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Sanitary quality of sashimis sold in Cuiabá, Brazil

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ABSTRACT

Fish consumption in Brazil has been steadily increasing, including raw dishes like sashimi. This study aimed to evaluate the freshness and microbiological quality of commercially available salmon sashimi in Asian cuisine establishments located in Cuiabá, Mato Grosso, Brazil. Samples from 14 establishments were evaluated by analysis of temperature, pH, total volatile nitrogen bases (TVB-N), *Staphylococcus* spp., total thermotolerant coliforms, Salmonella spp, aerobic psychrotrophic and mesophilic bacteria. We found that 100% of the samples were sold at temperatures between 8.2 ºC and 23.3 ºC, exceeding the limit of 5º C established by the Brazilian legislation. For pH, none of the samples disagreed with the legislation (7.0), and only 7.14% of them were above the limit for TVB-N (30 mg N/100 g). Counts were observed for all analyzed microorganisms, except Salmonella spp. The observed values were within the accepted range for consumption as determined in the literature and by the legislation, except for thermotolerant coliforms, for which three samples (21.43%) had values exceeding 10^2 MPN/g. Although the presence of potentially pathogenic microorganisms such as Staphylococcus spp. and thermotolerant coliforms, was detected, none of the values exceeded the limits set by the legislation. However, these results indicate that the sashimi was presented in unsatisfactory hygienic conditions, which could pose risks to the health of the consumer. We have concluded that there is need for greater inspection and compliance with the existing laws of Good Manufacturing Practices for food-related services, especially where raw fish is sold, and also investments in the training and supervision of handlers

PALAVRAS-CHAVE: asian cuisine; raw fish; freshness; microbiological quality; inspection.

INTRODUCTION

Recently, there has been a growing interest in seafood in Brazil, as shown by surveys conducted in the last few years with populations that use this product as a staple food. These researchers correlate the increase in consumption with information about its nutritional value and its association with improvements in health (MACIEL *et al*. 2012) and quality of life.

In Brazil, an average per capita fish consumption of 9.9 $Kg.hab^{-1}.year^{-1}$ was recorded (FAO, 2022). This consumption includes fish produced nationally and the import of a large variety of fish, especially of marine origin, like salmon (*Salmo salar*).

Salmon is a highly consumed species in Brazil, including raw, because of its nutritional benefits. Raw fish consumption grows every year, and, in this context, Asian cuisine stands out as one of the main factors responsible for this increase, providing an innovative form of presentation of this food (GERMANO AND GERMANO, 2008).

Asian food started to have from an exotic connotation — representing an eating style — to the significance of health-conscious fast food, since these dishes have a relatively short preparation time, meeting the needs of the modern world and at the same time representing an alternative to conventional fast foods, which are rich in calories, fats, and cholesterol (CWIERTKA, 2005).

Fish fillets served raw are a traditional delicacy known as *sashimi* in Japan that has become very popular around the globe. For a long time, however, there have been problems regarding its storage, due to the short shelf life of this product (CAO *et al*. 2015), which in some cases is influenced by the lack of quality of the raw material and improper handling throughout its farming-production process. This fact has encouraged many researchers, in different countries, to conduct studies evaluating the microbiological quality and freshness of sashimi sold in Asian cuisine restaurants to identify the main critical control points of this product (RODRIGUES *et al*. 2012; MUSCOLINO *et al*. 2014; CAO *et al*. 2015; MIGUÉIS *et al*. 2015; MONTANARI *et al*. 2015).

The fish intended for the preparation of sashimi should be obtained, handled, and stored under ice or freezing, in a hygienic manner, making this food safe for the consumer. It should be stressed that aiming at a better presentation, in the making of dishes, vegetables like lettuce, carrot, ginger, radish, and others are typically used. In addition to providing a nice visual appearance and a better-balanced meal (SIKORSKI *et al*. 1990), they can also be sources of contamination of this food.

The production of sashimi is a manual process that involves cleaning, evisceration, cutting, and arrangement. Because it is a manually prepared food, its direct contact with the hands of the handler may lead to an increased incidence of pathogens like *Staphylococcus aureus* and thermotolerant coliforms (JAY, 2000).

The traditional way of eating raw fish generates an increase in the risk of food poisoning from a variety of pathogenic bacteria. There is an alarming number of food poisoning outbreaks and hospitalizations around the world as a result of the consumption of Asian foods that do not undergo any thermal process to eliminate disease-causing microorganisms (CDC, 2012).

Thus, given the consumers' knowledge of the nutritional quality of salmon and the rising consumption of sashimi in Brazil, there is a need for further studies addressing the hygienic-sanitary quality of this product, since it may pose risks to the health of consumers for being consumed raw and because of the lack of specific legislations regarding its consumption.

