were established and their reliability verified.

Data Availability
The data used to support the findings of this study are included within the article. Raw data are available from the corresponding author upon request.

Conflicts of Interest
The authors declare that there are no conflicts of interest.

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**Table 5:** Predicted and observed values of TVB-N for dried shrimps at 293 K.

| SH     | Predicted value | Observed value | Relative errors (%) |
|--------|-----------------|----------------|---------------------|
| 7.092 ± 0.036 | 10.480 ± 0.187 | 0.00            |
| 6.374 ± 0.085 | 12.366 ± 0.266 | −8.38           |
| 5.694 ± 0.103 | 14.592 ± 0.288 | −14.15          |
| 5.733 ± 0.040 | 14.452 ± 0.298 | −5.98           |
| 5.683 ± 0.030 | 14.635 ± 0.312 | −2.2            |
| 5.564 ± 0.041 | 15.080 ± 0.361 | 1.74            |
| 4.929 ± 0.129 | 17.831 ± 0.409 | −6.64           |
| 4.946 ± 0.055 | 17.753 ± 0.237 | 4.10            |
| 4.425 ± 0.106 | 20.582 ± 0.170 | −9.74           |
| 4.360 ± 0.045 | 20.977 ± 0.114 | −4.11           |
| 4.319 ± 0.046 | 21.239 ± 0.240 | −1.12           |

Data are mean ± SD (n = 3).
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