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Agronegócio

Efeitos do estresse pré-abate na qualidade da carne do tambaqui armazenado no gelo

Effects of pre-slaughter stress on the quality of tambaqui meat stored on ice

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RESUMO: O objetivo deste estudo foi avaliar os efeitos do estresse causado por diferentes protocolos pré-abate na qualidade físico-química, sensorial e microbiológica da carne de tambaqui armazenada em gelo por 49 dias. Foram utilizados 144 tambaquis distribuídos em (DIC por seis tratamentos relacionados aos protocolos pré-abate (abate logo após a despesca, após 3h de transporte e após 6h, 12h, 24h ou 44h de recuperação após o transporte). Após a morte, os peixes foram armazenados em caixas térmicas com gelo e cada sete dias, três peixes de cada tratamento foram coletados das caixas de isopor para as análises. Tambaquis abatidos sob estresse logo após a despesca e após o transporte apresentaram filés com maiores taxas de crescimento bacteriano, piores resultados de pH, nitrogênio de bases voláteis totais-NBVT e substâncias reativas ao ácido tiobarbitúrico-TBARS, e coloração escura nos primeiros 21 dias de armazenamento, sendo consideradas impróprias para consumo após 42 dias de armazenamento. Tambaquis submetidos a períodos de recuperação após o transporte apresentaram melhor qualidade de carne, os filés apresentaram maior luminosidade e coloração vermelha inferior após 21 dias armazenados, além de crescimento microbiológico reduzidos e melhores valores para pH, NBVT e TBARS. Os resultados comprovaram que o uso de períodos de recuperação após o transporte e antes do abate melhora a qualidade da carne de tambaqui, aumentando sua vida útil quando armazenada em gelo até 49 dias.

Palavras-chave: Amazônia, *Colossoma macropomum.* Estresse produção animal. Qualidade do pescado.

ABSTRACT: The objective of this study was to evaluate the effects of stress caused by different pre-slaughter protocols on physicochemical, sensory and microbiological quality of tambaqui meat stored on ice for 49 days. A total of 144 Tambaqui fish were used. The experimental design was completely randomized constituted by six treatments related to the pre-slaughter protocols (slaughter right after harvesting, after 3h transport, and after 6h, 12h, 24h or 44h recovery period after transport). After confirming the death, fish were placed in styrofoam boxes with ice. Every seven days, three fish from each treatment were collected from the styrofoam boxes for physical, chemical, sensory and microbiological analyzes. Data collected were subjected to ANOVA and, subsequently, to the Tukey test at 0.01 and 0.05. Fish slaughtered under stress right after harvesting and transport presented fillets with higher bacterial growth rates, and worst values of pH, NBVT and TBARS, in addition to producing fillets with a darker color than other treatments in the first 21 days. These samples were considered unsuitable for consumption after 42 days stored on ice. Fish submitted to recovery periods after transport presented better meat quality, where fillets presented more luminous and less red color after 21 days stored on ice, as well as lower microbiological growth rates, and better values of pH, NBVT and TBARS. Our results proved that the use of recovery periods after transport and before the slaughter improves tambaqui meat quality and increases its shelf life when stored on ice up to 49 days.

Keywords: Amazon. Animal production. *Colossoma macropomum.* Fish meat quality, stress.

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INTRODUCTION

Front the current scenario of aquaculture market, it is essential to seek improvements in all points of the production chain, especially related to fish quality. Studies related to fish pre-slaughter practices since from the farm to the processing industry have very importance due to the negative effects that may occur in the fish meat quality at this stage, which also may provide many economic damages (Rahmanifarah *et al*., 2011; Mendes *et al*., 2015; Mendes *et al*., 2017, Oliveira Filho *et al*., 2021).

Pre-slaughter practices are known to cause high stress in fish, disrupting the animals' balance with the environment (homeostasis) and leading to stress responses (Barton, 2002; Oba *et al*., 2009; Inoue *et al*., 2010; Diniz; Honorato, 2012). Physiologically, this occur due to the fish are exposed to a sequence of stimuli that includes stages of chase and forced swimming, fish exposure to the air and abrasion of its body with the net and with other fish, in addition to the submission to high stocking densities (Digre *et al*., 2010; Lefevre *et al*., 2016). As a result, fish use all their energy reserves before slaughter, causing the acceleration of post-mortem biochemical changes that contribute to fast deterioration of the meat and, consequently, losses in its quality (Digre *et al*., 2017).

These biochemical changes in fish meat caused by pre-slaughter stress may be colorimetric changes, excessive water loss, increased proliferation of microorganisms that produce volatile compounds, and increase muscle pH. All these factors cause very decrease in the fish meat shelf-life (Erikson; Misimi, 2008; Gatica *et al*., 2010; Mendes *et al*., 2015; Mendes *et al*., 2017). The pre-slaughter practices are normally carried out aiming to the fish arrive alive in the industry, it is important to adopt a rest period after transport. Studies attested that this recovery time before slaughter is beneficial due to the animals may to restore or preserving their energy reserves, consequently causing a better quality of their meat (Mendes *et al*., 2015; Mendes *et al*., 2017).

