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The combination of microbiological, biochemical, and quality index methods in quality evaluation of Pacific white shrimps *(Litopenaeus vannamei)* preserved at 0°C

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Abstract:

In this study, the sensory, microbiological, and biochemical qualities were used to examine the quality of Pacific white shrimps (*Litopenaeus vannamei*) preserved at 0°C during a 10-day period. The sensory quality was evaluated by a quality index (QI) that resulted from a quality index method (QIM) scheme. Meanwhile, the total viable count (TVC) and K-value were used to assess the microbiological and biochemical qualities of the shrimp. On day 9, the results from TVC and QIM have shown that the shrimp showed signs of spoilage, corresponding to a log CFU/g of 6.4 and a QI of 21.37, which is unacceptable to consumers. The QI increased linearly with storage days therefore the remaining shelf-life of the shrimp was estimated from a linear regression equation. In particular, this study found a linear relationship between QI, K-value, and hypoxanthine content. Furthermore, hypoxanthine itself could be considered as an independent quality index like the K-index. In conclusion, the quality of Pacific white shrimp was categorized into four different classes: excellent, good, acceptable, and moderately acceptable, based on its sensory and biochemical quality indicators.

Keywords: **hypoxanthine, K-value, Pacific white shrimp, QIM.**

Classification number: **3.1**

Introduction

Shrimp are an important source of seafood with considerable nutrition and economic value in many countries around the world [1]. In Vietnam, Pacific white shrimp (*Litopenaeus vannamei*) have achieved a high export turnover rate in the seafood industry in recent years. However, the method used to assess the quality of shrimp in particular, and seafood in general, is not consistent with the current methods in other parts of the world. The quality of post-harvest shrimp decreases with storage time due to the impact of three main factors, which include the activity of endogenous enzymes, microorganisms, and chemical reactions. These activities alter the sensory state, chemical composition, as well as the total amount of aerobic microorganisms in shrimp [2, 3]. Therefore, methods that assess sensory, chemical, microbiological, and physical qualities are established based on these impact factors [2]. The organoleptic quality assessment method is based on variations of sensory properties including the color, smell, taste, and texture. The intensity rating method is considered the most popular at present, followed by the Torry scheme, quantitative descriptive analysis (QDA), and QIM, to name a few. Among those, QIM is predicted by Hyldig as the potential method for quality assessment in the European Community. QIM was developed by the Tasmanian Food Research Unit in Australia [4] and is continually being developed. QIM possesses many advantages such as short training time, short evaluation time, and high reliability in assessing the freshness of seafood preserved in ice [5]. The difference between QIM and other methods is that QIM is built to evaluate the sensory changes of specific species. QIM focuses on the correlation between the quality index

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and the storage time expressed via a linear regression equation. Thus, QIM allows an estimation of the remaining shelf-life of seafood. So far, QIM-Eurofish has developed 28 QIM schemes mainly for fish, shrimp, and squid species. However, the QIM scheme for Pacific white shrimp has not yet been developed [6]. In Vietnam, shrimp quality is assessed according to TCVN 3276-89 [7]. This standard is generally applied to all shrimp species and is not an intensity rating method, however, it is a semi-quantitative assessment that describes changes in the properties of shrimp. Therefore, quality results from TCVN 3276-89 do not resemble those obtained by the rating method used in importing countries. This leads to disputes over quality and financial losses for exporting enterprises. After shrimp die, the process of self-decomposition takes place immediately, which is followed by the process of decomposition [8]. This autolysis is caused by endogenous enzymes in the shrimp and results in the release of nucleotides and its derivatives. In 1959, Saito and his colleagues introduced the "K-value", which is calculated based on nucleotides and their derivatives. The "K-value" has been formulated as an index of freshness in seafood [9]. Unlike quality indicators such as TVB-N, TMA-N, or histamine that are suitable for assessing quality during the decomposition stage, the K-value tends to linearly correlate with storage time [10-15]. This allows researchers to develop a regression equation between the QI and K-value.

Therefore, this study aims to develop a complete quality assessment process including the analytical methods measuring the microbiological, biochemical, and sensory changes. The expected findings include the quality classification of Pacific white shrimp based on the QI and K-value as well as the linear regression equations between the quality indicators investigated.