The present study aimed to evaluate the freshness and microbiological quality of salmon-based sashimi sold in Asian cuisine establishments in the municipality of Cuiabá-MT, Brazil.

MATERIAL AND METHODS

Samples of salmon sashimi from 14 Asian cuisine establishments (S1 to S14) located in the municipality of Cuiabá-MT, Brazil, were evaluated. The establishments were chosen at random, consisting of 13 restaurants that sell sashimi *à la carte*, and 01 supermarket that sells the product in plastic trays, to-go only.

Collections took place in April and May 2015, by simulating the purchase of the product by an ordinary consumer, i.e., directly at the establishment and in the average amount of a seven-piece portion (± 200 g). Next, the sashimi samples were placed in labeled plastic bags and sterilized under UV light. Subsequently, they were packed in a cool box with ice (±1ºC) and transported to the Laboratory of Fish Hygiene and Technology at the Federal University of Mato Grosso, where the analyses were performed. Samples were evaluated according to their physicochemical and microbiological characteristics, as described below:

PHYSICOCHEMICAL ANALYSIS

The temperature of the samples was measured at purchase using an infrared thermometer (AK32) with laser sight. The results were evaluated by comparing them to the standards established for this type of product by Resolution RDC N° 216, of September 15, 2004 (BRAZIL, 2004), from the Brazilian Health Surveillance Agency (*Agência Nacional de Vigilância Sanitária* - ANVISA), which addresses the technical regulation of Good Practices for Food Services.

To evaluate the freshness of the samples, the hydrogenionic potential (pH) was determined by first grinding 10±0.005 g of muscle tissue in a domestic blender, which was then added with 100 mL of distilled water. The mixture was homogenized and the pH was read after 30 minutes using a Ms Tecnopon Instrumentação pH meter, model mPA210 (Zenebon, Pascuet and Tiglea, 2008). The total volatile basic nitrogen (TVB-N) was quantified by the distillation method (Savay-da-Silva et al., 2008), where 50±0.005 g of muscle tissue was crushed and homogenized with 150 mL of trichloroacetic acid ($C_2HCl_3O_2$) at 5% for 60 seconds. After filtering the homogenate, 10 mL of the filtrate was transferred to a 1000 mL Erlenmeyer flask, where 20 mL of distilled water and 1 g of magnesium oxide (MgO) were added and continued for distillation in THOTH equipment, model 2350.40. The distillate was recovered in 20 mL of 4% boric acid (H_3BO_3) with 5 drops of mixed indicator and titrated with 0.01 N sulfuric acid (H_2SO_4) to the turning point.

MICROBIOLOGICAL ANALYSIS

Among the microbiological analyses carried out, some are considered as recommended in normative instruction nº 161, of July 1, 2022, from ANVISA (BRASIL, 2022a), which establishes lists of food microbiological standards in item 7a, "Fish (fish, crustaceans, molluscs) raw, seasoned or not, fresh, chilled or frozen": Salmonella/25g, Staphylococcus coagulase positive/g, and Escherichia coli/g for products consumed raw and Resolution of the Collegiate Board - RDC No. 724, of July 1, 2022 (BRAZIL, 2022b) which Provides about standards microbiology of food and its application. In addition to these analyses, aerobic mesophilic and psychrotrophic microorganisms, total coliforms and thermorotating bacteria were counted based on the literature (Franco and Landgraf, 1996; ICMSF, 1998) and legislation in force at the time of the analysis (Resolution-RDC No. 12, Of January 2, 2001).

