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Analysis of the microbiological quality of yellow hake (*Cynoscion acoupa*) sold at farmers markets and supermarkets in São Luís, Maranhão, Brazil

Análise da qualidade microbiológica da pescada amarela (*Cynoscion acoupa*) comercializada em feiras e supermercados em São Luís, Maranhão, Brasil

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ABSTRACT: This study aimed to analyze the microbiological quality of yellow hake (*Cynoscion acoupa*) sold at farmers markets and supermarkets in São Luís, Maranhão, Brazil. Sixty yellow hake samples, collected at farmers markets and supermarkets, were analyzed at the Food and Water Microbiology Laboratory of the Universidade Estadual do Maranhão. In samples from farmers markets, we observed the presence of coliforms at 35°C (93.33%), coliforms at 45°C (80%), and *Escherichia coli* (13.33%). In samples from supermarkets, we observed the presence of coliforms at 35°C (86.67%) and coliforms at 45°C (46.67%), but not *E. coli.* The data indicate problems in handling fish throughout the yellow hake production chain, from capture to the point of sale. For both farmers markets and supermarkets, none of the samples showed contamination by coagulase-positive *Staphylococcus*, *Salmonella* spp., *Listeria* sp., and *Vibrio parahaemolyticus*. Regarding the genus *Aeromonas*, the most common species was *Aeromonas hydrophila*, found in 43 (81.13%) samples in farmers markets and supermarkets. We conclude that the samples presented unsatisfactory hygienic and sanitary conditions, presenting a potential risk of contamination of these foods available for sale.

Keywords: bacteria;*Escherichia coli*; microbiology; marine fish; Aeromonas.

RESUMO: Este estudo teve como objetivo analisar a qualidade microbiológica da pescada-amarela (*Cynoscion acoupa*) comercializada em feiras livres e supermercados em São Luís, Maranhão, Brasil. Sessenta amostras de pescada-amarela, coletadas em feiras livres e supermercados, foram analisadas no Laboratório de Microbiologia de Alimentos e Água da Universidade Estadual do Maranhão. Nas amostras, observamos a presença de coliformes a 35°C (93,33%), coliformes a 45°C (80%) e *Escherichia coli* (13,33%). Nas amostras dos supermercados, observamos a presença de coliformes a 35°C (86,67%) e coliformes a 45°C (46,67%), mas não de *E. coli*. Os dados indicam problemas no manuseio do pescado em toda a cadeia produtiva da pescada-amarela, desde a captura até o ponto de venda. Tanto em feiras livres quanto em supermercados, nenhuma amostra apresentou contaminação por *Staphylococcus* coagulase positiva, *Salmonella* spp., *Listeria* sp. e *Vibrio parahaemolyticus*. Em relação ao gênero Aeromonas, a espécie mais comum foi *Aeromonas hydrophila*, encontrada em 43 (81,13%) amostras em feiras livres e supermercados. Concluímos que as amostras apresentaram condições higiênico-sanitárias insatisfatórias, apresentando um risco potencial de contaminação desses alimentos disponíveis para venda.

Palavras-chave: bactérias; *Escherichia coli*; microbiologia; peixes marinhos; Aeromonas.

INTRODUCTION

More than 30 fish species within the *Cynoscion* genus have been described on the Brazilian coast. All of them are fish that have scales and the ability to produce acoustic signals through muscle tissue combined with the swim bladder, which characterizes this group. Among the main species of this genus is the yellow hake (*Cynoscion acoupa*) (Almeida *et al.*,2016; FISH BASE, 2024). The yellow hake can reach 1.0 m in length and weigh up to 30 kg. It is geographically distributed throughout the Brazilian coast. It has the following characteristics: elongated body shape; relatively large head; wide terminal mouth and jaws with small needle-shaped teeth; a rhomboidal caudal fin; dorsal fins with hard and soft rays, very high and developed; and general yellowish coloration, mainly on the back and at the end of the fins (Frazão, 2018).

