Journal of Food Safety

Journal of Food Safety ISSN 1745-4565

DEVELOPMENT AND APPLICATION OF THE QUALITY INDEX METHOD (QIM) FOR FARMED TAMBAQUI (*COLOSSOMA MACROPOMUM*) STORED UNDER REFRIGERATION

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Received for Publication January 6, 2016
Accepted for Publication March 10, 2016

doi: 10.1111/jfs.12288

ABSTRACT

This research aimed to assess the freshness of farmed tambaqui (*Colossoma macropomum*) eviscerated and stored in ice for 30 days. The changes observed in the fish over storage were monitored through sensory evaluation Quality Index Method (QIM), pH, total volatile basic nitrogen (TVB-N), thiobarbituric acid reactive substances (TBARS), and microbiological analyses (coagulase-positive staphylococcus, *Salmonella* spp., coliforms at 45C, and mesophilic and psychrotrophic bacteria counts). The Quality Index (QI) showed a linear increase, ranging from 0 (maximum freshness) to 34 (total loss of freshness), with a strong correlation ($R^2 = 0.988$) with storage time. The microbiological analyses showed that the psychrotrophic bacteria count went over the recommended limit after the 26th day. TVB-N ranged from 4.01 to 15.92 mg/100 g; pH, from 6.01 to 6.57; and TBARS, from 0.01 to 0.14 mg MDA/kg between the 1st and 30th days of storage. The sensory, physicochemical, and microbiological analyses established that eviscerated tambaqui stored in ice is appropriate for consumption up until the 22nd day.

PRACTICAL APPLICATIONS

The QIM takes into account the diversity among species and defines the sensory quality parameters for fish freshness. Sensory analysis has been shown to be an important tool to assess sensory quality of fresh fish and is largely used by sanitary inspection agencies. The QIM established that eviscerated tambaqui (*C. macropomum*) stored in ice at 0C remained proper for consumption until the 22nd day. The results of the application of QIM to marketed fish species can establish the product's shelf life which is a very important for the industry and for consumers. QIM: Development and Application of a Sensory Protocol for Farmed Tambaqui (*C. macropomum*).

INTRODUCTION

Worldwide aquiculture has been expanding more than any primary sector activity due to, among other factors, the growing population and increasing demand for healthy and nutrient-rich foods. Brazilian aquiculture production has been following this trend and produced 480,000 tonnes of fish in 2010, 24.5% of which of the so-called round fish: tambaqui (*Colossoma macropomum*), pacu (*Piaractus mesopotamicus*), butterfly peacock bass (*Cichla ocellaris*), tambaqui and pacu hybrid (tambacu), and others (Brasil 2012). Among those, tambaqui stands out, mainly in the country's north region, for being the most farmed fish species for its great psiculture potential, for successfully adapting to farming and industrialized feed, for consumer acceptance due to its flavor, and for its high market value (Santos *et al.* 2010).

Sensory analysis has been shown to be an important tool to assess sensory quality of fresh fish and is largely used by sanitary inspection agencies. The acceptance of fresh fish is determined by its sensory quality, hence, it is extremely important fish freshness be assessed right after slaughter so as to prevent or minimize the changes that may take place during storage, such as changes in skin, muscle firmness, odor and flavor (Huidobro *et al.* 2000).

In Brazil, the characteristics for fresh fish to be considered proper for consumption are established by several legislations such as the *Regulamento de Inspeção Industrial e Sanitária de Produtos de Origem Animal* (Regulation of Industrial and Sanitary Inspection of Animal Origin Products – RIISPOA) (Brasil 1997a), Ordinance no. 185 of the Ministry of Agriculture (Brasil 1997b), and the norms of the *Associação Brasileira de Normas Técnicas* (Brazilian Association of Technical Standards) (ABNT 1993). However, the criteria required by these legislations do not take into account the diversity among species and do not define sensory quality parameters for fish freshness.

The quality index method (QIM) is an evaluation methodology used to determine fish freshness and quality which assesses quality attributes such as appearance, texture, eyes, gills, and abdomen and the changes they undergo over storage. The QIM has some unique advantages such as maintaining sample integrity and estimating the period over which the fish stored in ice is proper for consumption (Sveinsdottir *et al.* 2002; Gonçalves 2011). Nevertheless, separate schemes must be developed for different species as each has its own quality standards and spoilage indicators (Sant'Ana *et al.* 2011).