For the analysis, the samples were first homogenized with 25g of sashimi and 225 ml of peptone saline water (SSP), obtaining the 10^{-1} dilution, and, from there, serial decimal dilutions up to 10^{-6} were obtained. For **Salmonella/25g**: the 10^{-1} dilution was incubated at 35 ± 1ºC for 24h, after which selective enrichment was carried out with tetrathionated broth (TT) (36ºC/24h)) and Rappaport Vassiliadis (RV) (42°C /24h), after this period, selective differential plating continued using Brilliant Green Agar (BGA) and Xylose Lysine Desoxycholate (XLD), incubating for 24h at the same temperatures as TT and RV, and, finally, carrying out if biochemical confirmation, with Triple Sugar Iron Agar (TSI) and Lysine Iron Agar (LIA) and serology; **Positive coagulase Staphylococcus/g**: Dilutions were sown on the surface of Baird-Parker agar, followed by incubation, counting of typical colonies and biochemical confirmation through catalase and coagulase tests, examination of morphology and Gram staining under a microscope; **Total coliforms**: Inoculated 1 ml of each dilution containing lauryl sulfate tryptose (LST) broth (3 tubes per dilution), incubating at 37ºC for 48h. After this period, the number of positive tubes was verified, characterized by the turbidity of the medium and the presence of gas in the Durhan tubes, thus moving on to the Brilliant Green Bile (BVB) broth, incubating at 37ºC for 48h, becoming positive through the same LST method and counting by Most Probable Number (MPN). **Thermotolerant coliforms**: From a positive LST, an Escherichia Coli broth was added and incubated in a water bath at 25ºC/24h, positive through the turbidity of the medium and the presence of gas in the Durhan tubes; **Escherichia coli/g:** From the positive tubes of EC broth, plates were inoculated by streaking with L-EMB agar (LevineEosin Methylene Blue), incubating under aerobic conditions at 35ºC/48h and subsequently, biochemical identification was carried out through citrate, Methyl Red/Voges Proskauer and indole tests; **Mesophiles:** dilutions were inoculated in Plate Count Agar (PCA), by depth and incubated for 48h at ±35ºC; **Psychrotrophs:** PCA was used using the surface method and incubated at ±3ºC for 7 days. After the incubation time, typical colony counts were made. All analysis were performed in triplicate and as described by Silva et al. (2017).

DATA ANALYSIS

To analyze the results, a descriptive analysis of the distribution of the obtained data was performed, and these data were expressed in percentage values. Further, they were compared with the standards, or maximum limits established for this

type of product by the current Brazilian legislation and as described in the literature.

RESULT AND DISCUSSION

PHYSICOCHEMICAL ANALYSES

The temperature, pH, and TVB-N values observed in the samples evaluated in this study can be viewed in Table 1. Table 2 shows the percentage of samples in conformity or non-conformity according to the physicochemical standards established by the current Brazilian legislation for each parameter assessed.

Table 1. Mean values for temperature, pH, and TVB-N (± standard deviation) observed in samples of salmon sashimi sold in Asian cuisine establishments in Cuiabá-MT, Brazil; and values established as quality standards by the Brazilian legislation.

SAMPLE	TEMPERATURE (º C)	PATTERN Legislation* (º C)	pH		TVB-N (mg N.100g^{-1})	
			MEAN ± SD	PATTERN Legislation**	MEAN ± SD	PATTERN Legislation**
S1	20.1	≤ 5	6.23 (0.016)	≤ 7	38.56 (0.212)	<30
S2	9.1	≤ 5	6.32 (0.025)	≤ 7	20.47 (0.071)	<30
S3	23.3	≤ 5	6.23 (0.032)	≤ 7	13.69 (0.071)	<30
S4	16	≤ 5	6.19 (0.017)	≤ 7	10.47 (0.141)	<30
S5	23.3	≤ 5	6.31 (0.026)	≤ 7	23.32 (0.071)	<30
S6	14.4	≤ 5	6.10 (0.026)	≤ 7	17.13 (0.000)	<30
S7	12.6	≤ 5	6.16 (0.055)	≤ 7	17.13(0.000)	<30
S8	11.9	≤ 5	6.19 (0.023)	≤ 7	24.73 (0.000)	<30
S9	15.1	≤ 5	6.26 (0.015)	≤ 7	26.15 (0.071)	<30
S10	9.3	≤ 5	6.17 (0.058)	≤ 7	24.74 (0.141)	<30
S11	8.2	≤ 5	6.18 (0.015)	≤ 7	25.66 (0.283)	<30
S12	10.7	≤ 5	6.13 (0.017)	≤ 7	23.80 (0.566)	<30
S13	14.2	≤ 5	6.06 (0.025)	≤ 7	20.47 (0.071)	<30
S14	12.6	≤ 5	6.15 (0.015)	≤ 7	23.32 (0.071)	<30

NOTE: TVB-N = total volatile basic nitrogen. Source: *(BRAZIL, 2004); ** (BRAZIL, 2020); SD = standard deviation.

Table 2. Distribution of conformities in samples of salmon sashimi sold in Asian cuisine establishments in Cuiabá-MT, Brazil, concerning the physicochemical standards.

Analysis	Non-conformity n (%)	Conformity n (%)
Temperature	14 (100)	0 (0)
pH	0 (0)	14 (100)
TVB-N	1 (7,14)	13 (92,86)

NOTE: n = number of samples. TVB-N = total volatile basic nitrogen. Source: own authorship.

