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ORIGINAL ARTICLE

Development of a quality index scheme and shelf-life study for whole tambaqui (*Colossoma macropomum*)

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ABSTRACT

The study developed a sensory scheme based on the Quality Index (QI) and estimated the shelf-life for whole tambaqui, *Colossoma macropomum* (Cuvier, 1818), stored in ice, assessing and determining the most appropriate chemical, physical, bacteriological and quality sensory parameters and their changes during storage time. Ninety six fish were evaluated at 1, 3, 5, 8, 10, 12, 15, 17, 19 and 22 days of ice-storage. The developed quality index (QI) showed four main quality attributes with a total of 29 demerit scores. The skin mucus and odor, as well as general appearance and ventral elasticity had a great importance for the statistical model applied, while eyes, gill mucus and dorsal elasticity showed lower significance for tambaqui QI. The pH showed few variations during the ice storage. Nitrogen bases, as well as the total count of specific spoilage bacteria, had a linear correlation with storage time. The QI proved to be efficient to assess tambaqui quality and loss of sensory quality over the storage period. The results suggest that whole, ice-stored *Colossoma macropomum* is fit for consumption until the 22nd day.

KEYWORDS: food quality, storage time, spoilage, sensory parameters

Desenvolvimento do protocolo do Índice de Qualidade e estudo da vida útil do tambaqui

RESUMO

O estudo desenvolveu um protocolo sensorial baseado no Método do Índice de Qualidade (IQ), estimando a vida útil do tambaqui, *Colossoma macropomum*, inteiro e conservado em gelo, avaliando e determinando as principais alterações físico-químicas, microbiológicas, os parâmetros sensoriais e suas alterações durante o armazenamento em gelo. Noventa e seis peixes foram avaliados no dia 1, 3, 5, 8, 10, 12, 15, 17, 19 e 22 de armazenamento em gelo. O índice de qualidade desenvolvido (IQ) apresentou quatro principais atributos de qualidade com um total de 29 pontos de demérito. O muco e odor da pele, assim como a aparência geral e elasticidade ventral, apresentaram uma maior importância para o modelo estatístico aplicado, enquanto que os olhos, muco das brânquias e elasticidade dorsal mostraram menor significância para o IQ do tambaqui. O pH apresentou poucas variações durante o armazenamento em gelo. As bases nitrogenadas voláteis totais, bem como a contagem total de bactérias específicas de deterioração, apresentaram correlação linear com o tempo de armazenamento. O IQ mostrou-se eficiente para avaliar a qualidade do tambaqui e as perdas da qualidade sensorial ao longo do período de armazenamento. Os resultados sugerem que o tambaqui, *Colossoma macropomum,* inteiro e conservado em gelo é considerado adequado para consumo até o 22° dia de armazenamento.

PALAVRAS-CHAVE: qualidade em alimentos, tempo de armazenamento, deterioração, parâmetros sensoriais

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INTRODUCTION

World aquaculture production continues to grow, according to the latest available statistics collected globally by FAO. Farmed fish contributed to 42.2% of 158 million tonnes of fish produced by capture fisheries and aquaculture in 2012. The world fish aquaculture production rose by 70.5 million tonnes in 2013 (FAO 2014). In Brazil, there was a significant growth of native fish species production in the last few years. In 2011, 111,084.1 tonnes of *Colossoma macropomum* were produced (MPA 2011). Native from the Amazon region (including Bolivia, Brazil, Colombia and Venezuela), this species has an efficient performance in intensive farming and reaches high market value (Val *et al.* 2000).

Tambaqui, or cachama (the common name in Spanish), has a high quality standard due to the relatively short time between harvest, slaughter and the product preservation in aquaculture production, and the possibility to control several parameters during the post-harvest stages. Therefore, it is essential to know the sensory and physicochemical changes that occur during storage of fish in ice for consolidation of fisheries supply chains, in order to avoid losses and increase its commercial potential (Borges *et al.* 2013). For this purpose, a quality index (QI) scheme or table was developed for tambaqui stored in ice boxes.

The QI is a tool for evaluation of fish freshness, based on the observation of fish quality attributes that receive a score ranging from zero to two or three (where zero is the best and two or three are the worst scores) (Bonilla *et al.* 2007). It uses a practical qualification system in which the fish is inspected and the corresponding demerit points are recorded. QI is specific for each species, so the European fisheries research institutes developed a strategic alliance called "QIM - EUROFISH" for the globalization of this method, especially with the publication of a manual in several languages containing the fish quality index scheme of various species (Do Amaral and Freitas 2013), especially for marine fish.

There are a few studies with freshwater species. Recently, QI protocols have been developed for pacu, P*iaractus mesopotamicus* (Holmberg, 1887), with 32 demerit points and a shelf-life of 11 days (Borges *et al.* 2013), for the hybrid tambacu, *Colossoma macropomum* x *Piaractus mesopotamicus*, with 26 demerit points and shelf-life of 11 days (Borges *et al*. 2014b), and for the hybrid tambatinga, *Colossoma macropomum* x *Piaractus brachypomus* (Cuvier, 1818), with 18 demerit points and a shelf-life of 10 days (Ritter *et al.* 2016). Tambaqui has a recently developed QI protocol for eviscerated fish, with 34 demerit points and a shelf-life of 26 days (Araújo *et al.* 2016). However, the whole fish (as it is traditionally marketed) has specific characteristics that lead to changes in sensory parameters over time, which require a specific protocol, not yet developed.