Tambaqui (*Colossoma macropomum*) is one of the most fish species produced in Brazil due to its great performance and meat quality highly appreciated, especially in the Brazilian Northern and Midwest regions (Santos *et al.,* 2009). In last decades, the tambaqui arrived in several new markets, even other countries. However, it is essential that studies about tambaqui keep being carried out aiming to improve its main qualities and processing by the industry. Thus, the objective of this study was to evaluate the effects of stress caused by different pre-slaughter protocols on physicochemical, sensory and microbiological quality of tambaqui meat stored on ice.

2 MATERIAL AND METHODS

2.1 EXPERIMENTAL DESIGN

A total of 144 Tambaqui specimens were used, with an average weight and length of 1,520±0.28 kg and 42.08±3.13 cm, respectively. Fish were obtained in a commercial farm located at km 27, Iranduba town, Amazonas, Brazil. The experimental design was completely randomized constituted by six treatments related to the pre-slaughter protocols (slaughter right after harvesting, after 3h transport, and after 6h, 12h, 24h or 44h recovery period after transport). In all treatments fish were stunned by hypothermia and bled from a cut in the gills.

The harvesting was carried out in the early hours of the day, when 24 fish were slaughtered in the farm being placed on styrofoam boxes with ice and sent to the INPA Food Technology Laboratory. Then, the other fish were placed in a 5000L transport box with aeration system and transported for 3 hours in an open truck to the INPA/COTEI Aquaculture Station. Soon arrival at INPA, another 24 fish were immediately slaughtered and placed on styrofoam boxes with ice and sent to Food Technology Laboratory.

The remainder fish were stored alive in four 2000L tanks with continuous water supply and constant aeration, simulating industry reception tanks processment. Each tank represented the other proposed treatments, where fish were slaughtered according to reaching the recovery periods proposed (6, 12, 24 and 44h after transport). After confirming the death of each treatment, fish were placed in styrofoam boxes with ice and taken to the INPA/COTEI Fish Processing Pilot Plant to monitor the post-mortem biochemical changes.

All styrofoam boxes with ice where fish where stored were identified according to the respective origin treatment. Every seven days, three fish from each treatment were collected from the styrofoam boxes for meat quality analysis. The collections occur at 0, 7, 14, 21, 28, 35, 42 and 49 days of storage, totaling eight weeks of experimental period. All fish were weighed using a scale with a capacity of 10 kg and the standard length was measured using a measuring tape (cm).

2.2 PHYSICAL ANALYZES

The pH determination was carried out in accordance with the analytical methods proposed by the Instituto Adolfo Lutz (2008), using a digital potentiometer device (Sensoglass, model: SP 1400) for precise determination using samples in triplicate. Instrumental color determination was performed in a Minolta colorimeter (model CR-300) using the CIELAB system (CIE, 1986), in CIELAB colorimetric space, defined by L*, a*, b*, with the L* coordinate corresponding to the luminosity, a* and b* refer to the green(-)/red(+) and blue(-)/yellow(+) chromaticity coordinates, respectively. Measurements were performed in triplicate on tambaqui fillets with the previously calibrated device.

2.3 CHEMICAL ANALYZES

The nitrogen determination from the total volatile bases (NBVT) was performed in triplicate, according to the method proposed by Wootlon and Chuah (1981). The TBARS determination was carried out according to methods proposed by Vyncke (1970). To TBARS values calculation, a straight line of the standard curve ($y = 48.946x + 0.0028$) was obtained with tetramethoxypropane, and the results were expressed in mg of malonaldehyde/kg sample. The samples were analyzed at the beginning and at the end of the experimental period.

2.4 SENSORY ANALYZES

For sensory analyses, it was used five trained evaluators and three fish at each treatment and storage period to evaluate physical sensory changes in general appearance (skin, scales, texture, hardness, elasticity, odor), eyes (transparency and shape) and gills (color and odor). This evaluation was performed using the Quality Index Method (MIQ) according to the sensory evaluation table proposed by Larsen *et al*. (1992). Points were assigned for each sensory characteristic analyzed to classify the quality of the evaluated fish.

2.5 MICROBIOLOGICAL ANALYZES

Standard counts of *Staphylococcus aureus*, *Salmonella sp*. and thermotolerant coliforms were performed according to the methos proposed by RDC number 12 (February 01, 2001; Brasil, 2001). The indicator groups were also evaluated to psychrophiles from 10 to 20º C, psychrotrophs from 0 to 7º C and mesophiles at 35º C according to the methods proposed by Silva *et al*. (2001).

2.6 STATISTICAL ANALYZES

Data collected were expressed as mean ± standard deviation (SD) and tested for normality (Shapiro-Wilk test) and homoscedasticity (Levene test). Data collected were subjected to ANOVA and, subsequently, to the Tukey test at 0.01 and 0.05. When the data did not meet the premise of parametric statistics, they were evaluated by the Kruskal-Wallis test.

The study was approved by the Ethics Committee on Animal Experimentation (protocol number 025/2017) of the Instituto Nacional de Pesquisas da Amazônia (INPA).