The storage and handling conditions of foods can be inferred based on the temperature at which they are sold. In the present study, a serious problem was detected regarding the serving temperature of sashimi at all establishments assessed, because all samples (100%) presented values above the range recommended by RDC 216, of September 15, 2004 from ANVISA, which addresses the technical regulation of Good Practices for Food Services (BRAZIL, 2004) This regulation recommends that products of this sort be sold at temperatures ≤ 5 ºC, i.e., at least refrigerated.

The samples from establishments S3 and S5 showed the highest temperatures (23.3 ºC), both with values approximately five times higher than the limit established by the current Brazilian legislation. The sample from establishment A11 had the lowest temperature (8.2 ºC); however, it was almost two times above this limit (Table 1).

In the same way, another study reported that 100% of the fish received at Japanese restaurants were at temperatures higher than 4 ºC (RODRIGUES *et al*. 2012).

Refrigeration is a cold-based preservation method in which the microorganism action and enzymatic activity are slowed. High temperatures affect both the sensory and microbiological characteristics of raw fish. Thus, it can be stated that, in the present study, the establishments used the cold chain improperly during the entire sale process of the salmon sashimi, thereby increasing the fish temperature, which affects its quality and shelf life.

Consuming sashimi at a temperature higher than that permitted by law can be risky, as it encourages the growth of pathogenic bacteria that can cause food poisoning; to accelerates the deterioration of fish, which can result in changes in texture, flavor, and aroma; making it sensorially unpleasant for consumption; loss of nutritional quality, since rising temperatures and even the presence of microorganisms can cause nutrient degradation.

Another important factor that might have influenced the temperature of the sold fish was the ambient temperature of the municipality of Cuiabá-MT, Brazil. The temperatures observed on the collection days were 31 ºC (04/13/2015), 29 ºC (04/27/2015), and 26 ºC (05/11/2015), according to the National Institute of Special Research (BRAZIL, 2015). The fish is a highly perishable food and is sensitive to heat; if the handler handles this food for too long at a high ambient temperature, like that in Cuiabá, he or she may wind up seriously affecting the freshness and microbiological quality of this product, due to the increase in the temperature of the fish itself.

The sashimi arrangement and positioning stages also deserve special attention to prevent cross-contamination of the foods, be it from the hands of the sushi chef, equipment, utensils, or countertops used. It is important that the ingredients be removed from refrigeration in small batches and be handled at room temperature for a maximum of 30 min. Seldom does one wait for the distribution of these dishes in Japanese restaurants, since they are prepared as per request of the consumer. However, in other places like supermarkets and common restaurants, some waiting may occur, and, in this case, the times and temperatures must be respected; namely 10 ºC for up to 4h, or 10 to 21 ºC for up to 2 h (PRADO *et al.,* 2015). In this experiment, we found that neither the sample obtained from the

supermarket (S6) nor the others met the time and temperature requirements mentioned by these authors.

To control the fish temperature, the use of equipment with a thermometer is necessary (PRADO *et al.,* 2015). In the present study, no thermometers were found in any of the evaluated establishments. Likewise, in a study conducted in the municipality of Presidente Prudente - SP, Brazil, the authors visited 52 Asian cuisine establishments and also did not find thermometers in most of them (VALEJO *et al*., 2003).

Hoel *et al.* (2015) also stressed the importance of high-quality ingredients and the control of the adequate temperature to ensure the stable quality and microbiological safety of this food product.

Despite the high temperatures observed in the salmon sashimi samples evaluated in the present experiment, no direct relationship was observed between these values and the microbiological quality of the samples; in other words, the samples that showed higher temperatures were not necessarily those that had higher microorganism counts. On the other hand, indirectly, although most of the samples showed presence of thermotolerant coliforms within the threshold tolerated by the legislation, the presence of this group is inadmissible in the food, since it suggests inappropriate handling and the possible presence of species pathogenic to humans.

The pH analysis revealed that all samples were in compliance with the limit established by current Brazilian legislation (BRASIL, 2020). Arruda and collaborators (2021) found similar results when they analyzed sashimi from restaurants in Cuiabá-MT. These findings contrast with data published by Rodrigues et al. (2012), in which the pH of 1.85% of sushi and sashimi samples was above this limit.

The pH is also an important parameter to measure the freshness and preservation of the fish that directly affects its microbiological quality. This is one of the most commonly performed assessments to check the quality of fish for being a fast and easy method.

The pH decline in the fish muscle is lower as compared with other slaughtered animals, due to its lower glycogen reserve. In general, it can be stated that the pH decreases from 6.9-7.0 to 6.2-6.3 in lean fish, though values can go as low as 5.5-5.7 in dark-meat fish (PEREDA *et al*., 2005).

