Hygiene Practices at Preparation and Bacterial Growth on Sushi in Southern Brazil

Luciana Meneghetti Gehrke[1], Paula Marques Rivas[2], Diego Chemello Muller [1] Gabriela Borges de Moraes[2], Jessica Moreira Cannavon[2], Susana de Oliveira Elias[1], Lucas Fallavena[1], Eduardo Cesar Tondo[1]

1 Laboratório de Microbiologia e Controle de Alimentos, Instituto de Ciência e Tecnologia de Alimentos da Universidade Federal do Rio Grande do Sul – ICTA/UFRGS. Avenida Bento Gonçalves, 9500 – Campus do Vale – Prédio 43212, Laboratório 205 – CEP 91501-970 - Porto Alegre/RS/Brasil.

2. Equipe de Vigilância de Alimentos de Porto Alegre – CGVS – SMS- PMPA Avenida Padre Cacique, 372. CEP 90810-240, Porto Alegre/RS/Brazil.

*Corresponding Author: Luciana Meneghetti Gehrke. E-mail: lucianamenege@yahoo.com.br

Postal Address: Avenida Bento Gonçalves, 9500 – Campus do Vale – Prédio 43212, Laboratório 205 – CEP 91501-970 - Porto Alegre/RS/Brasil

Phone/fax: 55-51-33086677

Abstract

Good Hygiene Practices and sushi preparation were followed in 29 sushi restaurants in Southern Brazil. The growth of *Escherichia coli*, *Staphylococcus aureus,* and *Bacillus cereus* were evaluated at different pH in order to identify proper pH of sushi pieces. After that, the growth of the same microorganisms was modelled at 7ºC and 25ºC in sushi pieces presenting pH 4.2. Results demonstrated that the majority of restaurants had adequate facilities, equipment, water supply, and pest controls, however, an expressive percentage of them demonstrated inadequacies concerning important food safety issues as temperature control and supplier's assurance of the raw fish. The pH of rice used to prepare sushi varied from 4.0 to 4.3 and was considered adequate, because no one of the evaluated microorganisms were able to expressively growth at pH ranging from 4.0 - 4.5 in BHI medium. Artificially contaminated hossomaki sushi pieces did not support growth of *E. coli*, *S. aureus*, or *B. cereus* at 7ºC and 25ºC, and this result was attributed to the pH 4.2. Based on the results, the most important control measures identified in sushi preparation were: 1) supplier assurance of raw fish, 2) temperature control of raw fish, and 3) pH control of sushi pieces.

Keywords: Japanese food, foodborne pathogens, pH, temperature

Highlights:

- 29 sushi restaurants were investigated about Good Hygiene Practices.
- Growth of *E. coli*, *S. aureus,* and *B. cereus* were evaluated at different pH on sushi.
- Majority of restaurants had adequate equipment, water supply, and pest controls.
- Important control measures were supplier assurance/temperature control of raw fish.
- The safety pH of sushi is under 4.5.

1. Introduction

In the last few years, an expressive increase of the consumption of sushi has been observed worldwide and also in Brazil. The increase of sushi market also occurred in Porto Alegre city, Southern Brazil, where, in 2012, there were 64 restaurants serving sushi, in 2013 were 97, and, in 2015, 119 (Sindicato de Hospedagem e Alimentação de Porto Alegre e Região-SINDHA, 2015). Concomitantly to the increase of consumption, several foodborne diseases involving sushi were also reported in Brazil and other countries (Jain et al., 2008; Manual integrado de vigilância, prevenção e controle de doenças transmitidas por alimentos, do Ministério da Saúde, 2010).

Although it is an old habit among Japanese population, sushi consumption was not as popular as it is nowadays worldwide. The sushi consumption only reached global scale around 1970, as a consequence of Japan's emergence on the global economic scene and a boom in sushi restaurants in the USA (Bestor, 2000). In Brazil, sushi consumption started around 100 years ago, when Japanese families arrived to work in coffee farms. These families have stablished residence especially in Southeast and Southern regions of Brazil, introducing new cultural habits and among them how to prepare sushi and sashimi.

Sushi is the combination of Japanese rice, added with rice vinegar, raw or cooked fish, sometimes, edible dry algae (*Porphyra* sp.), fruits, vegetables, kanikama (crab-meat food), wasabi, and ginger (Prado et al., 2014). There are at least 14 sushi variations, based on differences of ingredients, preparation and/or display kinds. There are also at least six types of sushi that are linked with regions of Japan that cannot be paired with those 14 variations (De Silva &

Yamao, 2006). Independently of the kind of sushi, its preparation involves a lot of manipulation and frequently the use of raw ingredients, increasing the risk of microbial contamination and consequently the occurrence of foodborne diseases. Pathogens may be introduced by handler's hands, raw material as fishes, or by cross contamination from utensils and there is no thermal processing step to eliminate microbial contamination. However, using adequate Good Hygiene Practices (GHP) and proper control measures during processing, sushi can be safe.

Among the main reasons for the involvement of sushi with foodborne diseases in Brazil are the usual large-scale production and the long-time exposure time of sushi pieces at environmental temperature, before consumption. In Japan, the volumes of sushi prepared in many traditional restaurants are much lower than those produced in Brazilian restaurants, and sushi pieces are immediately consumed after preparation, reducing the probability of microbial multiplication.

