

## Appraising the Shelf Life of Farmed Cobia, *Rachycentron canadum*, by Application of a Quality Index Method

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### Abstract

Freshness is the main concern of seafood quality, and the principal method to evaluate seafood freshness is sensory evaluation. The aim of this work was to study the quality changes of cobia, *Rachycentron canadum*, under ice storage through sensory and physical analysis as well as bacterial counting of specific spoilage organisms (SSOs). In particular, the utilization of a quality index method (QIM) scheme was proposed. Samples stored for 0–30 d were analyzed with the QIM. Ten panelists observed and registered the changes occurring in the fish starting on day zero and ending when the fish were spoiled. After developing the scheme, 11 sensory attributes were described in 23 points, which

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detailed the appearance of skin, eyes, abdomen, gills, and flesh deterioration. The volatile nitrogen compound measurements and microbiological data of the SSOs determined a shelf-life of 19 d for the samples. Sensory analysis showed a rejection point at 15.67 d of ice storage. The natural degradation of myofibrillar proteins and collagen was observed during ice storage. In conclusion, a shelf-life of 15 d was defined for raw cobia stored on ice.

Fish quality is a complex concept involving an entire range of factors that includes safety, nutritional quality, availability, convenience, and integrity for the consumer as well as freshness, eating quality, physical attributes of the species, size, and product type (Abbas et al. 2008). However, freshness is the most important quality factor for fishery because it is the first criterion for fish acceptance or rejection.

Sensory analysis has always played a key role in quality and freshness evaluation in the fish industry. This method has been considered rapid and inexpensive (Martinsdottir 2002), and it uses sight, taste, smell, and touch senses (Huidobro et al. 2000). However, sensory analysis quality depends on the sample, method used, and, primarily, the panelists (Stone and Sidel 1992), which are essential to determine fish shelf life (Huss et al. 2004).

The quality index method (QIM) was developed to evaluate cold fresh fish to improve the fish network in countries such as Australia, Norway, and Portugal (Bremner 1985). Currently, this method is applied to different products (Guillerm-Regost et al. 2006; Bonilla et al. 2007; Erikson et al. 2011; Mach and Nordvedt 2012). Based on a certain and systematic sensory analysis, QIM is a method to evaluate fresh fish worldwide (Martinsdottir et al. 2001). Because of the relevance of the QIM and its easy applicability, there are quality index protocols for 34 fish species and products (QIM-Eurofish 2012), and a free, digital quality index protocol for some of these species has been used for fish inspection in markets and industry.

Other techniques have been introduced to measure the physical and chemical parameters of fresh fish. Conducting a morphological analysis of food is important for evaluating the effects of storage on the microscopic structure of components that contribute to maintaining quality attributes. Recent studies have shown structural element changes of fish musculature promoted

by lytic processes that alter the biochemical composition of myofibrils (Pavlov et al. 2008) with losses in texture and meat quality.

Cobia, *Rachycentron canadum*, is considered a noteworthy candidate species for commercial aquaculture because it has rapid growth, excellent performance, good fillet quality, and high commercial prices. Shiao (2007) has examined cobia nutrition and performance, but focused on the quality of cobia fillets. The QIM scheme for cobia has only been reported in two publications, in which the authors developed a previous scheme for gutted cobia (Mach and Nordvedt 2012) and evaluated the applicability of a photochromic time–temperature indicator (TTI) to monitor the time and temperature history during the period of validity of the whole fish of the cobia specimen stored on ice (Brizio et al. 2014). No other studies have used the QIM to evaluate the physicochemical and microbiology properties of the whole fish.

Because of the relevance of the QIM and lack of information for some of the fish species, a more elaborate evaluation of cobia meat quality and freshness as well as the development of protocols of sensorial analysis has become necessary. The aim of this study was to develop and apply the QIM scheme for whole cobia stored on ice. Sensory evaluation, counts of specific spoilage organisms (SSOs), and physicochemical evaluations were performed to determine the shelf life of this product.

## Materials and Methods

### *Samples*

Three experiments were performed between January and September of 2011. The samples were obtained from a near shore production facility at Ubatuba, Sao Paulo, Brazil. The specimens of cobia were kept on ice (0–2 C) and transported to the laboratory in polystyrene boxes (within 24 h of harvest).

TABLE 1. Weight values, total length, and chemical composition of cobia, *Rachycentron canadum*, stored on ice.<sup>a</sup>

	Experiment 1	Experiment 2	Experiment 3
Weight (g) <sup>b</sup>	1513.02 ± 213.29	2587.00 ± 622.26	2634.64 ± 484.74
Total length (cm) <sup>b</sup>	47.60 ± 4.81	59.88 ± 4.44	60.25 ± 3.60
Moisture (%) <sup>c</sup>	72.18 ± 1.40	75.24 ± 0.62	77.05 ± 0.90
Crude protein (%) <sup>c</sup>	16.87 ± 1.73	17.19 ± 0.01	17.19 ± 0.01
Lipid (%) <sup>c</sup>	3.48 ± 0.32	5.82 ± 0.01	2.22 ± 0.13

<sup>a</sup>Mean + SD.