3 RESULTS

3.1 MUSCLE pH

The muscle pH values of tambaqui stored on ice were statistically different ($p<0.05$) between the treatments studied at all times evaluated (Figure 1). Lower initial pH values were observed in samples from fish collected right after harvesting and after transport. However, after seven days of slaughter and stored on ice and until the end of the period evaluated, these treatments showed higher pH values compared to fish samples submitted to different recovery periods after transport.

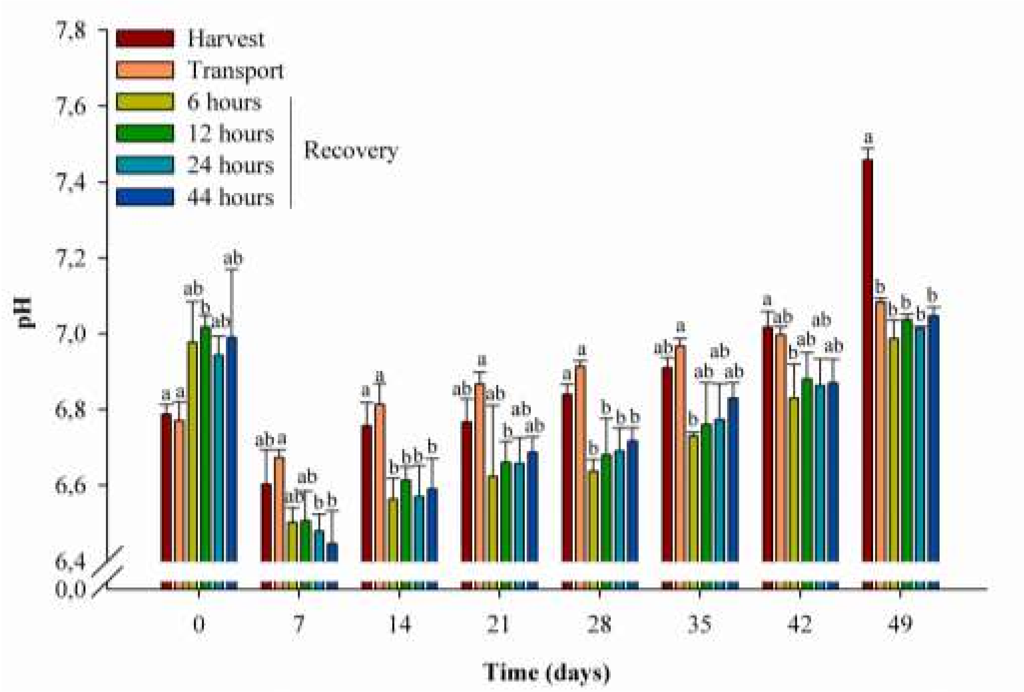

Figure 1. Results of muscle pH in tambaqui meat after different pre-slaughter protocols during 49 days stored on ice. Values are displayed as mean ± standard deviation (n= 3). Lowercase letters represent statistical differences between treatments within the sample time considering p<0.005.

3.2 INSTRUMENTAL COLOR

The meat samples of fish submitted to different recovery periods after transport presented significant higher values of luminosity (p>0.05) in the first 21 days of storage on ice when compared to the samples from fish collected right after harvesting and after transport (Table 1). After 28 days and until the end of the study, this parameter presented very similar values between all treatments. The values found in the parameters a* (red color intensity) were statistically different (p>0.05) between treatments with 0, 14 and 35 days of storage on ice (Table 1). The meat samples of fish collected right after harvesting and after transport presented higher values in the a* parameters up to 14 days of storage on ice when compared to the samples collected in fish submitted to the different recovery periods after transport. The values found in the parameters b* (yellow color intensity) remained similar between treatments during 42 days of the study. Only in the last period evaluated (49 days) there was a significant difference (p>0.05) between treatments, presenting higher values in the meat samples of fish collected right after harvesting (Table 1).

Table 1. Results of light intensity in tambaqui meat after different pre-slaughter protocols during 49 days stored on ice*