Materials and methods

Shrimp collection and storage

Healthy Pacific white shrimp were purchased from the Binh Dien market in Ho Chi Minh city. The shrimp samples were then washed with filtered water and packaged in polyethylene zipper bags (26.8×27.9 cm) (Alcoa Products Inc., VA, USA). These sample bags were placed directly in a polystyrene container containing shaved ice with a ratio of 1 shrimp to 2 shaved ices (w/w) and were delivered to the testing facility within 2 hours. At the testing facility, the sample bags were preserved at 0°C in a refrigerator for research purposes.

Reagents

Reagents including Adenosine-5'-triphosphate (PubChem CID: 5957), Adenosine-5'-diphosphate (PubChem CID: 6022), Adenosine-5'-monophosphate (PubChem CID: 6083), Inosinmonophosphate (PubChem CID: 8582), Inosine (PubChem CID: 6021), and hypoxanthine (PubChem CID: 790) were purchased from Sigma - Aldrich (Merck KGaA, Germany). Other reagents such as methanol (PubChem CID: 887), ethanol (PubChem CID: 702), and HPLC-grade water were bought from Merck Vietnam Ltd. Co.

Determination of TVC

TVC was measured according to Leboffe and Pierce (2015) [16]. In brief, 10 g of peeled shrimp were minced and mixed with 90 ml of 0.9% NaCl solution and then centrifuged at 3000 rpm at 30°C for 5 min. The pour-plate method using plate count agar was applied to determine the total plate counts. The extract from the samples were inoculated on agar plates and incubated at 30°C for 48 h before the colonies were counted. The experiments were repeated three times and the TVC values were expressed as log CFU/g (colony forming units).

Development of QIM scheme

The QIM scheme was developed based on three main steps according to Bernardi, et al. (2013) and Le, et al. (2017) [17, 18], which include: (1) identification of quality attributes, (2) main sensory evaluation, and (3) quantification of the developed (sensory) QI, as mentioned below.

Step 1 (preliminary program): three sensory experts observed all the changes in quality attributes (e.g. shape, odor, texture, eyes, shell color, and formation of black spots on the abdomen, body, tail). The terms were noted from direct observation [18-20]. The CATA (check all that apply) method was applied to select the most suitable descriptive terms from the preliminary terminology. Each attribute was then given a score from 0 to 3, with a low score suggesting a high quality level.

Step 2 (main program development and panel training): the shrimp samples were evaluated daily for ten consecutive days at 0°C. The start of day 0 was determined to be right after the shrimp arrived at the testing facility. The examination of the shrimp at different time points was conducted by six sensory panelists for one month. The panelists evaluated the samples and recorded the results without knowing the storage time. This process ensured accuracy, reliability, and minimized bias in data.

Step 3 (quantification of the recorded sensory QI): this step primarily involved quantification of the data collected from the QIM scheme. The regression equation between the days in storage and QI was developed to compare the projected shelf life with actual shelf life of shrimps. The data collected from ten different samples corresponded to each of the 10-day storage periods.

ATP, related compounds, and K-value

Nucleotides and related compounds including ATP, ADP, AMP, IMP, inosine, and hypoxanthine were measured by high-performance liquid chromatography with a diode array detector (HPLC-DAD). In this method, nucleotides and related compounds were extracted with 0.6 M perchloride acid and detected by the 2018 Cosmosil application (Nacalai Tesque, Japan) and Veciana-Nogues, et al. (1997) [21, 22]. In summary, 3±0.01 g of shrimp meat was blended with 10 ml of 0.6 M perchloride acid for 10 min by vortex. The mixture was then centrifuged at 3000 rpm for 10 min to collect the supernatant. This process was repeated three times. The extract was neutralized to pH 7.0 using 1 M KOH filtered through Whatman Grade 1 filter paper (Sigma-Aldrich, Germany) and diluted to 50 ml by volume. One milliliter of the diluted extract was uploaded into an SPE C18 column (Agilent Technologies, USA) and eluted with a 0.05 M K_2HPO_4 buffer to harvest 10 ml. Then, 50 µl of the harvested extract was injected into the column for the analysis of nucleotides and related compounds by HPLC. HPLC analyses were performed using an Agilent 1260 apparatus (Agilent Technologies, USA) equipped with diode array detector (Agilent 1260 detector), 5C18-PAQ column (size 5 µm, length 250 mm - 4.6 mm I.D) (Nacalai Tesque, Japan) at 30°C. The mobile phase of 0.05 M K_2HPO_3 was controlled at a flow rate of 1 ml/min. Nucleotides and related compounds were detected at a wavelength of 260 nm.

