Quality Index Method for Freshness Assessment of Chilled Japanese Flying Squid (*Todarodes pacificus*)

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Author's contribution

Author NTTM designed the study, performed the study, statistical analysis and interpretation of data, wrote the protocol, managed the literature searches and review, and wrote the manuscript.

Article Information

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ABSTRACT

Aims: To develop and apply a quality index method (QIM) scheme for evaluating the freshness and estimating the shelf life of raw whole Japanese flying squid (*Todarodes pacificus*) stored at low temperatures.

Study design: A QIM scheme was developed through pre-observation and formation of a preliminary scheme, finalization of the protocol and training of judges. The scheme was then applied to evaluate the freshness of squid in comparison with control methods, which were quantitative descriptive analysis (QDA), Torry scoring, and total viable count (TVC) determination.

Place and duration of study: Nha Trang University, Vietnam, between February and June 2012; and from November 2019 to January 2020.

Methodology: A total of 399 Japanese flying squids (*Todarodes pacificus*) of 50-70 g stored at 0-2°C were used in the study. Seven judges engaged in the QIM establishment and application. Control methods were QDA, Torry and TVC check. Principle component analysis (PCA) was used for studying the dataset main variance. Partial least square regression (PLS-R) was carried out to evaluate the possibility to predict storage time of the developed QIM scheme.

Results: The formulated QIM scheme for Japanese flying squid stored at low temperatures composed of 8 parameters, which are odour, eyes, skin pigment, skin elasticity, body shape, body texture, ink sac integrity, and head-body connection, forming a quality index (QI) range of 0-15.

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The PLS-R showed that the developed QIM protocol could give a shelf life estimation accuracy of $\pm$ 1.0 day if 7 squids from each batch were evaluated. The PCA showed that QDA parameters clustered into good and bad attribute groups, which characterised flesh and old/spoilt squid, respectively. QDA, Torry and TVC results revealed a maximal squid shelf life of 12 days at 0-2 °C. **Conclusion:** The QIM scheme for chilled Japanese flying squid (*Todarodes pacificus*) could be used to estimate the remaining shelf life of the product with the precision of $\pm$ 1.0 day, using at least 7 squids of the same storage time for assessment. Squid exhibited a shelf life of 12 days at 0-2 °C.

1. INTRODUCTION

Word capture of squid, cuttle fish and octopus in 2018 was 3,636,575 tonnes, in which 2,566,749 tonnes went to international export with a value of US$ 12,085,264,000, contributing 3.84% in volume and 7.32% in value to the total exportation [1]. Global squid trade has been increased and forecasted to be over US$ 11.6 billion in 2025. Main markets are the United States of America, European Union, China, Japan, Korea, India, and Southeast Asia. While frozen squid is common in the North, fresh squid is popular in Asian local markets [2].

Squid, cuttlefish and octopus are commercial cephalopods which greatly contributes to the export of Vietnam with the total export value of US$ 560 million in 2020 [3]. Japanese flying squid (*Todarodes pacificus*) is a popular marine species of Vietnam, which is mainly purchased in the form of fresh/chilled products. Therefore, it is important to find a good and convenient method for monitoring the freshness of whole chilled-stored squid to keep the seafood in good quality for human consumption.

Sensory assessment is a very common method of freshness determination. Quality Index Method (QIM) is a simple, rapid and reliable organoleptic tool to measure the freshness of aquatic materials and products [4]. The method was derived from Tasmanian Food Research Unit (Australia) [5] and later has been developed in European Union [4], as well as around the globe (such as in Argentina [6], Brazil [7], India [8], Vietnam [9,10], etc.) for many seafood species and products. A protocol of QIM contains a list of sensory attributes with detailed descriptions of freshness-spoilage degrees, which are corresponding to a demerit point range from 0 (for very fresh seafood) to a maximum of 3 (for spoilt samples) [4]. The total score of all attributes forms the so-called quality index (QI), which is linearly correlated with the chilled storage time, making the method convenient to estimate the remaining shelf life of aquatic products [4]. Every QIM scheme is unique and characteristic as it is established for each seafood species and product type [4,11]. Up to 2020, about 100 QIM schemes were reported [4,12–14]. Regarding cephalopods, QIM protocols have been established for several species and types, such as, raw whole common octopus (*Octopus vulgaris*) in ice [12]; raw whole, washed and unwashed European cuttlefish (*Sepia officinalis*) and broadtail shortfin squid (*Illex coindetii*) in crushed ice [15]; raw whole, unwashed European cuttlefish (*Sepia officinalis*) in crushed ice at 0°C [16]; and refrigerated octopus species *Octopus insularis* at 2 $\pm$ 2°C [17].

This study aimed to develop and applied a QIM scheme for assessing the freshness and estimating the remaining shelf life of raw whole Japanese flying squid (*Todarodes pacificus*) stored at low temperatures.