Several studies have been performed to develop QIM schemes for different fish species, such as *Salmo salar* (Sveinsdottir *et al.* 2002), *Oreochromis niloticus* (Albuquerque *et al.* 2004), *Solea senegalensis* (Gonçalves *et al.* 2007), *Morone saxatilis* and *Morone chrysops* (Nielsen and Green 2007), *Micropogonias furnieri* (Teixeira *et al.* 2009), *Litopenaeus vannamei* (Oliveira *et al.* 2009), *Sparus aurata* (Huidobro *et al.* 2000; Campus *et al.* 2011), *Pagellus bogaraveo* (Sant'Ana *et al.* 2011), *Boops boops L.* (Bogdanovic *et al.* 2012), *Merluccius merluccius* (Alonso *et al.* 2012), and *Panulirus argus* (Gonçalves *et al.* 2015).

The present study aimed to assess the sensory quality of eviscerated farmed tambaqui (*C. macropomum*) stored in ice by applying the QIM protocol.

MATERIAL AND METHODS

Sample Collection and Storage

The assays used 112 tambaqui (*C. macropomum*) specimens weighing on average 1.5 kg. The fish were acquired from a fish farm in the city of Terra Alta, PA, Brazil and were slaughtered through hypothermia by immersion in a 1:1 water: ice mixture in accordance with the animal ethics and well-being standards. After slaughter, the fish were stored in ice inside isothermic boxes at a 2:1 ice: fish ratio and transported to the laboratory within approximately 2 h.

The fish were eviscerated and washed in 5 ppm chlorinated water and stored between two layers of ice flakes in plastic crates covered with a plastic film. The crates were stored in a refrigerator (Whirlpool/BVR28G) at 0 ± 1C and were refilled with ice when needed. Fish temperature was monitored every 24 h with a digital thermometer (Incoterm/9791.16.1).

Quality Index Method

The team chosen to create the QIM protocol comprised five women and three men between 19 and 32 years old who attended 13 training sessions using the scheme developed by Bonilla *et al.* (2007) under standardized laboratory conditions. One tambaqui specimen was used per session.

To develop the QIM protocol, the fish were evaluated on days 1, 5, 9, 14, 19, 22, 26 and 30 of storage. The samples were removed from ice 30 min prior to each session and were presented to the referees in light-colored trays under white fluorescent light. The referees openly discussed the sensory attributes of appearance, odor and texture of the tambaqui at the different storage times. On the first day of evaluation, three specimens of the same batch were presented. Starting on the second day, the samples corresponding to that day of storage were presented along with three samples of another batch. In this stage, storage time was not informed so that the referees would relate the attributes to the fish conservation level. The QIM protocol was later employed by the trained team to evaluate 45 specimens, that is, three for each storage time.

Physicochemical Analyses

pH was analyzed following the methods by the AOAC (2002), total volatile basic nitrogen (TVB-N) was analyzed using the method described in Brazil (1999), and thiobarbituric acid reactive substances (TBARS) analysis employed the methodology proposed by Vyncke (1970).

Microbiological Analyses

The methodology described by Downes and Ito (2001) was used in the analyses of coagulase-positive staphylococcus, *Salmonella* spp., coliforms at 45C, and mesophilic and psychrotrophic bacteria counts.

Statistical Analysis

All analyses were carried out in triplicate and the values obtained were evaluated using the software XLSTAT for