Among the states in the Northeast region, Maranhão is considered one of the main producers of fish of extractive origin (Almeida *et al.*, 2011; Lopes *et al.,* 2012). In Brazil, yellow hake stands out as one of the most captured marine species (Silva, 2023). According to Pereira *et al.* (2010), yellow hake, like other fish, is received in São Luís, the capital of Maranhão, in vessels from various locations, or in refrigerated trucks from other states, and distributed to several establishments in the central region. The main distribution centers are farmers markets and supermarkets.

Yellow hake is commercially important mainly because it is a high-protein food; has a low saturated fat content; and is rich in vitamins, essential minerals, and omega-3 fatty acids. Given its high nutritional quality, its microbiological quality should be prioritized because it is a highly perishable food, due to its high water activity

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and pH close to neutrality, among other factors, that favors the development of pathogenic microorganisms that are harmful to the health of consumers. To ensure its quality, it is extremely important to consider time, hygiene, and temperature, avoiding rapid autolytic and/or bacterial reactions that interfere with the safety of the product (Alho *et al.*, 2023; Soares; Gonçalves, 2012).

Fish and other foods that do not meet microbiological standards can pose risks to public health, with foodborne diseases being a frequent problem in Brazil. Between 2014 and 2023, 6,874 outbreaks of foodborne diseases were recorded in Brazil, causing 121 deaths. The main etiological agents were *Salmonella sp., Escherichia coli*, *Bacillus cereus,* and *Staphylococcus aureus* (Brasil, 2024a). Therefore, in addition to sensory and physical-chemical tests, microbiological analysis should also be used to assess the quality of foods of animal origin, such as fish (Gonçalves, 2011). Although yellow hake has nutritional quality and great economic potential for Brazil and the state of Maranhão, there have been only a few studies on the microbiological characterization of this species (Alho *et al*., 2023; Oliveira *et al.*, 2022). This study aimed to obtain a better picture of the hygienic and sanitary conditions of yellow hake by analyzing the microbiological quality of slices sold at farmers markets and supermarkets in São Luís, Maranhão, Brazil.

MATERIAL AND METHODS

Sixty samples of yellow hake (*C. acoupa*) were obtained from the main commercial fish sale points in the municipality of São Luís, Brazil. Specifically, there were 30 samples collected from 5 supermarkets and 30 samples collected from 5 farmers markets. The samples (500-g pieces) were packaged in their own packaging, transported in isothermal polystyrene boxes with ice, and sent to the Food and Water Microbiology Laboratory, located at the Universidade Estadual do Maranhão, for analysis of microbiological quality. In the laboratory, the samples were removed from their original packaging, and 25 g of each sample were weighed aseptically for each analysis. The sample was placed in a vial with peptone water (dilution 10^{-1}), followed by serial dilutions up to 10^{-3}, and up to 10^{-4} for the analysis of *Vibrio parahaemolyticus.*

The microbiological analyses were performed following the methodology recommended by Normative Instruction No. 161 from Brazilian Health Regulatory Agency (Brasil, 2022), and the Manual of Methods for Microbiological Analysis of Food and Water (Silva *et al.*, 2021). *Aeromonas* spp. were identified according to the Aerokey II identification key (Carnahan *et al*., 1991). The analyses were performed in triplicate and the data were subjected to analysis of variance (ANOVA) and Tukey's test with 5% significance to determine significant differences in the means in Statistica 7.0.0.

RESULTS

Table 1 describes the results of the microbiological analyses for coliforms of 30 samples of yellow hake from farmers markets in São Luís, Brazil. We observed low counts of coliforms at 35°C (<3 most probable number [MPN]/g) in 2 (6.67%) samples; 27 (90%) samples presented counts in a range of 3-460 MPN/g, and 1 (3.33%) sample presented a count ≥1,100 MPN/g. Regarding the quantification of coliforms at 45°C, we observed values below 3 MPN/g in 6 (20%) samples, and 24 (80%) samples presented counts between 3 and 460 MPN/g. We also detected E. coli in 4 (13.33%) samples from farmers markets.