2. MATERIALS AND METHODS

2.1 Materials, Handling and Storage Conditions

Seven batches of a total of 399 Japanese flying squids (*Todarodes pacificus*) of 50-70 g, caught from the sea of Khanh Hoa, Vietnam (FAO 71-TB 1, average sea surface temperature 27.5-28.9°C), was used for pre-observation (60 squids), sensory panel training (105 squids) and shelf-life study (210 individuals for sensory evaluation and the rest for microbiological analysis). The batches were continuously numbered (batch 1-7) according to the harvest time. This was to have the samples of at least two different storage times at each sensory

evaluation session [18,19]. Batches 1 and 2, caught on 27/02/2012 and 12/03/2012, respectively, were used for pre-observation and formation of the primary QIM scheme. Batches 3 and 4, received 15/05/2012 and 18/05/2012, correspondingly, were utilised for panel training and finalisation of the QIM protocol. Batch 5, harvested on 30/11/2019, was employed for re-training of judges and re-check of the QIM scheme applicability. Batch 6 (caught on 20/01/2020) and batch 7 (22/01/2020) were used for the main shelf life study.

Live squid was purchased from a local port and transported in expanded polystyrene (EPS) boxes to Nha Trang University laboratories which was 500 m away. At the laboratories squid was put into polystyrene (PS) trays (3-4 squids per tray), then into polyethylene bag, air-tight sealed, placed with alternative crushed ice layers in 5-kg EPS boxes (with ice on top and at the bottom), and stored in a climatic chamber at 0-2 °C. The 5-kg EPS boxes, punched holes at the bottom for draining, were checked every 2-3 days and new ice was added as necessary.

Squid was stored up to 15 days, and samples were taken every 1-4 days for sensory analysis and/or TVC determination. Day 0 was the purchase day (same as harvest day).

2.2 Methods

2.2.1 Development of the QIM scheme for chilled Japanese flying squid

Seven people participated in the development of the QIM scheme and sensory evaluation. They are staffs of the Faculty of Food Technology, Nha Trang University (NTU). All members have been trained on using QIM schemes for other fish species (such as farmed cobia, farmed *Pangasius*, Asian seabass). Observations of the squid were carried out under standardised conditions at room temperature using electric light and with minimum disturbance. The squid was collected from the EPS boxes, taken out from the trays and placed on a stainless steel table, 20 min before the evaluation.

2.2.1.1 Preliminary observation and formation of a preliminary QIM scheme

Three sensory panellists, experienced with QIM for seafood, observed both side of each squid every 2-3 days (7 squids from each storage time). Changes noted in appearance, odour and texture of squid during storage were listed in a preliminary QIM scheme.

2.2.1.2 Finalisation of the QIM protocol and training of panellists

Three 2.5-h sessions were used for the development of the scheme and training of the panel. In each session, 7 squids of 2 disparate groups, which had been stored between 0 and 15 days, were observed. The preliminary scheme was explained to the judges during the first training while observing squids of different freshness (storage time). All suggestions of improvements by the panel members were included in the final protocol. Then the panellists were trained for other two sessions, where the storage time of squid samples was hidden until each session finished. The latest version of the scheme (Table 1) was completed by the panel leader and introduced to the judges on the last training session.

2.2.2 Application of the QIM scheme for shelf life study of chilled Japanese flying squid

The QIM scheme was used for assessing the chilled Japanese flying squid. Five 30-45-minute sessions were carried out. The assessors (n = 7), uninformed about the sample storage days, evaluated 7 squids from each of the 2 different batches in each session. Each squid was coded with three randomised digits, placed in an arbitrary order and evaluated individually.

2.2.3 Sensory control methods

Sensory evaluation of cooked samples using quantitative descriptive analysis (QDA) [20] was carried out in parallel as a control method and to determine the maximum storage time of the seafood [18]. Torry scoresheet of cooked squid, developed by the Torry Research Station [21], was also used for cooked samples. All works were done according to standard procedures. During formulation of the QDA profile, the judges made a list of parameters illustrating the cooked squid under the guidance of a panel leader. The panellists were then trained in using an unstructured scale (from 0-100%) to express the intensity of each attribute before the sensory sessions started. A total of 182 squids was used in vocabulary development and training (42 squids); and evaluation sessions (140 squids). Squid was placed in aluminium boxes and cooked by steam at 95-100 °C in 10 minutes.

Each assessor received duplicate samples from 2 different storage days. The samples were coded with three random digits. The QDA profile included 7 attributes of odour (boiled potato; ammonia; characteristic; musty; rancid; fishy upon cooling; and putrid); 3 attributes of appearance (light - dark; mucus; uneven colour); 7 attributes of flavour (sweet; meaty; salty; fatty; pungent/bitter; rancid; and spoilt/putrid); and 6 attributes of texture (flakiness; soft-tough; fibre; mushy; friable; and dry-juicy).

2.2.4 Microbiological analysis

Total viable counts in duplicates were determined by pour plate method in plate count agar according to NMKL 86, 2006, incubated at $30 \pm 1°C$ for 3 days.

2.2.5 Data analysis

Microsoft® Excel 2003 was used to calculate means and standard deviations, and to build graphs. Multivariate analysis was performed by a trial version of Unscrambler® 11.0 software package (CAMO A/S). Principle component analysis was conducted to study the main variance in the dataset by Panel Check V1.4.2 software (Ålesund, Norway). Partial least square regression was carried out to evaluate the possibility to predict storage time of the developed QIM scheme.