The K-value was calculated as the percent ratio of inosine (HxR) and hypoxanthine (Hx) to the sum of ATP and degradation products as shown below.

K-values(%)=100(inosine+hypoxanthine)/(ATP+ADP+AMP+IMP+inosine+hypoxanthine).

Data analysis

All experiments were conducted in triplicate. The obtained data were preprocessed with Microsoft Excel (version 2010) and analyzed using Statgraphics centurion software. A linear regression model was fit to the data. The significance level was at $p<0.05$.

Results and discussion

The change in TVC

The initial number of microorganisms in shrimp was mainly from the breeding environment [23]. The shrimp samples, after being washed with clean water, were stored at 0°C and the TVC with a log CFU/g value of 4.7 was recorded on day 1. The TVC values for the following days 2, 3, and 4 did not change significantly with 4.78, 4.89, and 5.01 (log CFU/g), respectively. However, starting from day 5, significant changes were observed ($p<0.05$). The TVC value was 5.55 on day 5, 5.78 on day 6, 5.77 on day 7, 5.89 on day 8, 6.4 on day 9, and 6.4 on day 10. The TVC values increased rapidly after day 4 for 10 days and exceeded 6 log CFU/g on day 9. The value of 6 log CFU/g is regarded as the limit allowed by the International Commission on Microbiological Specifications for Foods (ICMSF) for frozen shrimp. Thus, the shelf life of shrimps was set at day 8. Two recent studies by Naik, et al. (2014) [23] and Okpala, et al. (2014) [24] on black tiger and white shrimp samples have also shown that the shelf life are 8 days and 7 days, respectively. This difference in shelf life may be from different species, seasons, harvest techniques, age, and physiological conditions [2]. Microbiological changes in shrimp are closely related to sensory and biochemical changes. In the autolytic stage, as mentioned above, the main cause is due to the endogenous enzymes. Some components that are quickly metabolized during this period are ATP, glycogen that produces sweet IMP, and hypoxanthine derivatives that produce a bitter taste [2]. A decrease in pH, which is caused by lactic acid produced from glycogen digestion, facilitates the hydrolysis of the proteins of acidic enzymes. The hydrolysis of proteins forms basic substances such as TVB and biological amines [25]. This leads to the decomposition of proteins and lipids. This is the source of the unique flavors such as alcohols and aldehydes, especially aldehydes that have double bonds in the third position [26, 27]. Biochemical changes also strongly occur during the decomposition stage. TMA compounds have a fishy smell, which are formed by the reduction of TMAO and NH_3 resulting from the metabolism of amine acids by enzyme deaminases.

Development of QIM scheme for the quality evaluation of Pacific white shrimps

Changes in sensory qualities such as color, odor, and texture of head and tail were noted with preliminary descriptive terms. The CATA method was applied to select and arrange the terms in descending quality order as described in Table 1 [28].

Table 1. QIM scheme for Pacific white shrimp (*Litopenaeus vannamei*).

Characters		Description	Score
Odor	Meat	Fresh ocean smell	0
		Lightly fishy smell, smell of seashells	1
		Neutral or slightly trimethylamine (TMA)	2
		Strong trimethylamine (TMA)	3
Color	Head	Gray, gray - green, no spot	0
		Gray, gray - green, light yellow	1
		Light black, scattered black spots	2
		Black, large black spots	3
	Body	Shiny, gray, yellow, spotless	0
		Gray, green, some spots	1
		Gray, slightly pink, scattered spots	2
		Yellow, pink. Large black spots on the body and legs	3
	Tail	Gray, purple, spotless	0
		Gray, green, some spots	1
		Green, pink, large black spots	2
		Black	3
	Meat	Pearl color, translucent	0
		Translucent, silver-ish or gray-ish	1
		Milky white	2
		Milky white, pink, yellow	3
Texture	Shell	Hard, elastic	0
		Hard, slight elastic	1
		Slightly soft	2
		Soft	3
	Appearance	Head and body are intact. The head is tightly attached to the body. The tail is attached to the body	0
		Head is loose but body is still intact, and the tail is attached to the body	1
		Head is loosely attached to body; and flesh is loosely attached to shell	2
		Head is very loose, may not attached to the body; flesh is easily separated from shell	3
	Meat	Firm, springy, elastic	0
		Slightly less firm, less springy	1
		Soft, sticky, slightly elastic	2
		Soft, deformed	3

Other shrimp samples with different storage times were evaluated by the trained panel for ten consecutive days. Changes in sensory attributes, corresponding QI, and quality class from day 0 to day 10 are presented in Table 2.