Table 2 shows the data from the microbiological analyses for coliforms and *E. coli* in samples from supermarkets. We found low counts for coliforms at 35°C (<3 MPN/g) in 4 (13.33%) samples; 24 (80%) samples had counts ranging from 3 to 460 MPN/g, and 2 (6.67%) samples had high counts (≥1,100 MPN/g). Regarding coliforms at 45°C, we observed low counts (<3 MPN/g) in 16 (53.33%) samples, counts ranging from 3 to 460 MPN/g in 14 (46.67%) samples, and none of the samples had high counts (≥1,100 MPN/g). We did not detect E. coli in the samples obtained from supermarkets.

For both farmers markets and supermarkets, none of the samples presented contamination by coagulase-positive *Staphylococcus.* For coagulase-negative *Staphylococcus*, we found counts of <20 colony-forming units (CFU)/g in 7 (23.33%) samples from farmers markets. In general, the average count ranged from 3.8×10^3 to 1.4×10^5 CFU/g (Table 3). However, in supermarket samples, the bacteria counts were <20 CFU/g in 8 (26.67%) samples, and the average count ranged from 3.9×10^3 to 4.1×10^4 CFU/g.

Table 4 shows the average counts of coagulase-negative *Staphylococcus* in the samples. There was greater contamination by coagulase-negative *Staphylococcus* in farmers market samples compared with supermarket samples ($p < 0.05$).

We identified bacteria of the genus *Aeromonas* in 53 (88.33%) samples. We verified the occurrence of four species, namely: *Aeromonas hydrophila, Aeromonas caviae, Aeromonas schubertii*, and *Aeromonas veronii biovar veronii.* The most frequent was *A. hydrophila,* in 43 samples (81.13%), as shown in Table 5.

There were no colonies suggestive of *V. parahaemolyticus*. Of note, we used thiosulfate, citrate, bile, and sucrose (TCBS) agar, which is specific for identifying bacteria of the genus *Vibrio*. We did observe the possible occurrence of other species is reported, which was observed by the growth of colonies in the aforementioned culture medium. We observed low values (<3 MPN/g) for *Vibrio* spp. in 48 (80%) samples. In the 12 (20%) samples in which the genus was identified, the count ranged from 3 to 35 MPN/g. Finally, we also did not detect *Salmonella* spp. or *Listeria monocytogenes* in the analyzed samples.

DISCUSSION

Although Brazilian legislation (Normative Instruction No. 161 from Brazilian Health Regulatory Agency; Brasil, 2022; Santos, 2016) does not have standards for fresh fish, we analyzed coliforms at 35 and 45°C, considering that the presence of these microorganisms indicates inadequate hygienic-sanitary conditions of the fish. The possible reasons for the contamination of the analyzed samples by coliforms at 35 and 45°C may be related to a lack of personal protective equipment (PPE) by the handlers; exposure of fish on benches (without adequate storage on ice); exposed to the sun, traffic, people, and insects (flies); the absence of restrooms; and garbage disposed in inappropriate places. These reasons may have contributed to the contamination of the fish; therefore, we recommend to increase the health surveillance actions at these locations and that to

Table 1 – Minimum and maximum variation of the most probable number (MPN) of coliforms at 35 and 45°C in 30 samples of yellow hake (*Cynoscion acoupa*) sold at farmers markets in São Luís, Brazil.

MPN/g	Coliforms at 35°C		Coliforms at 45°C		*Escherichia coli*		Reference
	N	%	n	%	n	%	(BRASIL, 2022) (*Escherichia coli*)
<3	2	6.67	6	20	-	-	
3 to ≤460	27	90	24	80	-	-	5.0×10^1
≥1100	1	3.33	0	-	4	13.33	5.0×10^2
Total	30	100	30	100	4	13.33	-

Table 2 – Minimum and maximum variation in the most probable number (MPN) of coliforms at 35 and 45°C in 30 samples of yellow hake (*Cynoscion acoupa*) sold in supermarkets in São Luís, Brazil.