3. RESULTS AND DISCUSSION

3.1 The Developed QIM Scheme for Chilled Japanese Flying Squid

The final QIM scheme for Japanese flying squid consisted of 8 attributes of 1-2 demerit scores and a total score or quality index of 0-15 (Table 1). The QIM protocol included odour, eyes, skin pigment and elasticity, body shape and texture, ink sac integrity, and head-body connection description. The maximal score assigned to each parameter depended on the level of its distintive changes over time.

For the squid *Loligo plei*, smell of fresh seafood like seaweed and/or marine water is typical for fresh samples of 1 or 2 days post catch, while opaque eyes characterise spoiled squid after 7 days at 6 °C [22]. The squid *Loligo duvauceli* has been described with fresh seaweed odour, sheen and glossy skin, elastic and firm texture at day 0 of storage in direct and indirect ice [23].

The QIM protocol of whole raw squid (*I. coindetii*) stored in crushed ice includes 8 parameters, which are skin appearance/colour (0-2), odour (0-3), mucus (0-1); flesh texture (0-2); eyes shape/appearance (0-2), ocular tissue (0-2); mouth region odour (0-3) and mucus (0-1), making a QI of 0-16 [15]. The QIM scheme of whole raw cuttlefish (*Sepia officinalis*) in crushed ice contains 9 attributes such as skin appearance/colour (0-3), odour (0-2), mucus (0-1); flesh texture (0-2); eyes cornea (0-2) and pupil (0-2); mouth region odour (0-3); and connection bone/head (0-1), forming a QI of 0-17 [15]. The QIM protocol for whole raw octopus (*Cistopus indicus*) composed of 10 descriptors, namely skin appearance/colour (0-2), odour (0-2), mucus (0-1); flesh texture (0-1); eyes cornea (0-2) and pupil (0-2); mouth region colour (0-1), odour (0-1), and mucus (0-2); and material in the sucker of aims (0-2), formulating a maximal QI of 16 [8]. The QIM scheme of refrigerated octopus *Octopus insularis* included 8 attributes, namely colour of dorsal region (eye side) (0-3), colour of ventral region (opposite to eye side) (0-3), colour of mouth region (0-3), skin elasticity (0-3), odour (0-3), mucus (0-3), mantle (flesh firmness) (0-3), and eyes (0-3); and a total score of 0-24 [17].

The findings of this study, together with the above research, indicate that each QIM scheme is unique for certain seafood species and product type.

3.2 Application of the Developed QIM Scheme in Freshness Evaluation and Shelf Life Estimation of Chilled Japanese Flying Squid

The formulated QIM protocol (Table 1) was used for assessing the freshness of squid and results were shown in Figs. 1 and 2. All the attributes had the scores increasing over time (Fig. 1) and the QI approached the maximum score by day 15 (Fig. 2). The changes with time were most evident for the "Odour", "Eyes", "Texture", and "Ink sac integrity" attributes. The "Skin pigment", "Skin elasticity" and "Head-body connection" parameters started to change after 2 storage days. It is common to have different onset and growth pattern in the demerit points of QIM parameters, for example it was observed for cuttlefish (*Sepia officinalis*) in ice that skin appearance (dorsal side) and eyes descriptors varied earlier than other attributes [15].

Table 1. Quality Index Method (QIM) scheme for Japanese flying squid (*Todarodes pacificus*) stored at low temperatures

Quality parameter		Description	Score
Odour		Characteristic for fresh squid, fresh seaweed	0
		Somewhat characteristic, slightly fishy, or neutral	1
		Off-odour	2
Eyes		Convex, bright	0
		Flat, somewhat opaque	1
		Sunken, opaque	2
Skin	Pigment	Pigment sac integrated, clear colour	0
		Pigment sac broken, smudged or discoloured	1
	Elastiscity	Elastic, not broken down upon being stretched	0
		Somewhat elastic, not broken down upon being stretched	1
		Inelastic, broken down upon being stretched	2
Body	Shape	Round puff body cross-section, the distance between two opposite sides "back" and "belly" when placed on a flat surface about 10-20 mm	0
		Slightly deflated, the distance between two opposite sides "back" and "belly" when placed on a flat surface about 5-10 mm	1
		Collapsed, the "belly" side stick to the "back" side when placed on a flat surface	2
	Texture	Hard, recovering quickly after being pressed	0
		Firm, recovering after being pressed	1
		Soft, not recovering after being pressed	2
Ink sac integrity		Integrated, no ink spreading out on the body and packing tray	0
		Slightly broken, some ink spreading out on the body or packing tray	1
		Broken, ink spreading out on the body or packing tray	2
Head-body connection		Firm, no disconnection upon holding squid tentacles in the vertical direction, disconnected when being strongly stretched	0
		Somewhat firm, no disconnection upon holding squid tentacles in the vertical direction, disconnected when being slightly stretched	1
		Loosly, disconnected upon holding squid tentacles in the vertical direction	2
QI (Quality index) (0-15)			