	Days	Harvest	Transport	Recovery periods after transport				p-value
				6 hours	12 hours	24 hours	44 hours	
Light intensity (L>0)	0	25.70±1.56[a]	24.78±2.24[a]	35.81±3.04[b]	35.01±0.95[b]	37.07±1.27[b]	36.38±2.79[b]	<0.001
	7	24.43±1.63[a]	24.11±1.47[a]	33.21±1.45[b]	34.83±2.16[b]	35.10±2.53[b]	34.04±3.66[b]	<0.001
	14	24.46±1.43[a]	25.11±1.20[a]	34.82±1.21[b]	34.59±1.09[b]	35.19±3.26[b]	33.72±3.85[b]	<0.001
	21	27.06±2.25[a]	31.27±3.82[ab]	34.95±2.26[b]	34.00±2.15[b]	35.54±2.19[b]	33.52±1.42[ab]	0.011
	28	35.28±2.54	32.87±5.00	34.48±0.88	34.86±7.35	34.54±2.53	35.22±1.11	0.976
	35	35.00±3.47	34.84±1.77	35.32±2.05	34.30±1.16	34.07±3.88	35.53±1.09	0.974
	42	36.65±2.42	37.32±4.14	36.79±0.99	37.06±1.17	37.92±1.67	38.02±1.49	0.959
	49	43.91±2.38	44.09±1.32	41.12±2.32	41.57±1.92	41.43±2.43	40.87±3.13	0.382
Light intensity (a>0)	0	1.98±0.34[a]	2.02±0.18[a]	0.85±0.39[b]	0.88±0.14[b]	0.76±0.17[b]	0.69±0.25[b]	<0.001
	7	2.09±0.59	2.12±0.24	0.88±0.31	0.93±0.13	0.88±0.11	0.75±0.10	0.029*
	14	1.93±0.38[a]	1.93±0.49[a]	0.89±0.15[b]	0.85±0.17[b]	0.96±0.20[b]	0.67±0.19[b]	<0.001
	21	0.99±0.19	0.95 ±0.66	0.91±0.13	0.98±0.07	0.97±0.13	0.74±0.34	0.930
	28	0.52±0.61	0.79±0.35	0.92±0.08	0.83±0.31	0.97±0.19	0.69±0.28	0.663
	35	0.25±0.17[a]	0.77±0.14[b]	0.80±0.12[b]	0.89±0.10[b]	0.98±0.13[b]	0.87±0.13[b]	<0.001
	42	0.40±0.32	0.78±0.32	0.69±0.27	0.82±0.13	0.86±0.19	0.67±0.31	0.454
	49	0.09±0.79	0.54±0.54	0.67±0.34	0.69±0.04	0.70±0.24	0.68±0.28	0.519
Light intensity (b>0)	0	1.47±0.34	1.60±0.29	1.43±0.27	1.15±1.07	1.35±0.12	1.35±0.21	0.892
	7	1.63±0.35	1.75±0.19	1.58±0.51	1.12±0.09	1.32±0.88	1.31±0.21	0.561
	14	1.98±0.11	1.79±0.16	1.81±0.78	1.38±0.19	1.47±0.32	1.54±0.32	0.412
	21	1.94±0.74	1.77±0.89	1.99±0.29	1.48±0.38	1.64±0.29	1.73±0.37	0.863
	28	2.49±1.06	2.02±0.49	1.89±0.74	1.87±0.18	1.89±0.24	1.77±0.28	0.721
	35	2.29±0.27	2.42±0.14	1.81±0.61	1.80±0.20	1.82±0.20	1.88±0.32	0.128
	42	2.89±0.75	2.74±0.39	1.84±0.24	1.86±0.41	1.94±0.54	1.99±0.36	0.054
	49	3.23±0.37[a]	3.11±0.55[ab]	2.16±0.34[b]	1.94±0.26[c]	2.19±0.16[bc]	2.22±0.48[bc]	0.005

* Values are displayed as mean ± standard deviation (n= 3). Lowercase letters represent statistical differences between treatments within the sample time considering p<0.005.

3.3 NBVT

NBVT values were statistically different ($p<0.05$) between treatments after seven days of study (Figure 2). Higher values of NBVT were observed in samples from fish collected right after harvesting and after transport when compared to the treatments with different recovery periods after transport. This accelerated increase was demonstrated over the storage times by a regression curve for each treatment: harvesting ($F = 21.17 - 0.10x + 0.01x^2$; $R^2 = 0.98$); transport ($F = 20.51 + 0.02x + 0.006x^2$; $R^2 = 0.95$); Recovery 6 hours ($F = 17.44 - 0.15x + 0.0083x^2$; R^2

= 0.97), Recovery 12 hours ($F = 16.59 -0.002x + 0.0054x^2$; $R^2 = 0.95$), Recovery 24 hours ($F = 14.92 + 0.04x + 0.0052x^2$; $R^2 = 0.95$) and 44-hour Recovery ($F = 15.31 + 0.06x + 0.0046x^2$; $R^2 = 0.95$).