<sup>b</sup>*n* = 20.

<sup>c</sup>*n* = 6.

Three batches were captured (60 fish) from the same livestock. From each batch, 10, 5, and 2 specimens were randomly chosen for sensory, physicochemical, and microbiological analyses, respectively. The specimens were kept iced and boxed (0.2 + 0.7 C) in a room set at 17.60 ± 0.5 C, and ice was added daily. The initial morphometric and composition parameters of specimens in each experiment are presented in Table 1.

#### Sensory Evaluation

Ten trained assessors in a sensory test panel participated in the development of the QIM scheme. The assessors were employees and trainees of the Embrapa Fish Technology Laboratory. All members had prior training on fish sensory analysis and QIM schemes for other fish species.

Quality assessments of cobia were performed under standardized conditions at room temperature (25 C) using electric light and with as little distraction as possible. Cobia specimens were taken from the iceboxes and placed on a plastic tray with ice 20 min before the evaluation. Each fish sample was coded with a random three-digit number unrelated to storage time. In the preliminary observations, the experts evaluated daily the samples of cobia that were kept iced (0–2 C) for 15 d. Any changes observed in odor, eyes, gills, skin, and flesh deterioration were listed in a preliminary scheme. After these observations, 10 sessions of 1 h each were used to develop the scheme of the QIM sensory panel. In each session, 10 cobias, which had been stored on ice up to 24 d, were observed. Within the first 2 d of observing cobia freshness, the preliminary scheme was explained to the panelists. All suggested improvements by the panel members during the evaluation were included in the final

scheme. The final version of the scheme was finalized by the panel leader and presented to the panel on the last day of training.

The QIM application was made according to Sant'Ana et al. (2011). Ten cobia were assessed after 1, 3, 5, 7, 9, 11, 13, 15, 18, and 22 d of ice storage at the following three times: February, March, and September of 2012. The QIM scheme for whole cobia is presented in Table 2. The QIM scheme shows the quality attributes for flesh deterioration, gills, eyes, skin, and mucus, with scores ranging from zero to three points according to the changes in storage time.

#### Microbiological Analysis

Two cobia specimens were randomly chosen to perform the microbiological analysis. The specimens were used for mesophilic and psychrotrophic aerobic heterotrophic bacteria counting (Plate Count Agar – HIMEDIA<sup>®</sup>, LBS Marg, Mumbai, India), enterobacteria counting (Agar MacConkey – OXOID<sup>®</sup>, Basingstoke, Hampshire, UK), *Pseudomonas* spp (Pseudomonas Agar – OXOID<sup>®</sup>, Basingstoke, Hampshire, UK), and H<sub>2</sub>S-producing bacteria counting (Iron Agar – OXOID<sup>®</sup>, Basingstoke, Hampshire, UK) at intervals of 1, 4, 7, 11, 15, 18, 22, 25, and 29 d of storage using the technique of surface swabbing (Evancho et al. 2001). The plates with Pseudomonas Agar and Iron Agar media were incubated at 20 C for 24–48 h. Plates containing MacConkey Agar and Plate Count Agar for mesophilic bacteria counting were incubated at 35 C for 24–48 h, and the plates with Plate Count Agar for psychrotrophic bacteria counting were kept at 5 C from 7 to 10 d. After the incubation, the bacterial counting results were expressed as log<sub>10</sub>.

TABLE 2. *Quality index method score sheet developed for cobia, Rachycentron canadum, stored on ice.*

Parameter	Description	Score
Skin color/appearance	Iridescent pigmentation (black-silver), shining	0
	Shining reduction (some reduction on black)	1
	Distinct reduction, faded colors	2
Skin mucus	Transparent, not clotted	0
	Clotted and milky	1
	Clotted and yellow	2
Skin odor	Fresh	0
	Neutral to metallic, dry grass, grains	1
	Acrid	2
Eyes cornea	Rancid	3
	Translucent	0
	Slightly opalescent	1
Eyes pupils	Opaque, milky	2
	Black, clearly outlined (rounded)	0
	Slightly opaque, outline reduction	1
Eyes form	Gray, not outlined	2
	Convex, protuberant	0
	Flat, plane	1
Gills color	Sunken, concave	2
	Red/shining	0
	Pale red	1
Gills mucus	Gray-brown	2
	Transparent	0
	Yellow, clotted	1
Gills odor	Brown	2
	Fresh, seaweed	0
	Neutral, slightly seaweedy	1
Gills form	Slightly rancid	2
	Rotten	3
	Complete, well defined	0
Flesh deterioration/elasticity	Slightly deformed	1
	Deformed	2
	Finger mark returns quickly (<2 sec)	0
Quality index total	Finger mark returns slowly (between 3 and 2 sec)	1
	Finger mark returns slowly (>3 sec)	2
		0-23

### *Chemical Analysis*

Proximate composition analyses were conducted upon the arrival of the fish (day 1). Five randomly chosen fish were filleted and homogenized according to standard analytical methods (AOAC 2005). Moisture content was determined by the sample (2.0 g) weight difference before and after heating in an oven (QUIMIS, São Paulo, São Paulo, Brazil) for 24 h at 105 C. The total level of nitrogen was determined by the Kjeldahl procedure, and protein levels were estimated using a conversion factor of 6.25. Lipids were determined by extraction with petroleum

ether in a Soxhlet apparatus (SOLAB, Campinas, São Paulo, Brazil).