Due to the relevance of the QI and the lack of information for farmed whole tambaqui, the evaluation of its meat quality and freshness has become necessary. The aim of this study was to develop and apply the QI scheme for whole ice-stored tambaqui through sensory evaluation, counts of specific spoilage organisms (SSO) and physicochemical evaluations and to determine its shelf- life.

MATERIAL AND METHODS

A total of 132 tambaquis, *Colossoma macropomum*, were purchased at the aquaculture station "Ademar Braga" of Departamento Nacional de Obras contra a Seca - DNOCS (National Department of Works Against Drought), in Piripiri, Piauí State, Brazil. The fish were acquired in four batches, one of 36 fish (for protocol development) and three of 32 fish each (for the shelf-life study). All specimens were stored immediately in ice and transported in polystyrene boxes to the laboratory of Food Analysis and Technology, in Parnaíba, Piauí.

For development of the Quality Index (QI) scheme, a pilot experiment was conducted, and for QI application and validation, three experiments (I, II and III) were performed between March and September 2012. Fish were placed inside isothermal boxes with 1 kg of ice for every kg of fish (Borges *et al.* 2013). Temperature was kept close to freezing (0.2 ± 0.7 ºC), and ice was added daily. To ensure the safety of the samples, ice was made with UV-treated water, with sterility proven by water and ice microbiological analysis. The sampled fish had an average weight of 562.45 ± 134.75 g, 496.93 ± 78.70 g and 503.14 ± 48.16 g, respectively, for experiments I, II and III. For each experiment, 20 fish were randomly chosen for sensory analysis, 10 for physical and chemical analysis and 2 for microbiological analysis.

The QI assessment was done as recommended by Sant'Ana *et al.* (2011). The trained jury consisted of 10 (ten) people, 5 men and 5 women, selected by their experience in sensory analysis of fish. Each member evaluated 36 samples of tambaqui, divided into twelve batches of three fish, in each trial on days 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21 and 24, until spoilage defined by the sensory rejection, and when the amounts of volatile nitrogenous compounds and spoilage bacteria count exceeded the limits established by law (30 mg N.100 g^{-1} and 10^7 UFC.g^{-1}, respectively; Brasil 1997; Huss 1995). Thirty six fish were used for development of the Quality Index Scheme, in 12 sessions of one-hour each. The samples were removed from ice 30 minutes before the session, and the observations were conducted under ideal lighting and temperature (20 °C) conditions. After the sessions, the fish that had some injury were discarded to ensure that excessive manipulation did not accelerate deterioration of samples.

The jury described a list of quality attributes for appearance/texture, eyes, gills, skin, ventral and dorsal muscle and descriptions of how they change with storage time, based on a previous list of descriptors made under the jury leader guidance. After the individual analysis and group discussion, they selected the attributes and the intensity that better characterized the sensory parameters of tambaqui. They allocated the scale points for the attributes classification in intensity levels (0 for very fresh and 2 for rejection). If there was more than one well-defined intermediate level in the observations, the maximum range was set to 3. This attributes composed the Quality Index (QI) scheme used for shelf-life determination of whole tambaqui.

For shelf-life study, three experiments were conducted and fishes were evaluated in 1, 3, 5, 8, 10, 12, 15, 17, 19 and 22 days of iced-storage.

Bacteriological analysis was done as recommended by Sant'Ana *et al.* (2011) adapted from Cousin *et al.* (2001). Individual areas of 14.0 cm^2 (4.0 x 3.5 cm) of the skin (both sides of two fish) were swabbed with sterile cotton swabs, in duplicate. The swabs were transferred to tubes containing 5.0 ml of 0.85% NaCl solution. Appropriate series of decimal dilutions were performed, and surface inoculation was accomplished using the 20 µl drop method in Pseudomonas Agar (OXOID, Basingstoke, Hampshire, UK) to detect *Pseudomonas*; Nutrient Agar (OXOID, Basingstoke, Hampshire, UK) to count psychrophilic bacteria; MacConkey Agar (OXOID, Basingstoke, Hampshire, UK) for the Enterobacteriaceae counting; Plate Count Agar (PCA) (HIMEDIA, Mumbai, India) for mesophilic and heterotrophic aerobic bacteria; and Iron Agar for bacteria producing H_2S (OXOID, Basingstoke, Hampshire, UK). The plates containing the Pseudomonas Agar, Nutrient Agar and Iron Agar were incubated at 20 °C for 24 to 48 hours; MacConkey Agar was incubated at 35 °C for 24 to 48 hours, and PCA was incubated at 35 °C for 24 to 48 hours and also incubated at 5 °C for seven days (Table 1). Counts were performed in duplicate and expressed as logarithm of cfu $(cm^2)^{-1}$.

Ten samples were evaluated on day 1, 4, 8, 12, 15, 19 and 22. 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 inserted into the fish muscle. Total volatile base nitrogen (TVB-N) was measured by protein precipitation with trichloroacetic acid (TCA, 7.5%, Synth[@], Diadema, São Paulo, Brazil) and by evaluation of the TCA extract using the Micro Kjeldahl procedure according to an adapted method from Brasil (1981). The proximate composition was determined according to 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). Three determinations from the same sample were performed for all analyses.

The sensorial analysis data were evaluated for normality through the Shapiro-Wilk test (Zar 2010). Linear regression and Pearson's correlation between the QI score *versus* iced storage were measured using the OriginPro 8.1 software. Additionally, a partial least squares (PLS) regression was used to determine the uncertainty associated with QI prediction and the sensory attributes relevant for its development (Borges *et al.* 2014a), using the program XLSTAT for Windows version 2012.5 (Adinsoft, Paris, France).