In order to assure the safety of this kind of preparation, control measures should focus on the quality of raw ingredients, prevention of cross contamination and prevention of microbial multiplication. The former two aspects depending on the correct adoption of GHP, while the latter depends on the control of pH and temperatures. The objective of the present study was to evaluate the processing and sanitary conditions and bacterial growth on sushi prepared in Southern Brazil.

2. Material and Methods

2.1.Technical visits and official inspections

From August to December 2013, 29 Japanese restaurants specialized in preparation of sushi, were visited in Porto Alegre, the capital city of the State of Rio Grande do Sul, Southernmost State of Brazil. The visits aimed to evaluate the GHP and the flowchart of preparation of sushi. Among the visits, 12 were carried out by officers of Surveillance Service of Porto Alegre city and the other 16 visits were performed by a food safety expert. All the visits were carried out without previous information and each sushi establishment was audited one time.

Each technical visit or official inspection was done in two to four hours, using a risk-based instrument developed to evaluate the hygienic and sanitary conditions and grading of food services during 2014 FIFA World Cup, with some modifications (Brazil, 2013; Da Cunha et al., 2014). After audits and if necessary, official sanitary actions were adopted by Sanitary Surveillance officers, as notifications, food apprehensions, food discards, interruption of activities, among others.

The major aspects observed in audits were: 1) water supply; 2) structure and facilities; 3) sanitation procedures; 4) pest control activities; 5) food handlers behavior; 6) food preparation (ingredients used in sushi preparation; temperature of fish at reception; temperature of ready-to-eat sushi at distribution; pH of rice used for sushi preparation; duration of preparation); 7) storage, transportation and distribution; 8) technical responsibility, documentation, and records. The pH of sauce (sushizu) and sauced rice used for sushi preparation were analyzed using pH-measure strips (Merck). The temperatures of ready-to-eat raw fishes and sushi were measured using an infrared food thermometer (Akso) and a digital food thermometer (Incoterm).

2.2. Bacterial growth in different pH and on sushi pieces

2.2.1. Bacterial Strains

Aiming to study the bacterial growth on sushi, hossomaki sushi pieces were artificially contaminated with the following bacteria, separately. A strain of *B. cereus* (BCSST01) isolated from a rice sample involved in a foodborne outbreak occurred in the State of Rio Grande do Sul (RS), in 2014. *S. aureus* (SASFS02) isolated from a food sample involved in a foodborne outbreak occurred also in the State of RS, in 2003. *E. coli* ATCC 25972 from the Culture Collection of the Food Microbiology and Food Control Laboratory of the Institute of Food Science and Technology (ICTA/UFRGS). Outbreak strains were kindly provided by the Official Laboratory of Public Health of Rio Grande do Sul (FEPPS/IPB/LACEN/RS). Before the evaluation of growth on sushi pieces, all bacteria were tested in Brain Heart Infusion Broth (BHI Merck, Darmstadt, Germany) with different pH (4.0, 4.5, 5.0, 5.5, and 6.0), at 37ºC for 24 hours. Optical density of bacterial suspensions was measured at 630 nm using a UV/visible spectrophotometer (Ultraspec 3100 Pro, Amersham Biosciences, São Paulo).

2.2.2.Transport and preparation of samples

Hossomaki samples were acquired from a sushi restaurant of Porto Alegre city, where the production procedures were standardized, well controlled, and high levels of GHP procedures were verified. Control registers demonstrated adequate pH control and low variation in pH values among sushi pieces produced. All of the collected samples were transported by car inside thermal boxes, under refrigeration (7ºC), in less than 1 h, to the Food

Microbiology and Food Control Laboratory of the Institute of Food Science and Technology - ICTA/UFRGS for further analyses.

2.2.3. Microbial inoculation of hossomaki sushi

All the bacterial strains were cultivated in 5 mL of BHI, at 37℃, for 24h. This procedure was carried out twice, aiming to physiological activation of strains. After that, the cultures were centrifuged for 2 min at 5000 rpm, the supernatants were discharged and pellets were washed with 0.1% peptone water (Merck). This procedure was repeated 3 times. Cells were re-suspended with 0.1% peptone water and final cell concentrations of approximately 10^7 CFU/mL of *B. cereus* and 10^8 CFU/mL of *E. coli* ATCC 25972 and *S. aureus* were obtained. Decimal serial dilutions were prepared using 0.1% peptone water, and 1mL of appropriate dilution was inoculated on each hossomaki sample, reaching the final concentration of approximately 10^3 (3.0-3.8 log) CFU/g.

2.2.4. Microbiological counts

Artificially contaminated hossomaki sushi pieces were stored at 7ºC and 25℃, during different time periods. These temperatures were chosen, simulating two different scenarios: 1) inadequate refrigeration; 2) environmental temperature at distribution inside sushi restaurants. Sampling was carried out in varied time intervals, depending on the temperature of storage and the expected bacterial growth curve. At each time point, 10 g of sample were aseptically weighed, 90 mL of peptone water was added, and homogenized inside a sterile plastic bag using a stomacher, for 60s. After that, serial decimal dilutions were done in 0.1% peptone water, and 0.1 ml samples of the appropriate dilutions were spread on selective and non-selective agar plates.