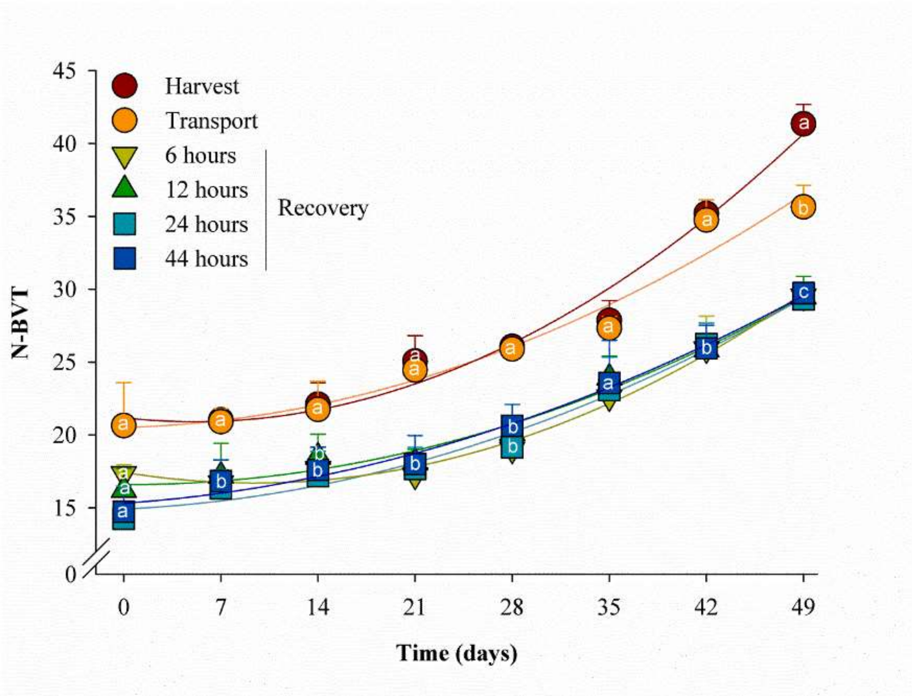

Figure 2. Evolution of NBVT levels in tambaqui meat after different pre-slaughter protocols during 49 days stored on ice. Values are displayed as mean ± standard deviation (n= 3). Lowercase letters represent statistical differences between treatments within the sample time considering $p<0.005$.

3.4 LIPID OXIDATION (TBARS)

The initial lipid oxidation values of tambaqui did not show significant differences ($p>0.05$) between treatments (Figure 3). However, there was significant differences ($p>0.05$) at 49 days stored on ice, presenting higher values of lipid oxidation in the samples of fish collected right after harvesting and after transport.

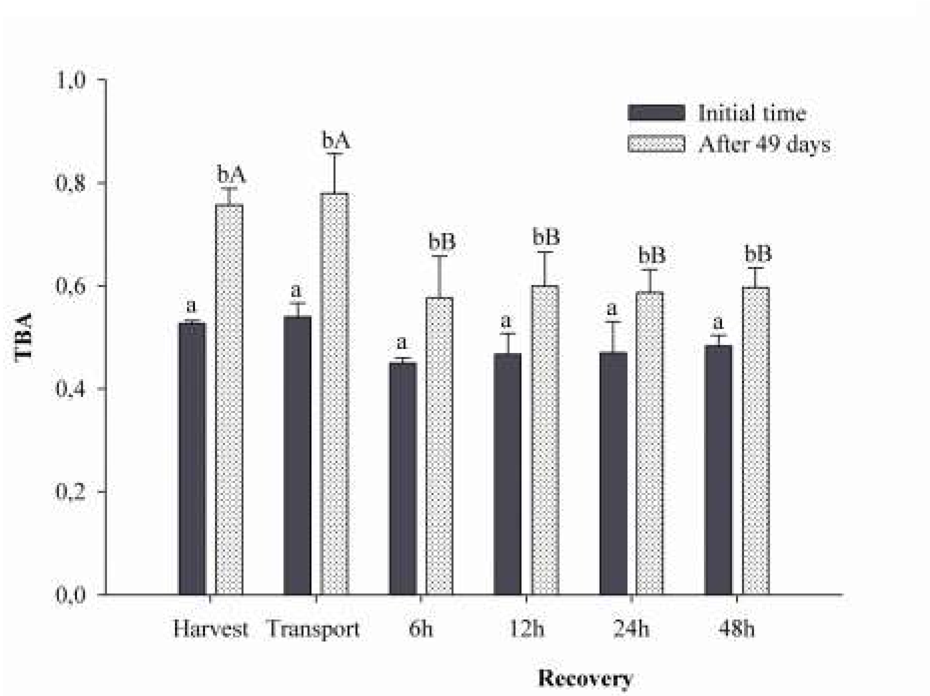

Figure 3. Evolution of TBARS values in tambaqui meat after different pre-slaughter protocols during 49 days stored on ice. Values are displayed as mean ± standard deviation (n= 3). Lowercase letters represent statistical differences between treatments within the sample time considering $p<0.005$.

3.5 SENSORY ANALYZES

The mean scores of the sensory parameters increased according to the storage time of tambaqui meat on ice. However, significant differences ($p>0.05$) were only observed between treatments after 21 days of storage (Figure 4). No significant effect of pre-slaughter stress was observed up to seven days of storage and all fish samples presented excellent quality during this period. After seven days, changes in meat quality were observed for each treatment. On 21 days of fish meat storage on ice, samples from fish collected right after harvesting and after transport presented regular quality, with characteristics such as strong smell, completely opaque eyes and presence of mucus in the dorsal region, when compared to the treatments with different recovery periods after transport that presented good quality. On 42 days of fish meat storage on ice, samples from fish collected right after harvesting and after transport were considered by the panelists as not suitable for consumption, while the meat of fish submitted to recovery periods after 49 days of storage on ice still had regular quality. This increasing loss of quality is demonstrated by the linear regression curve for the treatments harvest ($F = 1.87 + 0.24x$, $R^2 = 0.98$) and transport ($F = 2.19 + 0.24x$, $R^2 = 0.98$) and by the quadratic polynomial regression model for the 6-hour Recovery treatments ($F = 2.47 + 0.029x + 0.0026x^2$; $R^2 = 0.97$), 12-hour

Recovery ($F = 2.57 + 0.0013x + 0.0033x^2$; $R^2 = 0.95$), 24-hour Recovery ($F = 2.45 + 0.033x + 0.0027x^2$; $R^2 = 0.96$) and 44-hour Recovery ($F = 2.50 + 0.022x + 0.0030x^2$; $R^2 = 0.96$).