The Regulation for Industrial and Sanitary Inspection of Animal Products (*Regulamento da Inspeção Industrial e Sanitária de Produtos de Origem Animal* - RIISPOA (BRAZIL, 2020) establishes limits lower than or equal to 7,0 in the fish in order for it to be considered fresh. Therefore, all samples were ready for consumption and sale, in this regard.

In the literature, however, references indicate that pH is not a reliable parameter when evaluated separately to infer the freshness of fish, as values can vary due to numerous factors, including the fish species, handling, type of slaughter, and others (SOARES et al., 1998). Some authors suggest that this diminishes its scientific reliability, especially when this parameter is analyzed in isolation (REZENDE-DE-SOUZA et al, 2020).

Thus, even if the pH of the salmon sashimi samples evaluated in this experiment were in agreement with the limits defined by the legislation, this would not mean the samples would not pose a risk to the consumer, since the pH limit established by the legislation comprises all fish species, and there is no specific study for the evaluated product. Therefore, it is not recommended that the quality of the sashimi be determined only by the evaluation of this parameter.

In the TVB-N analysis, we found that 92.86% of the evaluated samples were in conformity with the maximum limit of 30 mg N.100 g^{-1} in the muscle (BRAZIL, 2020) established by the Ministry of Agriculture, Livestock and Food Supply (MAPA) for the sale of fresh fish. The only sample that did not conform to the legislation was that from establishment S1 (38.56 mg N.100 g^{-1}).

The TVB-N chemical test has the longest history of use as an indicator of fish freshness; it is based on the measurement of the amount of basic compounds recovered by distillation of the fish muscle, or extracts of fish muscle under alkaline conditions (HOWGATE, 2010a; REZENDE-DE-SOUZA; SAVAY-DA-SILVA, 2021). The determination of TVB-N is a parameter considered valid to check the fish preservation state, since it comprises compounds such as ammonium, trimethylamine, dimethylamine, and others. At the beginning of the degradation process, the most representative volatile base is the ammonium originating from the deamination processes of the ATP derivatives. Later, the ammonium originating from the degradation of other nitrogen compounds, e.g., amino acids, together with trimethylamine formed from the trimethylamine oxide, become present (OGAWA; MAIA, 1999).

The maximum TVB-N value established by the current Brazilian legislation is also a value established generally, with no specificity regarding the differences inherent to each species, which may generate some problems in the interpretation of results. In the literature, studies report that freshwater fish, in general, have a low TVB-N accumulation *post mortem*, because they have low trimethylamine oxide (TAMO) levels (EVANGELISTA *et al.* 2000), which naturally provide them with low TVB-N levels in the muscle when compared with marine fish in the same stage of decomposition, for instance.

For some time, the literature has presented critics about the reliability of results of TVB-N analyses with regard to their actual representation of the deterioration level of fish, given that there are different methods to determine the same variable and some significant changes between them (HOWGATE 2010a; HOWGATE 2010b; REZENDE-DE-SOUZA; SAVAY-DA-SILVA, 2021). Moreover, some studies have been conducted for specific species, aiming to standardize or optimize some of these methodologies, since it has already been identified that the results of this analysis may vary depending on the species analyzed and the methodology used. In some cases, the use of this parameter alone is not recommended to evaluate the freshness of fish (CASTRO *et al*. 2006; SAVAY-DA-SILVA *et al.* 2008; CÍCERO *et al.* 2014).

HOWGATE (2010b) also claimed that the TVB-N content is not an effective indicator to determine the deterioration of fish in the control of commercial quality, or in the official regulatory control, since the variations between measurements are large, and, additionally, there are variations between batches due to biological factors. However, other authors have affirmed that there is an evolution of volatile bases throughout the fish's shelf life (CASTRO *et al.*, 2006).

The literature has considerations about the TVB-N values, e.g.: fish in a completely fresh state is that whose TVB-N reaches 5-10 mg N.100 g^{-1} in the muscle, and fish with satisfactory freshness show up to 15-25 mg N.100 g^{-1}. At the onset of alterations, the N content may reach 30-40 mg.100 g^{-1}, and when the fish is spoiled, this content must be above 50 N.100 g^{-1} of muscle (OGAWA; MAIA 1999). If we consider the standards established by these authors, we can state that two (14.28%) samples (S3 and S4) were in complete freshness; 11 (78.57%) had satisfactory freshness (S2, S5, and S14); and one (7.14%) sample (S1) was at the onset of alterations.

In this sense, it was found that 7.14% of the samples presented TVB-N above the limit established by legislation, which could be a risk to the health of those who consume it, since this value is associated with the possible formation of biogenic amines, such as: histamine, tyramine and putrescine, which can cause food poisoning. According to FAO (2012), histamine poisoning, also called scombrotoxin, can result from the development of Enterobacteriaceae in fish. Furthermore, just like the consumption of sashimi at high temperatures, the consumption of this food with high TVB-N can compromise the sensorial quality of the product and corroborate the deterioration of the fish.