Mannitol Egg Yolk Polymyxin (MYP) Agar (Merck), ChromoCult® Coliform Agar (Merck) and Baird-Parker Agar (Merck) were used to grow *B. cereus* (30ºC for 24h), *E. coli* ATCC 25972 (37ºC for 24h) and *S. aureus* (37ºC for 48h), respectively. Total colony-counts (TCC) were enumerated using pour-plate method on plate count agar media (PCA; Merck), incubated at 30ºC, for 72h. All the bacterial counts were carried out in triplicate. The experiments were repeated at least twice and the results were expressed as CFU/g.

2.2.5. pH measurement

When the hossomaki samples arrived at Laboratory, a sample of 10 g was weighed inside a Beaker homogenized with 100 ml of distilled water, according to the methods preconized by Adolfo Lutz Institute (2005). The pH was measured directly in the solution with the QUIMIS pHmeter Q400AS, in duplicate. Furthermore, the pH of sushi components (rice, raw fish and seaweed) were measured, separately, using Macherey-Nagel Universal pH paper (in triplicate).

2.2.6. Modeling the growth data

Survival curves of *B. cereus*, *E. coli,* and *S. aureus* on sushi were obtained by fitting the experimental data with the log-linear model (Bigelow & Esty, 1920) with shoulder and tail from GInaFiT Excel add-in software (Geeraerd et al., 2005).

3. Results

3.1. Technical visits and official inspections

Technical audits and official inspections demonstrated different conditions among the 29 restaurants, depending on the item evaluated (Table 1). For example, all restaurants presented facilities with exclusive use of running potable water, appropriate connections to the sewer system and septic tank. However, 8 out of 29 did not demonstrate that water reservoir was cleaned and sanitized at intervals not exceeding six months, keeping records of this operation, as is mandatory by sanitary regulation of Southern Brazil.

The majority of restaurants demonstrated adequate facilities, including kitchens and consumptions saloons, and equipment for sushi preparation, and 21 out 29 presented appropriate toilets, equipped with all necessary items for personal hygiene, i.e. toilet paper, odorless antiseptic liquid soap or odorless liquid soap and antiseptic, paper collectors with lids operated without manual contact and non-recycled paper towels or other hygienic and safe system for drying hands. The unconformities observed in the 8 establishments ranged from the lack of individual items like toilet paper, liquid soap, antiseptic, etc. and lack of hygienic conditions of toilets. Even though the majority of establishments had appropriate facilities, in only 13 of them the facilities, equipment, furniture, and utensils demonstrated proper hygienic–sanitary conditions and only 16 demonstrated appropriate frequency of sanitization.

In relation to pest control, 25 establishments showed facilities, equipment, furniture and utensils free from the presence of animals and insects, probably because the majority (25) demonstrated that the pest control was performed by specialized companies. However, actions to prevent attraction, shelter, access, and dissemination of vectors and urban pests were deficient in 17 restaurants. These results means that establishments demonstrated one or more of the following problems: a) lack of closed drains; b) lack of rubber protection below doors; c) lack of anti-insect wets on windows, among others.

Audits demonstrated that food handlers did not work injured or presenting symptoms of diseases, and do not smoke, sing, whistle, sneeze, spit, cough, eat, handle money or speak unnecessarily, during food preparation in the majority of restaurants (22). However, in 19 restaurants people that handled raw foods did not wash and disinfect their hands frequently and at appropriate times, during food preparation.

At reception, approximately 30% of restaurants subjected foods to inspections and approvals or control the hygienic–sanitary conditions and temperatures. In those ones where temperature was controlled, the mean temperatures of raw fishes at reception was – 1.89 ± 4.46°C, varying from -12.3 to 5.7°C.

In 23 of 29 restaurants, the foods subjected to thawing were kept under refrigeration below 5°C, however in 14, perishable products were exposed to room temperature for long periods, what was considered inappropriate. This occurred due to long time of preparation, overloaded exposers, or exposers with inadequate temperatures or open doors.

Only 7 restaurants controlled the effectiveness of the heat treatment of foods (rice), and 17 of them presented a calibrated thermometer to measure the temperature of foods. 18 establishments kept cooked food refrigerated at temperatures below 5°C, conserved prepared food before consumption below 5°C and monitored the temperature of the equipment. The mean temperature of sushi and raw salmon at refrigerated distribution was 6.23 ± 3.86, varying from -1.2 to 12°C. At 2 restaurants, sushi pieces presented temperatures of 19°C. In 26 establishments there was a properly trained professional for controlling food safety activities, and 16 of them followed a Good Manufacturing Practices Manual and Standard Operating Procedures.

3.2. Bacterial Growth in different pH and on Sushi pieces

Optical density of bacterial suspensions did not chance in any of the tested pH during 6 hours at 37ºC. All the bacteria cultivated in BHI with pH 4.0 and 4.5 did not demonstrate significant increase of optical density, however the same microorganisms demonstrated expressive growth at pH 5.0 and 6.0 at 37ºC during 24 hours (data not shown).

Hossomaki samples used as controls did not show any *B. cereus* or *E. coli* contamination, before the experiments. However, the presence of *S. aureus* in low concentration (<10^2 CFU/g) was detected in some samples, probably due to handling during preparation. Hossomaki sushi pieces demonstrated mesophilic counts ranging from 4.0 to 5.0 log CFU/g in control samples (data not shown).

