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Quality assessment of amazonian fish from fish farming stored on ice

Avaliação da qualidade de pescados amazônicos provenientes de piscicultura armazenados em gelo

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ABSTRACT: The cultivation of natives Amazonian fish such as tambaqui (*Colossoma macropomum*), pirapitinga (*Piaractus brachypomus*), tambatinga (female, *Colossoma macropomum* X *Piaractus brachypomus*, male) and Pintando da Amazônia (female, *Pseudoplatystoma* sp. X *Leiarius marmoratus*, male) temmoratus, increased in recent years, making studies related to the validity of these foods after processing necessary. Therefore, the aimed of the study was to evaluate the sensory, microbiological (*Staphylococcus coagulase* positive count and presence of *Salmonella* spp.) and chemical (pH and Total Volatile Bases (TVB) quality of fresh gutted fish stored on ice. Nine specimens of each species were used, all cultivated in an excavated tanks. The fasting time ranged from 16 to 48 hours, depending on the species, the fish was slaughtered by means of cold shock. The fish was gutted and stored in the fish fridge. Sensory analysis was performed by a trained team available. For other analyses, it was sent to a laboratory specializing in food analysis. The sensory analysis of fresh eviscerated tambaqui was characteristic of the species until the 15th day of storage. Fresh eviscerated pirapitinga presented until the 16th day. The fresh eviscerated tambatinga hybrid was kept until the 14th day of storage. And for the Pintado da Amazônia, the organoleptic characteristics were presentable until the 12th day of storage. The microbiological and physical-chemical analyzes were in accordance with Brazilian legislation in all specimens of the analyzed species. It was observed that the longer the fasting period, there was a numerical increase in the pH of the fish meat.

KEYWORDS: *Colossoma macropomum*; *Piaractus brachypomus*; *Colossoma macropomum* X *Piaractus brachypomus*; *Pseudoplatystoma sp.* X *Leiarius marmoratus*

RESUMO: O cultivo de peixes nativos da Amazônia como tambaqui (*Colossoma macropomum*), pirapitinga (*Piaractus brachypomus*), tambatinga (fêmea, *Colossoma macropomum* X *Piaractus brachypomus,* macho) e o pintado da Amazônia (fêmea, *Pseudoplatystoma* sp. X *Leiarius marmoratus,* macho) tem aumentado nos últimos anos, fazendo com que estudos relacionados a validade desses alimentos após o processamento sejam necessários. Por isso, o objetivo do estudo foi avaliar os parâmetros de qualidade sensorial, microbiológica (contagem de *Staphylococcus coagulase* positiva e presença de *Salmonella* spp.) e química (pH e Bases Voláteis Totais (BVT)) deses pescados eviscerados frescos armazenados em gelo. Foram utilizados 9 exemplares de cada espécie, todos de origem de cultivo em viveiro escavado. O tempo de jejum variou de 16 a 48 horas, dependendo da espécie, o abate do pescado foi por meio de choque térmico. O pescado foi eviscerado e armazenado no frigorifico de pescado. Analise sensorial foi realizada por uma equipe treinada disponível. Para as demais análises foi enviado para um laboratório especializado em análises de alimentos. A análise sensorial do tambaqui eviscerado fresco estava característica da espécie até o 15°dia de estocagem. A pirapitinga eviscerado fresco apresentou até o 16°dia. O hibrido tambatinga eviscerado fresco foi até o 14°dia de estocagem. E para o pintado da Amazônia as características organolépticas estavam apresentáveis até o dia 12° dia de armazenamento. As analises microbiológicas e físico químicas estavam de acordo com a legislação brasileira em todos os exemplares das espécies analisadas. Foi observado que quanto maior o tempo de jejum, houve um aumento numérico no pH da carne dos pescados.

PALAVRAS-CHAVE: *Colossoma macropomum*; *Piaractus brachypomus*; *Colossoma macropomum* X *Piaractus brachypomus*; *Pseudoplatystoma sp.* X *Leiarius marmoratus*

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INTRODUCTION

Food from aquaculture plays a vital role in providing healthy diets and livelihoods for millions of people around the world (FARMERY; ALLISON, 2021). In this context, the production of fish of Amazon origin is inserted, which appears as a promising market nationally and internationally (SILVA, 2021; DANTAS FILHO et al., 2021). It is noteworthy that in the Amazon region, round fish and their hybrids play an important role in food production and in the local economy, among which the tambaqui (*Colossoma macropomum*) stand out, followed by pirapitinga (*Piaractus brachypomus*), and the hybrid a tambatinga (female, *Colossoma macropomum* X *Piaractus brachypomus*, male) (VALLADÃO et al., 2018). In addition to the round species, the hybrid pintado da Amazônia (female, *Pseudoplatystoma* sp. X *Leiarius marmoratus*, male) is highly appreciated because of the absence of thorns in the fillet and has excellent quality meat, due to its firm texture and light color (LABARRÈRE et al., 2013; VALLADÃO et al., 2018).

