# Microbiological and Physicochemical Qualities of Sushi and Sashimi from Japanese Restaurants in Brazil

**Camila Moura Batista[1], Maria Luiza Rezende Ribeiro[1], Mauricio Junio Franco de Souza[1], Liana Jayme Borges[1], Tânia Aparecida Pinto de Castro Ferreira[1], Maria Cláudia Porfirio André[2,*]**

[1]Faculdade de Nutrição, Universidade Federal de Goiás, Goiânia, GO, Brasil
[2]Instituto de Patologia Tropical e Saúde Pública, Universidade Federal de Goiás, Goiânia, GO, Brasil
*Corresponding author: mcporfirio@hotmail.com

**Abstract**  Japanese cuisine is known for its nutritional and sensory quality. Sushi and sashimi are highly perishable and widely consumed foods in Brazil. The production and storage of these foods under appropriate conditions are critical factors in ensuring consumer safety. This study was carried out to evaluate the microbiological and physicochemical conditions of sushi and sashimi sold in Japanese restaurants as well as their handling conditions. Samples of sushi (36) and sashimi (35) and 112 swabs of food handlers were collected from 18 Japanese restaurants of the central region, Brazil. Temperature, pH and Total Volatile Nitrogen Bases (in sashimi) of food samples were determined. Thermotolerant coliforms, coagulase positive *Staphylococcus*, *Vibrio parahaemolyticus*, *Bacillus cereus* (in sushi) counts and the detection of *Salmonella* sp. were also performed. *Staphylococcus aureus* and *Escherichia coli* were investigated in the swabs samples. The average temperature of the food samples at all the restaurants was higher than the recommended value as well as 63.9% of the sushi and 54.3% of the sashimi samples had above recommended pH. All sashimi samples had content within legal limits of Total Volatile Nitrogen Bases. Four samples (5.6%) were positive for thermotolerant coliforms, and in three of them (4.2%), *E. coli* was isolated. High counts of *Bacillus cereus* were found in 22.2% of the sushi samples. Coagulase positive *Staphylococcus*, *Vibrio parahaemolyticus,* and *Salmonella* sp. were not found in the samples analysed. In 9.0% of the clinical samples, thermotolerant coliforms were detected and *E. coli* was found in 5.4% (n = 6) of them. *Staphylococcus aureus* was not detected on any food handler. The high storage temperature of food, the lack of heat treatment and the microbial contamination found may lead the present microorganisms to reach high counts until consumption, and therefore represent risk to consumers. Thus, effective prevention by supervisory bodies is essential.

*Keywords: fish, microbiological quality, pH, Bacillus cereus*

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## 1. Introduction

The fish consumption in Brazil has increased in recent years and exceeds 12 kg/inhabitant/year, which is the minimum quantity recommended by the World Health Organization [1]. This is due mainly to its nutritional quality. Fish is an excellent source of animal protein, has anti-inflammatory action, and significant amounts of magnesium, phosphorus, and vitamins A and D. In addition, being a food rich in polyunsaturated fatty acids and lower cholesterol levels, it reduces the risk of developing cardiovascular diseases [2].

The globalization of eating habits has generated rapid spread of the raw fish consumption, a traditional Japanese food. Thus, several restaurants specialized in Japanese cuisine have sprung up in western cities. However, the restaurants do not always meet the minimum requirements of location, time, and temperature of food exposition [3].

Japanese cuisine consists mainly of fresh, natural foods, especially fish and vegetables. The main dishes are the sushi, seasoned and rolled in a filling with cooked rice, and the sashimi, fish fillets *in natura* cut into thin slices [2].

Fish meat and fish products are highly perishable. These foods are subject to contamination by pathogenic and spoilage bacteria from capture to consumption. Intrinsic characteristics are very important, as the high water activity, pH close to neutral and an environment rich in nutrients that is optimal for bacterial growth. Extrinsic factors also facilitate the deterioration of fish, such as the type of capture and transport, the extensive manipulation that these foods are subjected to, and hygienic conditions and temperature for storage and handling. Therefore may pose risk to consumers' health [4].