The pH and total volatile base nitrogen values of samples were investigated on days 1, 4, 8, 12, 16, and 20 of ice storage. The pH was determined using a digital pH meter (TESTO, model 205, Campinas, São Paulo, Brazil) equipped with a glass electrode (calibrated at pH four and seven), which was dipped into the fish. Total volatile base nitrogen (TVB-N) was determined by precipitating protein using trichloroacetic acid (TCA, 7.5%, Synth<sup>®</sup>, Diadema, São Paulo, Brazil) and evaluating the TCA extract using the

Micro Kjeldahl method according to a method adapted from Brasil (1981).

### *Histological Analysis*

Ten biopsy specimens were obtained from the dorsal muscles of two fish. The specimens were fixed in Carnoy's fixative, Bouin's fixative, and 10% neutral formalin. After removing the fixatives from the tissues using a water bath or ascending ethanol series, the material was cleared in xylene, soaked, and embedded in paraffin. Cross-sections of 6 µm were stained with hematoxylin-eosin (Ehrlich, Mumbai, India), and they were used to make permanent histological preparations (Vitanov et al. 1995). Each histological cross-section of all biological samples was studied with a light microscope.

### *Statistics Analysis*

The obtained sensorial analysis data were evaluated using the normality applied through the Shapiro–Wilk test (Zar 2010). Linear regression and Pearson's correlation between the quality index (QI) score versus iced storage were measured using the OriginPro 8.1 software.

Additionally, partial least squares (PLS) regression was used for determining the uncertainty associated with QIM prediction and the sensory attributes relevant for its development (Borges et al. 2013) using the XLSTAT for Windows version 2012.5 (Adinsoft, Paris, France).

## **Results**

The developed QI protocol showed 11 sensory attributes (Table 2) related to the skin (color/appearance, mucus presence, and odor), eyes (cornea, pupils, and form), gills (color, form, odor, and mucus), and flesh deterioration or elasticity of abdominal musculature (Fig. 1). The total of demerit points established was 23. However, some attributes did not show the highest score because their characteristics took longer to change.

The fish skin was not altered during the first 5 d of ice storage, and the distinctive color changed only after 10 d. The odor also changed slowly, scoring 2.68 with 22 d of storage. The skin

mucus was one of the attributes that showed a high variation due to leaching during manipulation and contact with ice, which became a less relevant attribute in the sensoria (Fig. 1).

The cornea and pupils showed noticeable changes, such as opalescence and an indistinct outline, during the first days of storage. The eye form was convex on the first day, and it was planar and flat for the 10 subsequent days, followed by a concave form during the rest of storage (Fig. 1).

The gill color and odor showed noticeable changes after the fifth storage day with highest scores on day 17 when the color was changed from red to gray-brown and the odor from neutral to rotten. After this period, the changes in the gill attributes occurred slowly. The gill form also changed slowly, following the color and odor pattern. The branchial arch formats were uniform and well defined during the first days of the experiment but became less defined, with the ends frayed and the arches agglutinated. Mucus was observed on day one, and it was transparent and not dense. On the following days of evaluation, the mucus became dense, darker, and yellow, with blood cuts on the 17th day of storage (Fig. 1).

Musculature elasticity took longer to show noticeable modification, which was flexible until day 10 of storage. The average scores for the quality attributes of the abdomen were low until approximately 8 d of storage, when they began to increase with the storage time (Fig. 1).

There was a linear relation between the total QI score and days of storage (Table 3, Fig. 2). This correlation was extremely high for whole cobia kept on ice ( $r^2 = 0.994$ ) during all experiments performed, thereby demonstrating that the developed QI protocol was consistent with all sensorial attribute changes of cobia during ice storage (Fig. 3). PLS regression (Fig. 4) described the correlation between cobia QIM quality parameters and showed those that have positive and negative importance in its development. The variable importance in the projection is relevant when attributes are  $>1.0$ . Thus, skin appearance, cornea, gills mucus, and abdomen elasticity had greater importance for cobia QIM. Attributes related to skin odor, form of eyes,