MPN/g	Coliforms at 35°C		Coliforms at 45°C		Escherichia coli		Reference
	N	%	N	%	N	%	(BRASIL, 2022) (*Escherichia coli*)
<3	4	13.33	16	53.33	-	-	5.0×10^1
3 to ≤460	24	80.00	14	46.67	-	-	
≥1100	2	6.67	0	-	-	-	
Total	30	100	30	100	-	-	-

improve the infrastructure of the sale points. To reduce contamination of fecal origin, it is also necessary to raise awareness among handlers regarding the personal hygiene requirements that must be followed. It is worth considering that this last criterion is essential to applied in various sectors of the yellow hake production chain, from the moment it is caught on board, unloaded at ports, during processing, until it reaches the end consumer with satisfactory microbiological quality.

Other studies have already confirmed the presence of coliforms at 35 and 45°C and *E. coli* in samples of hake unloaded in other cities of the state of Maranhão (Lopes *et al.*, 2012; Ferreira *et al.*, 2014) and in samples of fish sold in São Luís, Maranhão, Brazil (Oliveira *et al.*, 2022). These findings underscore the importance of adopting personal hygiene practices not only during marketing, but at the beginning of the fish production chain, for example, during capture and unloading in fishing ports throughout the state of Maranhão.

When comparing the coliform data presented in Tables 1 and 2, we recorded higher counts in a higher percentage of the samples from farmers markets. This finding can be explained by the local hygienic-sanitary conditions, which are precarious in the capital of Maranhão (Freitas, 2014). Regarding the samples from supermarkets, the results shown in Table 2 may have been influenced by the better infrastructure of the establishments compared with farmers markets. In addition, companies are required to provide PPE free of charge to workers, according to the labor regulatory standards of the Brazilian Department of Labor (Brasil, 2020, 2024b), contributing to the reduction in food contamination of fecal origin.

In turn, even though *E. coli* was absent in the yellow hake samples from supermarkets, some authors have shown that the ice used to preserve the fish can act as a vehicle for microorganisms, because the water used is not potable (Baldin, 2011; Lopes *et al.,* 2012; Ferreira *et al.,* 2014). This fact may be associated with the contamination observed in the samples we evaluated. The recommended amount of ice for preservation is a 1:1 ratio (fish/ice). The use of ice within potable standards and adequate refrigeration lead to a decrease in temperature, reducing and delaying the deterioration process, and prolonging the commercial validity of the fish and its state of freshness (Massaro; Yuri, 2016).

No sample showed contamination by coagulase-positive *Staphylococcus* (Table 3). Brazilian legislation (Normative Instruction No. 161 from Brazilian Health Regulatory Agency) has established a maximum count of 10^3 CFU/g (BRASIL, 2022). Although this legislation establishes standards for coagulase-positive *Staphylococcus*, it is important to draw attention to the count of coagulase-negative *Staphylococcus* in food. Lamaita *et al.* (2005) described the need to review Brazilian legislation to include standards for coagulase-negative *Staphylococcus*, given its importance for food safety, which may indicate hygienic failures associated with inadequate conservation processes (cooling, thawing, or storage). The toxigenic capacity of coagulase-negative *Staphylococcus* isolated from food has also been identified by polymerase chain reaction (Cunha, 2006;

Table 3 – Quantification of coagulase-negative *Staphylococcus* counts in samples of yellow hake (*Cynoscion acoupa*) sold in São Luís, Brazil.

	Farmers markets					Supermarkets					Reference
	A (CFU/g)	B (CFU/g)	C (CFU/g)	D (CFU/g)	E (CFU/g)	F (CFU/g)	G (CFU/g)	H (CFU/g)	I (CFU/g)	J (CFU/g)	(BRASIL, 2022)
Mean	7.9×10^3	7.3×10^4	1.4×10^4	3.8×10^3	1.4×10^5	3.9×10^3	6.5×10^3	1.3×10^4	7.1×10^3	4.1×10^4	Up to 10^3 CFU/g
SD	15481.30	92441.66	13946.82	4964.04	333528.4	3465.64	9549.17	11131.04	13382.94	97171.82	
CV(%)	195.14	125.98	102.42	127.82	239.56	87.37	145.42	82.45	187.17	232.93	

Table 4 – Average counts of coagulase-negative *Staphylococcus* in samples of yellow hake (*Cynoscion acoupa*) sold in São Luís, Brazil.