Table 2. Sensory changes, QI, and Pacific white shrimp quality classifications during storage at 0°C.

Days	Descriptions	QI	Class
1	Shrimp has fresh ocean smell, blueish-gray head. Roes begin to turn yellow. The head has no black spots. Head is intact and tightly attached to the body Body is shiny gray and intact. The back is yellow. Body has no black spots Tail is gray, a bit purple at the top end and has no black spots. Tail is attached to the body Shell is hard and tough Meat is translucent, firm and elastic	1.32	Excellent
2	Shrimp has fresh ocean smell. Head is blueish-gray. Roes are light yellow. Head has no black spots. Head is intact and tightly attached to the body Body is shiny gray and intact. The back is yellow. Body has no black spots Tail is gray, a bit purple at the tip of the tail, and has no black spots. Tail is attached to the body Shell is less tough Meat is translucent, firm and elastic	2.90	
3	Seashell smell, lightly fishy. Roes are yellow Head is blueish-gray, lightly loose from the body, and has some black spots Body is shiny, gray, and green, has no spots Body is intact Tail is gray and blue and has some black spots Tail is attached to the body Shell is less tough and less hard Meat is translucent and grayish. Meat is less firm	5.60	Good
4	Seashell and fishy smell. Roes are yellow Head is blueish-gray. Black spots appear on the swimming legs. Head is intact Body is shiny, gray, and green. The body has black spots. Body is intact Tail is gray and blue, has some black spots. Tail is attached to the body Meat is translucent, silver gray. Meat is less firm Shell is less tough	8.87	
5	Seashell and fishy smell. Roes are yellow. Head is gray. Black spots appear on the swimming legs. Head is loose from the body Body is gray, and green. The body has black spots. Body is intact but lightly stretched Tail is gray and blue, has black spots. Tail is attached to the body Meat is milky white and lightly soft Shell is less tough	10.53	Moderately acceptable
6	Fishy smell. Roes are light orange. Head is pinkish gray. Black spots on swimming legs Head is loose from the body Body is gray; light pink on the back. The body has black spots. Body is intact but lightly stretched Tail is blue and has dark spots. Tail is attached to the body Crispy shell Meat is silver gray and lightly soft	14.12	

7	Fishy, ammonia smell Roes are light orange. Head is pinkish gray Black spots on swimming legs and head. Head is loose from the body Body is gray and pink. The body has large black spots. Body is stretchy Tail is blue, pink and has dark spots. Tail is attached to the body Crispy shell Meat is milky gray and soft.	16.93	Just acceptable
8	Fishy, slightly trimethylamine odor Roes are orange and broken. Head is pinkish gray. The head has large black spots. Head is loose from the body Body is gray and pink. The body has large black spots. Body is stretchy Tail is blue, pink at the tip of the tail. Tail is attached to the body Crispy shell Meat is milky gray and soft, not elastic, sticky	18.50	
9	Fishy, trimethylamine odor Head is orange, and black. Large black spots Head is loose from the body. Roes are broken Body is yellow, pink. Body has large black spots Black spots appear on the abdomen. Body is stretchy Tail is black. Tail is attached to the body Soft shell Meat is milky white, yellow, pink. Meat is sticky	21.37	Rejected
10	Strong trimethylamine smell (rotten smell) Head is orange, black. Large black spots. Head is loose from the body. Roes are broken Body is yellow, pink. Body has large black spots. Black spots are on the abdomen and legs. Body is stretchy Black tail. Tail is attached to the body Soft shell Meat is milky white, yellow, pink. Meat is sticky	22.77	