In Brazil, the Ministry of Agriculture, Livestock, and Supply recommends physicochemical analysis to evaluate the quality and freshness of the fish. Determination of pH and Total Volatile Nitrogen Bases (TVNB) are very useful

[5]. Other methods commonly used to access the quality of fresh fish, include sensory and microbiological analysis [6].

The pH of fish and other marine products is neutral, which is a favorable condition for microorganisms to develop [7]. TVNB are volatile compounds present in fish meat and are indicative of the free nitrogen content in the food, which is a chemical index used for the quality control of fish [5].

Food handlers also affect the quality of the final product, especially raw or unprocessed products. More than 200 diseases can be transmitted to people through the ingestion of food contaminated with microorganisms and contamination can occur because of poor food-handling practices [8].

In view of the increased consumption of Japanese food and the lack of the information about the risks that it can represent, this study aimed to evaluate the microbiological and physicochemical aspects of sushi and sashimi sold in Japanese restaurants, as well as their preparation conditions.

## 2. Materials and Methods

### 2.1. Sampling

The study included all 18 Japanese restaurants (10 served *à la carte* and 8 were self-service buffets), located in the city of Goiania, capital of Goias State, Brazil, with an operating license from the Sanitary Surveillance of the city at the time of the survey.

From each restaurant, four types of food were selected such as Philadelphia sushi (with salmon and cream cheese); California sushi (with *kani*, cucumber, and mango); salmon sashimi and sea bass sashimi. Two hundred and fifty grams (250 g) of each sample were aseptically collected, when available, and transported in ice to the laboratory in sterile plastic bags. The temperatures of the samples were measured using digital thermometer ranging from -32°C to 380°C.

In addition, swab samples of nostrils and hands of food handlers directly involved in the preparation of sushi and sashimi were obtained after written informed consent from the participants. Then, the swabs were inoculated into tubes containing BHI broth (Brain Heart Infusion) and transported to the laboratory.

### 2.2. Physicochemical Analyses

The pH of sushi and sashimi samples was measured with a digital potentiometer (pH Meter TEc-2, Tecnal, Piracicaba, Brazil) in 5 g of crushed and homogenized sample with 50 mL of distilled water [9].

The values considered acceptable for fish, which were established by the Regulation for Industrial and Sanitary Inspection of Animal Products (RIISPOA) [10], are pH < 6.5 in the inner flesh. For evaluating the pH of sushi, pH < 4.6 was considered acceptable because of the acidification of rice used in the preparation [11].

The determination of TVNB content in sashimi was performed according to the methodology proposed by Saway da Silva et al. [12] and Brazilian legislation [5]. This method allows quantification of the free nitrogen content present in food (mg N/100 g of fish), and consists in the precipitation of protein nitrogen with trichloroacetic acid (Synth®). The liquid portion is separated after centrifugation, alkalized, and subsequently titrated with standard acid solution in the presence of mixed indicator.

The standards used were those established by the Brazilian Agricultural Protection Department of the Ministry of Agriculture, Livestock and Supply, which establishes the value of 30 mg N/100 g of sample as maximum TVNB for fresh fish [5]. To classify samples as having excellent freshness, the content of 5 to 10 mg N/100 g is required; as reasonable freshness state, TVNB between 15 to 25 mg N/100 g; as in the process of putrefaction, TVNB between 30 to 40 mg N/100 g; and as deteriorated, TVNB $\geq$ 50 mg N/100 g of sample, according Ogawa and Maia [13].

### 2.3. Microbiological Analyses

The sushi and sashimi microbiological analyses followed the procedures described by the Food and Drug Administration [14] and all analyses were performed in duplicate. For these samples, 25 g were diluted in 225 mL of 0.1% buffered peptone water and homogenized for 60s in a stomacher. Then, 10-fold dilutions of the homogenates were made in the same diluent [14].