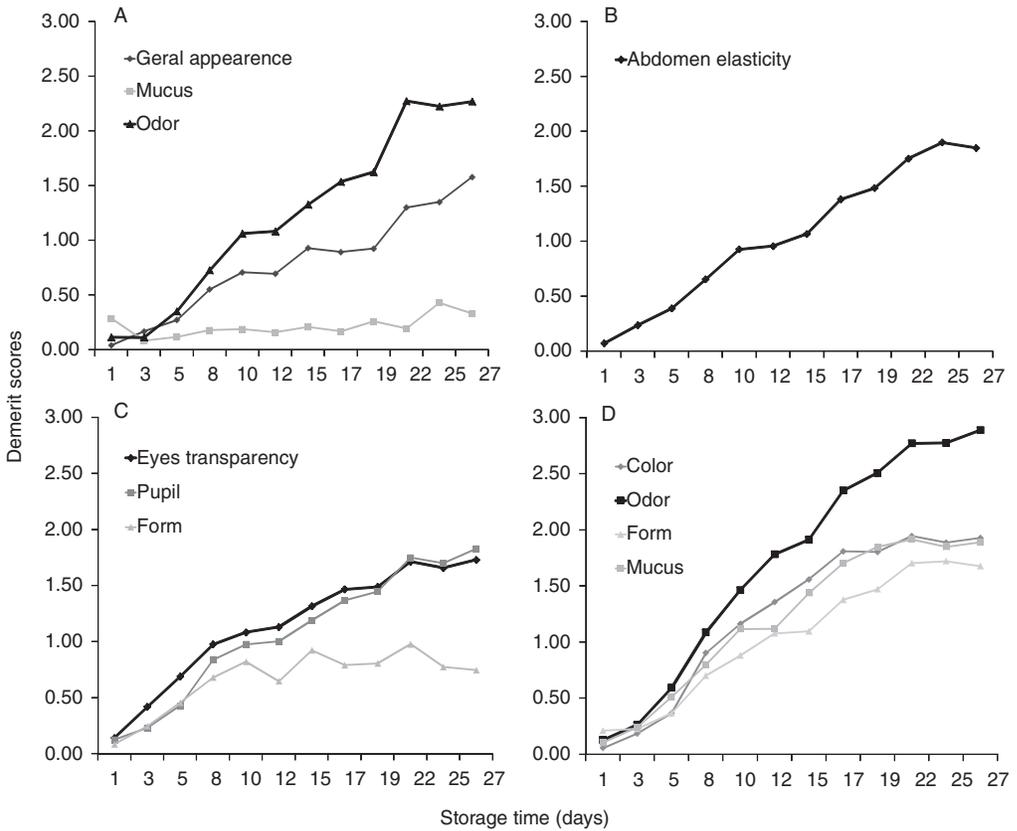


FIGURE 1. The mean demerit scores given on different storage days for each quality attribute of whole cobia, *Rachycentron canadum*, stored on ice: (A) skin; (B) flesh; (C) eyes; (D) gills.

TABLE 3. Pearson correlation and linear regression analysis of cobia, *Rachycentron canadum*, quality index versus storage time.

Experiment	I	II	III	All
Linear fit	$y = 0.739x + 3.673$	$y = 0.874x - 0.991$	$y = 0.876x - 0.467$	$y = 0.780x + 1.038$
Pearson's <i>R</i>	0.977	0.995	0.978	0.994
Adj. $R^2$	0.948	0.988	0.949	0.986
Residual sum of squares	8.309	2.543	11.317	1.389

and gills and color showed lower significance for the model (Fig. 4). These aspects do not express the true state of fish conservation and may lead consumers to reject a product fit for consumption.

Total volatile basic nitrogen (TVB-N) and pH measurements showed lower values for cobia. During the first 12 d of storage, cobia showed an average TVB-N value of 12.49 mg/100 g of muscle, which increased to 14.57 mg/100 g of muscle from days 15 to 29 of storage and lasted

until spoilage. The pH values ranged from 5.93 (24 h after slaughter) to 6.02 (after 18 d of storage) (Fig. 5).

Bacterial counting of SSOs showed, initially, low *Pseudomonas* spp, mesophilic bacteria, and  $H_2S$ -reducing bacteria counts ( $10^1$  CFU/cm<sup>2</sup>), while the psychrotrophic bacteria and enterobacteria counts ( $>10^3$  CFU/cm<sup>2</sup>) gradually increased from the first days of ice storage (Fig. 6). The bacteria numbers were low until day 12 of storage, but after this period, all