Artificially contaminated sushi pieces exposed to 7ºC demonstrated initial and final counts of *E. coli* and *B. cereus* of 3.09 ± 0.06 and 2.25 log ± 0.16 CFU/g, going to 3.69 ± 0.06 and 2.67 ± 0.16 log CFU/g, respectively, after 30 h (Figures 1 and 2). *S. aureus* demonstrated a constant profile in counts (3.88 ± 0.10 to 3,88 ± 0.16 log CFU/g) during 28 h. Mesophilic counts were carried out in parallel to each pathogen measurement and the counts remained practically the same (from 5.70 to 5.74 log CFU/g; from 5.03 to 5.41 log CFU/g; and from 5.87 to 5.74 log CFU/g), after 30h. At 25ºC, the initial and final counts of *E. coli* were 3.09 ± 0.08 and 3.07 ± 0.09 log CFU/g, respectively, after 30 h. Mesophilic counts increased from 4.60 to 5.93 log CFU/g in the same period, and the lag phase was 12 hours. The initial and final counts of *S. aureus* were

3.77 ± 0.10 and 3.15 ± 0.16 log CFU/g, respectively, after 30 h. Mesophilic counts of these samples increased from 5.11 to 6.32 log CFU/g, during this period, presenting lag phase of at least 12 hours. The initial and final counts of *B. cereus* reduced from 3.65 ± 0.06 to 1.65 ± 0.13 log CFU/g, respectively, during 34 h (Figure 3). Interestingly, it was observed that hossomaki sushi pieces exposed to 25°C reduced 2 log CFU/g of *B. cereus*, during 34 h. Mesophilic counts of these samples increased from de 4.73 to 7.23 log CFU/g in the same period, and lag phase was around 12 hours. The pH of hossomaki sushi samples remained constant (4.2) throughout the experiments.

4. Discussion

Food services are establishments where food meals are prepared and frequently consumed, including fast foods, hotels, school kitchens, restaurants, including those that prepare sushi. During the last years, food services have emerged as the main locals where consumers get their food (Yiannas, 2009) and, in order to fulfill this immense demand, there are several establishments working with adequate conditions, at the same time that others carry out their activities without ideal conditions or GHP procedures.

As expected, diverse scenarios were found in Japanese restaurants in Southern Brazil, where different GHP and process control levels were observed. For example, all restaurants were operating using adequate water supply, presenting potable water, sewer system, and septic tank, however, 27% of them were not able to prove cleaning procedures of water reservoir each 6 months, that is mandatory in Southern Brazil (RIO GRANDE DO SUL, 2009). Further, most of restaurants had adequate facilities, equipment and utensils necessary for sushi preparation, and the majority (72%) demonstrated appropriate toilets equipped with all necessary products for personal hygiene. This adequate basic sanitary and structure conditions of Japanese restaurants can be explained because this sector had expanded expressively and owner generally have resources to invest in facilities and infra-structure. Beyond this, in Porto Alegre city, practically all food services have access to public potable water, and the control of water and structure of restaurants have been one of the main focus of Sanitary Surveillance officers. This is especially true in those restaurants that receive several clients, as sushi establishments, because they may represent higher risk and affect higher numbers of people.

Audits and inspections also revealed that restaurants had adequate pest controls, because facilities, equipment and utensils were free of animals and insects, even though, procedures to avoid attraction, shelter, access and dissemination of insects had to be improved in more than a half of restaurants. The adequate pest control carries out by specialized companies is obligatory in Brazil and Southern Brazil by different regulations (BRAZIL, 2004; RIO GRANDE DO SUL, 2009), especially when chemical products have to be applied. Further, due to the expressive growing of sushi's restaurant in Southern Brazil, pest control and absence of any kind of insects or animals are important concerns of consumers, motivating owners to implement adequate pest controls and the use of chemical products. Other factor that probably motivate and make possible adequate structure and pest controls is the average price of Japanese meal in Porto Alegre city. While an average meal in a buffet food service costs around U$ 4 - 10, sushi meals may cost U$ 7 to 25, per person. With higher profits, restaurants can invest more in quality issues.

While general infra-structure and basic sanitary conditions of restaurants demonstrated to be adequate in the majority of restaurants, diverse inadequacies concerning procedures and process control were observed. For example, practically half of establishments demonstrated that facilities, furniture and utensils were not in proper hygienic-sanitary conditions and the frequency of sanitation was not adequate. In 19 restaurants, employees did not wash their hands at all adequate moments, i.e. at arriving, before handling food, after touching contaminated materials, after using toilets, and when needed. This is particularly preoccupant because preparation of sushi necessarily will involve a lot of manipulation and contaminated handler hands may result in sushi contamination. Supporting this possibility, some hossomaki pieces had *S. aureus* before artificial contamination. Appropriate food manipulation is very important in any kind of food service and failures may result in foodborne illnesses. Harada et al. (2013) described a foodborne outbreak during a 2-day traditional festival in Osaka, Japan. Among 126 customers who patronized a particular Japanese restaurant during the event, 102 developed symptoms of gastrointestinal disease. Strains of ETEC serotype O169:H41 were isolated from one food sample and from fecal samples collected from 19 of 34 patients and 2 of 4 food handlers. The cause of the outbreak was attributed to improper handling of raw materials or pre-prepared meals. Jain et al. (2008) described another outbreak due to sushi contamination by food handlers, in Nevada, USA. In August and November 2004, 2 clusters of diarrhea cases occurred among patrons of 2 affiliated sushi restaurants (A and B). In August 2004, a stool sample from one ill sushi restaurant A patron yielded enterotoxigenic *Escherichia coli* (ETEC). In December 2004, a third cluster of diarrhea cases was identified among sushi restaurant B patrons. One-hundred thirty patrons of sushi restaurant B reported symptoms. Illness was associated with consumption of butterfly shrimp, but only sushi restaurant B patrons reported diarrhea. Poor food-handling practices and infected food handlers likely contributed to this outbreak.

