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Microbiological quality of raw fish based food products

Qualidade microbiológica de produto alimentícío à base de peixe cru

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ABSTRACT
Sashimi is a food product widely produced in traditional Japanese restaurants. It has a high risk of microbial contamination because it comes from raw fish and due the lack of training in Good Handling Practices in most establishments that produce this kind of food. The objective of this work was to evaluate the microbiological quality of salmon-based sashimi produced and marketed in traditional Japanese restaurants in Vitória da Conquista – Bahia, Brazil. This is a quantitative, laboratory and explanatory study. PetrifimTM (3M) dishes were used to evaluate twelve samples of sashimi obtained from four restaurants. The microorganisms analyzed were: total coliforms, thermotolerant (Escherichia coli), Salmonella sp. and Staphylococcus aureus. The results indicated the presence of visible colonies of total coliforms in 10 of the analyzed samples. It was detectable the growth of bacterial colonies of Staphylococcus aureus only in samples A1 and D3. For Escherichia

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coli and Salmonella sp. there was no visible growth of colonies in the samples evaluated. In view of the results found, detection of bacterial contamination at considerable levels was observed. It is noteworthy that, even if it is within the values recommended by Brazilian legislation, the presence of such microorganisms may be related to the lack of hygiene in the place and/or the manipulator, poor conservation of fish and the lack of good handling practices.

Keywords: sashimi; contamination risk; hygiene

RESUMO

O sashimi é um produto alimentício largamente produzidos nos restaurantes de origem japonesa. Possui alto risco de contaminação microbiana por ser proveniente de peixe cru e pela falta de treinamento em Boas Práticas de Manipulação na maioria dos estabelecimentos que produzem o alimento em questão. Objetivou-se, com este trabalho, avaliar a qualidade microbiológica de sashimis à base de salmão produzidos e comercializados em restaurantes japoneses em Vitória da Conquista – BA. Trata-se de um estudo do tipo quantitativo, laboratorial e explicativo. Foram utilizadas placas Petrifilm® (3M) para avaliar doze amostras de sashimis adquiridas em quatro restaurantes. Os microrganismos analisados foram: coliformes totais, termotolerantes (Escherichia coli), Salmonella sp. e Staphylococcus aureus. Os resultados apontaram presença de colônias visíveis de coliformes totais em 10 das amostras analisadas. O crescimento de colônias bacterianas de Staphylococcus aureus foi detectável apenas nas amostras A1 e D3. Já para Escherichia coli e Salmonella não houve crescimento visível, estando ausentes nas amostras avaliadas. Diante dos resultados encontrados, foi observada detecção de contaminação bacteriana em níveis consideráveis. Ressalta-se que, mesmo estando dentro dos valores preconizados pela legislação brasileira, a presença de tais microrganismos pode estar relacionada à falta de higiene no local e/ou do manipulador, má conservação do pescado e à falta de boas práticas de manipulação.

Palavras-chave: sashimi; risco de contaminação; higiene.

1 INTRODUCTION

Brazil has an excellent aquaculture potential, which puts it in a prominent situation for fish production (FAO, 2016). Fish production in the country grew by 4.5% reaching 722,560 tons, according to data from the Brazilian Association of Pisciculture (2019).

Fishing products are an important ingredient in human feeding, rich in essential amino acids, contains low fat and high availability of omega 3 fatty acid, assisting in the control of cardiovascular diseases and in reducing LDL cholesterol levels and triglycerides in the blood. It presents liposoluble vitamins (A and D) and minerals such as calcium, phosphorus, iron, copper, selenium and, in saltwater fish, iodine (SARTORI; AMANCIO, 2012).

Raw fish-based foods, such as sashimi, originated in traditional Japanese cuisine, have been gaining prominence in Brazil (SANTOS et al. 2012). It is understood by sashimi, pieces of finely sliced raw fish (MORAES; DARLEY; TIMM, 2019).