It was also found that the only sample (S1) that showed TVB-N values above the threshold recommended by the legislation (BRAZIL, 2020) also presented thermotolerant coliform counts above the limit established by RDC no. 12, of January 2nd, 2001 by ANVISA (BRAZIL, 2001), and inappropriate serving temperature (BRAZIL, 2004).

To control fish temperature, pH and BNVT, it is necessary to adopt appropriate food safety practices, such as: storage in a sanitized location with temperature control that must be close to or below 0ºC and good practices in handling and preparing food, This includes washing hands frequently, cleaning and disinfecting surfaces and kitchen utensils, avoiding cross-contamination of raw fish with other foods, and training handlers.

Microbiological analysis

The fish safety regarding the microbiological standards is of paramount importance since foodborne diseases have always occurred due to lack of care from the acquisition of the raw material to its handling and processing (SOARES; GONÇALVES, 2012).

The results of microbiological analysis can be observed in Table 3. Table 4 describes the percentage of samples in conformity or not conforming to IN 161 (BRAZIL, 2022) and the literature (FRANCO; LANDGRAF, 1996; ICMSF 1998).

The evaluated microorganisms were within the limits established by the legislation and by the literature, except for thermotolerant coliforms, for which 21.43% (n = 3) of the evaluated samples were not in conformity with the legislation in force at the time of analysis (BRAZIL, 2001).

It was also found that 100% of the samples showed contamination with this microbial group, indicating a likely cross-contamination due to lack of hygiene of the handlers, utensils, or countertops. The presence of these microorganisms is inadmissible, as it may indicate failure in compliance with the Good Manufacture Practices (GMP) during the entire salmon sashimi making process.

Table 3. Microbiological quality of samples of salmon sashimi sold in Asian cuisine establishments in Cuiabá-MT, Brazil.

Samples	*Staphylococcus* spp. (CFU.g^{-1})	Total Col. (MPN.g^{-1})	Thermotolerant (Col. MPN.g^{-1})	Psychro. (CFU.g^{-1})	Mesophilic (CFU.g^{-1})	Salmonella spp.
S1	1.32 x 10^3	>1.1 x 10^3	1.1 x 10^3	6.27 x 10^3	2.28 x 10^2	Abs.25g^{-1}
S2	5.6 x 10^2	>1.1 x 10^3	2.3 x 10	3.98 x 10^3	2.04 x 10^2	Abs.25g^{-1}
S3	7.6 x 10^2	1.1 x 10^3	1.5 x 10	6.84 x 10^3	2.78 x 10^2	Abs.25g^{-1}
S4	3.8 x 10^2	>1.1 x 10^3	2.0 x 10	6.39 x 10^3	2.82 x 10^2	Abs.25g^{-1}
S5	8.4 x 10^2	> 1.1 x 10^3	3.5 x 10	3.28 x 10^3	1.54 x 10^2	Abs.25g^{-1}
S6	1.08 x 10^3	3.6	3.6	1.21 x 10^3	2.97 x 10^2	Abs.25g^{-1}
S7	1.82 x 10^3	92	3.6	2.53 x 10^3	1.44 x 10^2	Abs.25g^{-1}
S8	1.94 x 10^3	2.3 x 10	9.2	4.29 x 10^3	2.34 x 10^2	Abs.25g^{-1}
S9	5.24 x 10^3	2,3 x 10	9.2	6.87 x 10^3	1.87 x 10^2	Abs.25g^{-1}
S10	1.11 x 10^3	>1.1 x 10^3	1.1 x 10^3	2.67 x 10^3	5.67 x 10^2	Abs.25g^{-1}
S11	6.3 x 10^2	> 1.1 x 10^3	11	4.01 x 10^3	2.70 x 10^2	Abs.25g^{-1}
S12	3.3 x 10^2	1.1 x 10^3	2.4 x 10^2	3.21 x 10^3	1.53 x 10^2	Abs.25g^{-1}
S13	8,7 x 10^2	> 1,1 x 10^3	2,3 x 10	1,14 x 10^3	8,7 x 10^2	Abs.25g^{-1}
S14	2,7 x 10^2	1,5 x 10^2	3,6	5,07 x 10^3	4,17 x 10^2	Abs.25g^{-1}

NOTE: Col. = coliforms; Abs. = absence; Psychro= Psychrotrophic; CFU = colony forming unit; MPN = most probable number.