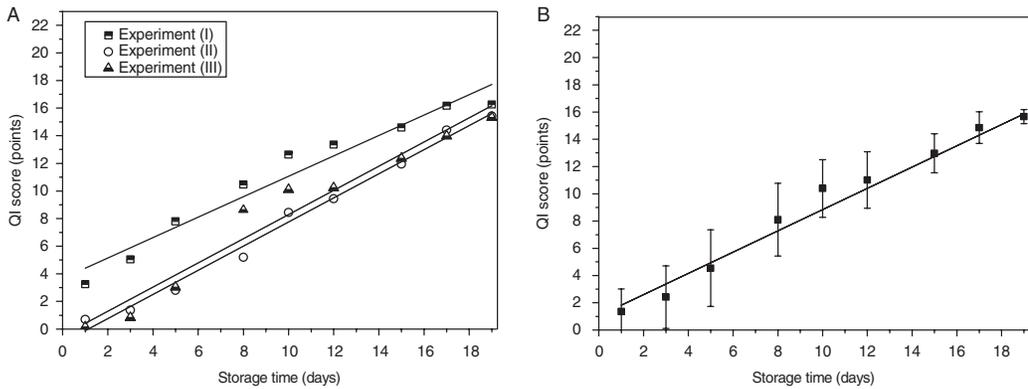


FIGURE 2. Quality index for whole cobia, *Rachycentron canadum*, stored on ice: (A) average of three experiments; (B) combined average.

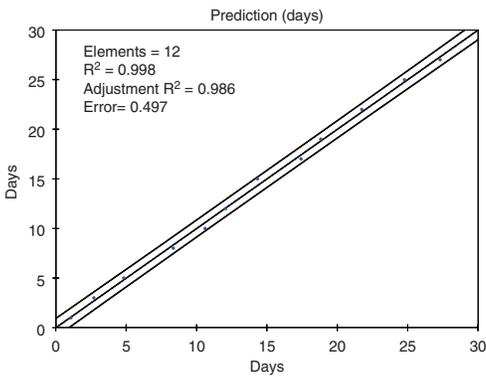


FIGURE 3. Partial least squares regression coefficient regression for quality index method parameters developed for whole cobia, *Rachycentron canadum*, during ice storage, with a 95% regression confidence.

analyzed bacteria showed a significant increase in the total counts. The highest count allowed of *Pseudomonas* spp ( $10^7$  CFU/cm<sup>2</sup>) was observed on day 17 of ice storage, and this same high count of enterobacteria was observed on day 20 of ice storage. Moreover, the highest counts allowed of mesophilic and psychrotrophic bacteria were observed on day 19 of ice storage, thus H<sup>2</sup>S-reducing bacteria did not show this same counting high (Fig. 6).

According to a model developed for predicting the quality of fish in the chill chain, values equal to or greater than 7.11 Log<sub>10</sub>CFU/g are related to the organoleptic rejection point (Koutsoumanis and Nychas 2000). Therefore, values next to 2.00 Log<sub>10</sub>CFU/g and 4.00 Log<sub>10</sub>CFU/g can

be considered low and moderate, respectively (Fig. 6).

The sensory results were corroborated by morphological analysis. During the first days of storage, the muscle fibers are well-defined hexagonal forms that have few extracellular spaces filled with collagen. After 7 d of storage, a loss of the muscle fiber structure was observed, which resulted in irregular and dehydrated muscle fibers. After 14 and 21 d of iced storage, the collagen fibrils in the pericellular connective tissue were disorganized and degraded, and the spaces between fibers increased in the muscle. Numerous cracks inside the fibers were also observed (Fig. 7). At this point, the flesh deterioration of sensory analysis showed that the muscle was soft because a finger mark returned slowly (Fig. 1).

## Discussion

The cobia specimens were fresh, and the odor was described as fresh seaweed or neutral from the beginning of the evaluation until 15 d of the storage time, which was similar to what has been observed for salmon (Sveinsdóttir et al. 2002). This observation could be related to the fact that freshly caught fish contains low levels of some volatile compounds which increase with the storage time, producing off-flavor. The sour and rotten odors, which are caused by short chain acids, amines, and sulfur compounds from microbial activity (Olafsdóttir and Fleurence

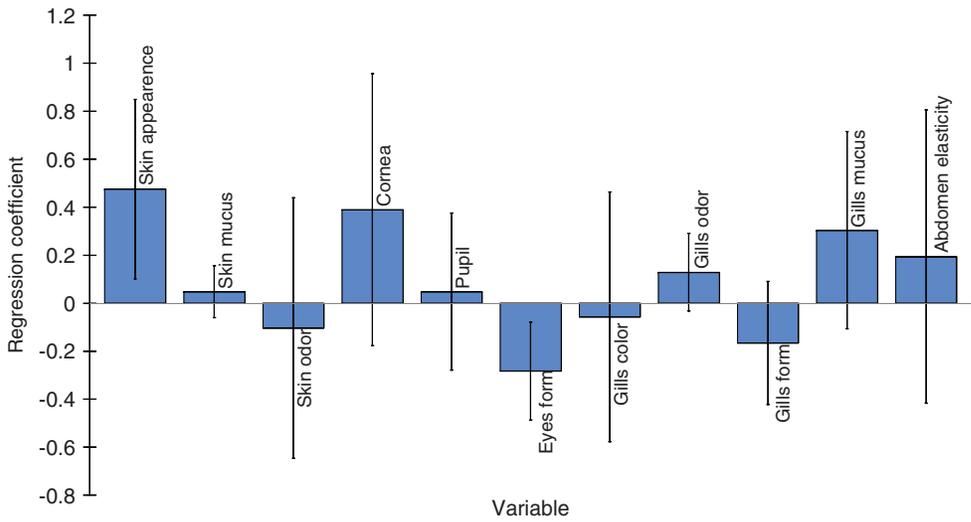


FIGURE 4. Partial least squares regression of quality index method with 23 demerit points versus the predicted scores. Traced lines represent 95% regression confidence limits.

