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Net cage tambaqui farming: microbiological quality, nutritional value and yield

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Additional keywords

Centesimal composition.
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SUMMARY

Tambaqui is traditionally consumed at a commercial size of 1.0 to 3.0 kg However, it is also consumed at between 0.350 to 0.450 kg, this size being referred to as tambaqui curumim (C). The objective of this study was to evaluate the microbiological quality of tambaqui at the commercial size (CS) and to characterize the nutritional value and yield of tambaqui belonging to the two size classes Curumim and CS originating from net cages. The effect of size on the centesimal composition and fish yield was evaluated in a completely randomized design with 12 repetitions while for the microbiological analysis one fish from each net cage was used. C showed protein, ash and moisture contents above those of CS, whereas the ether extract was higher for CS. The yields were 70.04% for C and 65.55% for CS. 50% of the samples showed contamination by *Staphylococcus aureus*, all values being within the Brazilian's regulation limits. *Salmonella* spp. was found in 91.67% of the samples while 75% showed contamination by total coliforms and 8.34% by *Escherichia coli*. C showed a suitable nutritional value and a better yield in relation to CS. The addition of C to the diet will depend on its acceptance in the markets where its commercialization is not yet established. The microbiological results suggest a tendency of the species to harbor *Salmonella* spp. on the body surface.

Palavras chave adicionais

Composição centesimal.
Tambaqui curumim.
Salmonella spp.
Staphylococcus aureus.

Tambaqui de tanque-rede: qualidade microbiológica, valor nutricional e rendimento

RESUMO

O tambaqui é consumido tradicionalmente em tamanho comercial (1 a 3 kg), mas também entre 0,350 a 0,450 kg, sendo denominado Curumim(C). Objetivou-se avaliar a qualidade microbiológica do tambaqui em tamanho comercial (TC) e caracterizar o valor nutricional e rendimento de tambaquis em duas classes de tamanho: Curumim (C) e TC, provenientes de tanques-rede. O efeito do tamanho sobre a composição centesimal e rendimento do peixe foi avaliado em delineamento inteiramente casualizado com 12 repetições, enquanto para as análises microbiológicas, utilizou-se um peixe de cada tanque-rede. C apresentou teores de proteína (19,08%), cinzas (2,91%) e umidade (71,58%) superiores aos de TC, 17,83%, 2,00% e 68,25%, respectivamente, enquanto extrato etéreo foi maior para TC (9,25%), contra 5,16% para C. O rendimento foi de 70,04% para C e 65,55% para TC. Cinquenta por cento (50%) das amostras apresentaram contaminação por *S. aureus*, entretanto, dentro dos limites estabelecidos pela legislação. Constatou-se presença de *salmonella* em 91,67% das amostras, enquanto 75% delas apresentaram contaminação por coliformes totais e 8,34% por *E. coli*, denotando contaminação da água de cultivo. O C apresentou valor nutricional adequado e melhor rendimento em relação ao TC. A inserção do C nas dietas dependerá da aceitação pelos mercados onde sua comercialização não esteja estabelecida. Os resultados das análises microbiológicas realizadas com o tambaqui em tamanho comercial sugerem predisposição da espécie para o desenvolvimento de *Salmonella* spp. na superfície corporal.

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INTRODUCTION

The increasing production of fish raised in net cages is considered to result from the growing demand for fish, the reduction of natural fishing stocks and the need for more efficient production technologies (Santiago, 2013). Tambaqui (*Colossoma macropomum*), a Brazilian native fish species, is the second most farmed and consumed fish species in Brazil. It was responsible for 28.1% of the national fish production in 2015, second to tilapia. The Northern Region of Brazil notably accounts for 47.7% of the national tambaqui production (IBGE, 2016). Although tambaqui is traditionally sold at weights between 1.0 and 3.0 kg, there is also a market for this fish at 0.350 to 0.450 kg, when it is called "tambaqui curumim" (FRANCO, 2013). The cultivation time in net cages can vary from 4-6 months (tambaqui curumim) to 15 months (traditional commercial size) (Gandra, 2010).