Table 4. Distribution of conformities of samples of salmon sashimi sold in Asian cuisine establishments in Cuiabá-MT, Brazil, regarding microbiological parameters.

Analysis	Non-conformity n (%)	Compliance n (%)	Standard of law or temperature
Salmonella spp.	0 (0)	14 (100)	Absence.25g^{-1}*
Staphylococcus spp.	0 (0)	14 (100)	10^6 CFU.g^{-1}**
Coliforms totais	0 (0)	14 (100)	10^6 MPN.g^{-1}**
Thermotolerant col.	3 (21.43)	11 (78,57)	10^2 MPN.g^{-1}***
Psychrotrophic	0 (0)	14 (100)	10^7 CFU.g^{-1}****
Mesophilic	0(0)	14 (100)	10^7 CFU.g^{-1}****

NOTE: Col. = coliforms; n = n = number of samples; CFU = colony forming unit; MPN = most probable number; Source: *(BRAZIL, 2022); ** (FRANCO AND LANDGRAF ,1996); ***(BRAZIL, 2001); **** (ICMSF,1998).

In another study also with salmon sashimi, the authors observed that 46.66% of 15 samples showed presence of thermotolerant coliforms above the limit established by the legislation, which is a similar value to that observed in the current study (MONTANARI *et al*. 2015). Arruda and collaborators (2021) analyzed samples of salmon sashimi in five restaurants in Cuiabá-MT and found values above the recommended level in two of them, one with 50% non-conformity and the other, 100%. These authors reinforce that this group of microorganisms may contain potentially pathogenic strains that cause foodborne diseases.

Consuming salmon sashimi containing thermotolerant coliforms represents a health risk, as it may indicate contamination of fecal origin and the possible presence of harmful pathogens. The presence of this microorganism is directly related to failures in Good Manufacturing Practices, mainly in the hygiene of hands and uniforms of handlers, incorrect use of personal protective equipment and contamination of surfaces.

In the present study, the contamination by Salmonella spp. was not a matter of concern, because the presence of this bacterium was not detected in any of the samples of 25 g evaluated, as provided by the current Brazilian legislation (BRAZIL, 2022). A similar result was found by Muscolino *et al*. (2014). Other authors, however, analyzing 10 pairs of sashimi from two restaurants in Fortaleza/CE, Brazil, observed that 50% of the samples presented high indices of Salmonella spp (MENEZES *et al.* 2006). In another study conducted in a town in the backlands of Bahia, the authors analyzed salmon sashimi and found the presence of Salmonella spp. in 100% of the samples (SANTOS et al., 2022).

The same observation was true for Staphylococcus spp. analyses; none of the samples presented counts above the maximum limit suggested by the literature, which is 10^6 UFC.g^{-1} (FRANCO; LANDGRAF, 1996) and there was no sample confirmed for Staphylococcus aureus, a result similar to that found by Arruda and collaborators (2021). In an investigation conducted by MIRANDA; BAIÃO (2011), however, unlike the present study, 25% of the samples were unsatisfactory for Staphylococcus spp., that is, they presented counts higher than 10^6 for this microorganism.

Contamination by *Staphylococcus* spp. should always be considered of great relevance, as it is a hygienic-sanitary indicator of the raw material, from its import, in the case of salmon, to the sale of the final product. This microorganism is usually transmitted to the food through handlers or occasional animals that may come into contact with the product, because they are naturally present in the skin and mucosal microbiota. Besides, if their presence is detected in the foods, the probability of food poisoning outbreaks is elevated.

According to the limits established in the literature, all samples also displayed acceptable counts for total coliforms, which were below the limit of 10^6 CFU.g^{-1} (FRANCO AND LANDGRAF 1996).

The results of this research were similar to those found by Matos et al. (2020), who, when investigating sashimi samples in Vitória da Conquista, Bahia, obtained results considered within the allowed limits for coliforms and the absence of *Escherichia coli* and Salmonella spp.

We emphasize that the fact that the samples presented results in conformity with the limits established by the literature for both *Staphylococcus* sp. and total coliforms does not annul the possibility that their consumption may pose a risk to the consumer's health, since the literature does not establish a specific limit for these microorganisms in products derived from raw fish, but rather a general limit for pathogenic microorganisms present in animal products.

In this way, even if the values found in the analyzed samples did not extrapolate the limits suggested by the literature, it should be stressed that the presence of these microorganisms in these samples may be indicative of failures complying with the Good Manufacture Practices by these establishments, especially with respect to the hygiene of handlers, as well as storage at inappropriate temperatures. All of this can increase the risk of cross-contamination, compromise the quality of the product served and put the health of the consumer at risk, since the safety of this food is compromised, potentially causing infections and food poisoning. It is worth noting that by purchasing fish of

good origin and having controlled storage and handling temperatures, as well as good manufacturing practices, these risks are minimized.