The commercialization of fish in the Amazon region is usually sold fresh, cooled with ice. The Regulamento da Inspeção Industrial e Sanitária de Produtos de Origem Animal (RIISPOA) considers fresh fish to be that which has not been subjected to any conservation process, except for the action of ice (BRASIL, 2020). However, fish are highly perishable after slaughter due to the rapid appearance of cadaveric changes and bacterial invasion of the viscera with deteriorating action (ORDONEZ, 2005). The speed of appearance of such changes occurs depending on factors such as the species of fish, the way in which they were captured and slaughtered, and storage temperature (KOBLITZ, 2016). Fish can be evaluated for quality through sensory, physicochemical and microbiological analysis (KOBLITZ, 2016). The mechanisms of intrinsic alterations after death can alter the organoleptic characteristics of fish, observed by sight, smell and touch by man. By vision, the characteristics of color and brightness of the eyes, gills and integument can be observed. By smell, you can notice the characteristic odors of fresh fish. By touch, the texture of the musculature and fins can be perceived, as well as the adherence of the scales to the integument (KOBLITZ, 2016, BRASIL, 2020).

Physicochemical analyzes aim to indicate the freshness of the fish and must coincide with the sensory appreciation (KOBLITZ, 2016). Through these analyzes it is possible to quantify degradation compounds in fish (OLIVEIRA et al., 2019). For this, measurements of pH, total volatile bases (TVB) and others are performed (TAVARES; MORENO, 2005; LIMA et al., 2019). The perishability of fish can be accelerated by the presence of spoilage microorganisms (HERNÁNDEZ et al., 2009; GONZAGA JUNIOR, 2014). In addition, the monitoring of pathogenic microorganisms must be carried out to ensure that the food is safe for consumption and free from infectious and toxinogenic diseases (LIMA et al., 2019). Some factors can accelerate the development of microorganisms, and thus, reducing the shelf life of fish. Among these factors are pH, water activity, nutrient content and temperature (GONZAGA JUNIOR, 2014; SLEDER et al., 2015). The evaluation of the presence of *Salmonella* spp. and *Staphylococcus coagulase* positive count in fish may indicate both that the raw material arrived contaminated in the industry and that the processed food was contaminated by hygiene failures during processing (LIBRELATO; SHIKIDA, 2005; OLIVEIRA et al., 2019).

Therefore, in order to commercialize fish on the national or international market, the products must be microbiologically within the limits established by legislation (LIMA et al., 2019). Therefore, the quality and shelf life of fish is influenced by the amount of microorganisms present and by adequate storage (SABAINI et al., 2015 DURANI et al., 2021). However, currently there is little research on the shelf life of fresh gutted Amazonian fish. Therefore, the topic remains current and of importance given the potential of fish production in the Amazon.

Given the assumptions, the aimed of this study was to evaluate the meat quality of tambaqui (*Colossoma macropomum*), pirapitinga (*Piaractus brachypomus*), tambatinga (female, *Colossoma macropomum* X *Piaractus brachypomus*, male) and pintado da Amazônia (female, *Pseudoplatystoma* sp. X *Leiarius marmoratus*, male) as a function of sensory, microbiological and chemical parameters.

MATERIAL AND METHODS

As the study was carried out with animals slaughtered by the fish processing unit, it was not necessary to submit the research to the Committee on Ethics in the Use of Animals (CEUA). However, ethical principles were respected in the research.

The research was carried out in a fish processing unit, located in the Rondônia state. The analyzes were performed at the Food Analysis Laboratory, in Ji-Paraná city, RO. To carry out the experiment, data were obtained from fish from the region of Vale do Jamari, RO. Thirty-six specimens were used, nine of each species, tambaqui (*Colossoma macropomum*), pirapitinga (*Piaractus brachypomus*), tambatinga (female, *Colossoma macropomum* X *Piaractus brachypomus*, male) and pintado da Amazônia (female, *Pseudoplatystoma* sp. X *Leiarius marmoratus,* male). It is worth emphasizing that each of the species belongs to the same lot. Following the prescriptions described by Oliveira et al. (2014), the harvest date, fasting time and suppliers were different for each species evaluated (Table 1).