The changes in the sensory quality of Pacific white shrimp were divided into five stages corresponding to five storage periods: from day 1 to day 2; from day 3 to day 4; from day 5 to day 6; from day 7 to day 8; and from day 9 to day 10. In stage 1 (from day 1 to day 2), the shrimp underwent a period of stiffness and there were almost no changes in color, texture, and odor. The QI scores of day 1 to 2 went from 1.32 to 2.9, respectively. In stage 2 (from day 3 to day 4), the sensory characteristics of the shrimp were similar to those on days 1 and 2, however, the color of head and tail began to change and therefore the QI scores were 5.60 and 8.87, respectively. In stage 3, (from day 5 to day 6), the shrimp shell lost its glossiness and became slightly opaque. At this point, the shrimp were still quite fresh, milky white, and slightly soft, but they had lost their elasticity and had a few black spots on the head and tail. Thus, the QI scores were between 10.53 and 14.12. In stage 4 (from day 7 to day 8), the color of the head and tail changed considerably compared to the initial samples. The head and tail were slightly black and the head was attached to the loose body. The shrimp had a reddish body with a moderate density of black spots and opaque shell. The meat was not attached to the skin. It was slightly pink, soft, and had slightly sour smell, which acquired QI scores of 16.93 and 18.50, respectively. In stage 5 (from day 9 to day 10), the head was almost detached from the body, the shrimp had a foul and sour smell, soft meat, a lot of black spots, and no legs. The QI scores during this stage were 21.37 and 22.77, respectively. A linear relationship between storage days and QI score was found by a simple linear regression equation: QI=2.4×day-0.79 (R^2=0.990). The shelf life of Pacific white shrimp at 0°C was projected to 8 days corresponding to QI of 18.5. Le, et al. (2017), Okpala, et al. (2014), Nirmal, et al. (2009), and Hanpongkittikun, et al. (1995) [18, 24, 29, 30] also obtained a shelf-life for black tiger and white shrimp ranging between 7 and 8 days. In reference to previously published studies, we categorize the quality of Pacific white shrimp into 4 classes: Excellent (QI from 0-2.9), Good (QI 2.9-8.87), Moderately Acceptable (QI 8.87-14.12), and Just Acceptable (QI 14.12-18.50). The established QIM scheme was validated by comparison between its projected shelf-life and the actual shelf-life of the shrimp samples. Ten shrimp samples with different storage times were randomly selected and subjected to the QI evaluation. The QI score for each sample was the average score given by the sensory panel. The estimated shelf life was calculated using the linear regression equation (QI=2.41×day-0.79; R^2=0.990). The results in Table 3 show that the actual shelf life was not significantly different from the estimated shelf life ($p<0.05$). Table 3 describes the data from the 10 experimental samples.

Table 3. Validation of the QIM scheme with the projected and the actual shelf life.

Samples	QI	Equivalent number of storage days in ice	Projected shelf life according to QIM scheme	Actual shelf life (n=3)
1	1.14	0.8	7.2	7.07±0.06
2	2.82	1.5	6.5	6.5±0.10
3	4.27	2.1	5.9	5.97±0.12
4	6.43	3.0	5.0	5.03±0.12
5	10.04	4.5	3.5	3.57±0.15
6	13.17	5.8	2.2	2.23±0.15
7	2.58	1.4	6.6	6.50±0.10
8	12.93	5.7	2.3	2.23±0.12
9	6.92	3.2	4.8	4.97±0.15
10	9.80	4.4	3.6	3.50±0.10

Table 3 shows that the remaining shelf-life of the samples were nearly equal to the shelf-life calculated from the linear regression equation between QI and storage days. This validated the efficiency of the QIM scheme for Pacific white shrimp and proved that this scheme can be used to evaluate the quality and the classification of shrimp quality as shown in Table 2.

The change in K-value

Shrimp undergo the autolysis process quickly after harvest. ATP is one of the first metabolites in fisheries after death. The changes in nucleotide and related compounds are presented in Fig. 1. From this figure, the changes of ATP, ADP, AMP, IMP, and inosine became quickly prone due to the endogenous enzymes possess in fish and shellfish. In contrast, the transformation from hypoxanthine to uric acid occurred slowly. Therefore, the amount of hypoxanthine increased with the storage time. Fig. 1 depicts the ATP content and its derivatives resulting from the ATP autolytic process. The ATP components and their derivatives are expressed in μM/g. The contents of ATP, ADP, AMP, IMP, HxR, and Hx measured on day 1 were 0.4, 1.07, 7.03, 0.4, 0.44, and 0.59 μM/g, respectively. This proved that the metabolism from ATP to AMP occurred quickly at the beginning. The amount of ATP and ADP from the 3rd day onward had very low values in the remaining days. The amount of AMP decreased rapidly until day 4 (4.12 μM/g), meanwhile, the amount of IMP, Hx, and HxR increased gradually to 1.24, 1.09 , and 0.84 μM/g, respectively, on day 4. However, there were differences in these components in the following days: the amount of AMP decreased steadily; IMP increased until day 6 then decreased slightly to day 10; HxR almost did not change; while Hx increased gradually over the storage time. Thus, among the components considered, the Hx value increased linearly with the storage time and the linear regression equation between Hx and the storage day was Hx=0.177×day+0.33 (R^2=0.974). The K-value calculated from the equation increased with time of storage. The linear regression equation between the K-value and storage day was K-value=3.05×day+1.8 (R^2=0.992) (Fig. 2). Studies [10-15, 31] also show that the K-value and hypoxanthine amount increase linearly with storage time. This suggests that it is possible to use the amount of hypoxanthine to assess the quality change of Pacific white shrimps preserved at 0°C using the linear regression procedure between the K-value and hypoxanthine presented above.