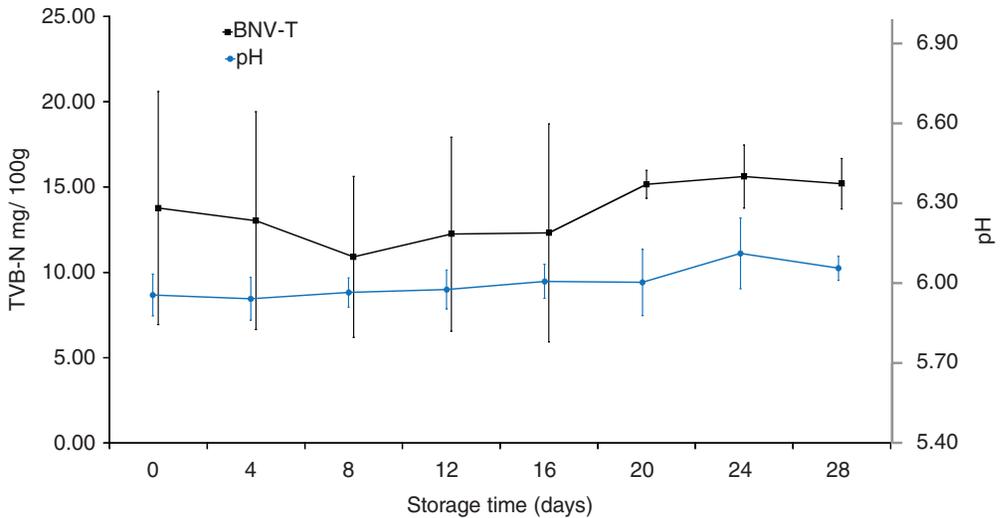


FIGURE 5. Nitrogen total volatile bases (TVB-N, represented by squares) and pH (represented by circles) of whole cobia, *Rachycentron canadum*, during ice storage.

1997), were observed after only 20 d of iced storage.

The eye form changes observed depend on the fish storage position. For example, the eyes are flattened when the fish is horizontally placed and convex if the fish is placed standing up or vertically (Campos et al. 2005).

The gill area is considered to be the part of a fish that is the most susceptible to microbial putrefaction. Therefore, the color and smell

change as the fish starts to spoil. A bright red color of the gills can be easily observed as it changes to brown and then to gray-brown, depending on the degree of spoilage (Lauzon et al. 2010). The change in blood color is a blood heme chemical breakdown that correlates with other symptoms of spoilage.

Musculature elasticity was flexible until day 10 of storage, when average scores for the QI began to increase with the storage time (Sveinsdóttir

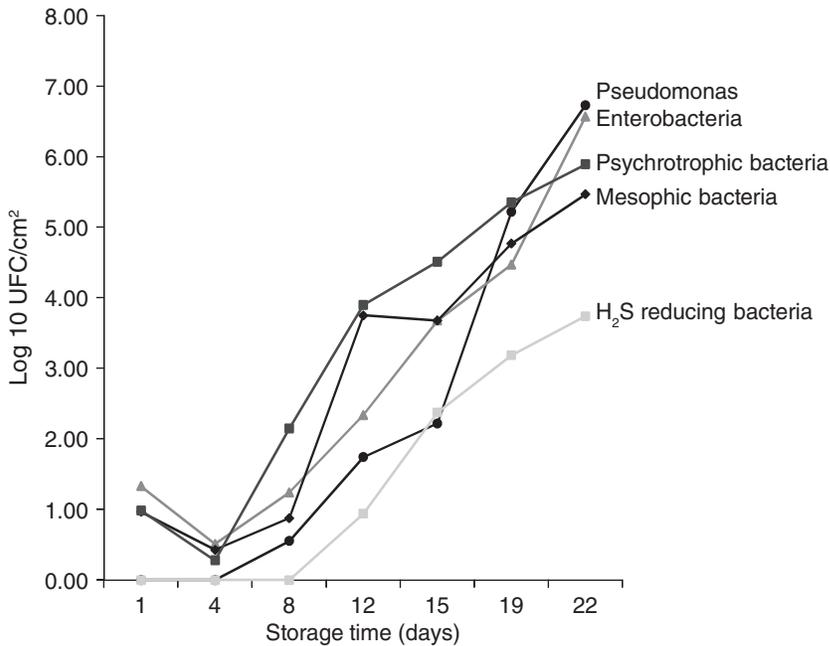


FIGURE 6. Mean  $\pm$  SD of bacterial results of *Pseudomonas*, *Enterobacteria*, *Psychrotrophic bacteria*, *Mesophilic bacteria* and *H<sub>2</sub>S reducing bacteria*, *Rachycentron canadum*, stored on ice for 22 days (n = 8). CFU = colony forming units.

et al. 2002). After 10 d of iced storage, the flesh became soft due to autolysis caused by fish muscle and microbial enzymes (Nielsen 1995).