Total and thermotolerant coliforms were enumerated on Violet Red Bile Agar (VRBA) incubated at 37°C for 24-48h and subsequent confirmative tests were performed in brilliant green bile 2% lactose broth (Oxoid) at 37°C for 24h. Thermotolerant coliforms were determined in EC broth (Oxoid) incubated at 45.5°C for 24 h and *E. coli* confirmation was performed by Gram staining and the IMViC tests [14].

Coagulase positive *Staphylococcus* were enumerated on Baird-Parker agar (Oxoid) supplemented with egg yolk tellurite emulsion, incubated at 37°C for 48 h. After growth, presumptive *S. aureus* colonies were counted and tested for catalase, coagulase and thermonuclease (TNase) production and anaerobic fermentation of glucose and mannitol [14].

For determination of *V. parahaemolyticus* the MPN technique was performed in alkaline peptone water and subsequent inoculation in TCBS (Thiosulfate Citrate Bile Sacarose) agar. After incubation at 35-37°C for 24h, typical colonies were purified and the confirmatory tests were performed [14].

All samples were analysed for the presence of *Salmonella* spp. after pre-enrichment in buffered peptone-water and enrichment in tetrathionate broth (Oxoid) and Rapapport-Vassiliads broth (Oxoid). Enrichment cultures were streaked on xylose lysine deoxycholate (XLD) agar, bismuth sulphite agar and Hektoen enteric agar (Oxoid) and the typical colonies were identified by biochemical tests [14].

For sushi samples the *Bacillus cereus* counts were enumerated on mannitol-egg yolk-polymyxin (MYP, Oxoid) agar incubated at 37°C for 48h and biochemical tests [14].

To evaluate the microbiological quality of sushi and sashimi, Brazilian legislation [15] establishes limits of $10^2$ CFU/g for thermotolerant coliforms; 5 x $10^3$ CFU/g for coagulase-positive *Staphylococci*, $10^3$ CFU/g for *Vibrio parahaemolyticus*, and absence in 25g for *Salmonella* sp.

The presence of *Escherichia coli* and *Staphylococcus aureus* was also determined in food handlers by inoculating samples of hand and nasal swabs previously incubated at 37°C for 24 h in BHI broth, on EMB agar surface and Salt Mannitol agar, respectively. Confirmation was performed by standard tests [16].

## 2.4. Ethics

This study was approved by the Ethics Committee of the Federal University of Goias (CEP/UFG) under the number 360/11.

## 3. Results

The 71 collected samples included 36 sushi (Philadelphia and California types) and 35 sashimi (salmon and sea bass).

The average temperature of the sushi and sashimi obtained from 18 restaurants was 14.1°C (Figure 1). The temperatures of sushi ranged from 11.6°C to 27.5°C. No sample was at the temperature recommended by the Brazilian legislation (5.0°C) [17].

From 35 samples of sashimi tested, 30 of them (85.7%) were at temperature above the permissible limit. The temperature of the sashimi samples from the 18 restaurants ranged from -1.2°C to 19.2°C, but no restaurant presented the average temperature of the samples below the limit established by law (5°C).

Of the 18 restaurants evaluated, in 13 (72.2%) (Figure 2) and 10 (55.6%) (Figure 3), the average pH of sushi and sashimi, respectively, was above the recommended by legislation. Of the 36 samples of sushi evaluated, the average pH was 5.04 and ranged from 4.35 to 6.94 (Figure 2).

Twenty-three samples (63.9%) showed pH > 4.6, which is above the limit set for classification of this food as suitable for consumption [11]. In 35 sashimi samples, pH values ranged from 5.06 to 8.73 with average of 6.59 (Figure 3), and 54.3% of these samples showed pH > 6.5; therefore, above the maximum limit established by the law [10].