Sale point	Number of samples	Average CFU/g of coagulase-negative *Staphylococcus*	Reference (BRASIL, 2022)
Farmers markets	30	$4.8 \times 10^4 \pm (5.9 \times 10^4)^a$	10^3 CFU/g
Supermarkets	30	$1.4 \times 10^4 (\pm 1.5 \times 10^4)^b$	10^3 CFU/g

The data are presented as the mean (± standard deviation)
[a,b] A means followed by a different letter indicates a significant difference (Tukey's test).
Coefficient of variation of the farmers markets = 123.06%
Coefficient of variation of the supermarkets = 106.93%
CFU/g, colony-forming unit per gram

Table 5 – *Aeromonas* spp. isolated from samples of yellow hake (*Cynoscion acoupa*) sold at farmers markets and supermarkets in São Luís, Brazil

Species	Sale point										Total	Percent (%)
	Farmers markets					Supermarkets						
	A	B	C	D	E	F	G	H	I	J		
Aeromonas hydrophila	4	3	6	6	4	5	5	4	2	4	43	81.13
Aeromonas veronii biovar veronii	2	1	-	-	-	1	-	-	2	-	6	11.32
Aeromonas schubertii	-	-	-	-	-	-	1	-	-	-	1	1.89
Aeromonas caviae	-	-	-	-	1	-	-	2	-	-	3	5.66
Total	**6**	**4**	**6**	**6**	**5**	**6**	**6**	**6**	**4**	**4**	**53**	**100**

Lopes *et al*., 2012).

The average count of 3.8×10^3 to 1.4×10^5 CFU/g for coagulase-negative *Staphylococcus* in the farmers market samples and 3.9×10^3 to 4.1×10^4 CFU/g for the supermarket samples (Table 3) indicate inadequate handling of yellow hake at these sale points. Considering that a significant percentage of the population is an asymptomatic carrier of *Staphylococcus* sp., hygienic habits should be adopted when handling fish. Hand contact with the skin, nasal, and oral mucous membranes, without subsequent hygiene, contaminates the fish during handling (Alho *et al*., 2023).

Lopes *et al.* (2012) did not detect coagulase-positive *Staphylococcus;* however, they reported an

average count of 2 × 10^3 to 3.1 × 10^5 CFU/g for coagulase-negative *Staphylococcus* in yellow hake unloaded at the port of Cedral, Maranhão, Brazil. Therefore, as the current study suggests, the greater contamination by coagulase-negative *Staphylococcus* found in farmers market compared with supermarkets (Table 4) is probably due to inadequate hygienic-sanitary conditions at the farmers markets, poor personal hygiene among most handlers (vendors and/or fishermen), a lack of refrigerated counters used for handling, inadequate handling of money, and inadequate storage of fish for sale.

We found a higher percentage of *A. hydrophila* isolates in the samples we analyzed compared with *A. caviae, A. veronii biovar veronii, and A. schubertii* (Table 5). The presence of *Aeromonas* spp. may be due to possible cross-contamination (contact with the surface of the vessel and plastic or polystyrene boxes), because their habitat is the aquatic environment, or to inadequate refrigeration and/or the use of insufficient ice and water without adequate potability standards in their production. Due to the psychrotrophic characteristics of *Aeromonas* spp., storing fish at 4°C is not a safe method to inhibit the growth of these microorganisms (Visentini, 2013). Our findings corroborate the study by Lopes *et al.* (2012), who reported that 19 samples (45.24%) of yellow hake from Cedral, Maranhão, Brazil were contaminated by *A. hydrophila,* probably due to inadequate contact of the food with fishermen carrying this bacterium, because *Aeromonas* spp. can be associated with various skin infections.