Food Surveillance reports about foodborne diseases of Porto Alegre city, occurred from 2004 to 2014, demonstrated that there were thirteen outbreaks involving the consumption of sushi. In total, 35 people became sick, presenting one or several of the following symptoms: nausea, vomits, diarrhea, abdominal pain, sickness, headache, and fever. In the majority of outbreaks, sushi pieces were consumed inside restaurants where they were prepared, however in 5 outbreaks the consumption occurred at private residences or at workplaces of victims, as well. Among the sick people, eleven were men and twenty-four were women with ages ranging from 10 to 50 years old. Etiological agents were not identified in the majority of outbreaks, except in two of them that the identified microorganisms were *E. coli* and *B. cereus*. The causal factors were not always identified, but when it was possible, they were identified as cross contamination due to failures in manipulation of food handlers, inadequate hygienic conditions of equipment and utensils, maintenance of sushi pieces for long time at environmental temperature or inadequate refrigeration temperature.

One important issue of concern was that most of the irregularities in sushi restaurants of Southern Brazil were observed in controls of raw materials and control process, during food preparation. According to Tondo and Bartz (2014), even though adequate installations are very important to produce safe food, the use of potable water and the implementation of process controls, as the control of temperature, time of exposure, pH, etc., are priorities in order to prevent foodborne diseases. The adequate process controls are essential because they may eliminate, reduce or control the bacterial contamination occurred due to non-unconformities of infra-structure, facilities, manipulation, and even contamination of raw materials. Our results demonstrated that among the 29 establishments, approximately 30% did not perform inspections and approvals of raw fish at reception and the temperatures were not checked. Practically the same percentage of restaurants presented inadequacies in relation to the integrity of primary packaging, hygienic-sanitary conditions of raw materials and ingredients received, and lack of information on labels of raw materials at reception. This is other important issue to be improved because raw fish is a frequent ingredient of sushi and microbial contamination depends on controls implemented by suppliers. Actually, suppliers are responsible for the microbial assurance of raw fish received by sushi restaurants, and these establishments must to guarantee the adequate storage at appropriate temperature, from reception to consumption. According to Yiannas (2009) in order to dramatically reduce risk, future strategies must focus on eliminating the presence of pathogenic organisms on raw and processed products before they enter food services, rather them eliminating them at the restaurant. This is particularly important for sushi establishments, because there is no step in the flowchart of production able to eliminate microbial contamination present on raw fish of sushi. According to Codex Alimentarius (CAC/RCP 52/2003), hazards of fish and fishery products are *Salmonella, Shigella, E. coli, Vibrio cholerae, Vibrio parahaemolyticus, Vibrio vulnificus,* and Parasites of public health significance as trematodes, nematodes, cestodes. In some products, *S. aureus* and *Clostridium botulinum* can be included, as well. In order to control all of them, preventive measures must be applied at suppliers. According to (Food Standards Agency, 2012) that provides guidance for the legal requirements of England, Wales, and Northern Ireland about freezing for fishery products intended to be eaten raw or lightly cooked, fishery products that are subject to a freezing treatment must be frozen at -20℃ for not less than 24 hours, or -35℃ for not less than 15 hours, and the freezing must reach all parts of the product. The same document also reported that fishery products that were commercially frozen at -18ºC for at least 4 days for storage, transport and distribution are exempt from the freezing requirements referred above. Other exemption is in the case of farmed fish when cultured from embryos and fed on a diet that cannot contain viable parasites and were cultivated in environments that are free of viable parasites. Similar parameters were proposed by ICMSF (2015) who reported that raw fish should be cultivated under GHP/GMP and must be stored at least at – 18 ºC or – 20 ºC in order to eliminate parasites before use in sushi preparation.

Several failures on temperature controls were verified by our study. For example, practically a half of restaurants exposed perishable foods to environmental temperature for long periods; thawing was not always carried out adequately (ex. under refrigeration); the cooking temperature was not always controlled or the effectiveness of heat treatment was evaluated; there was no calibrated thermometer in several restaurants, suggesting that the control of temperature was not a priority; prepared food was stored at inadequate temperatures; and the reduction of temperature of prepared food was not always performed adequately. Analyzing the flowchart of preparation of sushi in