According to the average values of TVNB (mg TVNB/100 g) observed, all the sashimi samples analysed were within the recommended legal limits, with free nitrogen level < 30 mg N/100 g [5]. There was no significant difference between the levels of free nitrogen found in the two types of evaluated sashimi, ranging from 5.09 to 10.49 mg N/100 g in sea bass sashimi and from 5.49 to 8.69 mg N/100 g in salmon sashimi. The samples also presented excellent freshness (between 5 to 10 mg N/100 g) [13].
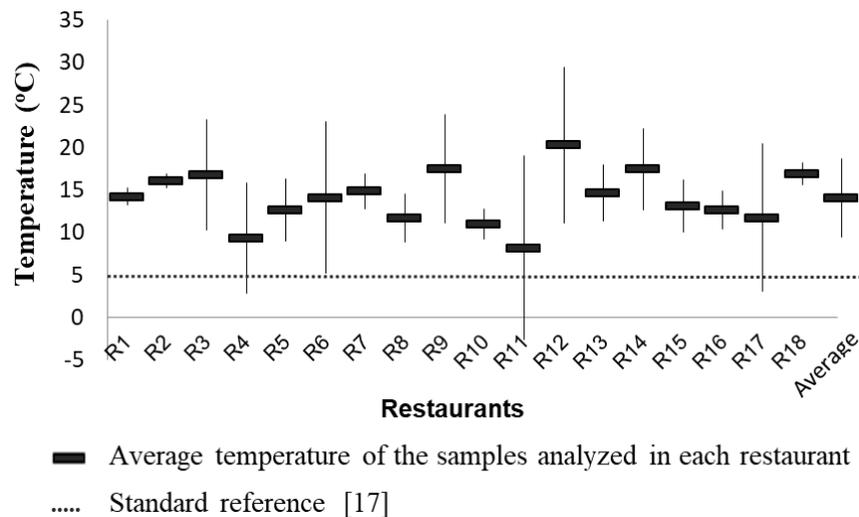


**Figure 1.** Average temperatures (°C) of sushi and sashimi obtained from Japanese restaurants
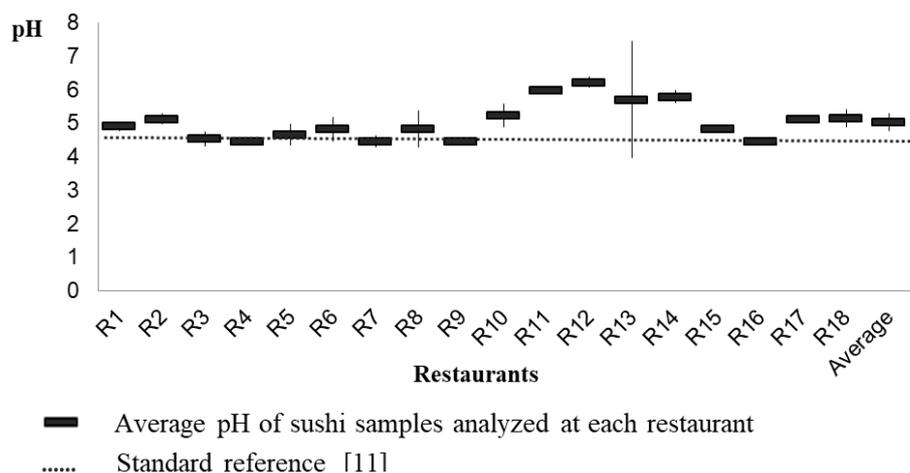