The yellow hake sold in São Luís, Maranhão, comes from artisanal fishing in several coastal cities of the state, including Cedral, Cururupu, Humberto de Campos, Raposa, São José de Ribamar, and Tutóia, among others. Therefore, finding a high frequency of *Aeromonas* spp. in the samples probably indicates the ability of these bacteria to withstand salinity. According to Visentini (2013), these results may explain the distribution of Aeromonas in marine waters and their development and maintenance in foods with NaCl as a preservative.

Although we did not investigate virulence factors in the *A. hydrophila, A. veronii biovar veronii, A. caviae,* and *A. schubertii isolates,* it is important to emphasize that Abbott *et al.* (2003) consider them potentially pathogenic, for both humans and fish, due to the virulence factors found. According to Peixoto *et al.* (2012), *Aeromonas* spp. secrete amylase, chitinase, gelatinase, lecithinase, elastase, aerolysin, nuclease, lipase, and protease. These proteins are virulence factors that cause diseases in fish and humans.

The absence of *V. parahaemolyticus, Salmonella* sp., and *L. monocytogenes* represents a positive point regarding the microbiological quality of the samples analyzed, because the presence of these bacteria implies public health problems (Busch, 2022). Sample collection during the rainy season in the state may have been contributed to the absence of *V. parahaemolyticus*. The samples would have been influenced by the low concentrations of this microorganism in the marine environment during this period. Rosa *et al.* (2017) showed that even though it is a halophilic species, *V. parahaemolyticus* is not easily found in places where the water temperature drops below 15°C, with a correlation between the presence of this bacterium and the temperature and salinity of the water. During rainy periods, the coast of Maranhão has reduced temperature and salinity, which may have interfered with the levels of this microorganism. In Brazilian legislation (Normative Instruction No. 161 from Brazilian Health Regulatory Agency) (Brasil, 2022), there is no reference value for *V. parahaemolyticus* in fresh fish; limits are established only for ready-to-eat dishes based on raw fish (10^3 CFU/g). However, the data presented may support revisions in legislation, with the possibility of including this pathogen.

The analyzed samples do not pose a risk of transmitting *Salmonella* spp. to consumers, which qualifies them as within the standards of Brazilian legislation. Specifically, Normative Instruction No. 161 from Brazilian Health Regulatory Agency (Brasil, 2022) establishes the absence of this bacterium in 25 g of fresh fish. Similar results regarding the absence of *Salmonella* sp. have also been reported in other studies in fish (Silva *et al*. 2008, Lopes *et al.* 2012; Ferreira *et al.* 2014).

Regarding *Listeria* sp., the analyzed samples presented satisfactory hygienic-sanitary conditions. Brazilian legislation (Normative Instruction No. 161 from Brazilian Health Regulatory Agency) (Brasil, 2022) does not establish a reference value for this bacterium in fish, but it is worth noting that, according to Silva (2009), the presence of *Listeria* sp. in fish poses a risk to consumer health, especially the species *L. monocytogenes*, which has emerged as one of the most important foodborne pathogens. The results of this study are similar to those of Santos (2016) who, when analyzing 60 samples of tambaqui (*Colossomamacropomum*) sold in farmers markets and supermarkets in São Luís, Maranhão, also did not find *Listeria* sp. in any of the samples analyzed. Even though this bacterium was absent in the studied samples, the possibility of these microorganisms being present in fish or fishery products cannot be ruled out.

CONCLUSION

The samples of yellow hake sold at farmers markets and supermarkets showed high coliform counts at 35 and 45°C, as well as the presence of *E. coli, A. hydrophila, A. caviae, A. veronii biovar veronii,* and *A. schubertii.* Based on these findings, we concluded that the samples presented unsatisfactory hygienic and sanitary conditions, demonstrating a potential risk of contamination of these foods available for sale. Therefore, it is important to intervene by training handlers to adopt personal hygiene practices at the beginning of the production chain, from catching the fish to distribution and marketing at the sale point.

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