Table 1. Fish harvesting and slaughter date, with their respective weights and fasting period.

Fishes	Dates	Average Weight*	Fasting Period (Hours)
Tambaqui	01/14/2020	2.5kg	48
Pirapitinga	11/23/2019	1.5kg	24
Tambatinga	11/25/2019	2.0kg	16
Pintado da Amazônia	11/23/2019	1.5kg	24

*Weight class considered by the fish industry as ideal for commercialization.

All fishes harvestings were carried out in an excavated tanks, before which the fish were fasted. The fasting period is determined for each species by the fish Industry. After capture, the fish were immediately immersed in water at a temperature close to 0° C for 20 minutes, resulting in slaughter by cold shock according to the adapted methodology by Rodrigues et al., (2013). Subsequently, the fish were stored and transported in ballast in a 2:1 ratio (ice/fish) to the fish company where the fish were washed with ice cold water and stored with ice treated in scales. Then, the fish were eviscerated and placed in trays with ice treated in scales, in the proportion of 800g for one kg of fish, thus keeping the fish at a temperature always close to 0.0 ± 1.0° C, of according to RIISPOA (BRAZIL 2020). During the storage of the product, the amount of ice on the surface of the fish was checked twice a day. When the ice was insufficient, more ice was placed in scales, to maintain the temperature (GONÇALVES, 2011).

Over the storage time in the processing unit, a sensory evaluation of the fresh gutted fish was carried out, by completing a spreadsheet of the self-control plan established by the company and approved by the Ministério da Agricultura, Pecuária e Abastecimento (MAPA), and the appearance, scale, skin, mucus, eyes, operculum, gills, abdomen/muscle, odor and color of fresh gutted fish. In this form, the option in conformity or not in conformity with the fish was marked, in relation to the item evaluated. By a team from the processing unit, which was trained and composed of nine people. The training was based on the Brazilian legislation RIISPOA (BRASIL, 2020) and international standards (ISO 8586, 2012).

Following the prescriptions described by Oliveira et al. (2014), for chemical and microbiological analyses, the fish were sent to the Food Analysis Laboratory, located in Ji-Paraná city, RO, Brazil. Triplicate samples were sent with different days of storage (Table 2).

It is worth emphasizing that the tambaqui on the 22nd day of storage did not arrive at the proper temperature for analysis in the laboratory, and samples of this species and shipping date were discarded. Meanwhile, the pH was determined according to Ordinance No. 1, of October 7, 1981 and the TVB by Normative Instruction No. 20, of July 21, 1999. As for the microorganisms evaluated, for *Staphylococcus coagulase* positive the methodology of according to ABNT NBR ISO 6888-1:2016 and for *Salmonella* spp. the AFNOR BIO 12/16-09/05 was used.

Table 2. Fish sent to the Laboratory in three different storage periods.

Fishes	Storage periods (days)		
	Week 1	Week 2	Week 3
Tambaqui	6	15	22
Pirapitinga	3	12	20
Tambatinga	3	9	20
Pintado da Amazônia	3	12	20

After confirming the assumptions of normality and homoscedasticity by the Shapirow-Wilk and Levene's tests (α=0,05), respectively, a two-way ANOVA was performed to assess the effect of time on the analyzed species, in the same way, the species was used as the second independent variable for the interspecies comparisons.

RESULTS AND DISCUSSION

Sensory analysis

At the beginning of the storage, all the fish analyzed presented a tegument with metallic shine and bright pigmentation, the fins were resistant, the flesh firm, the eyes convex and shiny and with a well-defined black pupil. The scales were well attached and the odor was seaweed. However, the tambatinga from the 16th of storage showed loss of metallic shine, less resistant fins, more flexible body and slight opacity in the eyes, the same was observed in pirapitinga on the 18th day and in tambaqui on the 20th day.

Studies carried out by Silva et al. (2018), observed in the same species, tambaqui and pirapitinga, changes in organoleptic characteristics from the 22nd day stored on ice, with a time longer than that shown in this study. At the same time, shorter periods of conservation on ice were observed in studies carried out with the entire pirapitinga with changes in characteristics after the 13th day. Murthy (2015), as well as Ritter et al. (2016) and Siqueira et al. (2015), observed organoleptic alterations in the eviscerated tambatinga stored on ice after 10 days.

As for the pintado da Amazônia, changes in organoleptic characteristics were observed from the 15th day onwards, with loss of metallic shine, less resistant fins, more flexible body and slight opacity in the eyes. In a study carried out by Oliveira (2015), the captive pintados da Amazônia ones acquired three days after slaughter, and eviscerated only at the time of analysis. In addition, they showed physical changes in the carcass between 8 and 10 days of storage on ice or between 11 and 13 days after slaughter, being considered rejected after the 15th day after slaughter, similar to what was observed in this study.