**Figure 2.** Dispersion of the averages and standard deviation of pH of the sushi collected in Japanese restaurants
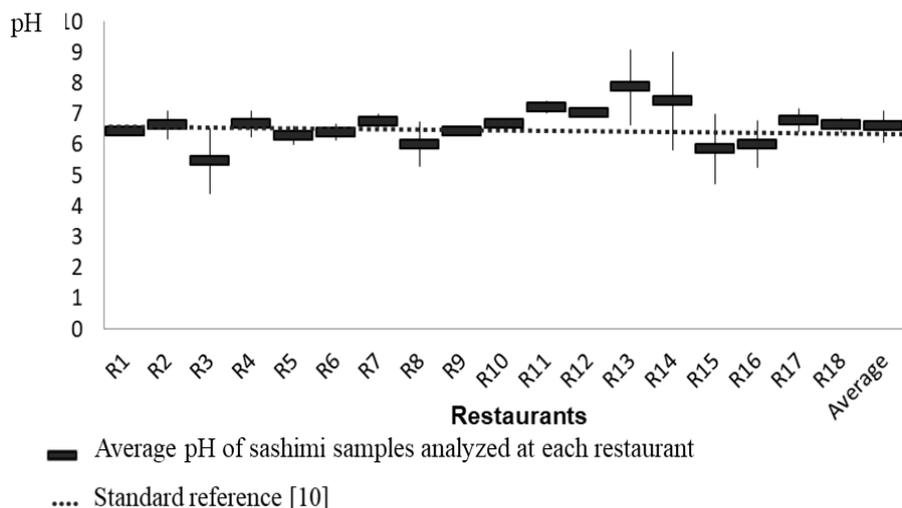
**Figure 3.** Dispersion of the averages and standard deviation of pH of sashimi collected in Japanese restaurants

**Table 1. Frequency of Indicator Microorganisms in Sushi and Sashimi of Japanese Restaurants**

| Type of sushi/sashimi | Samples collected | Total coliforms | | Thermotolerant coliforms | | *E. coli* | | *B. cereus* | |
|---|---|---|---|---|---|---|---|---|---|
| | | n* | % | n | % | n | % | n | % |
| Philadelphia sushi | 18 | 9 | 50.0 | 2 | 11.1 | 2 | 11.1 | 5 | 27.8 |
| California-type sushi | 18 | 5 | 27.8 | 0 | 0.0 | 0 | 0.0 | 3 | 16.7 |
| Salmon sashimi | 18 | 8 | 44.4 | 1 | 5.6 | 1 | 5.6 | - | - |
| Sea bass sashimi | 17 | 7 | 41.2 | 1 | 5.9 | 0 | 0.0 | - | - |
| Total | 71 | 29 | 40.8 | 4 | 5.6 | 3 | 4.2 | 8 | 22.2 |

*n = number of positive samples.

The frequency of indicators and pathogenic microorganisms isolated from sushi and sashimi are shown in Table 1. Of the 18 restaurants evaluated, 88.9 % (n=16) presented at least one sample with high counts of total coliforms. Of the 71 sushi and sashimi samples collected, 40.8% (n=29) were positive for this indicator microorganism, with counts ranging from $10^3$ to $8.8 \times 10^5$ CFU/g.

Of samples with high scores for coliforms, 5.6% (n=4) were positive for thermotolerant coliforms, which included two Philadelphia sushi, one salmon sashimi, and one sea bass sashimi. The scores ranged from $4.8 \times 10^2$ to $8.8 \times 10^5$ CFU/g. Of these, *E. coli* was isolated in three (4.2%).

Thirty-six sushi samples were analysed, of these, eight (22.2%) showed positive results and high score of *Bacillus cereus* ranging from $10^3$ to $5.0 \times 10^4$ CFU/g. Among the contaminated preparations, five (27.8%) were Philadelphia sushi and three (16.7%) were California-type sushi. Coagulase-positive *Staphylococcus*, *Vibrio parahaemolyticus* and *Salmonella* sp. were not identified in any sample analysed.