TABLE 1. QIM SENSORY ANALYSIS PROTOCOL DEVELOPED FOR EVISCERATED TAMBAQUI STORED IN ICE

Parameters	Attributes	Characteristics	Scores
Overall aspect	Surface aspect	Intense glisten, intense pigmentation	0 ( )
		Shiny, slight loss of color, transparent mucus	1 ( )
		Little glisten, loss of color, opaque mucus, small lesions	2 ( )
		No glisten, opaque color, yellowish opaque mucus, lesions	3 ( )
	Meat firmness	Firm and elastic	0 ( )
		Reduced firmness and elasticity	1 ( )
		Elastic	2 ( )
		Flaccid, body deformations	3 ( )
Eyes	Transparency (eyeball)	Transparent, clear	0 ( )
		Slightly opaque	1 ( )
		Opaque	2 ( )
	Pupil	Visible, well defined	0 ( )
		Foggy, defined	1 ( )
		Grayish, undefined	2 ( )
	Shape	Convex	0 ( )
		Flat	1 ( )
		Concave	2 ( )
		Concave, deformed, volume loss	3 ( )
	Blood	Absent	0 ( )
		Slightly bloody	1 ( )
		Bloody	2 ( )
Gills	Color	Bright red to purple	0 ( )
		Less-bright red	1 ( )
		Less-bright red to brown, with pale edges	2 ( )
		Discolored	3 ( )
	Odor	Algae	0 ( )
		Neutral, less intense algae	1 ( )
		Slightly metallic, acrid or rancid	2 ( )
		Rancid, characteristic of putrefaction	3 ( )
	Shape	Whole	0 ( )
		Slightly misshapen	1 ( )
		Misshapen	2 ( )
Abdominal cavity	Color	Light rosy	0 ( )
		Rosy, slightly opaque	1 ( )
		Rosy, slightly dark	2 ( )
	Odor	Algae	0 ( )
		Neutral	1 ( )
		Slightly acrid and rancid	2 ( )
		Acrid and rancid	3 ( )
Skin	Scales	Firm, strongly adhered	0 ( )
		Adhered	1 ( )
		Slightly loose	2 ( )
		Loose	3 ( )
Fins	Elasticity	Very elastic (returns immediately under tension)	0 ( )
		Elastic (returns more slowly under tension)	1 ( )
		Little elastic (does not completely return under tension)	2 ( )
		No elasticity (does not return under tension)	3 ( )
Score 0 to 34			

Windows, version 2012, with the following statistical methodologies: analysis of variance (ANOVA) at 5% significance according to *F*-test, Tukey's test ($P \leq 0.05$), simple linear regression, and partial least squares (PLS) regression analysis.

RESULTS AND DISCUSSION

Developing the Quality Index Method

The team trained in the QIM developed a specific protocol to assess the sensory quality of eviscerated tambaqui stored

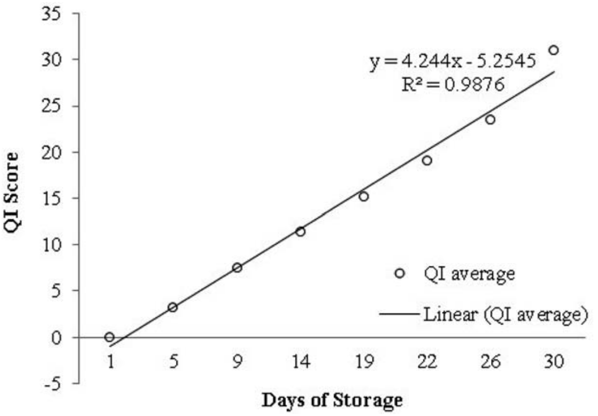

FIG. 1. EVOLUTION OF THE MEAN QIM SCORES OBTAINED FOR EVISCERATED TAMBAQUI STORED IN ICE

in ice. The specific attributes considered relevant to assess tambaqui freshness and that characterized the sensory changes over storage in ice were listed from a consensus among the referees and are shown in Table 1.

The QIM sensory protocol developed for eviscerated tambaqui stored in ice scored 13 quality attributes and established a quality index (QI) ranging from 0 (maximum freshness) to 34 (spoiled) demerit points for the description of the tambaqui on days 1, 5, 9, 14, 19, 22, 26 and 30 of storage.

The QI obtained by applying the QIM protocol to tambaqui at different storage times showed a linear increase, ranging from 0.00 ± 0.00 to 31.00 ± 1.75, with a strong correlation ($R^2 = 0.988$) between the mean QI on each day of storage and the time of storage in ice (Fig. 1).

The quality parameters assessed during storage are shown in Fig. 2. The surface aspect and meat firmness (Fig. 2a) and the attributes of the parameter "eyes" (Fig. 2b) significantly differed ($P \leq 0.05$) over the storage period. The eyes became flat and then concave starting on the fifth day, while changes in transparency were observed after the ninth day, when opacity and loss of pupil edge definition began. Blood was only observed on the 26th day onward.