It is worth mentioning that due to favorable tissue conditions and high-water activity, fish is susceptible to enzymatic, microbiological and oxidative changes, with microbial activity being the main form of deterioration assessment (FR; LANDGRAF, 2008).

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Good Practices are procedures aimed at the production and marketing of safe foods (MELO et al, 2018). Many establishments commit irregularities due to ignorance about the legitimate health action. In traditional Japanese cuisine restaurants, there is great handling of food products (RODRIGUES et al., 2017). However, these places have been understudied in Brazil, especially in relation to sanitary aspects and food safety, which does not allow to profile the adequacy of these establishments in terms of hygienic-sanitary aspects and compliance with current legislation.

The food product in question is considered a high-risk preparation, especially when prepared by people who do not have adequate training, and may raise the risk of the incidence of quality indicator microorganisms such as total coliforms and thermotolerant coliforms, in which *Escherichia coli* is the most relevant species of the group, and pathogens such as *Staphylococcus aureus* and *Salmonella sp*., which are commonly associated with foodborne diseases (NASCIMENTO et al, 2019; SOUZA et al. , 2015).

According to Tortora, Funke and Case (2012), the ingestion of food contaminated by bacteria can cause gastroenteritis and intestinal disorders, caused by the multiplication of microorganisms that may or may not produce toxins. This factor intensifies when preparations are served without any cooking process, which poses an even greater health risk (SOUZA et al., 2015).

The objective of this study was to evaluate the microbiological quality of salmon-based sashimi produced and marketed in Japanese restaurants in Vitória da Conquista – Bahia, Brazil.

2 METHODOLOGY

2.1 TYPE OF STUDY

This research can be classified as analytical quantitative, basic and experimental. (FONTELLES et al., 2009). As for its objective, it is classified as explanatory and has a laboratory character, referring to technical procedures in the laboratory (GIL, 2002).

2.2 SAMPLE COLLECTION SITE

The samples were collected at traditional Japanese cuisine restaurants in Vitória da Conquista - Bahia, Brazil. In this regard, four restaurants were selected and to safeguard the identity of the evaluated companies, the samples were coded by letters A, B, C and D.

2.3 ANALYSIS OF SAMPLES

Microbiological analyses of sashimi were performed according to DRC 12 of January 2, 2001 (BRASIL, 2001), for ready-to-use dishes (ready-made food from kitchens, restaurants and alike), meat, fish and raw similar foods (raw kibbeh, carpaccio, sushi, sashimi), etc. The presence of

microorganisms analyzed were: total coliforms, *Escherichia coli*, *Salmonella sp*. and *Staphylococcus aureus*.

For the analyses, 120g of salmon sashimi fillets were collected and these were acquired anonymously in four restaurants in the city of Vitória da Conquista - Bahia, Brazil, collecting three samples per restaurant on alternate days, totaling twelve samples. The samples were placed separately in packages free of microbial contamination, and packed inside a thermally insulated box (4 °C) containing frozen gel bags and analyzed according to the standards of the Brazilian Pharmacopoeia (BRASIL, 2010).

The packages containing the samples were taken and kept under freezing to the University Center of Technology and Sciences Laboratory within a maximum of 30 minutes. At the time of the analyses, the packages were externally disinfected with 70% alcohol, before opening. It was then removed and cut into small fragments of representative samples to perform serial dilution, with properly sterilized material.

2.4 SERIAL DILUTION

After homogenization of the sample, 25g of sashimi were weighed in an analytical scale, using properly sterilized glass. Then, the sample was diluted in 225 mL of buffered peptone water 0.1% previously sterilized, in Erlenmeyer vial of 250 mL, corresponding to the dilution of 10^{-1}. From the first dilution, 1.0 mL was homogenized in 9 mL of peptone water, the second dilution was performed denominating it from 10^{-2}. To compose the third dilution, 1.0 mL of dilution 10^{-2} was homogenized to in 9 mL of buffered peptone water, corresponding to 10^{-3}.