The mucus on the mouth region of broadtail shortfin squid (*Illex coindetii*) was perceived only after 5 days in ice, while other parameters changed sooner [15]. For refrigerated *Octopus insularis*, such parameters as colour of dorsal, ventral and mouth regions, skin elasticity, odour, and eyes were highly correlated with storage time, while those as mucus and mantle (flesh) firmness were not [17]. Discolouration of the skin has been known as a typical issue of cephalopods due to the breakdown of the skin chromatophores during storage and/or because of improper handling [8,24]. Endogenous proteases are major contributors to the tissue softening of cephalopods [8,25], however, the increase in autolytic activity at later stage has been explained by the microbial proteases [8,26].

The Quality Index (QI) was calculated for each storage day of sampling and formed a linear relationship with storage time (Fig. 2): QI = 0.9415*day + 0.1114 (R^2 = 0.9806). This is in accordance with the findings for other cephalopods, such as, for iced whole broadtail shortfin squid (*I. coindetii*) (R^2 = 0.9954) and iced whole cuttlefish (*Sepia officinalis*) (R^2 = 0.9939) [15]; and iced common octopus (*Octopus vulgaris*) (R^2 = 0.9954) [12].

The results were analysed with PLS-R to examine how the QI predicted the storage time of chilled squid (Fig. 3). The standard error of cross validation (SECV) may be used to evaluate the precision of predictability. As QI was the sum of 8 attributes evaluated in the QIM scheme,
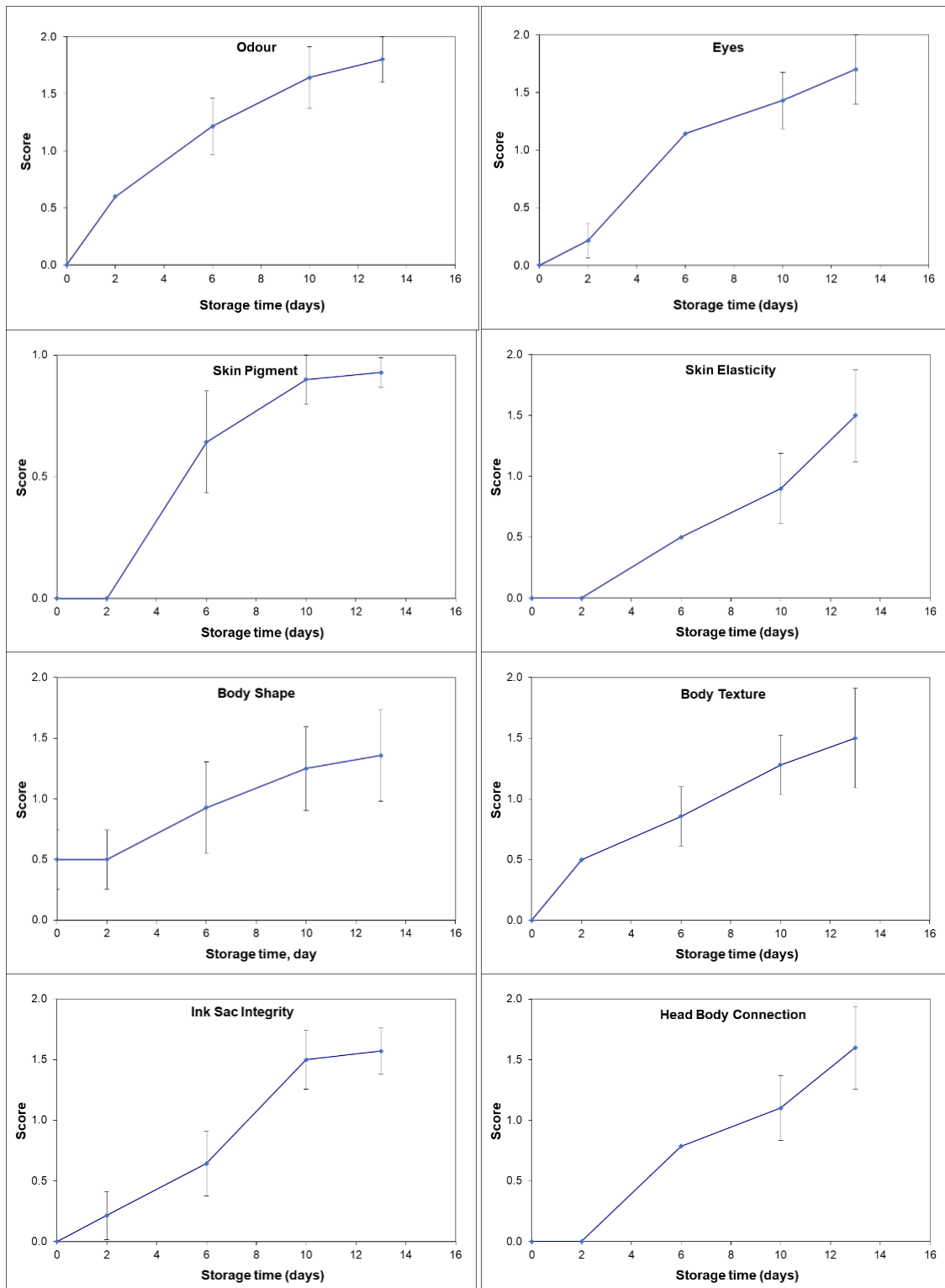
**Fig. 1. Changes in sensory scores of QIM attributes of Japanese flying squid
(*Todarodes pacificus*) versus days at 0-2 °C**
Mean scores and standard deviations of seven squids of a same batch (batch 7) assessed by seven panelists
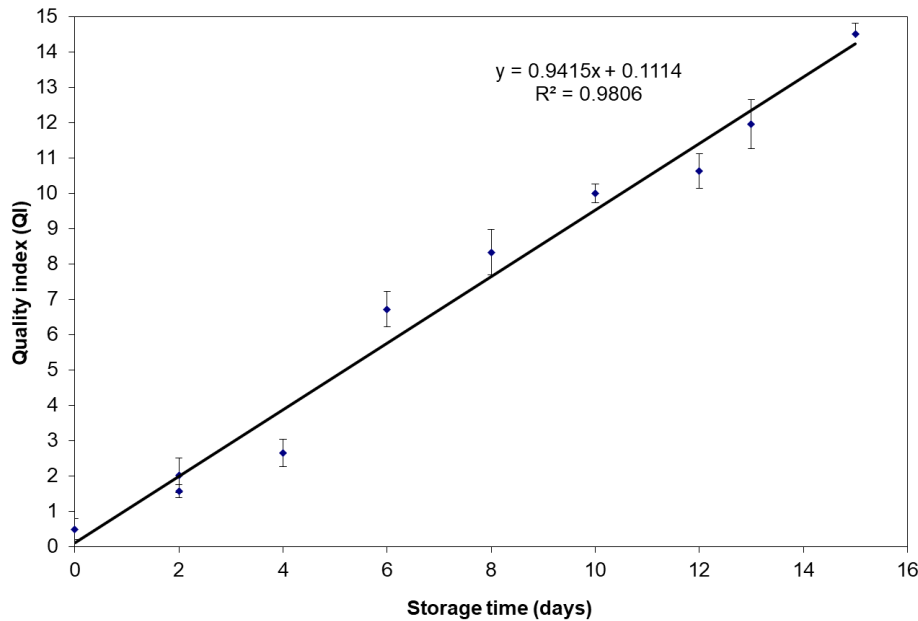
Fig. 2. Changes of Quality Index (QI) of Japanese flying squid (*Todarodes pacificus*) stored at 0-2°C
Mean scores and standard deviations of seven squids assessed by seven panellists