Southern Brazil it was possible to observe that the preparation of sushi generally begins on mornings, when rice is cooked and cooled. At several restaurants, rice is prepared one day before, highlighting the necessity of appropriate storage. After cook, rice is then mixed with rice vinegar, what have to be done property in order to guarantee complete pH reduction of the whole batch of rice. After that, sushi pieces are prepared by hand of sushiman, what may take several hours, depending on the quantity of sushi produced. In Brazil, due to the current high consumption, big amounts of sushi are prepared in restaurants, and after sushi pieces are ready, they can remain several hours waiting for distribution and consumption. Based on this, problems with the control of temperature during the preparation can be critical, and it may result on multiplication of microorganisms especially if the pH of rice is not controlled. Our results demonstrated that no one of the evaluated microorganisms were able to growth at pH 4.0 and a slightly increase of OD_{630nm} was observed at pH 4.5 after 24 hours at 37°C. Corroborating these findings Jay (2005) Cotter and Hill (2003) Hocking (2003), and Lambert and Stratford (1999) have reported that foods with pH around 4.0 - 4.5 may prevent microbial multiplication, because most of food pathogens do not multiply at this pH range. Highlighting the necessity of pH control in rice for sushi, the food regulation of New South Wales (NSW) Food Authority advocates that cooked rice added with vinegar have to present a pH of 4.6 or less (NSW, 2007).

In the present study, Further, hossomaki sushi pieces presenting pH 4.2 were artificially contaminated with *E. coli, S. aureus,* and *B. cereus.* Contaminated sushi pieces were exposed to 7°C and 25°C, simulating an abuse refrigeration temperature (7°C, being <5°C the preconized temperature) and assuming that 25ºC is a possible and frequent environmental temperature inside sushi restaurants. The microorganisms were chosen because *E. coli* and *S. aureus* are well known Gram-negative and Gram-positive food contaminants, respectively, and *B. cereus* is a common pathogen involved with foodborne outbreaks due to consumption of rice meals (Oh et al, 2015). According to the microbial results, no growth was detected at 7ºC, during 30 hours, and the counts of *E. coli* and *B. cereus* even decreased. At 25ºC, *E. coli, S. aureus*, and *B. cereus* did not demonstrate significant growth after 30h, and this can be attributed to the pH because these microorganisms are mesophylls that easily multiply at this temperature. Corroborating these findings, counts of mesophilic microorganisms on sushi presented lag phase of at least 12 hours at 25ºC. The pH control of rice mixed with vinegar seems to be an effective measure to control microbial multiplication in sushi. Rusul and Yaaccob (1995) have tested 194 *B. cereus* isolates for growth temperature, pH profile and enterotoxin production. 91.8% of the strains were enterotoxigenic and 50% of the enterotoxigenic strains were capable of growing at 5ºC. However, enterotoxigenic strains did not grow at pH 4.0, but 42.0% of the strains were able to grow at pH 4.5, after 7 days, at 37℃. This period is much longer than periods of sushi preparation and consumption in Southern Brazil and worldwide. Based on this information, if sushi pieces present pH around 4.0 – 4.5 it is possible to conclude that most food pathogens will not growth. Further, we contaminated whole hossomaki pieces including those that had raw fish, and pathogens did not growth as well, suggesting that the pH also prevent microbial growth on fish. In order to verify this result, raw fish inside hossomakis were took off and pH was measured, demonstrating values of 4.0 – 4.2. The pH barrier can also be important to control microbial multiplication in sushi that are exposed to consumption. Currently, the preconized temperature for refrigerated foods exposed to consumption in RS is < 5.0ºC (RIO GRANDE DO SUL, 2009). This temperature is not always ease to be reached in sushi pieces because transference of temperature is difficult in this kind of food and equipment is not always as good as those able to decrease the temperature. Our results demonstrated that microorganisms did not growth on hossomaki pieces, presenting pH 4.2, at 7ºC, and this result suggests that even higher temperatures than <5.0ºC could be used at exposure of sushi if pH is controlled.

Based on the results, it is possible to conclude that several of the Japanese restaurants presented adequate general hygienic and sanitary conditions, concerning infra-instructure, water supply and pest control. However, during the evaluation of flowchart processing, the majority of inadequacies were verified in process controls, as inadequate temperatures. Analyzing the characteristics of sushi, its probable microbial hazards (ex. *B. cereus, S. aureus, Diphyllobothrium* spp., etc.) and flowchart of preparation, we indicated the necessity of safety assurance from suppliers of raw fish and temperature control, during the entire process of raw fish. Further, it is also necessary the pH control of rice < 4.5 in order to prevent bacterial multiplication. These measures may contribute significantly to the safety of sushi.

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Table 1. Risk-based instrument and respective results after evaluation of hygienic and sanitary conditions of 29 sushi restaurants of Southern Brazil.

Item Evaluated	CONFORM	NOT CONFORM	NOT APPLICABLE
1. Water supply			
1.1 Exclusive use of drinking water for food handling (public water supply or alternative solution with semiannual tests confirming that the water is safe to drink according to laboratory reports).	26	3	0
1.2 Facilities with running water	29	0	0
1.3 Facilities have connections to the sewer system or septic tank.	29	0	0
1.4 Reservoir in proper hygienic condition.	22	7	0
1.5 Reservoir properly covered and maintained (absence of cracks, leakage, infiltrations and peeling, among other defects).	22	7	0
1.6 Water reservoir sanitized at intervals not exceeding six months, keeping records of the operation.	21	8	0