The attributes of the parameter "gills" significantly differed ($P \leq 0.05$) over the 30 days of storage (Fig. 2c). Initial gill color ranged from light red to dark red, in a gradient from the edges to the center, until they became discolored or brown. The gills showed changes in odor starting on the fifth day and volume loss was observed on the 19th day. The changes in the parameter "abdominal cavity" (Fig. 2d) were only significant ($P \leq 0.05$) starting on the ninth day, with the color varying from light pink to dark pink and the odor shifting from algae-like to rancid.

The scales were evaluated regarding adherence to the skin, which went from strongly adhered to loose, with a significant difference ($P \leq 0.05$) starting on the 14th day of storage (Fig. 2e). The changes in fin elasticity began on the ninth day and significantly differed ($P \leq 0.05$) starting on the 19th day (Fig. 2f).

The attributes assessed were linearly correlated, ranging from R^2 0.80 to 0.98 during storage in ice, except for blood presence in the eyes, which was moderately correlated at $R^2 = 0.69$.

The evolution of the quality attributes of visual aspect, eye transparency and shape, gills, and abdominal cavity assessed in the QIM protocol for eviscerated tambaqui on days 1, 14 and 30 of ice storage can be seen in Fig. 3.

The results obtained from the application of the QIM protocol were analyzed using PLS regression to verify the efficacy of the QI during tambaqui storage in ice (Fig. 4).

Figure 4a shows that, on the first days of storage (1, 5 and 9 days), the QI scores attributed by the referees were close. The variation increased over the storage time, which indicated that it was easier for the referees to assess the fresh tambaqui. A similar result was observed by Sveinsdottir *et al.* (2001) when developing the QIM for Atlantic salmon (*S. salar*).

The standard error of performance (SEP) can be used to assess the precision of the QI predictability. The SEP obtained in this research for QI of tambaqui was 2.9, a result similar to that found by Gonçalves *et al.* (2015), who obtained SEP of 2.745 when developing the QIM protocol for lobster (*Panulirus argus*) with 11 attributes. Sveinsdottir *et al.* (2002) obtained SEP of 2.023 for salmon (*S. salar*). The authors considered that normal distribution could be obtained with a 95% confidence interval and that this value could be reduced by including more samples in each batch, as observed by Sveinsdottir *et al.* (2001), who included five samples per day of storage and obtained SEP of 1.4.

According to Donadoni *et al.* (2012), the variables with importance projection (VIP) above 1.0 are considered relevant for the statistical model used. In Fig. 4b, the quality attributes that stood out the most for the QIM scheme of tambaqui were: surface aspect, gill color, abdominal cavity odor, eye transparency, fins, eyes, firmness, eye shape and pupil.

Changes in Quality Regarding the Physicochemical Parameters of Tambaqui during Ice Storage

The results of the physicochemical parameters used to monitor the degradation of the tambaqui specimens during ice storage for 30 days are shown in Table 2.

The changes in pH were significant ($P \leq 0.05$) starting on the ninth day and only by the 22nd day of storage did it go beyond the limit of 6.5 established by the current legislation (Brasil 2001a). Almeida *et al.* (2008) studied the postmortem changes of tambaqui stored in ice and found medium pH

