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What Happens to the Microflora of Retail Sushi in the Warm Season?

E. Tirloni*, C. Bernardi, G. Gandolfi, P. Cattaneo, S. Stella

University of Milan, Department of Health, Animal Science and Food Safety, Via Celoria 10, Milan, Italy
*Corresponding author: erica.tirloni@unimi.it

Abstract Sushi is a perishable ready to eat product composed by several raw ingredients, and the storage temperature is crucial in the maintenance of satisfactory hygiene. Aim of this study was the microbiological characterization of sushi mimicking a thermal abuse likely occurring in the summer season. Mixed sushi (rolls and nigiri) produced in a small scale factory in Northern Italy was stored for 2 h at 12°C ("transport") and subsequently for 4 days at 8°C ("home storage") and daily submitted to microbiological analyses coupled with the control of organoleptic quality. Total viable Count was above 5 Log since the production day and was mainly constituted by *Pseudomonas* spp.; the values increased during storage overcoming the 6 Log level from day 3, and reaching level above 8 Log CFU/g at the last sampling time. From a sensorial point of view, from the second day a decay in odour and colour was observed. LAB showed a gradual increase never overcoming 6 Log CFU/g, while *Enterobacteriaceae* increased and overcame 4 Log CFU/g after 2 days. Yeasts showed a moderate growth (always <5 Log CFU/g) while *Bacillus cereus*, Staphylococci and Clostridia were generally below the detection limits. *Listeria monocytogenes* was never detected. A reduction of shelf-life from 3 to 2 days should be applied especially in particular warm months in order to limit bacterial replication.

Keywords: *sushi, thermal abuse, spoilage microorganisms, ready-to-eat fish products*

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1. Introduction

In recent years a significant structural change in the European population, including the Italian one, occurred: the combination of different cultures and traditions led to the development of new lifestyles and consequently to a change in food consumption patterns. Not only restaurants and canteens, but also the Large Scale Retailers encouraged the development of proposals aimed to satisfy ethnic demands and needs. All these efforts resulted in an increase of the consumption of "not Mediterranean foods" such as oriental specialties, kebab, cous cous, Latin food and sushi, especially among the younger population. Sushi is a traditional Japanese ready-to-eat (RTE) seafood product that mainly consists of cold cooked vinegared rice parallelepipeds topped with raw or cooked fish and rice rolls filled with fish, vegetables, egg and often wrapped in seaweed. Thanks to the healthy perception felt by consumers and to the diffusion in different sale channels (on the Italian market sushi could be found already packed on display counters or directly assembled in-store kiosks in front of the consumers), this product has become in the last years a trendy meal in Europe. For example, almost 30% of adult people from Norway and France eat sushi at least a couple of time per month [12].

The worldwide diffusion of sushi consumption is also due to the accessibility of the prices. However, the low costs could be an alarm bell especially in terms of raw materials quality, training of staff and application of hygienic manufacturing practices. Several studies have already showed that sushi can be the source of severe foodborne diseases due to pathogenic microorganisms such as *Salmonella* spp., *Bacillus cereus*, *Staphylococcus aureus*, *Vibrio parahaemolyticus* and *Escherichia coli* [3,4,5,8,19,27,29]. Moreover, *Listeria monocytogenes* should be carefully considered as potential biological risk in retail fresh sushi due to the high prevalence in the raw materials and in the production plants: as a matter of fact the presence of this microorganism was already found in sushi in several previous studies with prevalence from 1.2% to 6.4% [1,30].