1.7 Material that internally coats the water reservoir does not compromise the water quality.	22	7	0
2. Structure			
2.1 Toilets have hand sinks and products for personal hygiene (toilet paper, odorless antiseptic liquid soap or odorless liquid soap and antiseptic, collectors with lids operated without manual contact and non-recycled paper towels or other hygienic and safe system for drying the hands).	20	8	1
2.2 Presence of physical separation or another effective means of separation of different activities to prevent cross-contamination.	18	11	0
3. Sanitization of the facilities, equipment, furniture and utensils			
3.1 Facilities, equipment, furniture and utensils kept in proper hygienic–sanitary condition.	13	16	0
3.2 Appropriate frequency of sanitization of equipment, furniture and utensils.	16	13	0
3.3 Utensils used for the sanitization of the facilities different from those used for the sanitization of the equipment and utensils in	27	2	0

contact with food.			
3.4 Dilution, contact time and method of use or application of sanitizing products follows the manufacturer's instructions.	22	7	0
3.5 Sanitizing products regularized by the Ministry of Health.	22	7	0
4. Integrated control of vectors and urban pests			
4.1 Control of vectors and urban pests performed by a specialized and properly legalized company.	24	5	0
4.2 Existence of a set of effective and continuous actions to prevent attraction, shelter, access and dissemination of vectors and urban pests	12	17	0
4.3 Constructions, facilities, equipment, furniture and utensils free from the presence of animals, including vectors and urban pests.	25	4	0
5. Food handlers			
5.1 Handlers removed from the food preparation when they have injuries or symptoms of diseases.	25	4	0
5.2 Employees wash hands thoroughly when arriving at work, before and after	10	19	0

handling food, after any interruption of work, after touching contaminated materials, after using the toilet and whenever it is needed.			
5.3 Food handlers do not smoke and speak when unnecessary and do not sing, whistle, sneeze, spit, cough, eat, handle money or engage in other activities that may contaminate food.	22	7	0
6. Raw materials, ingredients and packaging			
6.1 Subjected to inspection and approval at reception.	18	11	0
6.2 Raw materials, ingredients and packaging used for preparation are maintained under proper hygienic–sanitary conditions.	20	9	0
6.3 Intact primary packaging of raw materials and ingredients.	20	9	0
6.4 Use of raw materials and ingredients within the expiration date, taking into consideration the order of receipt.	16	13	0
6.5 Raw materials properly portioned, packaged and labeled with at least the following information: name of product, date of portioning and expiration date after	14	15	0

opening or removal of the original packaging.			
6.6 Temperature of raw material and ingredients verified at reception and storage.	19	10	0
6.7 Ice used in food made from drinking water and kept under hygienic–sanitary condition.	22	2	5
7. Food preparation			
7.1 Sinks in the food preparation area equipped with products for hand hygiene (odorless antiseptic liquid soap or odorless liquid soap and antiseptic product, non-recycled paper towels or other hygienic and safe system for drying the hands).	23	6	0
7.2 During the food preparation process, people that handle raw foods wash and disinfect their hands before handling prepared food.	19	7	3
7.3 Perishables products exposed to room temperature for only the minimum time required for food preparation.	15	14	0
7.4 Thawing performed as directed by the manufacturer and using one of the following techniques: refrigeration at a temperature	23	6	0

below 5 ℃ or in microwave oven when the food is to be cooked immediately.			
7.5 Foods subjected to thawing are kept under refrigeration if they are not immediately used and not refrozen.	25	4	0
7.6 Heating of foods to ensure that all parts of the food reach a temperature of at least 70 ℃, or other combination of time and temperature that ensures the hygienic–sanitary quality of food.	14	12	3
7.7 The effectiveness of the heat treatment is evaluated.	7	13	9
7.8 Availability of a properly calibrated thermometer with which to measure the temperature of food.	17	12	0
7.9 After cooling, prepared food kept refrigerated at temperatures below 5 ℃ or frozen at temperature equal to or below –18 ℃.	21	7	1
7.10 Food to be consumed raw, when applicable, subjected to a sanitization process with an appropriate certified product applied in such a way as to avoid residues.	22	6	1
7.11 Avoidance of direct or indirect contact	25	3	1

between raw foods, semi-ready and ready-to eat food.			
7.12 Temperature of the prepared food reduced from 60 ℃ to 10 ℃ within 2 hours during the cooling process.	14	8	7
8. Storage, transportation and display of the prepared food			
8.1 Prepared food stored under refrigeration or freezing is labeled with at least the following information: name, date of preparation and expiration date.	16	13	0
8.2 The maximum period for the consumption of prepared and refrigerated food is 5 days if the storage temperature is equal to or below 4 ℃. When temperatures above 4 ℃ and below 5 ℃ are used, the maximum period for consumption is reduced.	18	10	1
8.3 During food display, handlers follow procedures that minimize the risk of contamination of prepared food by hand antisepsis and by the use of utensils or disposable gloves, if applicable.	19	10	0
8.4 Prepared food kept refrigerated at a temperature equal to or below 5 ℃.	18	9	2

8.5 Prepared food kept at temperatures above 60 ℃	12	6	11
8.6 Temperature of the display equipment regularly monitored.	21	8	0
8.7 Prepared foods maintained in the storage area or awaiting transportation are labeled (name of product, date of preparation and expiration date) and protected from contaminants.	13	5	11
8.8 Storage and transportation occur under time and temperature conditions that do not compromise the hygienic–sanitary quality of the prepared food.	3	2	24
8.9 Food preserved at hot temperatures maintained at temperatures above 60 ℃; time from preparation to display of the food not to exceed 6 hours.	11	9	9
9. Responsibility, documentation and records			
9.1 Presence of a properly trained individual responsible for food handling activities (technical manager, owner or designated employee).	26	3	0
9.2 The company follows the Manual of Good Practices and the Standard Operating	16	13	0

Procedures.			