A knowledge of the chemical composition and body yield of the carcasses is important to aid the formula-

tion of human diets calculated based on the nutritional contribution of foods (Gonçalves, Almeida & Borges, 2003; Van Cleef et al., 2017). Fish meat presents high quality protein rich in essential amino acids, complex B vitamins and minerals (such as phosphorus, magnesium, iron and zinc) (Goes et al., 2016). Among the benefits provided by the consumption of fish meat is a reduction in the risk of cardiovascular diseases due to the ingestion of polyunsaturated fat (Oliveira, Lourenço, Sousa, Joele, & Ribeiro, 2015). Thus, obtaining and disseminating data on the chemical composition and yield of fish are essential for the formulation of balanced human diets with the inclusion of fish and to improve the competitiveness of fish in relation to other protein sources (Sales & Maia, 2013; Souza, Macedo-Viegas, Zuanon, Carvalho, & Goes, 2015).

Besides determining the nutritional value of a product, it is important to guarantee its microbiological and sanitary quality. The technical resolution RDC Nº12, implemented on January 2nd 2001 by the Brazilian Health Regulatory Agency (ANVISA), establishes microbiological and sanitary standards for specific foods and a set of criteria for the obtainment and interpretation of results for microbiological analysis of foods destined for human consumption. This regulation determines the tolerance limits for contamination by *Staphylococcus aureus* in 10 g of product and a required absence of *Salmonella* spp. in 25 g of the product for fish in natura, fresh or frozen (assuming the fish will not be consumed raw).

The objective of this study was to evaluate the microbiological quality of tambaqui of commercial size and to characterize the nutritional value and the yield of tambaqui belonging to the two size classes (curumim and commercial size), cultivated on farms in net cages.

MATERIAL AND METHODS

In order to determine the effect of the two size classes on the centesimal composition and fish yield, the experimental design was completely randomized with 12 repetitions. The sample unit was comprised of three fish from each net cage, totaling 72 fish: 36 tambaqui curumim (0.350 to 0.450 kg) and 36 commercial size tambaqui (1.0 to 3.0 kg). The statistical analysis was carried out with the aid of the programs Bio Estat 5.3 (Ayres, Ayres Jr, Ayres & Santos, 2007) and ASSISTAT (Silva & Azevedo, 2002) and the data were submitted to the Shapiro-Wilk test to examine the normality of the variables.

The data obtained for all of the parameters evaluated were found to be statistically normal, except for the crude protein, which was transformed using the square root. The t-test was then applied for the comparison of the averages of the centesimal composition of the two sizes, considering a significance level of 5% ($P<0.05$)

When normality was confirmed, the analysis of related samples was carried out with the application of the Student's t-test, considering a significance level of 5% ($P<0.05$). The fish were raised in 12 net cages with a volume of 18 m^3 (3.0 x 3.0 x 3.0 m), in a water reservoir located in the southeast region of the state of Tocantins, Brazil, by April/2014 until September/2015. The feeding consisted of commercial feed, and the quantity and frequency of the supply varied according to the fish weight. Three fish of each net cage were captured at 6 months of age, weighing between 300 and 500 g (tambaqui curumim) and four fish of each net cage at 12 months of age, weighing between 1.0 kg and 1.5 cage kg (commercial-size tambaqui), totaling 84 fish.

The fish were killed by cerebral concussion, immediately after their removal from the net cages. They were then placed in autoclavable sterile sampling bags, kept cool in thermal boxes containing crushed ice and transported to the Experimental Field Center for Fisheries and Aquaculture (CEAq) at Embrapa Pesca e Aquicultura located in Palmas, in the state of Tocantins. One commercial-size tambaqui from each net cage was used for the microbiological analysis and the other three were used for the determination of the centesimal composition. The sample preparation and the microbiological analysis were carried out at the Laboratory of Health for Aquatic Organisms at CEAq, while the centesimal analysis was carried out at the Laboratory of Kinetics and Modeling Processes (LACIMP) at the Universidade Federal do Tocantins (UFT).

For the carrying out of the centesimal analysis, a composite sample comprised of 3 fish was evaluated for each net cage. The fish were weighed individually and the head, fins and viscera were removed and the rest of the body, which is the part generally eaten, was cut into steaks. The grinding of the three fish was then carried out in two stages (1st grinding and 2nd grinding) until the obtainment of a homogenous mass without pieces of bone being apparent. The mass was then pre-dried at 60 °C in a drying and sterilizing oven with circulation and renewal of air until constant weight was established.