**Table 2. Frequency of indicator microorganisms in nasal and hand samples of food handlers from Japanese restaurants**

| Samples origin | Samples collected | Thermotolerant coliforms | | *E. coli* | |
|---|---|---|---|---|---|
| | | n* | % | n | % |
| Nasal swabs | 56 | 5 | 8.9 | 2 | 3.6 |
| Hand swabs | 56 | 5 | 8.9 | 4 | 7.1 |
| Total | 112 | 10 | 8.9 | 6 | 5.4 |

n* = number of positive samples.

Nasal and hands swabs were collected from 56 food handlers, totalizing 112 samples. In 10 (9.0%) samples obtained from eight (14.3%) food handlers, thermotolerant coliforms were detected, with 50% in nasal samples and 50% in hands samples. In six (5.4%) samples, *E. coli* was identified (Table 2). *Staphylococcus aureus* was not detected in any sample obtained from handlers.

## 4. Discussion

This study found an average temperature of the evaluated preparations (14.1°C) much higher than the temperature recommended by Brazilian legislation (5°C) [17]. Similar results were observed in other Brazilian cities such as Fortaleza/CE, where 100% of sushi and 90% of the sashimi evaluated were above the recommended temperatures [18]. In Porto Alegre/RS, the average of exposure temperatures reached 16.6°C [19], and in Rio de Janeiro/RJ an average temperature was observed of 27.15°C and 15.8°C in samples of sushi and sashimi, respectively [20]. High storage temperatures of sushi (18.0°C) were also observed in studies conducted in Taiwan [21] and in Australia (15°C and 9.6°C) [22].

Maintaining food highly manipulated such as sushi and sashimi at recommended temperature by Brazilian legislation proved to be a challenge in this study. The exposure of these foods at improper temperatures favors the microbial multiplication and increases the risk of foodborne diseases (FBD) outbreaks.

It was observed that 63.9% of the sushi samples were inadequate as regards the pH (4.6 maximum) with an average of 5.04. The average pH was also high on analysis carried out in Taiwan, which obtained pH from 4.51 to 6.11 [23], and in the state of Rio de Janeiro, which obtained pH of 5.75 in sushi and sashimi from the city Duque de Caxias and 5.92 in the city of Rio de Janeiro [20].

The average pH of the assessed sashimi was 6.59, and the pH of 54.3% of the samples were above the value recommended (6.5) [10]. In Portugal, 40% of fresh fish analysed showed pH above the allowed values [24]. Damasceno [25] detected values closer to the recommended in Belo Horizonte/MG, Brazil, where 18.0% of fish samples showed pH > 6.5.

The determination of pH is an important factor in assessing the quality and conservation status of fish and its derivatives. If the pH is near to neutrality (pH=7.0), the risk of microbial multiplication increases, whereas microorganisms multiply preferably at pH 6.5 to 7.5 [9].

As for the physicochemical deterioration measured by free nitrogen concentration (TVNB) of sashimi samples, these samples were found to have good freshness, with values lower than 30 mg N/100 g [5]. Most of the nitrogenous component of fish is part of proteins and reflects the characteristics of the skeletal muscle of the fish. Other studies evaluating this parameter also found satisfactory results including Damasceno [25] who analysed salmon, and Cartonilho and Jesus [26] who analysed tambaqui.

Coliforms bacteria in high amounts do not necessarily indicate that this is a pathogenic microorganism and that there is a risk of FBD. However, its determination in high amounts indicates improper hygienic and sanitary conditions as well as failures in food processing. Coliform contamination was identified in 40.8% of the samples, a result that corroborates with the study conducted in Taiwan. [21] In a study conducted in Recife/PE, 100.0% of the evaluated sashimi samples were contaminated by these bacteria [27].

The presence of thermotolerant coliforms is indicative of recent fecal contamination and the possible presence of pathogens. This group consists mostly of *E. coli* but some other enterics such as *Klebsiella* can also ferment lactose at these temperatures and therefore, be considered as fecal coliforms [14]. This study detected the presence of these bacteria in 5.6% of the samples, values much lower than those found in other studies in Brazil, such as 25.0% in Brasilia/DF [28], and 66.7% in Porto Alegre/RS [19], as well as in other countries like Taiwan (68.2%) [23].