RESULTS

The resulting QI scheme had four quality attributes (general appearance, gills, eyes and texture) divided into 12 parameters (Table 2). According to the tambaqui sensory characteristics, parameters were assigned a score ranging from 0 to 2 or 0 to 3. Up to 2 demerit points to "scales", "general appearance" and "fish odor", 2 points for "color", "odor", "form" and "mucus" of gills, 3 points for "pupil transparency" and "shape of eyes", 2 points for "back elasticity" and 3 for "ventral elasticity" (Figure 1).

The scores given for all the quality attributes are summarized by the Quality Index (Figure. 2), which increases linearly with the storage time in ice. However, some attributes did not show the highest score during the shelf-life study. That's because some sensory characteristics showed a slow rise while other parameters accused higher values which established the rejection point. The "odor" received lower average scores, with a demerit score of 0.03 (at day 1) and 1.05 (at day 22) when the jury already classified tambaqui as unfit for consumption. On the contrary, the attributes related to the eyes and gills presented the highest loss of freshness. At day 5, the average score of gills had passed one (1.0) point. Also, the attributes "transparency", "pupil" and "eye shape" scored 1.08, 1.38 and 1.33 points, respectively. These last attributes were close to 3 points at day 22 (rejection point) (Figure 1).

The gills (color and odor) changed faster, varying from shine red to dark red and from mild /delicate odor of seaweed to sweetish acrid on day five (5) (Table 2). On the 19th day, the gills showed brown color and bloody mucus. The odor, considered

Table 1. Description of microbiological media, temperature and time of incubation used in the study of shelf-life for whole tambaqui, *Colossoma macropomum* storage in ice.

Media	Bacteria	Temperature/ Incubation time
Pseudomonas Agar	*Pseudomonas*	20°C / 24 to 48 hours
Nutrient Agar	Psychrophilic bacteria	20°C / 24 to 48 hours
MacConkey Agar	Enterobacteriaceae	35°C / 24 to 48 hours
Plate Count Agar (PCA)	Mesophilic aerobic bacteria	35°C / 24 to 48 hours
	Psychrotrophic aerobic bacteria	5°C / 7 days
Iron Agar	Bacteria producing H_2S	20°C / 24 to 48 hours

Table 2. QI scheme for whole tambaqui, *Colossoma macropomum*, ice-storage assessment.

Qualities/ attributes		Descriptors	Score
General appearance	Superficial aspects	Bright colors, golden on dorsal region and black on ventral one	0
		Slight loss of color/ opaque colors	1
		Marked loss of staining	2
		Green coloration on the white sections by bacterial colonization	3
	Odor	Fresh fish smell / suitable odor	0
		Sweetish/acrid	1
		Rancid	2
	Scales	Firm and well attached	0
		Few attached / dropping scales	1
		An easy dropping	2
Eyes	Eyeball	Transparent/ bright	0
		Lightly opaque	1
		Whitish	2
		Opaque/ reddish	3
	Pupil	Dark/rounded/well defined	0
		Lightly opaque/ rounded/ well defined	1
		Loss of design/foggy/opaque/unfit	2
		Opaque/ unfit	3
	Shape	Protuberant/occupying all eye socket/lightly convex	0
		slightly convex	1
		Concave/partially occupying the eye orbit	2
		Concave/ out of the eye orbit	3
Gills	Color	Vibrant red/bright	0
		Dark red/washed-out parts	1
		Brown/ colorless	2
	Odor	Algae	0
		acrid/ metallic/ sweetish	1
		Lightly rancid	2
	Shape	Uniform	0
		Slightly misshapen	1
		Misshapen / shredded	2
	Mucus	Absent/reddish	0
		Yellowish	1
		With blood/ brown coloration/ thick	2
Texture	Ventral elasticity	Rigid	0
		Good elasticity	1
		Lightly firm	2
		Loss of elasticity/soft	3
	Dorsal elasticity	Good elasticity	0
		Lightly firm	1
		Loss of elasticity/soft	2
Quality index (QI)			0-29