An appropriate preparation and the storage at low temperatures are the main control points in order to assure a satisfactory hygienic quality of this product. In particular, temperature is one of the most relevant factors that significantly influence microbial growth rates: the control of this parameter through all the sushi production chain is important in order to evaluate and play down the risk of spoilage or pathogenic microbial growth. In particular, if sushi is not produced, handled and stored at proper refrigeration temperature, it could be more susceptible to the replication of the natural spoilage microflora and to secondary contaminations. The European legislation doesn't lay down any specific temperature requirement, although refrigeration during display is mandatory, while some national guidelines have been issued. For example, the Food Hygiene Regulations guidelines adopted in UK

refer that chilled RTE food "*may not be stored at a temperature above 8℃ if it is likely to support the growth of pathogenic organisms or the formation of toxins*" [9]. Moreover, according to Manitoba State (Canada) guidelines, during display, sushi must be protected from direct sunlight and kept under refrigeration (at 5℃ or less), and, according to New South Wales (Australia) specific sushi preparation guidelines, "*Once prepared, sushi should be placed under refrigeration (at 5℃ or less) unless it is being displayed for sale immediately*" [22,26].

As well as for the retail, particular attention and control should be used in home settings: Azevedo et al., [2] demonstrated that the 71% of domestic refrigerators in Portugal operated at temperatures higher than 6℃ and Marklinder et al. [23] showed that the maximum temperatures recorded in Swedish refrigerators ranged from 11.3 to 18.2℃.

In the present study, fresh sushi produced by an Italian factory was microbiologically evaluated mimicking a possible thermal abuse occurred in the warm season: spoilage and potential pathogenic microorganisms were considered during settled times. A sensorial evaluation of the product was also performed in order to highlight a change that could be perceived by the consumers.

2. Materials and methods

2.1. Production of Sushi

Sushi was produced in a small scale factory in Northern Italy. A mixed product typology was chosen for the present study, in order to evaluate the most representative sale unit. It was composed as follows: scallop nigiri, salmon nigiri, egg nigiri, maki roll with cooked salmon and courgette, maki roll with peppers and cream cheese, hosomaki with salmon and hosomaki with courgette. (Figure 1). Fresh or, in some cases, frozen ingredients were prepared and assembled with cooked rice; as highlighted by the flowchart (Figure 2) the raw materials, both for the production of rolls and for nigiri, were stored in dedicated refrigerated cells before the preparation and assembling of each ingredient used. Frozen smoked salmon was thawed before the preparation and assembly with the other ingredients. A "use by" date of 3 days was assigned by the producer. After production, sushi samples were immediately transported in refrigeration conditions and stored as reported in section 2.2.

2.2. Thermal Profile

Sushi samples were stored mimicking the hypothetical purchase and consumption in the summer season. To match this aim, samples were submitted to an intense thermal abuse for two hours (12℃) simulating bringing them home unrefrigerated from the retail store, that is often made by car without particular attention. Afterwards, the samples were stored at a slight thermal abuse (8℃) for 4 days, simulating the home refrigeration, taking into account the difficulty for old fridges to maintain low temperatures, especially in the warm months.

2.3. Experimental Design

All the samples were stored 2 hours at 12℃, then maintained at 8℃ and sampled after a total of 24, 48, 72 96 hours from the production. Microbiological samples were analysed in triplicate for the parameters reported in section 2.4 and for sensorial properties; a total number of 36 samples were processed.