the measurement error may be assumed to be normally distributed, and the prediction can be considered as a t-distributed. Therefore, SECV x t (df = 8) = 0.87 x 2.306 = 2.0 could be regarded as a 95% confidence interval. So, it can be assumed that the QI (if 7 squids were assessed) could be used to predict storage time with ± 1.0 day. Based on the value of SECV, it is advisable to use 7 squids or more from each batch in the assessment, as using fewer individuals might reduce the accuracy of evaluation and predictability. The prediction precision of this QIM protocol is similar to those of other seafood species, such as European cuttlefish (*Sepia officinalis*): ± 1 days using 5 cuttlefish per lot [16], farmed Atlantic salmon (*Salmo salar*): ± 1.5 days with 5 salmon from each batch [18]; Arctic charr (*Salvelinus alpinus*): ± 1.3 days with 5 samples assessed [27], *Pangasius* fillets: ± 1.67 days using 3 fillets per lot [28].

There was a linear correlation between Torry score and storage time: $y = -0.227x + 8.4771$; $R^2 = 0.9095$ (x - Chilled storage days; y - Torry score) (Fig. 4). At day 13 of storage the score approached 5 which indicated slight sourness [21]. It was reported that *Todarodes pacificus* muscle refrigerated at 0-3 °C was in the passable stage with no smell and firm texture

after 6 days, lost its sensory quality after 8 days, and entered the advanced degradation stage with putrid odour and very soft texture after 10 days [29]. For the squid species of *L. plei* sensory quality became unacceptable after 7 days in contact and non-contact ice at 0-1 °C [22].