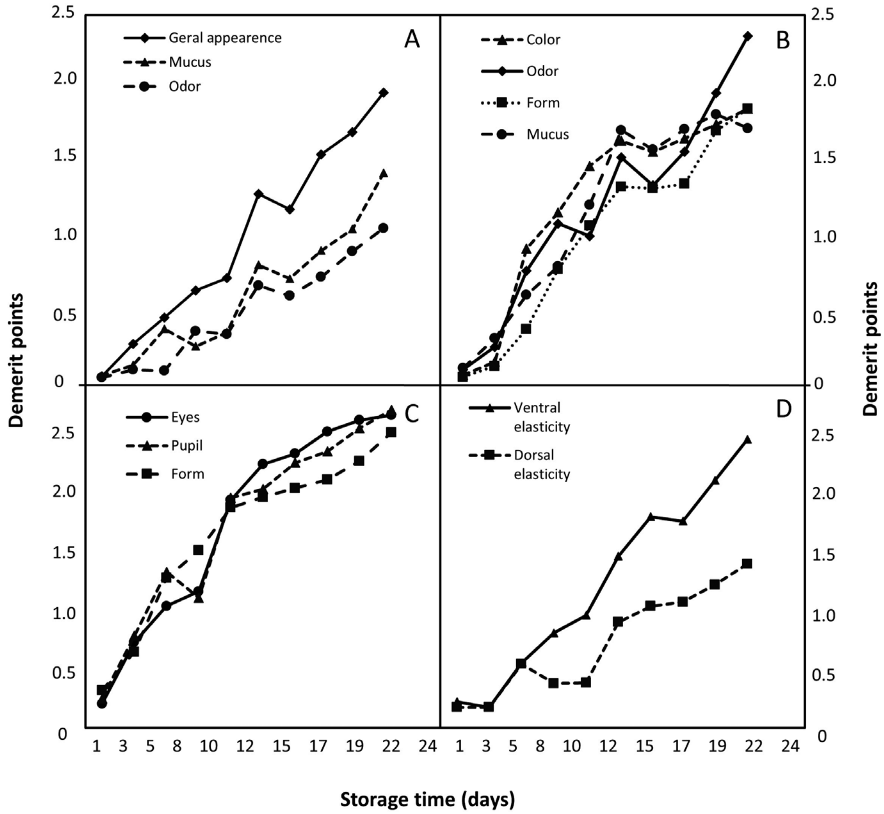

Figure 1. Average demerit scores given on different storage days for each quality attribute of ice-stored whole tambaqui, *Colossoma macropomum*: (A) appearance; (B) gills; (C) eyes; (D) texture.

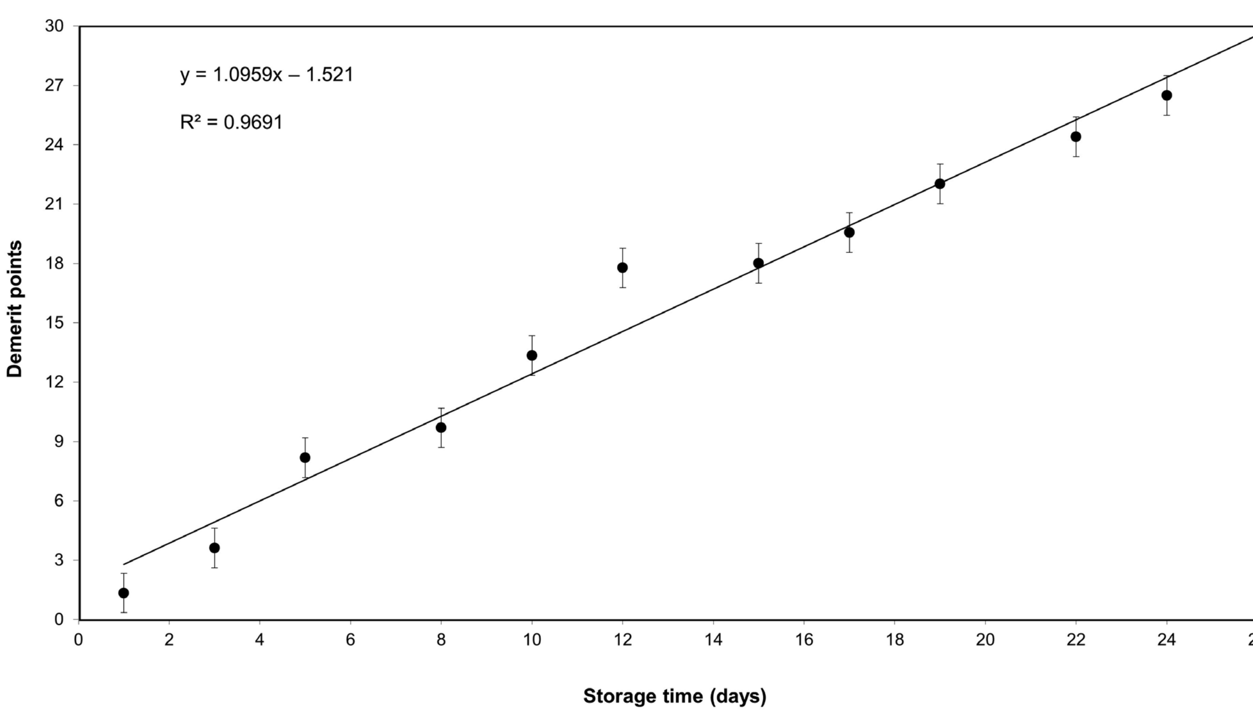

Figure 2. Linear correlation between QI and the 29 demerit points for ice-stored whole tambaqui, *Colossoma macropomum*. Bars represent the daily standard deviation.

the most important attribute in fish sensory analysis, changed gradually throughout storage time, and rancid smell only had higher demerit points on the 22nd day (Figure 1).

The elasticity losses observed by the jury in tambaqui muscle could be related to the reduction of 21.68% in muscle protein from day 1 to 9, and 1.30% from day 9 to 18 (Table 3).

At rejection (22nd day), the fish received 26 demerit points, 86% of the QI maximum score (Figure 2). At this point of evaluation, gills presented rancid odor, eyes were opaque and red, and texture was flaccid from lost elasticity (Figure 1), which led the jury to reject the samples for consumption.

The Quality Index is based on a statistical model that correlates sensory analysis with storage time. The correlation between tambaqui QI parameters and predicted values can be observed in the partial least squares (PLS) regression, which had a strong correlation coefficient (R^2=0.981) between demerit points and the prediction model (Figure 3). The contribution of each parameter is demonstrated on Variable Importance in the Projection (VIP) (Figure 4), which showed positive and negative importance in its development through standardized coefficients. All the QI attributes were considered significant by the model (supporting the jury's observations), even gill mucus and color, which showed lower significance in the model. The standard deviation estimated for the QI was 1.099 days according to the PLS regression with the 95% regression confidence limits (Figure 3), while the standard error of performance (SEP), used to assess the precision of the QI prediction in days, was 0.845, meaning that shelf-life may vary by one or more days.

TVB-N values increased from 15.23 to 23.17 mg N 100 g^{-1} from day 1 to 22, while pH increased from 6.52 to 6.71 in the same period (Figure 5). The mesophilic, psychrophilic and psychotropic bacteria remained under 10^6 CFU during the study period, and H_2S producing bacteria showed reduced growth rate throughout the experiment (Figure 6).