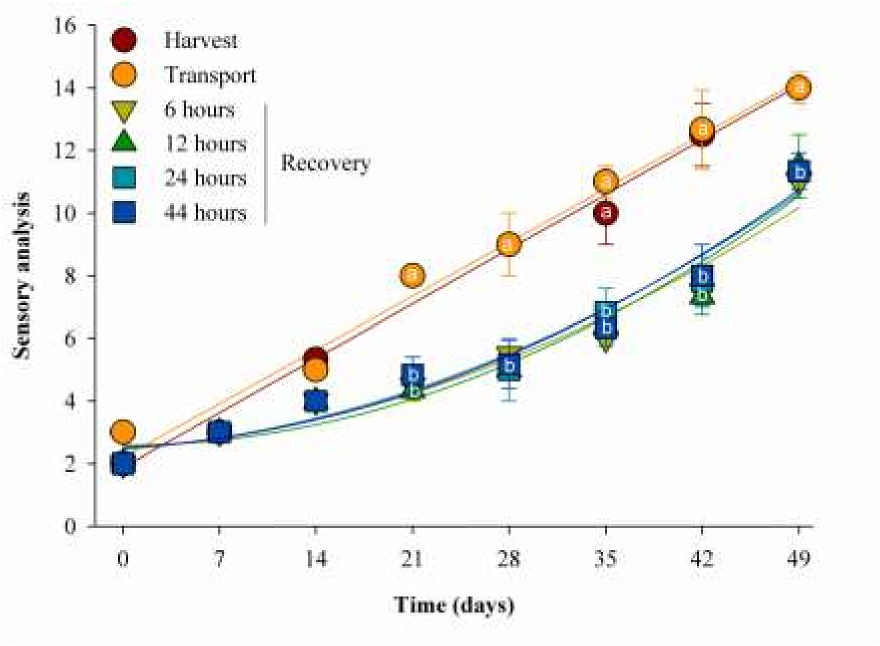

Figure 4. Evolution of sensory characteristics in tambaqui meat after different pre-slaughter protocols during 49 days stored on ice. Values are displayed as mean ± standard deviation (n= 3). Lowercase letters represent statistical differences between treatments within the sample time considering $p<0.005$.

3.6 MICROBIOLOGICAL ANALYZES

It was not identified the presence of *Salmonella* sp., thermotolerant Coliforms and *Staphylococcus aureus* in the tambaqui meat samples evaluated, remaining within the standards established by current legislation (BRASIL, 2001). On seven days of storage on ice, samples from fish collected right after harvesting and after transport presented higher counts of mesophilic bacteria, remaining with higher counts until the end of the experimental period when compared to the treatments with different recovery periods after transport (Table 2). These treatments also presented higher counts for piscotrophic and psychrophilic bacteria groups (Table 2) after 14 days of storage on ice, remaining with higher counts until the end of the experimental period. The treatments of the recovery periods of 24h and 44h presented the lowest values in the count of the piscotrophs and psychrophils (CFU/g) during the experimental period.

Table 2. Mesophilic, Piscotrophs and Psychrophils bacteria count (CFU/g) in tambaqui meat after different pre-slaughter protocols during 49 days stored on ice

	Days	Harvest	Transport	Recovery periods after transport			
				6 hours	12 hours	24 hours	44 hours
Mesophilic bacteria	0	$3x10^2$	$5x10^2$	$1x10^2$	Absent	$1x10^2$	$1x10^2$
	7	$8x10^2$	$7x10^3$	$1x10^2$	$1x10^2$	Absent	$1x10^2$
	14	$7x10^3$	$11x10^3$	$6x10^2$	$1x10^2$	$1x10^2$	$5x10^2$
	21	$7x10^3$	$13x10^3$	$8x10^2$	$3x10^2$	$1x10^2$	$9x10^2$
	28	$8x10^3$	$17x10^3$	$3x10^3$	$6x10^2$	$7x10^2$	$4x10^3$
	35	$9x10^3$	$25x10^3$	$6x10^3$	$3x10^3$	$3x10^3$	$5x10^3$
	42	$12x10^3$	$35x10^3$	$8x10^3$	$5x10^3$	$8x10^3$	$8x10^3$
	49	$17x10^3$	$28x10^3$	$9x10^3$	$7x10^3$	$10x10^3$	$9x10^3$
Piscotrophs bacteria	0	$1x10^2$	Absent	$1x10^2$	Absent	Absent	$1x10^2$
	7	$1x10^3$	$3x10^3$	$1x10^2$	$1x10^2$	$1x10^2$	Absent
	14	$8x10^3$	$6x10^3$	Absent	Absent	$1x10^2$	Absent
	21	$27x10^3$	$47x10^3$	$3x10^3$	$2x10^3$	$9x10^2$	$9x10^2$
	28	Countless	Countless	$5x10^3$	$4x10^3$	$4x10^3$	$6x10^3$
	35	Countless	Countless	$9x10^3$	$11x10^3$	$11x10^3$	$16x10^3$
	42	Countless	Countless	$37x10^3$	$32x 10^3$	$33x10^3$	$35x10^3$
	49	Countless	Countless	Countless	Countless	Countless	Countless
Psychrophils bacteria	0	Absent	Absent	Absent	Absent	Absent	Absent
	7	Absent	$1x10^2$	Absent	Absent	Absent	Absent
	14	$3x10^3$	$2x10^3$	Absent	Absent	Absent	Absent
	21	$37x10^3$	$43x10^3$	$4x10^3$	$4x10^3$	$6x10^3$	$5x10^3$
	28	Countless	Countless	$9x10^3$	$16x10^3$	$14x10^3$	$17x10^3$
	35	Countless	Countless	$28x10^3$	$25x10^3$	$26x10^3$	$26x10^3$
	42	Countless	Countless	$41x10^3$	$34x10^3$	$27x10^3$	$35x10^3$
	49	Countless	Countless	Countless	Countless	Countless	Countless