Figure 1. Typology of sushi analysed and ingredients

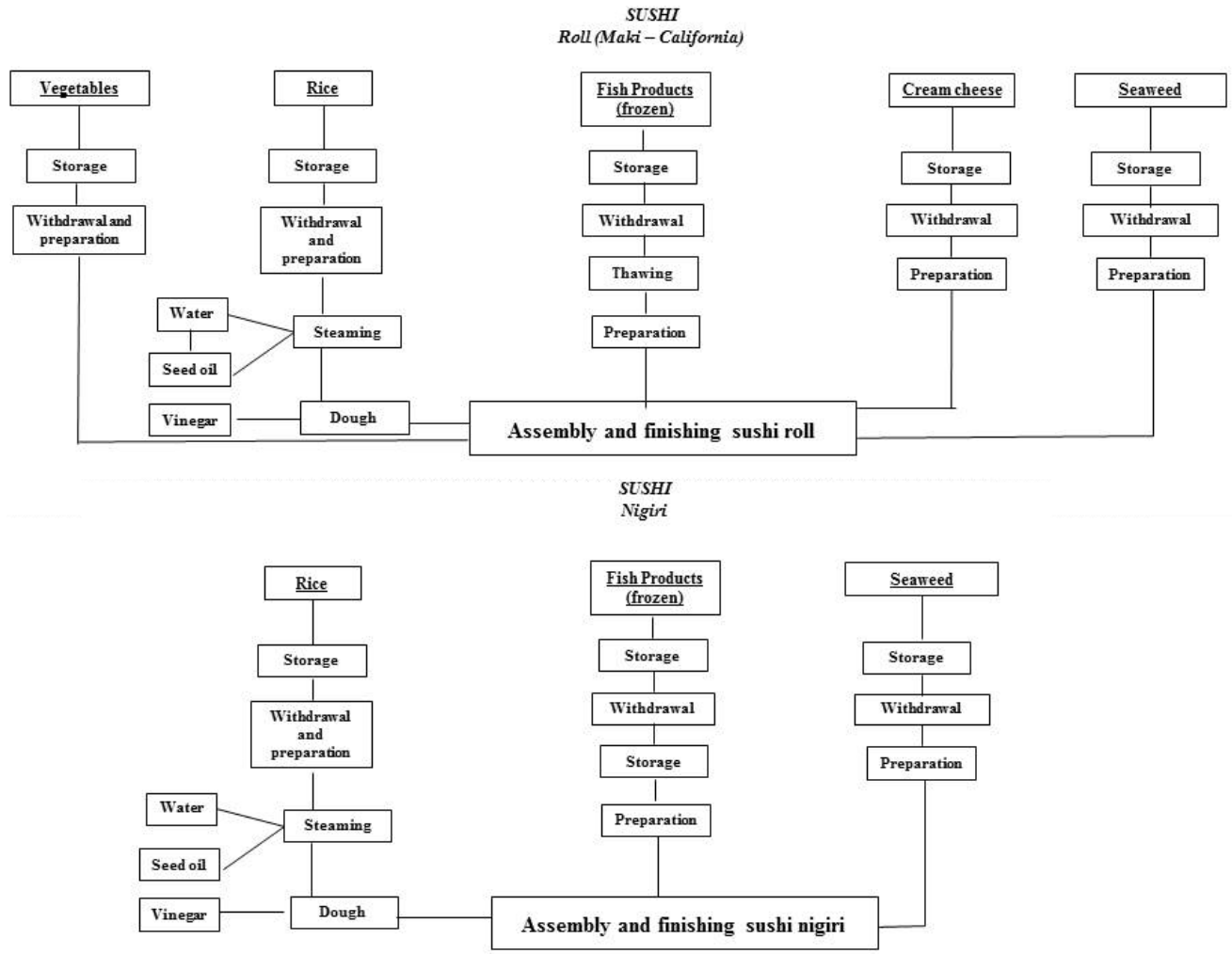

Figure 2. Flow sheet of roll and nigiri sushi production

2.4. Analyses

In order to evaluate the organoleptic quality of the product after storage at thermal abuse, at each sampling time colour, odour and aspect were evaluated by a panel of 8 people.