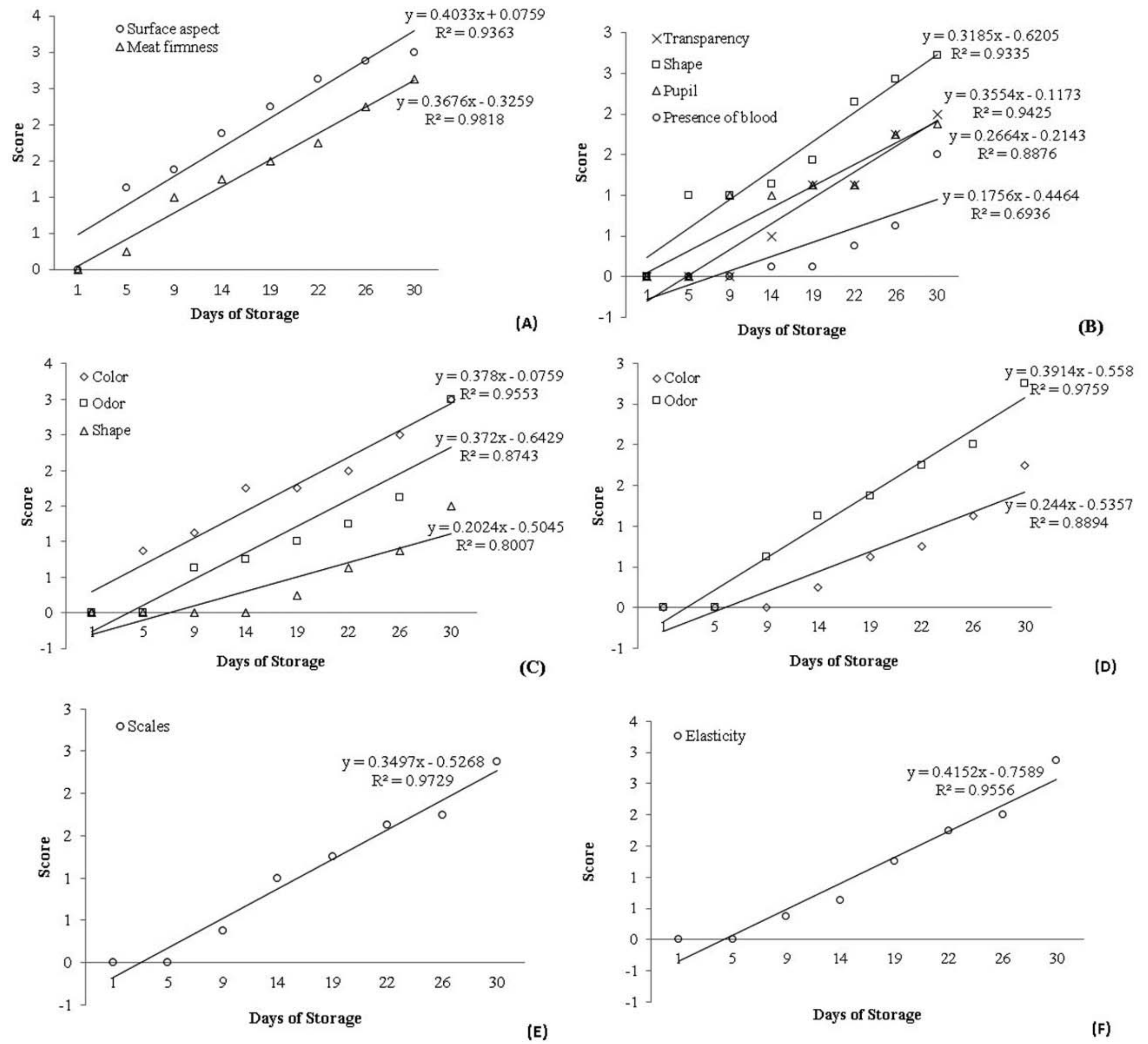

FIG. 2. EVOLUTION OF THE MEAN SCORES OF THE PARAMETERS ASSESSED IN THE QIM PROTOCOL FOR EVISCERATED TAMBAQUI STORED IN ICE FOR 30 DAYS
(A) Overall aspect; (B) Eyes; (C) Gills; (D) Abdominal cavity; (E) Skin; (F) Fins.

values (6.07 to 6.66) during 49 days. Borges *et al.* (2013) conducted a similar research with pacu (*P. mesopotamicus*) and found pH between 6.06 and 6.57 over the 17 days of storage.pH values tend to rise over storage due to the accumulation of TVB-N formed from autolytic and bacterial activities (Gonçalves *et al.* 2015). pH and TVB-N had a strong linear correlation ($R^2 = 0.97$ and $R^2 = 0.99$, respectively) with ice storage time.