PCA results of QDA data are shown in Fig. 5. The first two principal components (PCs) explained in total 94.8% of the variation between the samples. The fresh squid data located on the left hand side of PC1, while those of the samples with longer storage time moved further to the right of this PC. This shows that the fresh squid was characterised by the following "good" attributes: mushy, soft and fibre texture (T-Mushy, T-Soft and T-Fibre), sweet and meaty flavour (F-Sweet and F-Meaty), characteristic and boiled-potato odour (O-Characte and O-BoiledPo), whereas old squid was characterised by "bad" attributes such as dark colour/appearance (A-Light-Da), flaky texture (T-Flaky), bitter, rancid and spoilage flavour (F-bitter, F-Rancid and F-Spoilt), musty, ammonia, rancid, fishy upon cooling and putrid odour (O-Musty, O-Ammonia, O-FishyUpo and O-Putrid). The PCA also shows that the attribute dry-juicy texture (T-Dry-Juicy) was not good enough
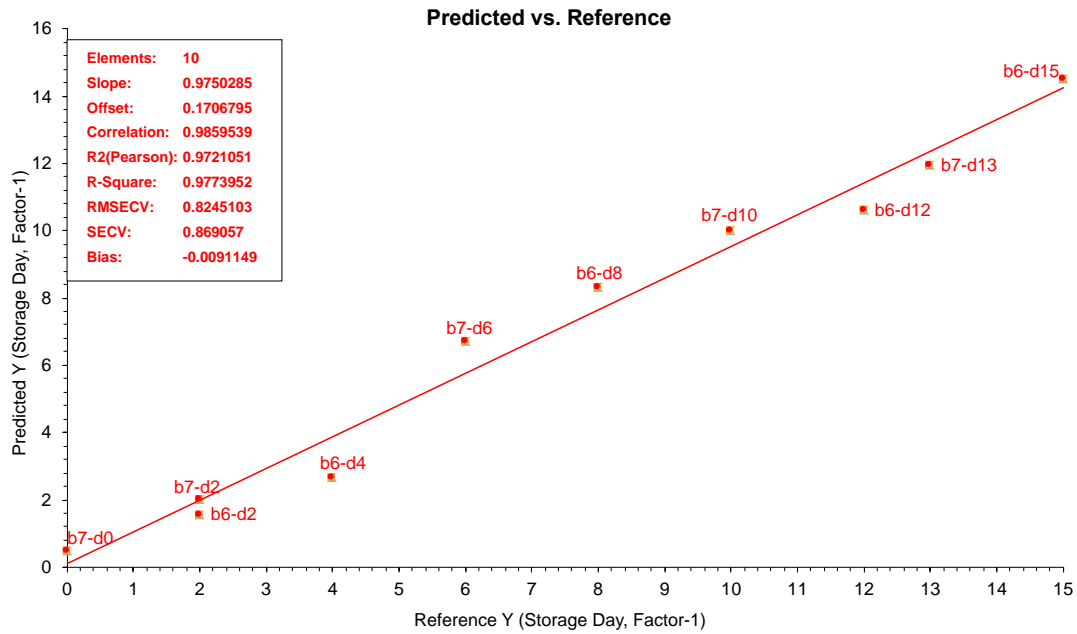
Fig. 3. PLS-R modelling of QIM data of Japanese flying squid (*Todarodes pacificus*) stored at 0-2 °C using full cross validation: Predicted against measured Y values, that is storage time from harvest

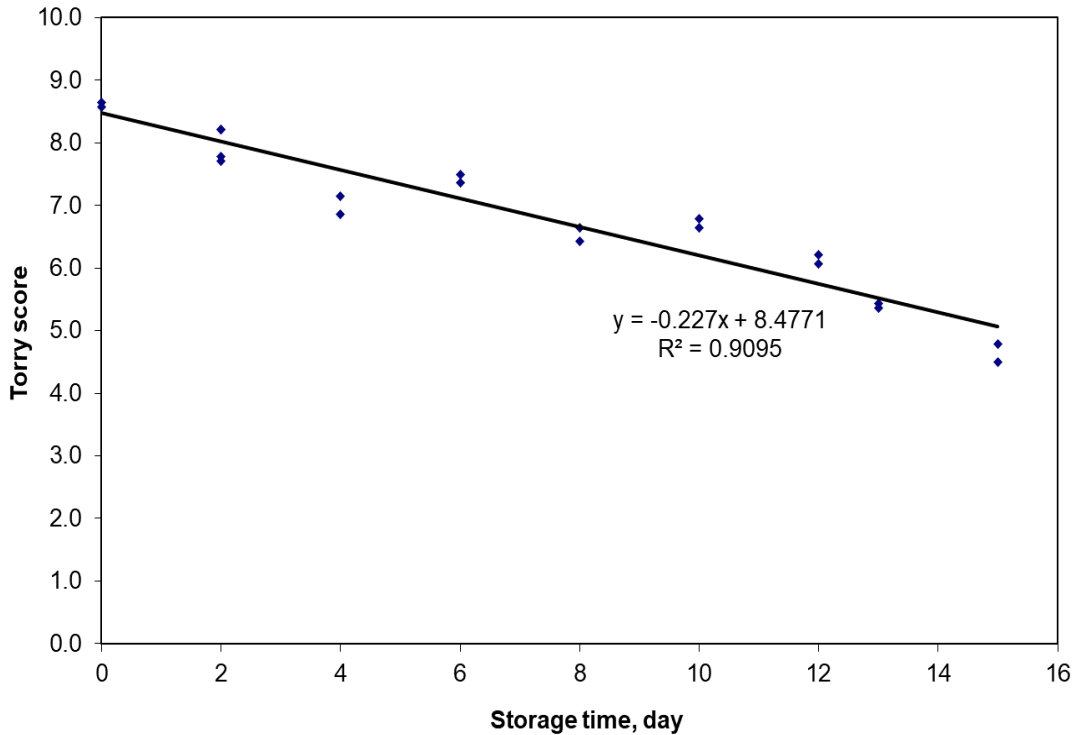*"b" stands for batch number and "d" for storage day*