For the microbiological determinations, 20-25 g of product were 10-fold diluted in chilled sterile diluent solution (0.85% NaCl and 0.1% peptone) and homogenized for 60s in a Stomacher 400 (Seward Medical, London, UK). Then, appropriate 10-fold dilutions of the homogenates were made in chilled saline solution. Total mesophilic and psychrotrophic viable counts were determined onto Plate Count Agar (PCA, Biogenetics, Ponte San Nicolò, I) and incubated at 30°C for 48 h [15] and at 10°C for 7 days, respectively. *Pseudomonas* spp. were enumerated on CFC agar (Biogenetics) incubated at 30°C for 48h, lactic acid bacteria were enumerated on De Man Rogosa Sharpe (MRS) agar (Biogenetics), incubated in anaerobiosis at 30°C for 48h [13], *Enterobacteriaceae* were enumerated on Violet Red Bile Glucose Agar (VRBGA, Biogenetics) incubated at 37°C for 24h [17], yeasts and moulds were enumerated on Sabouraud Agar (SAB, Biogenetics) incubated at 30°C for 96h [18], coagulase positive staphylococci were enumerated on Baird Parker Agar (Biogenetics) incubated at 37°C for 48h [14], presumptive *Bacillus cereus* were enumerated onto PEMBA, Biogenetics) incubated at 37°C for 48h. Spores of *B. cereus* and reducing sulphite *Clostridia* were enumerated after pasteurization of the samples at 80°C for 10 min respectively onto PEMBA incubated at 37°C for 48h and on Tryptose Sulfite Cycloserine (TSC, Biogenetics) [16] incubated at 37°C for 48h. Detection and enumeration of *Listeria monocytogenes* were performed according to AFNOR methods (AFNOR BRD 07 / 4–09 / 98 and AFNOR BRD 07/05–09/01, respectively).

2.5. Statistical Analyses

Data from microbiological analyses were analyzed by a one-way univariate analysis of variance using SAS procedure with MIXED procedure of SAS software (SAS Inst. Inc., Cary, NC, 2006) to test variable differences for each parameter by time. For all statistical evaluations, threshold levels of $P \leq 0.05$ and $P \leq 0.01$ were considered for significance.

3. Results and Discussion

Sushi is a very perishable ready-to-eat product as it is composed mainly by fresh materials and no further heat treatments are foreseen before consumption. Thus, each potential contamination can affect the final product; for the rice, that is the only component that is cooked before the assembly, just a post-contamination could occur. The

trial simulated a likely purchase – non-refrigerated transport - home storage scenario in the most critical season, resulting in an evident thermal abuse. The storage at inappropriate temperature, as already stated, is one of the main cause of alteration of the product and in some occasions could also promote the growth of potential pathogenic microorganisms. It was already stated that the rates of unsatisfactory sushi samples analysed raised up during summer season if compared to the winter one, especially for faecal coliforms/*E. coli* and staphylococci [27].

The values of mesophilic and psychrotrophic total viable counts are presented in Figure 3. The starting Total Viable Count was above 5 Log CFU/g both for mesophilic and psychrotrophic bacteria and the microbial population was mainly composed by *Pseudomonas* spp.: their presence in sushi could be explained by the presence of vegetables (peppers, courgettes). In fact, *Pseudomonadaceae* represent the main microbial population of these substrates, sometimes resulting in an evident spoilage (soft rot), thanks to the their pectinolytic and proteolytic activities [20] that can be exerted also at temperatures below 2°C. The growth of *Pseudomonas* spp. in sushi was already observed in previous studies [7,25]. Total Viable Count, and at the same time *Pseudomonas* spp. count, increased gradually during the storage in thermal abuse, reaching loads close to 8 Log CFU/g at the last sampling time. At the last two sampling times a statistically significant increase of TVC and *Pseudomonas* spp. from T0 was revealed. Mesophilic and Psychrotrophic microbial counts were equivalent for the entire period considered. The results were evaluated according to the microbiological guidelines for ready-to-eat food issued by the Center for Food Safety in Hong Kong in 2014 [8] and to the guidelines for assessing the microbiological safety of ready-to-eat foods placed on the market issued by Health Protection Agency in United Kingdom in 2014. When gram-negative bacteria predominate in the aerobic colony counts of RTE foods, the UK Guidelines indicate, the possibility of manifest spoilage from 7 Log CFU/g (TVC), resulting in smears, discolouration and slime formation, mainly due to *Pseudomonas* spp. replication. Moreover, in accordance with the same guidelines, for ready-to-eat fish and cold-smoked fish foods that can be assimilable to sushi, total viable count values could be considered satisfactory if below 6 Log CFU/g and borderline if between 6 and 7 Log CFU/g. In this study, TVC and *Pseudomonas* spp. overcame the threshold value of 6 Log CFU/g from the third day, that is the last day of "use by" date assigned by the producer; nevertheless, considering the general aspect at the opening of the package, a shorter "sensorial shelf life" can be estimated, odour and colour were stable throughout the first day, while from the second day a decay of colour and odour, especially in the vegetable component was clearly evidenced.