Microbiological analysis

No differences were observed in microbiological analysis for *Staphylococcus coagulase* positive and for *Salmonella* spp., on different days of storage (Table 3).

The results (Table 3) demonstrate that all samples are within the minimum limits established by Normative Instruction No. 60, of December 23, 2019, which establishes the lists of microbiological standards for foods. According to this Normative Instruction in fresh fish for *Staphylococcus coagulase* positive there is a lower limit of 10^2 CFU/g and an upper limit of 10^3 CFU/g. Since, of five samples, only two can fall between these limits, being considered with marginal quality. However, for

Salmonella spp. in this same product, the minimum limit is considered as Absence, that is, if there is a positive unit, the five samples are rejected (BRASIL, 2019).

Chemical analysis

For all samples analyzed, the pH remained below the limit of 7.0 established by RIISPOA in Article 211 (BRASIL, 2020). There were no statistical differences ($p>0.05$) between the different storage times of the species, but when comparing the species, it is observed that tambaqui had a less acidic pH than the other species analyzed, as shown in the Table 4. According to Gaíva-Carmona (2014), unbled tambaqui meat stored on ice (until the 15th day) may express a pH above 6.50, which corroborates the present study.

For pirapitinga, the pH ranged from 6.29 on the 5th day of storage, reaching 6.25 on the 20th day of storage. For tambatinga the pH ranged from 5.86 on the 3rd day of storage, reaching 5.93 on the 18th day of storage. In the study by Ritter et al. (2016) with gutted tambatinga stored on ice, the pH value found ranged from 6.25 on the first day to 6.74 on the 28th day. Siqueira et al. (2015) observed that eviscerated tambatinga stored on ice, expressed a pH variation between 6.00 and 6.73, in 21 days of storage. According to Bermejo-Poza et al. (2020) in his study, he identified that the longer the fasting time, there is an increase in muscle pH. In the present study, the tambaqui fasted for 48 hours, while the tambatinga fasted for 16 hours, comparing the pH of the two species, the pH of the tambaqui is higher than the fasting time of the tambatinga.

The pH is related to the amount of glycogen available in meat and the decrease in this factor is due to the conversion of glycogen into lactic acid *post mortem*. Because when fish are alive the pH is close to neutral in their muscle tissue (HUSS, 1998). This conversion occurs during *rigor mortis* and is important for meat conservation, as the presence of acids in meat can limit the growth of microorganisms (SILLA-SANTOS, 1996; CORRÊA et al., 2016).

For the hybrid pintado da Amazônia, the pH ranged from 6.31 on the 5th day of storage, reaching 6.36 on the 20th day of storage. In the study by Lanzarin et al. (2016) with the eviscerated pintado da Amazônia stored on ice, found a pH variation from 6.11 to 6.63 during 28 days of storage. The different pH results may be due to several factors such as the fish's nutritional diet and the stress caused at the time of harvesting (HUSS, 1998).

Total Volatile Bases (TVB) comprise ammonia, trimethylamine (TMA), small amounts of dimethylamine (DMA) and methylamine. The increase in this parameter coincides with bacterial decomposition (KOBLITZ, 2016). In Brazilian legislation, the acceptable limit is 30mg of N/100g for TVB in the fish (BRASIL, 2020). For all samples analyzed, the TVB values were below the acceptance limit established by RIISPOA. For tambaqui the samples presented 5.74mg of N/100g in the two shipments on the 6th and 15th days. Gaíva-Carmona (2014) found the value of 7.79 mg of N/100g for the unbled tambaqui stored on ice on the 15th day, being a value above that found in this study, but still within the limits established by legislation. In the research by Silva (2018) there was a variation from 15.23mg of N/100g to 23.17mg of N/100g from the 1st to the 22nd day. It was observed that the tambaquis analyzed by Silva (2018) showed greater degradation on the first day compared to this study.

Regarding the other species analyzed in this study, in the second shipment of samples, the results for TVB showed higher values than the first shipment and the third shipment. This result may be due to the handling of transport to

Table 3. Average values of *Staphylococcus coagulase* positive count and the presence of *Salmonella* spp. in fresh gutted fish on different days of storage at 0.0 ± 1.0° C.