*E. coli* was isolated in 4.2% (n=3) of samples, similar to other studies [23,27,29]. However, it was lower than that observed by Basti et al. [30], who isolated this microorganism in 30.8% of the evaluated food.

This study found no statistically significant relationship between the high temperatures of the samples and contamination by thermotolerant coliforms as observed in other studies [3,19].

*Bacillus cereus*, due to the resistance of its spores, is widely distributed in nature. For this reason, easily contaminated foods such as cereals can trigger episodes of food poisoning. High levels of these bacteria indicate survival during rice cooking and storage at incorrect temperatures that allow spores to germinate and multiply. Contamination was observed in 11.3% of the sushi, more than found in Seattle (4.7%) and similar to that of Taiwan, 18.2% (n=4) [23].

The Brazilian health legislation does not establish parameters for *B. cereus* in sushi, so the studies developed in Brazil did not investigate the presence of this microorganism. However, we observed in this study the importance in considering the determination of this microorganism in the routine analysis of this food. In all restaurants evaluated, the sushi was not stored at the recommended temperature and 63.9% of the samples did not have proper pH. This demonstrates the risk that consumers of this food are exposed daily in the various establishments that sell this kind of food.

The absence of coagulase positive *Staphylococcus*, *Vibrio parahaemolyticus,* and *Salmonella* sp. is a positive factor, because these microorganisms are pathogens and their intake can cause FBD in consumers. The absence of these microorganisms has also been observed by other authors in Brazil and in other countries like Australia [22,28,31].

The acidification of the rice by vinegar and the reduction of water activity by the addition of salt and sugar inhibit the multiplication of microorganisms. However, as shown by other authors, even at acidic pH, the sushi showed higher contamination than sashimi. This fact can be explained by excessive handling of rice, most of the time without using gloves and without proper hand hygiene, as well as the inadequate storage temperature of rice [18].

There are no data on the Brazilian legislation on maximum tolerance for thermotolerant coliforms and *E. coli* in clinical samples of handlers, but the presence in hands and nostrils reveals poor hygiene, fecal contamination, and transferability of this and other enteropathogens to highly manipulated foods [32].

It should be noted that during the visit and sample collection in the presence of inspectors of the Health Surveillance, the food handlers washed their hands and disinfected extensively with 70% alcohol before sample collection. Despite this, coliforms and *E. coli* were found, which indicates that the contamination could be much larger in routine working conditions.

The correct washing and hands hygiene are the most effective measures to eliminate pathogenic microorganisms. Microbiological analyses of handlers' gloves in Teixeira de Freitas, BA found thermotolerant coliforms on 73.3 % of gloves and *E. coli* on 6.7% of the samples [32].

This highlights the need to improve the Good Manufacturing Practices to ensure consumer safety. It was observed that there is an urgent need for compliance with the basic conditions of food preservation, which in the case of sushi and sashimi refers to the control of temperature and pH.

## 5. Conclusions

The most significant microbiological contamination found in the samples was by coliforms, indicating, once more, conditions of inadequate handling; and *B. cereus*, whose detection is not required by current legislation. This

result points to the need to recognize the potential hazard of high scores of this microorganism in sushi and to the re-evaluation of microbiological standards required for this food.

Considering the absence of heat treatments that are characteristic of the evaluated products, and the high storage temperatures found that favor the multiplication of the microorganisms, it is evident that the consumption of these foods in these conditions represents risks to the consumer. It is necessary to adopt educational measures that address personal hygiene and good handling practices, as well as greater accuracy in controlling the storage temperature of the products in commercial establishments.

## Acknowledgements

## Statement of Competing Interests

The authors have no competing interests.

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