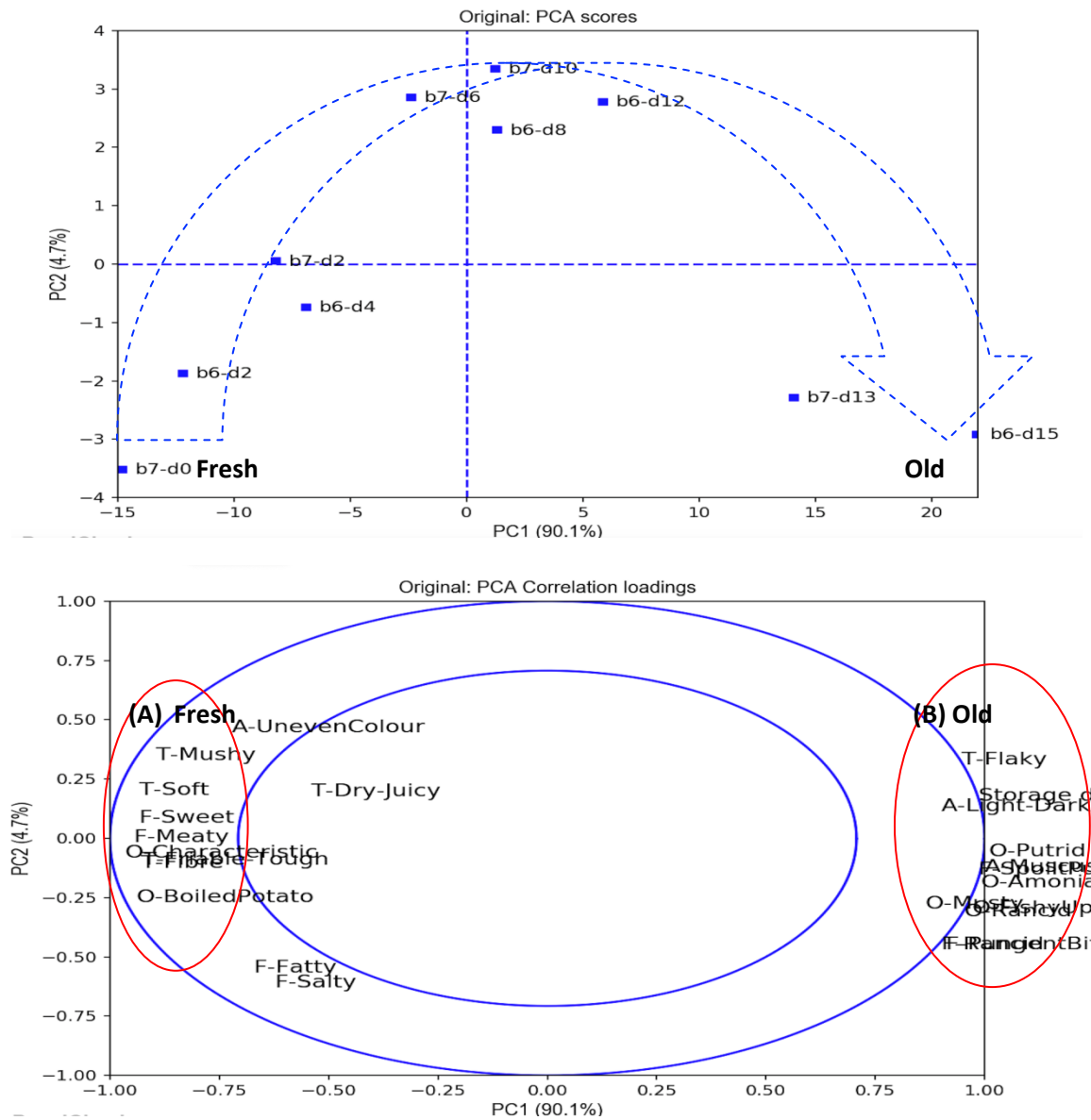**Fig. 4. Torry scores of Japanese flying squid over chilled storage time**