TVB-N values significantly differed ($P \leq 0.05$) over the storage period. The Brazilian legislation (Brasil 2001a) sets a maximum acceptable limit for fish of 30 mg N/100 g TVB, which was not reached over the 30 days of storage. Almeida *et al.* (2008) analyzed TVB-N in tambaqui stored in ice and found that the maximum value allowed by the legislation was reached on the 36th day. Lira *et al.* (2001), when analyzing fresh smalltooth sawfish (*Pristis pectinata*), obtained a value of 22.32 mg N/100 g TVB. The TBARS values found in the present study are considered low since tambaqui has a low muscle lipid content. However, these values progressively increased over storage, with a strong correlation ($R^2 = 0.96$). The changes in TBARS were significant ($P \leq 0.05$) starting on the ninth day of storage.

Borges *et al.* (2013), when analyzing the shelf life of pacu (*P. mesopotamicus*) stored in ice for 17 days, also found

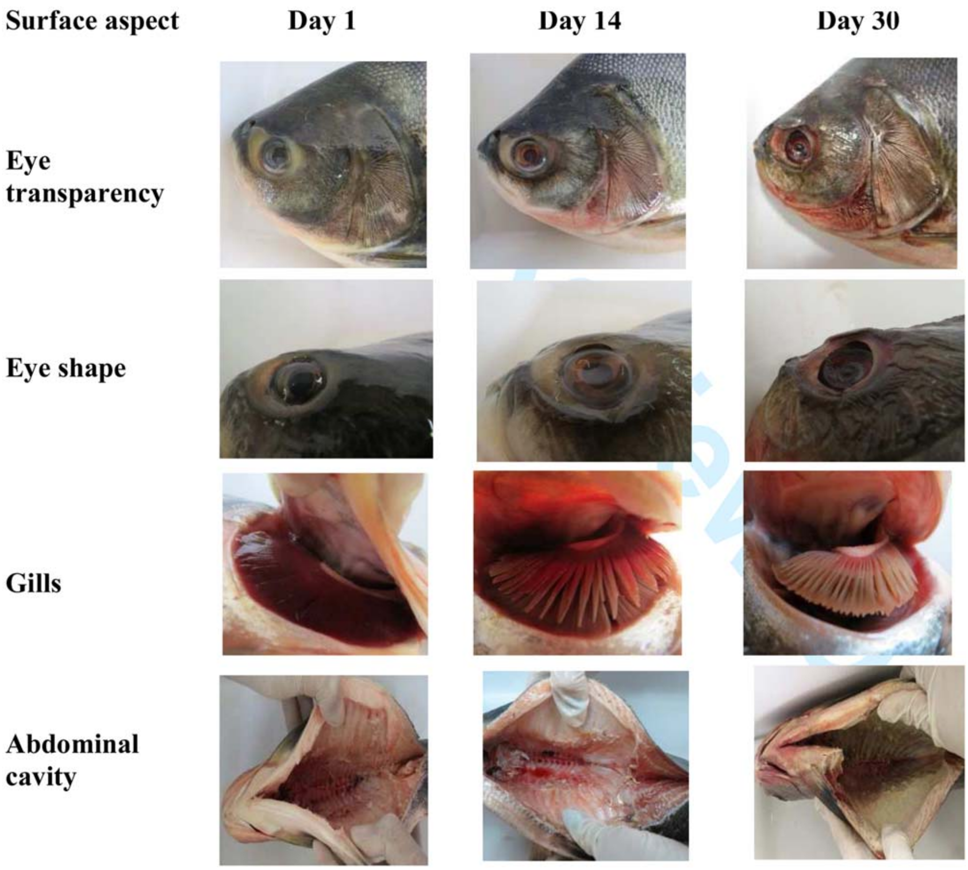

FIG. 3. QUALITY ATTRIBUTES ASSESSED IN THE QIM PROTOCOL FOR TAMBAQUI (*C. MACROPOMUM*)