The moisture was determined by means of complete drying in an oven with air circulation at 135 ºC for 2 h (Silva & Queiroz, 2002). The contents of crude protein and ether extract were determined by the Dumas and Soxhlet methods, respectively, as described in the Compêndio Brasileiro de Alimentação Animal (2009). The yield was obtained through the percentage calculation of the weight of the edible portion of the fish in relation to the weight of the whole fish. The percentage values of the viscera were calculated in the same way. In the calculation of the edible portion (tambaqui curumim), the weight of the gutted fish without the head and viscera were considered, whereas for the commercial-size tambaqui this portion was considered as the weight of the gutted fish without the head, fins and tail.

One tambaqui with commercial size (1.0 kg to 1.5 kg – 15 months old) from each net cage was analyzed for the detection of *Salmonella* spp. and to obtain the S. aureus, total coliforms and *Escherichia coli* counts, according to resolution RDC N° 12, January 2nd 2001, of ANVISA. The laboratory material and the material used in the fish capture and transportation were previously sterilized along as well as the media for the solutions. All the required sanitation care was taken

during the procedures so as to avoid cross contamination during the analysis.

For the detection of *Salmonella* spp., 25 g of fish was removed from the dorsal region and transferred to a 500 ml sterile sampling bag to which 225 ml of *Salmonella* enrichment broth was added. Each sample was homogenized in a Stomacher piston homogenizer and incubated at 41.5 ºC for 24 h. After this period, 0.1 ml of incubated solution was inoculated in a tube with 9 ml of Rappaport. The tubes were then incubated for a further 8 h and 1 ml of the tube content was inoculated on a 3M™ Petrifilm™ Salmonella Express (SALX) plate which was incubated for a further 20-24 h at 41.5 ºC. After the incubation of the plate, the reading was carried out for the detection of the presence or absence of Salmonella based on the kit methodology.

For the *Staphylococcus aureus* (coagulase positive) counts, 10 g of fish was removed from the dorsal region and transferred to a 500 ml sterile sampling bag to which 90 ml of sterile water was added. Each sample was then homogenized in a Stomacher and then diluted in the proportion 1:10 (0.1 ml of sample and 0.9 ml of sterile water). An aliquot (1 ml) of this solution was inoculated on a plate of the 3M™ Petrifilm™ Staph Express (STX) kit. In the next step, the plates were incubated at 37 ºC for 24 h. After the incubation period, the reading was carried out following the kit instructions.

The *Escherichia coli* counts were carried out using 10 g of fillet extracted from the lumbar region of each fish and transferred to a 500 ml sterile sampling bag to which 90 ml of sterile water was added. Each sample was then homogenized in Stomacher and 1 ml of this solution was inoculated on a plate of the 3M™ Petrifilm™ (EC) kit for the *E.coli* and coliforms counts and incubated at 35 °C for 24 h. After the incubation period the reading was carried out following the kit instructions.

For the analysis of fish composition, the fish were first collected with 6 months in October/2014 and later with 12 months in September/2015, in the latter were collected those for microbiological analyzes. Microbiological analyzes were carried out one day after collection and those of centesimal composition carried out until December/2015. The study reported herein was approved by the Ethics Commission of Embrapa Pesca e Aquicultura for the Use of Animals in the Fisheries and Aquaculture Sector (certificate nº15).

RESULTS AND DISCUSSION

Centesimal composition results of tambaqui in two sizes classes are presented in **Table I**.

A previously published table showing the composition of foods from the Amazon (Aguiar, 1996) provides values for raw whole commercial-size tambaqui (without viscera, scales and bones) of approximately 72.7% moisture, 19% protein, 6.9% lipids and 1.4% ash. The values obtained for the tambaqui curumim in this study, except for the ether content, was very close to those published in Table 1. They were also consistent with values reported by Rocha, Aguiar, Marinho & Shrimpton. (1982), who analyzed the main fish species consumed in the state of Amazonas and other native fish species, including tambaqui weighing up to 3.0 kg, with regard to aspects related to energy, protein and zinc. However, no publications could be found in the literature which report data for the centesimal composition of tambaqui curumim. It is known that there are intrinsic and exogenous factors correlated to the variation in the chemical composition and yield of fish, such as: time of year (climate, season), feed (type, quality and quantity) and other rearing and environmental conditions (such as water temperature), species, genetics, age, gender, sexual maturation phase and part of the carcass analyzed (Macedo-Viegas, Scorvo, Vidotti & Secco, 2000; Souza et al., 2003; Sales & Maia, 2013).