2.5 INOCULATION, INCUBATION TEMPERATURE AND IDENTIFICATION

For microbiological quality analysis, the methodology described by Nero, Beloti and Barros (2000) was used, which uses analyses in Petrifilm™ (3M) dishes for specific microorganisms. All Petrifim dishes were properly identified with the letters A, B, C and D, corresponding to each restaurant. Each analysis was performed in triplicate and the sample units were labeled as: A1; A2; A3; B1; B2; B3; C1; C2; C3; D1; D2; and D3.

2.6 DETECTION OF TOTAL COLIFORMS, *Escherichia coli* AND *Staphylococcus aureus*

Using Petrifilm dishes specific to each species of microorganism, the dilutions were inoculated. The referred top film of the plate was suspended using the pipette subsequently adding 1.0 mL of dilution 10^{-1} in the center of the lower film, and finally leaving the top film fall slowly on the inoculated dilution. The plastic diffuser was positioned in the center of the plate and the sample was equally distributed. The procedure was performed in the other dilutions (10^{-2} and 10^{-}

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^{3}).

Then the plates were incubated in an incubator considering the optimum temperature for total coliforms and *Staphylococcus aureus* as 37 °C for 24 to 48 hours and *E. coli* at 45 °C for 24 hours. For identification and counting of the colonies, the total coliforms were considered for red staining and *Escherichia coli*, colonies with red coloration and lighter halo formation around the colonies. For counting the *Staphylococcus aureus*, their colonies were identified in the Petrifilm dishes by red or blue coloration surrounded by a rosy area.

2.7 DETECTION OF *Salmonella sp.*

The current legislation demands absence of *Salmonella sp*. in 25 g samples to be considered as secure (BRASIL, 2001). For the analysis of absence or presence of *Salmonella sp*., 25g of sashimi were weighed and transferred to 225 mL of buffered peptone water 0.1%. Subsequently, the samples were incubated at 37 °C for 24 hours. Inoculation occurred after this period. Using Petrifilm™ (3M) plate specific for *Salmonella sp*., the top film of the plate was raised and 1.0 mL of dilution 10^{-1} was added in the center of the lower film, leaving the top film to fall on the inoculated dilution. The plastic diffuser was positioned in the center of the plate, so that the sample was evenly distributed. Then the plates were incubated in incubator at 37 ºC for 24 hours. *Salmonella sp*. identification occurred by the growth of colonies that present dark green to black coloration.

2.8 DATA ANALYSIS

The results of the analyses were recorded in scientific notation and expressed in CFU/g (colony-forming units per gram) according to the literature for each evaluated microorganism and the presence or absence of *Salmonella sp*. using 25g of each sample.

3 RESULTS AND DISCUSSION

The presence of *Staphylococcus aureus* was identified in the samples of sashimi A1 and D3, as can be observed in Table 1, while in the other samples no growth of bacterial colonies was observed. In the results of the total coliform tests, there was no visible colonies in samples B1 and D2 and presence in other samples presented. In the analyses of *Escherichia coli* and *Salmonella sp*., the result was negative for all samples.

Table 1: Microbiological detection (CFU/g) obtained from prepared and marketed sashimi in Vitória da Conquista – Bahia, Brazil, restaurants. Abs: Absence; Source: Search data.