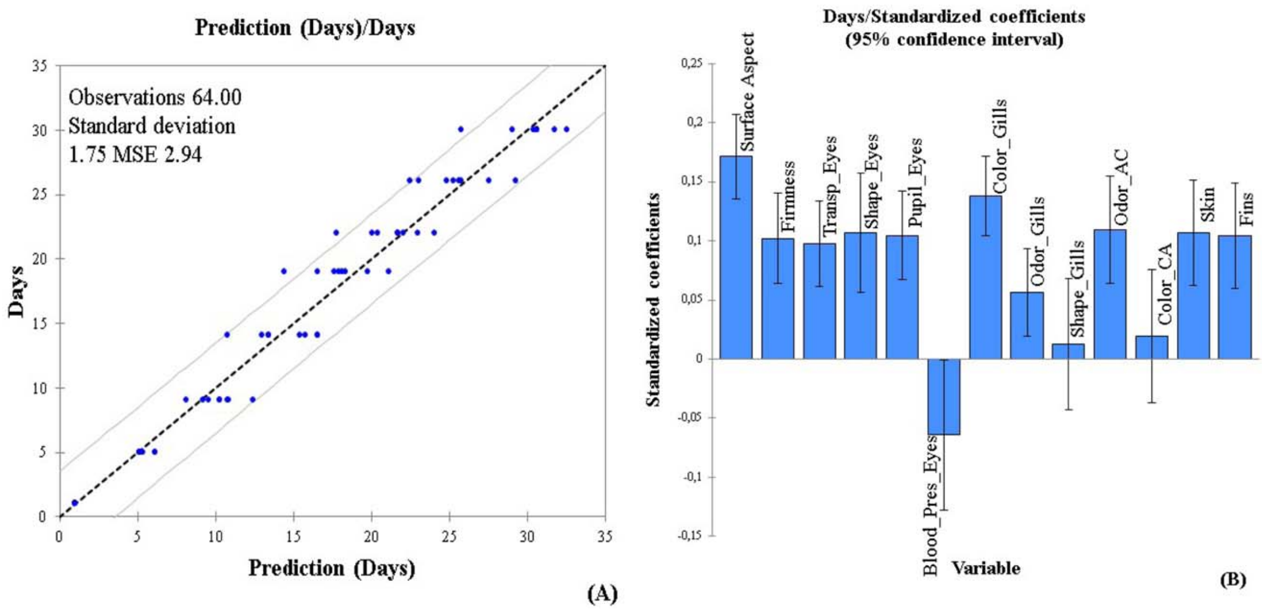

FIG. 4. PARTIAL LEAST SQUARES REGRESSION OF THE QIM PARAMETERS DEVELOPED FOR EVISCERATED TAMBAQUI STORED IN ICE (95% REGRESSION CONFIDENCE).

TABLE 2. RESULTS OF THE PHYSICOCHEMICAL ANALYSES DURING ICE STORAGE OF EVISCERATED TAMBAQUI

Time	pH	TVB-N (mg N/100 g)	TBARS (mg MDA/kg)
1	6.01 ± 0.01^{a}	4.01 ± 0.00^{a}	0.01 ± 0.00^{a}
5	6.09 ± 0.02^{a}	5.98 ± 0.11^{b}	0.01 ± 0.00^{a}
9	6.16 ± 0.01^{ab}	7.87 ± 0.00^{c}	0.04 ± 0.00^{b}
14	6.34 ± 0.01^{bc}	8.58 ± 0.00^{d}	0.05 ± 0.00^{c}
19	6.41 ± 0.01^{cd}	11.04 ± 0.00^{e}	0.06 ± 0.00^{d}
22	6.45 ± 0.02^{cd}	12.83 ± 0.34^{f}	0.08 ± 0.00^{e}
26	6.51 ± 0.02^{cde}	13.93 ± 0.11^{g}	0.12 ± 0.01^{f}
30	6.57 ± 0.02^{def}	15.92 ± 0.34^{h}	0.14 ± 0.01^{g}

*Values represent means ± standard deviation.
†Different letters in the same column indicate statistical difference ($P < 0.05$).

TBARS values between 0 and 0.1653 mg MDA/kg. Bogdanovic *et al.* (2012), when studying the shelf life of sardine (*Sardine pilchardus)* and bogue (*Boops boops*), suggested limits of 5–8 mg MDA/kg for sensory acceptance of fish. Chytiri *et al.* (2004) observed that the TBARS values in noneviscerated trout slowly increased over 18 days of storage and reached a final value of 19.41 mg MDA/kg, while filleted trout samples reached a final value of 16.21 mg MDA/kg on the 18th day of storage.