The tambaqui curumim had a higher protein content than the commercial-size fish. This phase (weight up to 600 g) is characterized by intense growth, when the fish present a higher growth rate/maintenance requirements ratio compared to larger fish and the nutrients are preferably destined to the muscular growth process rather than fat accumulation (Kubitza, 2004; Fernandes, Doria & Menezes, 2010). The protein content of curumim was very close to the value given in the table for the composition of foods from the Amazon, that is, 19% for raw whole tambaqui (Aguiar, 1996).

Curumim showed lower ether extract content (4.08% less than the commercial-size fish), which was expected since the fish in this growing phase accumulate less body fat than adult fish. According to the classification of Stansby & Olcott (1968), the samples analyzed in this study showed average fat (5-15%) and high protein (15-20%) contents.

The ash content was higher for curumim, which may be due to the differences in the edible portion of the two size classes of fish evaluated. In the case of the curumim only the head and viscera were removed whereas for the commercial-size tambaqui, besides the head and viscera, the fins and tail were also removed. Due to the higher proportion of bone in the curumim there was a higher ash content in relation to

Table I. Centesimal composition of tambaqui in two size classes (Composição centésimal de tambaqui em classes de dois tamanhos)

Centesimal composition (% body weight)	Tambaqui curumim (0.350 to 0.450 kg)	Commercial-size tambaqui (1.0 to 3.0 kg)
Moisture	71.58 ± 0.99[a]	68.25 ± 1.71[b]
Crude Protein	19.08 ± 0.66[a]	17.83 ± 0.83[b]
Ether Extract	5.16 ± 0.20[b]	9.25 ± 0.35[a]
Ash	2.91 ± 0.28[a]	2.00 ± 0.00[b]

Averages followed by different letters on the same line differ according to the Student's t-test considering a level of 5% probability (P<0.05).

the weight of the fish. The fins and tail of the curumim were maintained due to the way curumim is consumed as a typical dish of the Amazon, that is, only the head and viscera are removed (Gandra, 2010).

The commercial-size tambaqui presented an average yield of 65.55% while the corresponding result obtained for curumim was 70.04%, which again could be due to differences in the composition of the edible portion of the two fish size classes: commercial-size fish (gutted without the head, fins and tail) and curumim fish (gutted without the head). Thus, since less of the curumim was discarded to obtain the edible portion of the fish, the yield was increased, while in the case of commercial-size tambaqui, besides the head and viscera, the fins and tail were discarded, reducing the yield. The values found in this study were consistent with that reported by Souza & Inhamuns (2011), i.e., approximately 62% yield for tambaqui without viscera, head, scales and fins. The viscera represented 6 and 7% of the whole fish weight for the commercial-size tambaqui and curumim, respectively.

In relation to the microbiological analysis (**Table II**), only the results for coagulase-positive staphylococci remained within the acceptable limits established by the resolution RDC Nº 12 (ANVISA, 2001). The presence of *Salmonella* spp. was observed in the samples analyzed in this study. Only one other study evaluating the quality of farm-raised tambaqui (gutted, washed with water containing 5 ppm chloride and stored under ice) could be found in the literature prior to the carrying out of this study; however, this was related to fish ponds (Araújo, Lima, Joele, & Lourenço, 2017). The authors reported results analogous to those obtained in this study regarding coagulase positive staphylococci. However, they reported the absence of Salmonella in the samples analyzed, which may be due to the level of fish processing in that study. In contrast, the samples in the current study (fresh whole fish) were analyzed soon after being captured and no processing was applied.

Of the 12 samples submitted to microbiological analysis, 50% showed contamination by *S. aureus*, however, the values were within acceptable limits established by the resolution RDC Nº 12 (ANVISA, 2001): tolerance of up to 10^3 UFC/g of coagulase-positive staphylococci for fish in natura. It is important to highlight that the analysis was carried out on fish after being captured, with no washing with sanitizer or direct contact with ice (only with the sterile sampling bags). Soares et al. (2014) in their study of Nile Tilapia (*Orechromis niloticus*), from fish caught in cages, observed an absence of *S. aureus* in all simples.