The number of quality attributes and the total demerit points depend on each studied fish species because the sensorial characteristics are species specific. The main QIM protocol that was recently developed for whole gutted cobia contains 11 attributes covering 25 demerit points (Mach and Nordvedt 2012). However, the rejection point for whole gutted cobia is achieved with 13.8 total points and 15 d of ice storage. In this study, the total score was 15.67 with 19 d of storage (Fig. 2), with an accuracy of  $\pm 0.49$  d (Fig. 4). These different results can be attributed to the different areas of production (Brazil and Vietnam) and lots (or stocks) of fish. These differences may also be due to the use of gutted fish by Mach and Nordvedt (2012), which can favor microorganism entry into the muscle and stimulate the spoilage process.

Other studies using fish species have described a total of 30 demerit points for blackspot seabream, *Pagellus bogaraveo*, with a shelf life of 12–13 d (Sant'Ana et al. 2011); 22 demerit

points for Senegalese sole, *Solea senegalensis*, with 14 d of storage (Gonçalves et al. 2007); 15 points for farmed gilthead sea bream, *Sparus aurata*, with 14 d of storage (Šimat et al. 2012); and 14 demerit points for hybrid striped bass, *Morone saxatilis*  $\times$  *Morone chrysops*, with a shelf-life recommendation of 14 d (Nielsen and Green 2007).

The changes of sensorial attributes are the results of fish spoilage caused by complex biochemical, physical, and microbiological processes. Therefore, the sensorial analysis must be performed along with physicochemical and microbiological evaluations.

TVB-N and pH measurements are physicochemical aspects that allow the fish quality to be inferred along with sensorial attribute correlations. After the fish die, the pH of muscles decreases due to a biochemical reaction that produces ATP through glycogen degradation and lactic acid production. However, the pH decrease during rigor mortis is low, with values near 6.0, which is caused by low glycogen concentration. According to other studies, cobia filets show low pH values in the muscle (approximately

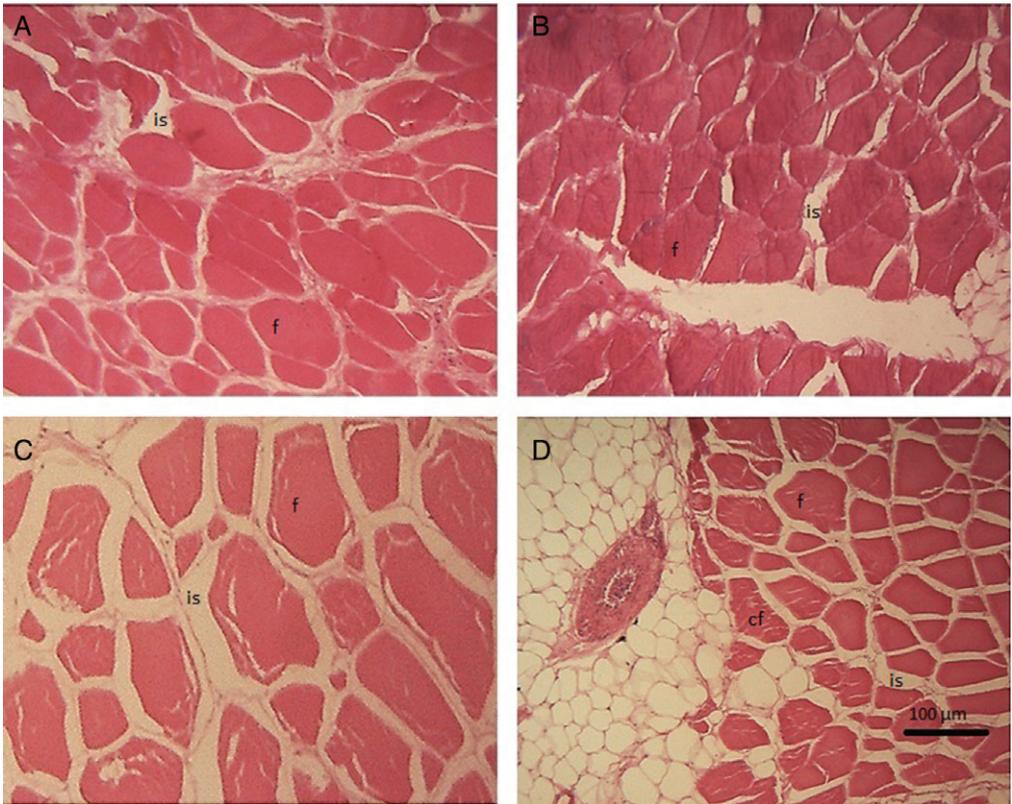


FIGURE 7. Longitudinal sections of cobia muscle tissue at (A) 1 d, (B) 7 d, (C) 14 d, and (D) 21 d of iced storage. Bars represent 100  $\mu\text{m}$ ; f = fibers; is = intercellular space; cf = crack in fibers.