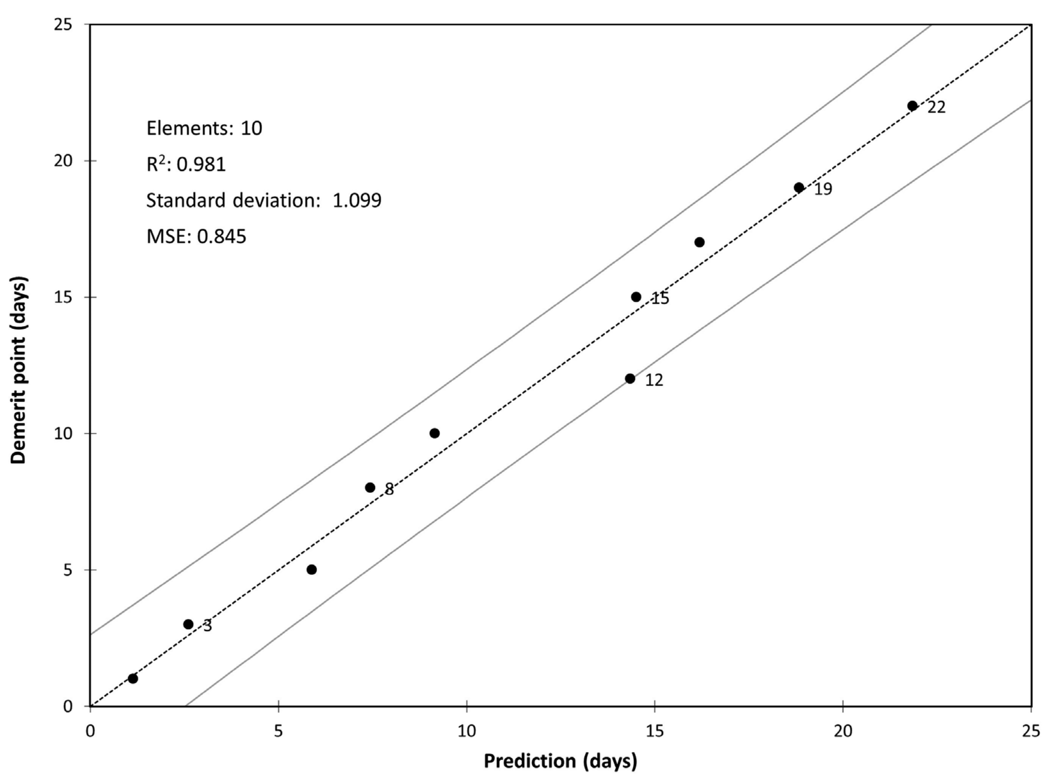

Figure 3. Partial Least Square (PLS) regression of QI with 29 demerit points versus the predicted scores. Traced lines represent 95% regression confidence limits

Table 3. Proximate composition (moisture, protein and lipids) of whole ice-stored tambaqui, *Colossoma macropomum*, on day 1, 9 and 18 of its shelf life.

Day	1º	9º	18º	CV (%)
Moisture (%)	79.90^a	81.26^b	81.56^b	0.79
Crude Protein (%)	22.37^a	17.56^b	17.52^b	9.62
Lipids (%)	0.48^a	0.37^a	0.51^a	61.86

Means on the same line followed by different letters are significantly different (p<0.05), n = 12.