Changes in Microorganism Counts in Tambaqui during Ice Storage

No *Salmonella* spp. was detected in the tambaqui samples analyzed over the 30 days of storage and the counts of coliforms at 45C and coagulase-positive staphylococcus were below the threshold (10^3) allowed by the current legislation (Brasil 2001b). These microorganisms are not part of the fish's regular microbiota and, when present, may indicate

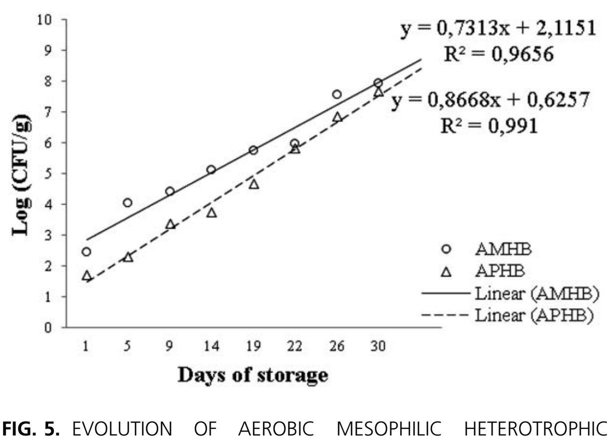

FIG. 5. EVOLUTION OF AEROBIC MESOPHILIC HETEROTROPHIC BACTERIA COUNTS (AMHBC) AND AEROBIC PSYCHROTROPHIC HETEROTROPHIC BACTERIA COUNTS (APHBC) IN THE SAMPLES OF EVISCERATED TAMBAQUI (*C. MACROPOMUM*) STORED IN ICE FOR 30 DAYS.

contamination of the fishing ground and inappropriate handling in the productive chain, which includes the ice, equipment, tools and so forth that have come into contact with the fresh fish (Santos *et al.* 2008; Kumar *et al.* 2009).

The results of aerobic mesophilic heterotrophic bacteria counts (AMHBC) and aerobic psychrotrophic heterotrophic bacteria counts (APHBC) in the samples of eviscerated tambaqui stored in ice for 30 days are shown in Fig. 5.

The Brazilian legislation does not set a limit for AMHBC or APHBC in fish, however, the International Commission on Microbiological Specification for Foods (ICMFS 2005) recommends a maximum limit of 7 log CFU/g for standard plate counts of mesophilic and psychrotrophic aerobic bacteria in refrigerated fish.

The mesophilic bacteria count in tambaqui ranged from 2.44 to 7.95 log CFU/g over the 30 days of storage and was within the limits recommended by the ICMSF (2005) up until the 22nd day of storage. The psychrotrophic bacteria count ranged from 1.70 to 7.67 log CFU/g and was above the limit recommended by the ICMSF after the 26th day of storage.

Morton (2001) stated that aerobic microorganism counts are not necessarily correlated with the presence of pathogens and/or toxins. Borges *et al.* (2013), when assessing the shelf life of pacu (*P. mesopotamicus*) stored in ice for 17 days, found psychrotrophic bacteria counts between 2.47 and 10.04 log CFU/g and mesophilic bacteria counts between 3.30 and 10.30 log CFU/g and concluded that eviscerated pacu stored in ice was proper for consumption until the 11th day of storage.

CONCLUSIONS

The QIM developed for eviscerated tambaqui stored in ice highlighted the freshness indicators eye color and shape, gill odor, surface aspect and meat firmness, with a strong linear correlation at R^2 ranging from 0.80 to 0.98. The quality index (IQ) obtained for the different storage times increased linearly, with a strong correlation ($R^2 = 0.988$) between the mean QI of each day and the time of ice storage, which indicates a decrease in fish freshness over storage.

The values of TVB-N and TBARS during eviscerated tambaqui storage in ice are not safe indicators to assess freshness because, when pH and mesophilic and psychrotrophic bacteria counts reached the maximum limits set by the international commission on microbiological specification for foods, those parameters were still within standard values. The QIM established that eviscerated tambaqui stored in ice at 0C remained proper for consumption until the 22nd day, according to the results of pH, mesophilic bacteria count, and sensory aspects, mainly meat firmness, eye shape, and gill odor and color.

ACKNOWLEDGMENTS

The authors thank CNPq for the scholarship granted and to PROPESP/UFPA for funding the article translation.

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