Fig. 5. PCA scores and correlation loadings plots of the QDA data
"b" stands for batch number and "d" for storage day
T = Texture; F = Flavour; O = Odour; A = Appearance; O-FishyUpo = Fishy upon cooling odour; O-Characte = Characteristic odour; O-BoiledPo = Boiled-potato odour; A-UnevenCo = Uneven colour appearance; A-Light-Da = Light - dark appearance
Circle (A) includes: T-Mushy, T-Soft, T-Fibre, F-Sweet, F-Meaty, O-Characte and O-BoiledPo. Circle (B) includes A-Light-Da, T-Flaky, F-bitter, F-Rancid, F-Spoilt, O-Musty, O-Ammonia, O-FishyUpo and O-Putrid

to characterise the changes of squid with time. Similarly, QDA parameters of Atlantic salmon also grouped into positive attributes (such as, seaweed odour and flavour; cucumber odour; sweetish, mushroom, and fish oil flavour; juicy and firm texture) and negative ones (such as, amine odour and flavour; sour flavour; rancid odour and flavour) [18]. Analogously, QDA descriptors of cooked tilapia clustered into freshness attributes (such as, sweet, arctic charr, and metallic flavour) and spoilage indicators (such as, musty, pungent, rancid, and spoilage flavour) [30].
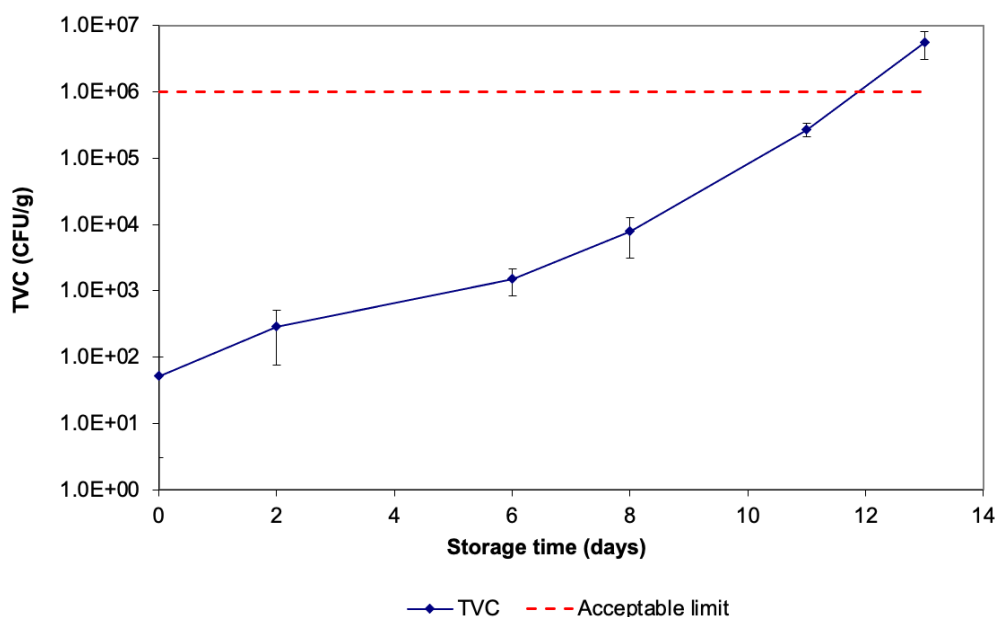
Fig. 6. Evolution of TVC in Japanese flying squid during chilled storage
Means and standard deviations of two replicates

The QDA results revealed that the maximum storage time of Japanese flying squid at 0-2 °C was 12 days, after which (day 13) rancid and putrid odour, bitter, rancid and spoilt flavour were detected with the scores higher than 20 (data not shown). The difference in rancid flavour between samples of day 13, day 15 and the rests could be seen by distinguished groups on the scores plot of Fig.5. This agreed well with the Torry result, which reached the value below 5.5 at day 13.

Microbiological results (Fig. 6) indicated that the TVC increased with time and surpassed the allowable limit for human consumption (10^6 CFU/g according to the Vietnam Ministry of Health) at day 13, which confirmed the maximum shelf life of chilled squid was 12 days at 0-2 °C. Psychrotrophic TVC of squid species *L. plei* was found to remain below 10^6 CFU/g after 16 days stored in contact and non-contact ice at 0-1 °C [22]. Likewise, the total count of psychrotrophic bacteria in red octopus (*Octopus maya*) stored at 4 °C showed a decrease from 6.4 log CFU/g at hour 18 (day 0-1) to 4.7 log CFU/g at hour 72 (day 3). Aerobic plate count of octopus (*Cistopus indicus*) stored in ice was 4.023 ± 0.14 log CFU/g at the beginning and became 3.096 ± 0.21 log CFU/g after 9 days [8]. These differences in microbiological loads over time might be due to the effect of seafood grounds, harvesting, handling and storage conditions, as well as

cephalopod species and microbial natures (mesophilic, psychrotrophic or else).

QDA, Torry and TVC results were in good agreement with the QIM outputs where the QI surpassed 3/4 of its maximal value after 13 storage days (Fig. 2). According to Sykes et al. [16], the acceptability score should be 3/4 of the total QI. This indicated promising application of the QIM scheme for freshness evaluation and shelf life estimation of Japanese flying squid (*Todarodes pacificus*) stored at low temperatures.

4. CONCLUSION

A QIM scheme was built and applied to evaluate the quality of Japanese flying squid (*Todarodes pacificus*) stored at 0-2 °C. A linear correlation between the QI and storage days was found. The QIM protocol could be used to estimate the remaining shelf life of the product with the precision of ± 1.0 day, if at least 7 squids of the same storage time were assessed. QDA, Torry scores and TVC evoked a maximal squid shelf life of 12 days.

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