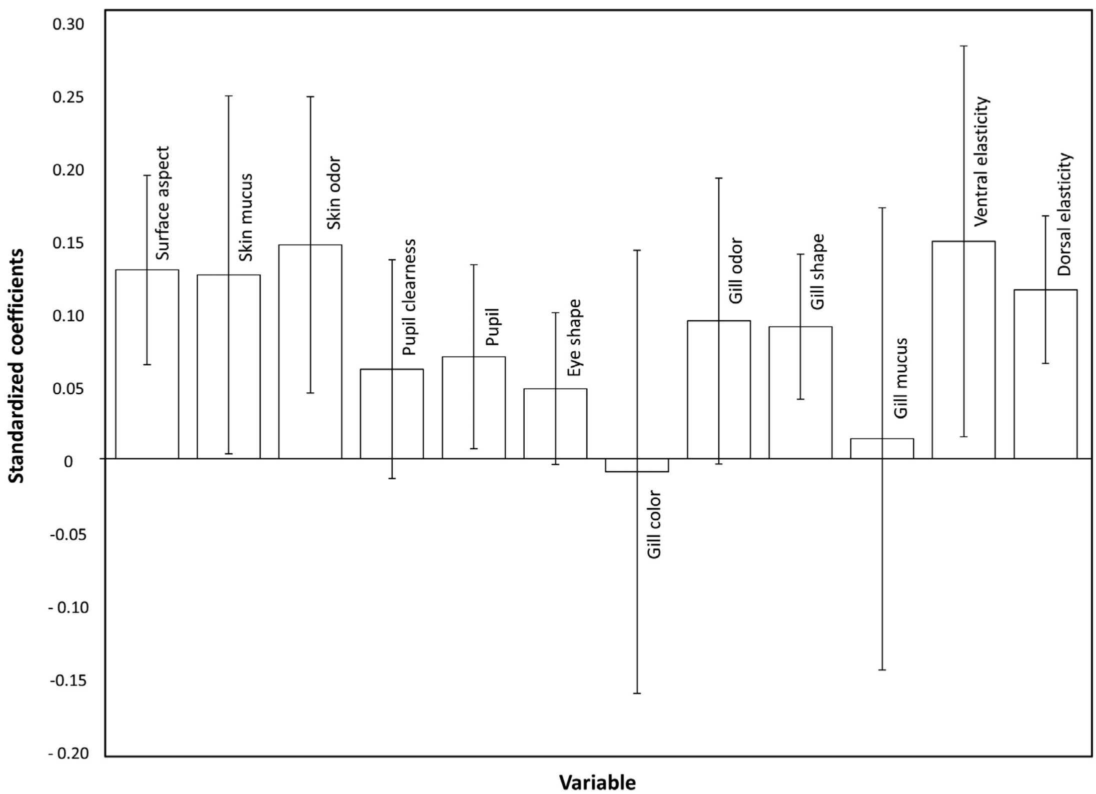

Figure 4. The variable importance in the projection (VIP) for QI parameters developed for ice-stored whole tambaqui, *Colossoma macropomum*, with a 95% regression confidence.

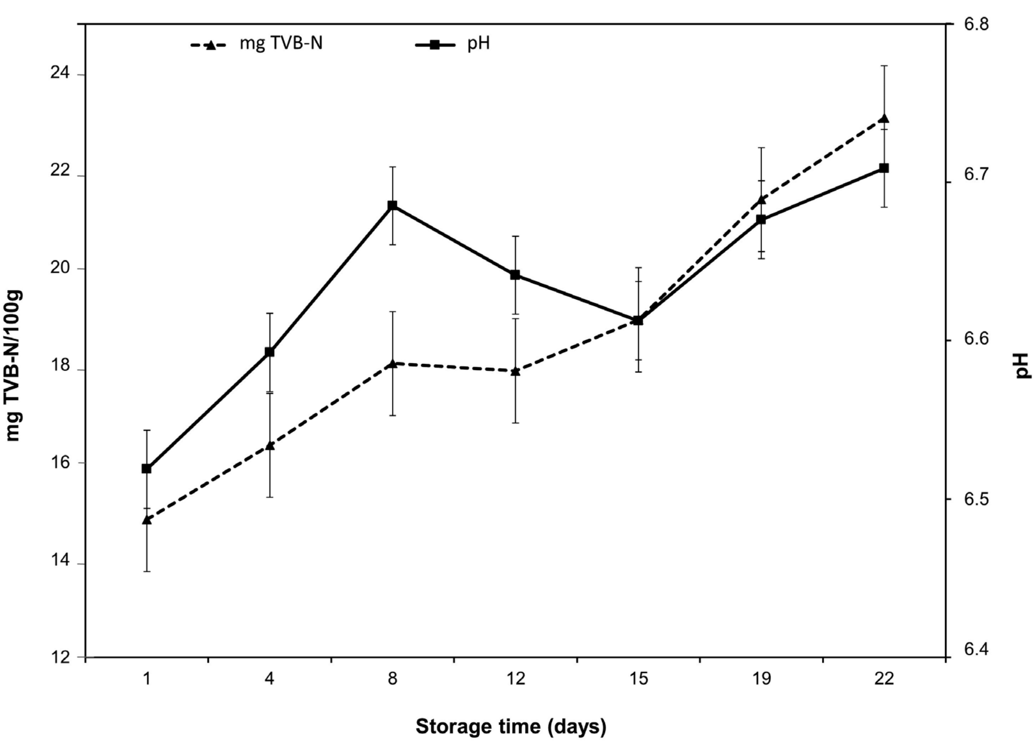

Figure 5. Variation of pH and TVB-N (mg 100 g^{-1}) for whole tambaqui, *Colossoma macropomum* stored in ice.