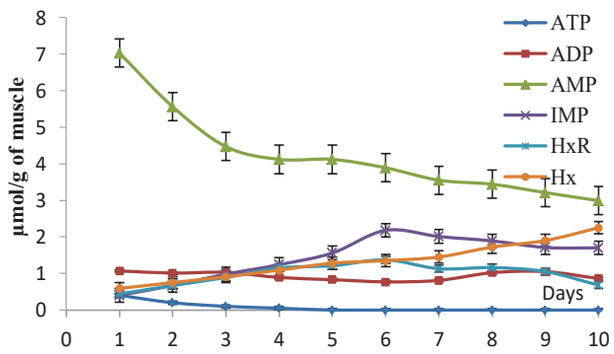

Fig. 1. Changes in nucleotide content and the composition of derivatives during 10-day storage. Adenosine triphosphate - ATP; Adenosine diphosphate - ADT; Adenosine monophosphate - AMP; Inosine monophosphate - IMP; Xanthine - HxR; Hypoxanthine - Hx.

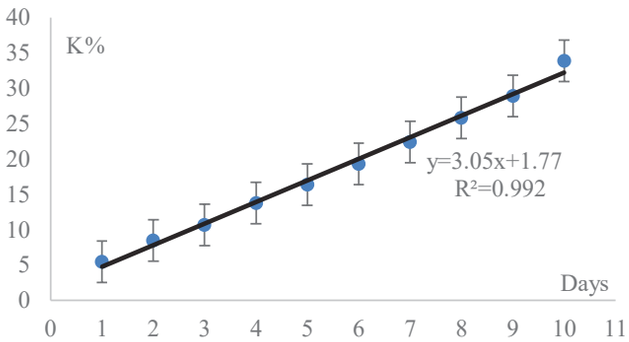

Fig. 2. Changes of K-value during 10-day storage.

Correlation between the quality indices and classification of shrimp quality

The results showed that the QI, K-value, and hypoxanthine correlated linearly with storage time. Therefore, these quality indicators correlate linearly with each other. The linear regression equation between the indicators are as follows: QI=0.79K-9.49 (R^2=0.956); QI=13.54Hx-7.44 (R^2=0.979); Hx=0.06K-0.13 (R^2=0.950). Table 4 presents the results of a combined quality classification of Pacific white shrimp between the QIM and chemical indicators including K-value and hypoxanthine.

Table 4. Quality classification of Pacific white shrimps based on QI, K-value, and hypoxanthine.

Quality class	QI	K-value	Hypoxanthine (Hx)
Excellent	0≤QI≤2.9	0≤K≤18.36	0≤Hx≤0.75
Good	2.9<QI≤8.87	18.36<K≤24.30	0.75<Hx≤1.09
Acceptable	8.87<QI≤14.12	24.30<K≤29.34	1.09<Hx≤1.35
Moderately acceptable	14.12<QI≤18.5	29.34<K≤ 34.11	1.35<Hx≤1.72

Conclusions

A QIM scheme has been developed for Pacific white shrimp (*Litopenaeus vannamei*) stored at 0°C. The scheme employs the descriptive terms for quality changes in sensory attributes in accordance with the quality requirements required by QIM (from 0 to 3). The quality of the shrimp was categorized into four classes, including Excellent, Good, Acceptable, and Moderately Acceptable, which are based on the QI score. The QIM scheme for Pacific white shrimp was validated for its accuracy and efficiency. This scheme can be combined with the K-value or hypoxanthine value for the quality assessment of Pacific white shrimp.

COMPETING INTERESTS

The authors declare that there is no conflict of interest regarding the publication of this article.

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