4 DISCUSSION

The literature report that fish with stress caused by slaughter process tends to present faster post-mortem biochemical changes when compared to unstressed fish and, consequently, fast degradation and earlier quality loss of meat (Goes *et al*., 2015; Mendes *et al*., 2015; Mendes *et al*., 2017). The results confirm this affirmation, because worst results were found for fish slaughtered after pre-slaughter stress in all analyzes evaluated. Lower muscle pH levels in the first hours after slaughter are due to post-mortem biochemical changes, which produce lactic acid in the muscle and lower pH (Rahmanifarah *et al*., 2011). The time for this pH decrease is totally related to the amount of energy reserves that the fish had before dying (Matos *et al*., 2010).

In the present study, a slower pH decrease was observed in meat samples from fish submitted to recovery periods after transport, while samples from fish collected right after harvesting and after transport presented lower pH values at time 0, confirming that the post-mortem biochemical changes occurred more accelerated for these treatments according to reported by Oliveira-Filho (2021), who observed low pH levels after 5 hours of pacu slaughter using different slaughter methods. Melo *et al*. (2018) also observed a decrease in muscle pH in tilapia slaughtered by different electronarcosis voltages after 5 hours of slaughter. Erickson *et al*. (2016) also observed lower initial pH values in stressed Atlantic cod when compared to the control. This sudden drop in pH accelerates the meat degradation process, where a series of changes in fish quality faster happen caused by the increase in volatile compounds released by microorganisms (Digre *et al*., 2011; Mendes *et al*., 2017).

The results demonstrated a faster increase in pH values along the 49 days of storage on ice of meat samples from fish collected right after harvesting and after transport when compared to fish submitted to recovery periods after transport. The increase in pH values is a consequence of bacterial growth, which produces volatile substances and raises the pH, as evidenced by the NBVT results and the microorganism counts found in this study. These results corroborate those reported by Mendes *et al*. (2017), who observed higher pH values in tambaqui stressed during slaughter. Higher levels of total volatile compounds in fish muscle indicate deterioration, mainly caused by the number of microorganisms present in the muscle, which are responsible for the production of these volatile sulfur compounds, gradually increase with storage time (Mendes *et al*., 2017).

This indicates that fish collected right after harvesting and transport presented faster degradation when compared to the fish submitted to recovery periods after transport, a fact that was also confirmed by the values found in the microorganisms counts that were indicative in this study, as well as the sensory evaluation that demonstrates a greater loss of quality. In this sense, Mendes *et al.* (2017) reported a gradual increase in nitrogenous volatile bases for tambaqui stored on ice, which was higher in fish slaughtered by asphyxiation shortly after transport. Vargas, *et al.* (2013), using different slaughter methods, found no significant difference ($p>0.05$) in NBVT values for matrinxã stored on ice for 435 hours.

The sensory parameters evaluated in this study presented a faster loss of quality in fish submitted to stress, where fish collected right after harvest and transport were rejected for consumption after 42 days stored on ice. These results were later than those found by Mendes (2013), who considered tambaqui slaughtered right after harvesting and transport unsuitable for consumption after 30 days stored on ice, and also by Silva *et al*. (2018) who rejected tambaqui for consumption after 22 days stored on ice. In this study, fish submitted to different recovery periods after transport were not considered unsuitable for consumption until 49 days of storage, showing better quality and longer shelf life. Other authors have also reported faster quality losses for stressed fish in slaughter when compared to fish submitted to recovery periods after transport, such as for cod (Digre *et al*., 2011; Hultmann *et al*., 2012), tilapia (Viegas *et al*., 2015; GOES *et al*., 2018), pacu (Oliveira Filho *et al*., 2021) and tambaqui (Mendes *et al*., 2017).