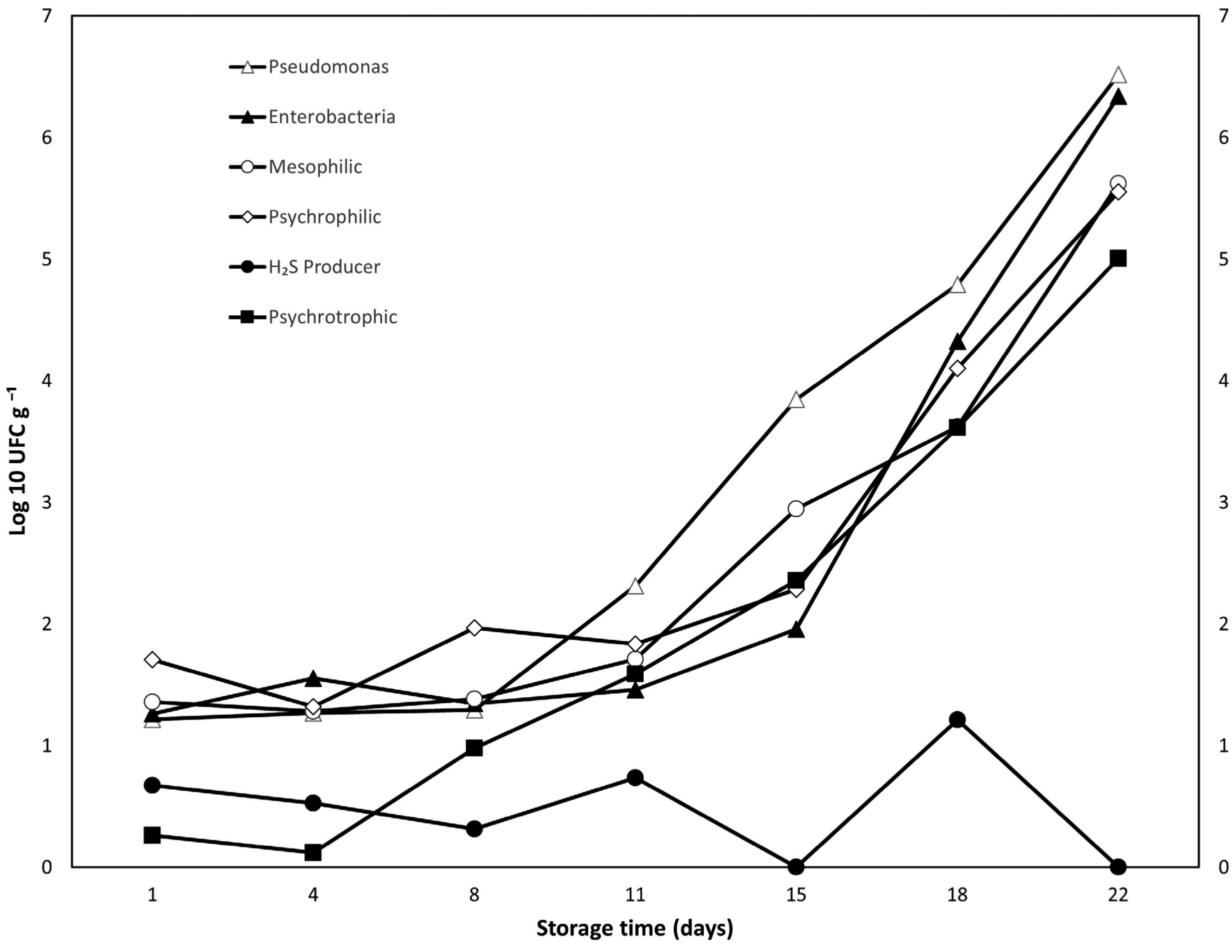

Figure 6. Mean counts of bacteria Pseudomonas, Enterobacteriaceae, H_2S Producers, mesophilic, and psychrotrophic (log CFU g^{-1}), during the period in ice storage (days) of whole tambaqui, *Colossoma macropomum*.

DISCUSSION

The QI scheme in this study had four quality attributes divided into 12 parameters and 29 demerit points, which was almost 20% lower than that developed for eviscerated tambaqui (36 demerit points) (Araujo *et al.* 2016). However, similar results were obtained for pacu, *Piaractus mesopotamicus*, with 16 parameters and 32 demerit points (Borges *et al.* 2013) and for the hybrid tambacu (*C. macropomum* and *P. mesopotamicus*), with 11 parameters and 26 demerit points (Borges *et al.* 2014a). That similarity is due to pacu and tambacu belonging to the Characidae family (Ritter *et al.* 2016). For species such as seabream, *Sparus aurata* Linnaeus, a maximum score with only 15 demerit points was obtained (Simat *et al.* 2012). The different results show the importance of QI to evaluate fish quality, because it is species-specific and considers the different perceptions of the jury (Lanzarin *et al.* 2016).

The Quality Index parameters increased linearly with the storage time. Odor and general appearance increased slowly, while the attributes related to the eyes ("transparency", "pupil" and "eye shape") and gills ("color" and "odor") presented the highest loss of freshness. These results agree with Almeida *et al.* (2006) who observed that the first changes in tambaqui stored in ice included a larger amount of mucus on the gills and a smooth deformation of the eyes caused by friction with the ice. The autolytic reactions caused by the increased storage time, combined with the water absorption from the melting ice, make the observation of the eyes and evaluation of their quality attributes more difficult and confusing to the jury (Ritter *et al.* 2016). Similarly, the eyes of Amazonian pintados, *Pseudoplatystoma fasciatum* (Linnaeus, 1766) x *Leiarius marmoratus* (Gill, 1870) remained only slightly opaque until the 16th storage day, then turning opaque, with a progressive increase in the demerit points with increasing storage time (Lanzarin *et al.* 2016). In the piramutaba or Laulao catfish, *Branchyplatystoma vaillant* (Valenciennes, 1840), the opacity of pupil and cornea, including the eye flatness, were reported on the tenth storage day (Marinho *et al.* 2014). A similar evolution of the eye attributes was observed for the Atlantic salmon, *Salmo salar* L. (Sveinsdóttir *et al.*2002) and the croaker, *Micropogonias furnieri* (Desmarest, 1823) (Teixeira *et al.* 2009).

The changes of gill color and odor seems to be characteristic for the Characidae fish, since in the hybrid tambatinga, the color of gills was also initially red blood, becoming brownish red and reaching a brownish color throughout the ice storage (Ritter *et al.* 2016). Off odor is the primary cause of acceptability reduction of fish by consumers and it is due to large amounts of non-protein nitrogen, high content of fat and autolytic enzymes in the fish tissues (Huss 1995).

Because of these rapid changes of sensorial parameters, the total of demerit points did not reach the maximum score during the shelf-life study. The QI for quality evaluation of cod, *Gadus morhua* (Linnaeus, 1758), stored on ice at 0 ± 1 °C during 14 days, reached 18 points as maximum score, which resulted in a shelf-life of eight days (Bonilla *et al.* 2007). The QI for pacu reached a total of 18 demerit points (56.2% of the maximum score) (Borges *et al.* 2013).

Our QI for whole ice-storaged tambaqui presented statistically sound parameter values. The variable importance in the projection (VIP) shows relevance when attributes are ≥1.0 (Donadoni *et al.* 2012). Therefore, skin mucus and odor, as well as general appearance and ventral elasticity had the highest importance for tambaqui QI. For eviscerated tambaqui all the quality attributes, except for blood press eyes, were relevant for the QI scheme (Araujo *et al.* 2016).