Figure 1: Survival of *E. coli* on hossomaki sushi stored at 7 °C (■) and 25 °C (◆), fitting data to the GlnaFiT Excel add-in software. Each symbol represents a mean of duplicate results.

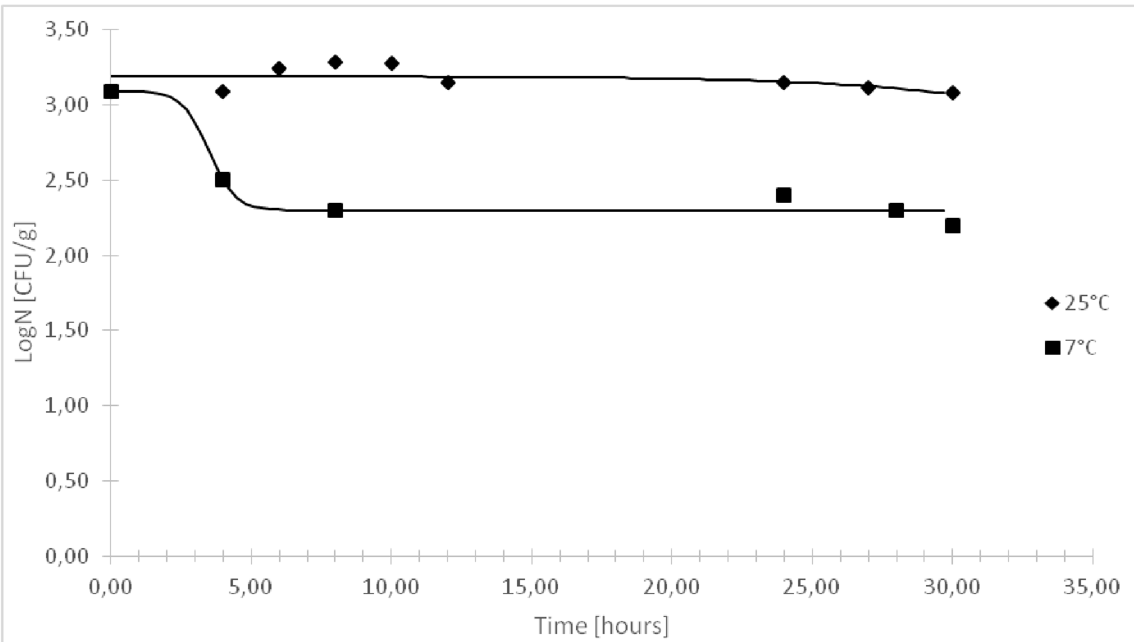

Figure 2: Survival of *S. aureus* on hossomaki sushi stored at 7 °C (■) and 25 °C (♦), fitting data to the GInaFiT Excel add-in software. Each symbol represents a mean of duplicate results.

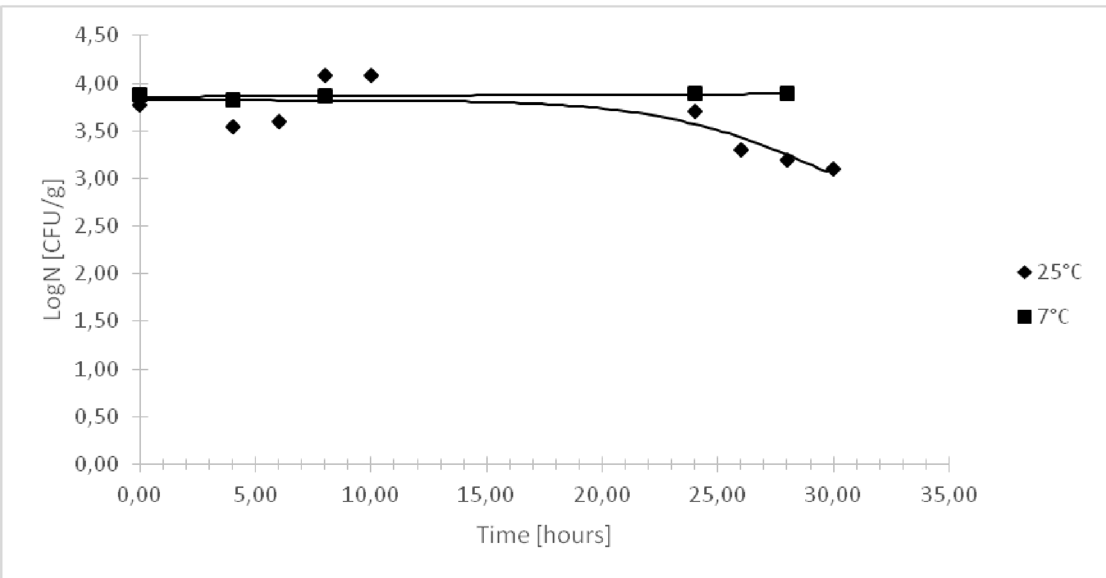

Figure 3: Survival of *B. cereus* on hossomaki sushi stored at 7 °C (■) and 25 °C (♦), fitting data to the GInaFiT Excel add-in software. Each symbol represents a mean of duplicate results.