Psychrotrophic and mesophilic aerobic bacteria can also be indicative of the hygiene conditions in which the salmon was obtained and stored. If we consider only these microorganisms to evaluate the samples, we can assert that they were under satisfactory conditions, since 100% of them showed counts for these microorganisms below the upper limit established in the literature (10^7 CFU.g^{-1}) (ICMSF 1998), even if their serving temperatures did not reach the values recommended by the legislation (BRAZIL, 2004). Arruda and collaborators (2021) found a similar result when they analyzed spoilage bacteria in salmon sashimi also sold in Cuiabá-MT, that is, the counts were lower than what the literature (ICMSF 1998) establishes.

Among the main problems stemming from the high number of psychrotrophic bacteria in foods, the following ones stand out: gelation, alterations in consistency and texture, unpleasant tastes and smells, and rancidification due to the production of thermostable proteases and lipases (ORDONEZ, 2007) and also the pathogenicity of the microorganism. The samples evaluated in this experiment did not have significant sensory alterations, probably because of the low presence of these microorganisms.

The fish meat has a peculiar chemical composition that provides it with nutritional richness, but with a high potential for deterioration. In this regard, the nutritional benefits of this food group can only be fully exploited when the safety and quality factures are ensured. This makes it essential that tools be adopted to prevent the deterioration mechanisms, such as the cold chain technique (SOARES AND GONÇALVES, 2012), application of Hazard Analyses and Critical Control Points (GOLDING *et al*., 2014), and use of the GMP in all stages of the production chain.

CONCLUSIONS

Although the majority of the samples analyzed demonstrated compliance with the standards established by Brazilian legislation, the identification of high values in some parameters raises concerns regarding the quality and durability of the products.

In this sense, maintaining the cold chain is crucial to ensuring the safe consumption of these products, as inadequate storage and transportation can compromise not only the sensorial quality, but also the microbiological integrity of the food. Given this scenario, companies are recommended to exercise greater caution when handling, storing and selling raw Asian culinary products, requiring substantial investments in training and supervision of handlers, in order to preserve not only quality, but also health , safety and consumer well-being.

Finally, the need for more rigorous inspection and strict compliance with current standards for products from food services becomes evident, highlighting the importance of Good Manufacturing Practices.

Qualidade sanitária de *sashimis* comercializados em Cuiabá, Brasil

RESUMO

O consumo de peixes no Brasil vem crescendo de forma constante, inclusive em pratos que são servidos crus, como o sashimi. O objetivo deste estudo foi avaliar o frescor e a qualidade microbiológica de sashimis de salmão comercializados em estabelecimentos de culinária asiática localizados em Cuiabá, Mato Grosso, Brasil. Amostras de 14 estabelecimentos foram avaliadas por meio de análises de temperatura, pH, bases nitrogenadas voláteis totais (BNVT), *Staphylococcus* spp., Coliformes termotolerantes totais, *Salmonella* spp, bactérias aeróbias psicrotróficas e mesófilas. Verificou-se que 100% das amostras estavam sendo comercializadas em temperaturas entre 8,2 ºC e 23,3 ºC, superiores ao limite de 5 ºC estabelecidos pela legislação brasileira. Para pH, nenhuma das amostras discordou da legislação vigente (7,0) e apenas 7,14% delas estavam acima do limite para BNVT (30 mg N/100 g). As contagens foram observadas para todos os microrganismos analisados, exceto *Salmonella* spp. Os valores observados encontram-se dentro da faixa aceita para consumo determinada pela literatura e pela legislação brasileira vigente, exceto para os coliformes termotolerantes, para os quais três amostras (21,43%) apresentaram valores superiores a 10^2 NMP/g. Embora tenha sido constatada a presença de microrganismos potencialmente patogênicos como *Staphylococcus* spp. e coliformes termotolerantes, nenhum dos valores ultrapassou os limites estabelecidos pela legislação. Porém estes resultados indicam que os sashimis se apresentaram em condições higiênicas insatisfatórias, podendo levar riscos à saúde do consumidor. Concluímos que há necessidade de maior fiscalização e cumprimento das legislações existentes de Boas Práticas de Fabricação para serviços relacionados à alimentação, em especial onde há comercialização de peixe cru, e também investimentos na capacitação e fiscalização dos manipuladores.

PALAVRAS-CHAVE: Cozinha asiática, Peixe cru, Frescor, Qualidade microbiológica, Inspeção.

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