The values determined for standard deviation (1.099 days) and standard error of performance (0.845) were within the estimated range for others species. SEP was 1.184 for pacu (Borges *et al.* 2013), 1.057 for tambacu (Borges *et al.* 2014b) and 2.9 for eviscerated tambaqui (Araújo *et al.* 2016), usually at intervals of up to 1 and 5 days. Cyprian *et al.* (2008) developed a QI for arctic char, *Salvelinus alpinus* (Linnaeus), a freshwater fish, with results similar to those found in the present study: quality attributes and storage time had a high linear correlation and the SEP was only 1.48 (one day).

The TVB-N values for whole tambaqui on ice over 22 days complied with the Brazilian legislation (maximum of 30 mg N.100 g^{-1}; Brasil 1997). Changes in TVB-N values found for Amazonian pintado were within 9.45 (on the first day of ice storage) and 13.37 N.100 g^{-1}, with a significant linear increase of 41.48% after 28 days of ice storage ($p<0.01$) (Lanzarin *et al.* 2016). The perception of odor in fish can be related to the gradual increase of TVB-N caused by bacterial activity (Huss 1995). The metabolites produced by mesophilic bacteria were probably responsible for the decrease in the sensory odor quality of tambacu (Borges *et al.* 2014b) that was also observed for tambaqui in this study.

The increase in pH measured throughout the storage of whole tambaqui can be explained by *post mortem* biochemical reactions in fish muscle. After slaughter, a small decrease in pH occurs as a result of disruption of the oxygen supply to the muscle tissue, where muscle glycogen becomes anaerobic and converted to lactic acid by glycolysis (Mendes *et al.* 2017). The gradual increase in pH values after harvesting also reflected the production of alkaline bacterial metabolites in spoiling fish and coincided with the increase in total volatile basic nitrogen (Abbas *et al.* 2008). Similar results were reported for tambatinga, whith a linear increase in pH from 6.25 to 6.74 at day 28 (Ritter *et al.* 2016), and for eviscerated tambaqui, whith an increase in pH from 6.01 (first day) to 6.45 at the end of shelf-life (22th day) (Araujo *et al.* 2016). The Industrial Inspection and Sanitary Regulation of Animal Products - RIISPOA (Brasil 2001) establishes that the maximum pH for fresh fish should be below 6.8 in external meat and 6.5 in the inner flesh (Cartonilho and Jesus 2011). For marine fish, pH values in the muscle can be very low (approximately 5.50) during the beginning of storage (Shiau 2007) and increase with time.

The pH and the microbial development and activity are proportionally related. Increased pH leads to greater microorganism growth (Simat *et al.* 2012). In the present study, pH increased from the 12th day on concomitantly with bacteria counting (mesophilic, psychotropic, psychrophilic and Pseudomonas). Microorganisms are very important for the evaluation of fresh fish, because many of the volatile compounds are produced from the metabolism of specific spoilage organisms (SSO), including volatile sulfur compounds typical of the deterioration of fish (Huss 1997) and loss of muscle elasticity (Ando *et al.* 1999).

In this study, Pseudomonas and Enterobacteria counting showed an increase close to the limit recommended by the World Health Organization (WHO) (10^7 Colony Forming Units - CFU). Even under lower temperature, a strong growth of heterotrophic aerobic psychrotrophic bacteria was observed in tambatinga (Ritter *et al.* 2016). This growth may also be due to the use of gutted tambatinga, which can favor the entry of microorganisms into the muscle, which then stimulate the spoilage process. The samples analyzed in this study can be considered satisfactory in relation to hygiene of storage conditions, since they did not exceed the acceptable limits until the 22nd day of storage (Huss 1997).

All the observation points were very close to the abscissa in the regression line, except for day 12, exactly when the bacteria count increased. Judges were in better agreement when evaluating the fresh fish using the QI protocol, as compared to spoilage fish (Sveinsdóttir *et al.* 2002). This was observed in the shelf-life study: the judges sensed the sensory changes caused by bacteria growth. These differences demonstrate that QI is important for fish quality evaluation, but the most appropriate assessment should include a set of methods (microbiological, physico-chemical and sensory analysis) in order to achieve more concise and relevant results (Ritter *et al.* 2016).

The elasticity losses observed by the jury in tambaqui muscle and the reduction in muscle protein were also observed in super chilled salmon fillets, which showed a loss of 16% up to the 7th storage day, and a 2% reduction from the 7th to 14th day (Kaale and Eikevik 2016). The biological proteins decrease because of reactions with the amine groups of lysine, the oxidation of methionine, and other amino acids changes (Tironi *et al.* 2009), and it is also related to texture modifications by cross-linking polypeptide chains (Aubourg 1999). Thus, proteases and microorganisms cause natural degradation of myofibrillar proteins and collagen (Chéret *et al.* 2006) and explain how the muscle becomes weak during ice storage (Ando *et al.* 1999). The collagen degradation of muscle fibers of cobia (*Rachycentron canadum*) was as certained by sensory and histological analyses (Fogaça *et al.* 2016). After death, collagen was degraded through a progressive breakdown of the collagen junctions between the myocommata and the muscle fibers (Delbarre-Ladrat *et al.* 2006) caused by microbial and enzyme reactions that are promoted by pH increases (Huss 1997).

CONCLUSIONS

The IQ protocol developed for whole tambaqui stored in ice was efficient and reliable for sensory, chemical and microbiological changes. Under the experimental conditions of this study, the analysis of TVB-N cannot be considered a good index for evaluating the loss of freshness of tambaqui stored in ice. Therefore, it is suggested that whole tambaqui *Colossoma macropomum* stored in ice has a shelf-life of 22 days.

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