Stocking week	Microbiological Analysis		
	Fishes	S. coagulase positive	Salmonella spp.
W1*	Tambaqui	<1.0X10^2 UFC/g est	Absent
W2**	Tambaqui	<1.0X10^2 UFC/g est	Absent
W1	Pirapitinga	<1.0X10^2 UFC/g est	Absent
W2	Pirapitinga	<1.0X10^2 UFC/g est	Absent
W3***	Pirapitinga	<1.0X10^2 UFC/g est	Absent
W1	Tambatinga	<1.0X10^2 UFC/g est	Absent
W2	Tambatinga	<1.0X10^2 UFC/g est	Absent
W3	Tambatinga	<1.0X10^2 UFC/g est	Absent
W1	Pintado da Amazônia	<1.0X10^2 UFC/g est	Absent
W2	Pintado da Amazônia	<1.0X10^2 UFC/g est	Absent
W3	Pintado da Amazônia	<1.0X10^2 UFC/g est	Absent

Storage weeks W1* (Week 1), W2** (Week 2), and W3*** (Week 3).

Table 4. Average and standard deviation values for pH and TVB contents in fresh gutted fish on different days of storage at 0.0 ± 1.0° C.

Stocking week	Chemical analysis				
	Fishes	pH		TVB (mg of N/100g)	
		Average	SD[1]	Average	SD[1]
W1*	Tambaqui	6.38^{aA}	± 0.03	5.74^{aB}	± 0.01
W2**	Tambaqui	6.38^{aA}	± 0.03	5.74^{aB}	± 0.01
W1	Pirapitinga	6.29^{aA}	± 0.05	5.74^{cB}	± 0.00
W2	Pirapitinga	6.18^{aA}	± 0.03	19.07^{aA}	± 0.04
W3***	Pirapitinga	6.25^{aA}	± 0.03	11.46^{bA}	± 0.03
W1	Tambatinga	5.86^{aB}	± 0.11	11.47^{bA}	± 0.00
W2	Tambatinga	5.87^{aB}	± 0.09	19.09^{aA}	± 0.07
W3	Tambatinga	5.93^{aB}	± 0.24	11.46^{bA}	± 0.04
W1	Pintado da Amazônia	6.31^{aA}	± 0.02	5.74^{cB}	± 0.00
W2	Pintado da Amazônia	6.14^{aA}	± 0.07	19.08^{aA}	± 0.04
W3	Pintado da Amazônia	6.36^{aA}	± 0.05	11.47^{bA}	± 0.03

Storage weeks W1* (Week 1), W2** (Week 2), and W3*** (Week 3). Average and deviation values (± SD) for pH and TVB through the three weeks of storage (W1, W2 and W3) for each species. Lowercase letters represent intra-species comparisons, upper-case letters represent inter-species comparisons for each week, different letters indicate differences ($p<0.05$).

the laboratory, but even so, this degradation did not reach the acceptable limit, which is 30mg of N/100g, therefore, it is in accordance with the standard established by legislation. In Murthy's research (2015), fresh pirapitinga *in natura* obtained on the 13th day the value of 19.97mg of N/100g and fresh eviscerated pirapitinga reached 23.23mg of N/100g, showing that greater manipulation can cause more accelerated degradation. The value found by the author for fresh eviscerated pirapitinga is higher than the value of 19.07mg of N/100g on the 12th day of storage in relation to this study.

Ritter et al. (2016) observed in tambatinga the TVB values between 7.525mg of N/100g on the 1st day to 13.125mg of N/100g on the 28th day of storage on ice. At the same time, Siqueira et al. (2015) observed that eviscerated tambatinga stored on ice varied from 10.78 to 18.29 mg/100g in 21 days. Lanzarin et. al. (2016) when studying the Pintado da Amazônia, obtained results for TVB that ranged from 9.45mg of N/100g on the 1st day to 13.37mg of N/100g on the 28th day of ice storage. The result found in this study of 19.08mg of N/100g on the 12th day is greater than the result found by Lanzarin et. al. (2016) on the 28th day.

CONCLUSIONS

Sensory analysis of fresh eviscerated tambaqui was characteristic of the species until the 15th day of storage. Fresh eviscerated pirapitinga presented until the 16th day. The fresh eviscerated tambatinga hybrid was kept until the 14th day of storage. And for the pintado da Amazônia, the organoleptic characteristics were presentable until the 12th day of storage. The microbiological and physical-chemical analyzes were in accordance with Brazilian legislation in all specimens of the analyzed species.

It was observed that the longer the fasting period, there was a numerical increase in the pH of the fish meat. Studies on these fish are still very scarce in the region, which demonstrates the importance of evaluation parameters to determine the quality of this food source. Also emphasizing the establishment of adequate fasting times for Amazonian fish in future works, with the intention of preserving the quality of the meat.

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