5.50), which is similar to the values observed in pelagic fishes, and the pH decreases during the beginning of storage (Shiau 2007). In this study, cobia stored on ice showed pH values ranging from 5.75 24 h after slaughter to 6.10 after 18 d of storage (Fig. 5). The pH increase can be affected by fish species, microorganism type, microorganism amount, handling, and storage (Teodoro et al. 2007). In this work, the low muscle pH observed at the beginning of iced storage reflected good quality of the fish (Abbas et al. 2008). The increase in pH values after day 5 reflected the production of alkaline bacterial metabolites, such as TVB-N (Šimat et al. 2012).

TVB-N is represented by substances such as ammonia and trimethylamine, which are produced when the fish is decomposing (Scherer et al. 2006). TVB-N values are low during capture, slaughter, and rigor mortis periods,

when the autolytic process results in a slight loss of flavor and smell. Higher TVB-N values are observed during the postrigor period, with higher microorganism activity and nitrogen compounds, which lead to an unpleasant flavor and odor of the product (Contreras-Guzmán 1994).

The higher values during the first days of storage are caused by an initial 40% degradation of free amino acids, which results in ammonia production in the cobia muscle (Shiau 2007). On the following days, the lower TVB-N values are related to the low activity of  $\text{H}_2\text{S}$ -producing microorganisms in cobia stored on ice during the experiments tested in the laboratory (Fig. 6). A similar study of quality of grass carp, *Ctenopharyngodon idella*, storage on ice (Scherer et al. 2006) showed no significant differences in the TVB-N during 20 d of storage.

The initial levels of TVB-N were close to the limit established for freshly caught fish (20 mg

per 100 g), but the values at the end of storage did not remain below the recommended limit of 30–35 mg TVB-N/100 g. At the sensory rejection point of the samples (15 d), the content of volatile amines did not indicate spoiled fish. Similar results have been observed for trout, *Onchorrhynchus mykiss*, caught from fish farms, where whole gutted fish and trout fillets were kept on ice, resulting in TVB-N/100 g values of 18.11 and 26.06, respectively; these values are not adequate to indicate the quality deterioration of fish during ice storage (Chytiri et al. 2004) when compared to the human threshold for increases in off-flavor and the deteriorating appearance quality.

Fish is considered a perishable food. The loss of quality is triggered by the autolytic enzymes of the gills and muscles, followed by the growth of skin microorganisms. *Pseudomonas*, *Shewanella*, or H<sub>2</sub>S-producing bacteria are some of the most important organisms that are responsible for fish spoilage (Franco and Landgraf 2008). However, the psychrotrophic, mesophilic, and enterobacteria total counting is also recommended to evaluate sanitary conditions of the products.

According to Huss (1997), total bacterial counting is used to indicate the sanitary conditions of raw material, several stages of the food process and the efficiency of the procedures, as well as the storage and distribution conditions. A maximum count of 10<sup>7</sup> CFU/g or cm<sup>2</sup> for fish stored on ice is recommended, and the fish product is considered unhealthy with counts above this limit.

Cobia showed a maximum count of *Pseudomonas* spp, enterobacteria, mesophilic, and psychrotrophic bacteria between 17 and 20 d of ice storage (Fig. 6), when the sensorial analysis described a sour milky skin odor, rotten gill odor, and soft musculature. These observations are related to the presence of *Pseudomonas* and *Shewanella* (Sant'Ana et al. 2011), as demonstrated by a prior study performed using blackspot seabream.

Despite the low bacteria count, the rejection point of 15 d of storage observed in the cobia sensorial analysis can be related to other deteriorating reactions, such as lipid oxidation

triggered by the presence of reactive oxygen species generated by autolytic reactions (Sant'Ana et al. 2011).

The natural degradation of myofibrillar proteins and collagen due to proteases and microorganisms are linked to decreased hardness during ice storage (Chéret et al. 2006). This degradation explains how the muscle became relatively weak and showed gapping effects during ice storage (Ando et al. 1999).

Fish muscle cells run in parallel and are connected to sheaths of connective tissue (myocommata), which are anchored to the skeleton and skin. In the fish connective tissue, collagen was degraded after death, as shown by the histological images of muscle fibers (Fig. 7) as a progressive breakdown of the collagen junctions between the myocommata and the muscle fibers during ice storage (Delbarre-Ladrat et al. 2006).

In conclusion, a shelf life of 15 d was defined for whole cobia stored on ice, determined by sensory scores, pH measurements, and bacterial counting. The TVB-N values were not considered because they are not reliable quality indicators during ice storage. Histological analysis confirmed the cobia muscle changes observed in the sensorial evaluation.

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