The presence of *Salmonella* spp. was detected in 91.67% of the samples. The resolution RDC Nº 12, January 2001, recommends that fish in natura, chilled or frozen, which is not destined to be consumed raw, must not have the presence of Salmonella in 25 g of the processed product. When there is the presence of Salmonella, the whole lot must be condemned and considered inacceptable for consumption (ANVISA, 2001). The detection is a general indicator of the hygiene level of the product, which may offer health risks for consumers. The temperature limits for the growth of this bacterium vary between 5 and 43 ºC (ICMSF, 1996). The fish analyzed in this study were captured during the warmest period of the year when the maximum air temperature registered was 35 ºC. Castilho (2012) found contamination by *Salmonella* in only 0.6% out of 170 tilapia fish samples in natura originated from net cages where the water temperature varied between 20.9 and 23.9 º C. The conditions of capture and preparation of the samples reported by these authors were similar to those of the current study where the fish samples did not undergo any level of processing. Salmonella is a type of bacteria naturally present in bird feces, which can contaminate the cultivation water and consequently the farmed fish. It was expected that the flow of water through the net cages would be sufficient to avoid the contamination of the fish. However, this was clearly not the case in this study, suggesting that

Table II. Results for microbiological analysis to determine the presence of *Salmonella* spp. and the counts for *S. aureus* (coagulase positive), total coliforms and *E.coli* for adult tambaqui (Resultados da análise microbiológica para determinar a presença de *Salmonella* spp. e as contagens para *S. aureus* (coagulase positiva), coliformes totais e *E.coli* para tambaqui adulto).

Samples	*S. aureus* (UFC/g)	*Salmonella* spp.	Total Coliforms (UFC/g)	*E. coli* (UFC/g)
1	$8x10^2$	PRESENT	0	0
2	$6x10^2$	PRESENT	0	0
3	0	ABSENT	6x10	0
4	0	PRESENT	$3.1x10^2$	0
5	10^2	PRESENT	1x10	0
6	10^2	PRESENT	3x10	0
7	0	PRESENT	3x10	0
8	10^2	PRESENT	$5x10^2$	0
9	0	PRESENT	$4x10^3$	0
10	10^2	PRESENT	$12.6x10^2$	2x10
11	0	PRESENT	0	0
12	0	PRESENT	$4x10^2$	0

tambaqui might have a predisposition to harbor this type of bacteria on the surface of their bodies.

Furthermore, according to **Table II**, around 75% of the analyzed samples showed contamination by total coliforms and 8.34% by *E. coli*. ANVISA does not establish limits for coliforms in fish in natura or fresh fish, however, this group is very important since its presence is an indicator of fecal pollution in waters (Vieira, 2004). Cardoso, Tessari, Castro & Kanashiro (2001) noted that the water is the main source of coliform contamination in pisciculture. Araújo et al. (2017) presented data on coliform counts observed in farm-raised tambaqui which were similar to those obtained in the current study. However, they analyzed samples of farmed tambaqui raised in fish ponds, which had been gutted and stored under ice. The coliform counts for the samples reported in the cited reference were below 10^3 UFC/g (Araújo et al., 2017).

Santos et al. (2018) evaluated the microbiological quality of 15 units of eviscerated caranha (*Piaractus-mesopotamicus*) packed in ice collected in a processing unit. They did not identify contamination by *E. coli* and *salmonella*, and the *Staphylococcus aureus* count was within the limit established by the current legislation of, say, 103 UFC/g. While Afonso (2016) analyzed 51 fresh and frozen eviscerated corvine (*Argyrosomusthorpei*) fish units obtained after being unloaded at the fishing port of Beira in Mozambique. No samples showed contamination by *salmonella*, and for *S. aureus* it was below the limit established by the legislation, whereas for faecal coliforms one sample presented contamination.

CONCLUSION

Tambaqui curumim showed suitable nutritional value and the possibility of a greater global exploitation of this fish in relation to the commercial size, providing a better yield. However, its contribution to the feeding of the population will depend on the acceptance of this product in markets where its commercialization is not yet established. The results for the microbiological analysis of commercial-size tambaqui suggest a predisposition of this species for the development of *Salmonella* spp. on the surface of the fish body. Further investigation is required to clarify this matter. In addition, the presence of total coliforms in 75% of the samples may indicate water contamination at the site where these fish were raised.

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