Samples	*Staphylococcus aureus*	Total Coliforms	*Escherichia coli*	*Salmonella sp.*
A1	1×10^3	1×10^2	Abs	Abs
A2	Abs	1×10^2	Abs	Abs
A3	Abs	1×10^2	Abs	Abs
B1	Abs	Abs	Abs	Abs
B2	Abs	$2{,}0 \times 10^2$	Abs	Abs
B3	Abs	$1{,}0 \times 10^2$	Abs	Abs
C1	Abs	12×10^2	Abs	Abs
C2	Abs	$4{,}0 \times 10^2$	Abs	Abs
C3	Abs	$4{,}0 \times 10^2$	Abs	Abs
D1	Abs	63×10^2	Abs	Abs
D2	Abs	Abs	Abs	Abs
D3	$1{,}0 \times 10^3$	67×10^2	Abs	Abs

The results obtained in the 12 samples showed two (17%) samples with results of 1.0×10^3 CFU/g for *Staphylococcus aureus* (samples A1 and D3). This result is in accordance with the maximum established by resolution - RDC no. 12 (BRASIL, 2001), which establishes a maximum limit of up to 5×10^3 CFU/g. In the other 10 samples (83%) there was absence of *Staphylococcus aureus*. These results are indicative that the manipulation of food products as well as the cleaning and hygiene of utensils and surfaces were performed in accordance with food safety standards for human consumption.

A similar result was observed in a study developed by Montanari et al., (2015) in which microbiological quality was evaluated in 15 samples of fresh salmon marketed in the city of Ji-Paraná – Rondônia, Brazil, all samples presented contamination by *Staphylococcus aureus*, however, the values found were within the limits allowed by the legislation.

In *Salmonella sp*. analysis of sashimi samples, it was found that all samples were absent of visible colonies in the tests using 25g of samples, being within the standards established by current legislation (BRASIL, 2001). This fact is indicative that the original product has not been contaminated by this pathogen at any time of fish processing. Lima (2009), in his studies in the city of Recife – Pernambuco, Brazil, analyzed 40 samples of sashimi from different restaurants, 20 of which were specialized establishments and 20 of non-specialized establishments, also verified the absence of the related bacterium.

Regarding the analysis of total coliform, the results indicate that 12 of the 10 samples (82.3%) showed characteristic colonies, while two (17.7%) showed negative results for total coliforms. It is worth noting that there are no rules established by current legislation in this aspect. However, the

presence of total coliforms is related to several factors, such as the lack of hard hygiene in the face of the processing of food and the hands of food manipulators, inadequate clothing, poor hygiene of the place and the conservation of fish. Such factors can directly influence food contamination, as this bacterial species does not belong to the external natural microbiota of fish (SOARES; GERMANO, 2004).

In the analysis of *Escherichia coli*, all evaluated samples did not present any CFU/g detection, being in accordance with the standards established by current legislation, which recommends a maximum value of 1.0×10^2 CFU/g (BRASIL, 2001).

In the analysis carried out by Resende, Souza and Oliveira (2009) in 87 sashimi samples, 25% of them were contaminated by *Escherichia coli* with populations above the permitted by legislation. This fact indicates flaws in hygienic-sanitary control at some stage of the processing or marketing of the food product.

The results found in this study for *Staphylococcus aureus*, *Escherichia coli* and *Salmonella sp*. were within the standards established by the current legislation throughout the samples analyzed. Regarding the presence of total coliforms, there was present in most samples, but there are no values established in the legislation.

Silva et al. (2013) reported the need for professionals linked to the production and processing of food products to adopt daily hygiene practices, actions aimed at the quality control of food and hygiene procedures in the processing, storage and conservation of food.

4 CONCLUSION

In most of the sashimi samples analyzed, bacterial contamination was detected at significative levels, but all values were within that recommended by the legislation. It is noteworthy that, even if within the recommended values, the presence of such microorganisms may be related to the lack of hygiene in the place and/or the manipulator, poor conservation of fish and the lack of good handling practices.

It is recommended that professionals responsible for the manipulation and distribution of fish, follow the measures recommended by Good Manufacturing Practices (GMP) in order to minimize the risks of possible food toxinfection of consumers and minimize the chances of contamination of these foods.

Therefore, more research on the subject is suggested, including in other types of establishments that commercialize this type of product and thus promote partnerships between

institutions and food market. This type of cooperation will promote the implementation of Good Hygiene Practices and ensure the marketing of safe food for consumption.

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