The results also confirmed higher values of TBARS in fish slaughtered right after harvesting and transport when stored on ice for 49 days. This fact may be explained by the number of spoilage bacteria found in these treatments, which were possibly responsible for the faster degradation in the proteolytic and lipolytic activities, increasing the lipid oxidation. Mendes *et al*. (2017) observed that tambaqui slaughtered after the stress of pre-slaughter practices and frozen are more susceptible to lipid oxidation, which may be related to the fast degradation of nucleotides caused by this stress (Amaral *et al.,* 2018).

These results are according to those reported by Nathanailides *et al.* (2011), where the authors observed an increase in lipid oxidation of European bass slaughtered under stress. Although Matos *et al*. (2010), evaluating pre-slaughter harvesting stress, did not observe significant differences in TBARS values for dourado. However, the values found in this study were not harmful to the quality of tambaqui meat, because according to Al-Kahtani *et al*. (1996) meat products can be considered in good condition when the TBARS levels are below 3 mg of malonaldehyde per kg.

Some fish species, when subjected to the stress of pre-slaughter practices, develop low light in the meat coloration (Digre *et al*., 2011; Goes *et al*., 2018). Generally, darker fillets are observed right after slaughter due to the excessive effort that the animals are subjected to before their death, conducing a greater amount of blood to the muscles (Vargas-Baldi *et al*., 2018). This hypothesis is based on the results obtained in this study, once tambaqui slaughtered after pre-slaughter stress (right after harvest and transport) presented meat samples with lower luminosity after 21 days of storage on ice when compared those submitted to recovery periods after transport. Similar results were reported by Digre *et al.* (2017), who observed slightly darker cod loins (lower L* values and higher a* values) when stored alive for 6 h. Goes *et al*. (2019), studying the stress at different stocking densities in Nile tilapia, observed fillets with higher luminosity for the highest evaluated density.

The results of initial values of a* (redness) were higher in fish submitted to stress, as found by Concollato *et al*. (2014) evaluating the stress caused by slaughtering methods applied to salmon using carbon dioxide for 20 minutes. Viegas *et al*. (2015) also observed higher values of a* and lower values of L* in tilapia fillets, when fish were slaughtered by stressful methods. In this study, the values of b* (yellowing) presented higher values in fish submitted to stressed up to 49 days of storage on the. The increase in b* parameters of tambaqui fillets can be related to the lipid oxidation, which proves the increase in lipid oxidation in fish submitted to pre-slaughter stress. Similar results were reported by Lerfal *et al*. (2015), where the authors did not observe significant differences in the b* values of salmon fillets after 13 and 19 days stored on ice. However, higher values were observed in the b* parameters in meat samples from fish submitted to pre-slaughter stress. Goes *et al*. (2015) observed higher values of b* parameters in tilapia fillets when subjected to high stocking densities.

The results of the microbiological analyzes recommended by the RDC indicate that the fish meat samples evaluated were according to the standards established by the Brazilian legislation. The absence of *Salmonella sp*., thermotolerant coliforms and *Staphylococcus aureus* proves the effectiveness in the hygienic care used at the time of handling in each treatment evaluated, avoiding cross-contamination. These cares during handling of fish meat are very important to avoid the occurrence of these microorganisms considered as pathogenic (Scherer *et al*., 2004).

In the counts of the indicator groups, higher values were observed in meat samples from fish submitted to pre-slaughter stress. This higher number of bacteria found in these treatments may be related to several factors, such as the fast drop in pH, caused by the fast accumulation of lactic acid in the tissue that stimulate the enzymatic process and, consequently, the microbiological growth becomes faster in stressed fish (Mendes *et al*., 2017). Naturally, the fish has bacteria on the surface of its body. Right after the death, these bacteria proliferate on the fish muscle tissue, accelerating the decomposition of its meat (Ogawa *et al*., 1999). Front this, it is important to preserve the energy reserves of the animals before their death to cause more slow deterioration process, as observed in the samples of fish submitted to recovery periods after transport. Results obtained by Martins *et al*. (2002) reported a high concentration of mesophilic bacteria in the first days of storage, emphasizing that this high number of bacteria found in the muscle possibly occur due to the stress caused by pre-slaughter practices. Oliveira *et al*. (2014) also observed an increase in mesophilic bacteria after 10 days of storage. Brazilian legislation does not establish a limit for the groups of psychrophilic and psychrotrophic bacteria in fish, however, high counts of these spoilage microorganisms reduce the shelf life of fish, due to the growth capacity that these bacteria have in proliferating on the fish muscle at low temperatures (Lanzarin *et al*., 2016), as observed in this study.

5 CONCLUSION

Tambaqui slaughtered under stress right after harvesting and after transport presented fillets with higher bacterial growth rates and worst values of pH, NBVT and TBARS, in addition to producing fillets with darker coloring after 21 days stored on ice, being considered unsuitable for consumption after 42 days. Tambaqui submitted to recovery periods after transport from 6 up to 44 hours presented lower sensory results, not being considered unsuitable for consumption after 49 days of storage on ice, in addition to presenting fillets with greater luminosity, less redness after 21 days, smaller microbiological counts, and low pH, NBVT and TBARS values, proving that recovery periods after transport improves tambaqui quality and increases its shelf life when stored on ice.

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