In this study, the loads reached resulted to be high: the thermal abuse that was applied in order to verify the activity of the microflora present in sushi as it was purchased in the summer season, was influenced by the hypothetical consumer's behaviour. The data obtained are in agreement with the results of Miguéis et al. [24], who found a variation of the deteriorative microflora of sashimi sampled in different seasons. As expected, a fast deterioration occurs when fish food is subjected to temperatures above the optimal refrigeration.

Lactic Acid Bacteria can be considered a part of the natural microflora of some ingredients used for the sushi preparation. LAB counts showed a gradual increase (about 1 Log) during the trial. However, the values observed were not very high, never overcoming 6 Log CFU/g, that is considered a precautionary threshold for the initial spoilage of fresh seafood. This limit is often used in food industries to indicate the end of shelf-life of a fish product, although different limits (above 7-8 Log CFU/g) are indicated for sensory rejection [28].

Considering *Enterobacteriaceae*, a gradual increase of loads was observed in the product, overcoming after 2 days the 4 Log CFU/g level, recognized as "threshold" for specific spoilage [11] (Table 1). The initial contamination could be due to their presence in the vegetables as well as a contamination by food handlers. A careful attention should be paid to assure a low initial contamination, as several species, including psychrotrophic ones, could be responsible of spoilage or potential risk for the consumers.

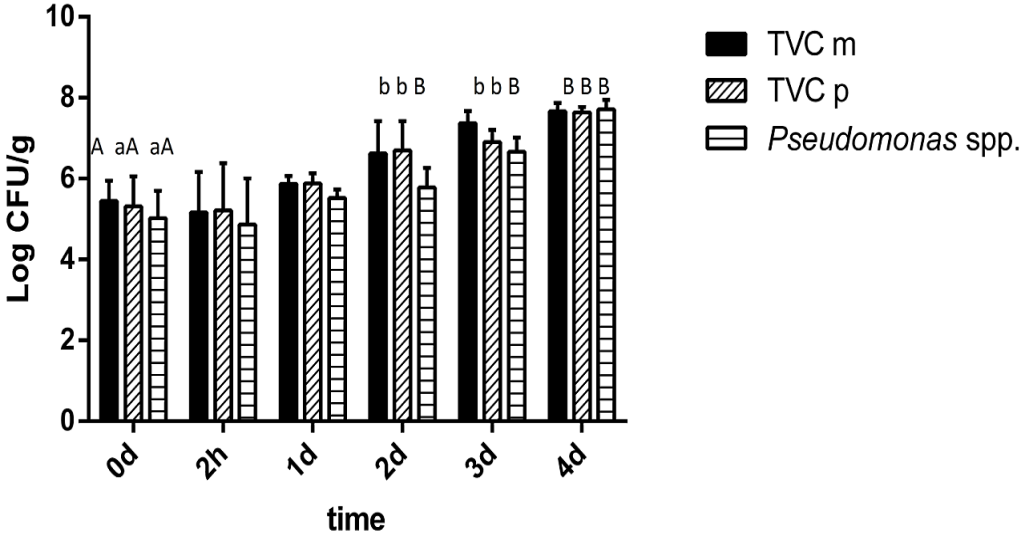

Figure 3. Mesophilic and psychrotrophic Total Viable Count (TVC) and number of *Pseudomonas* spp. along the sampling time considered. a,b=P≤0.05; A, B=P≤0.001

Table 1. Microbial loads (expressed as Log CFU/g) detected during the sampling times (d=day, h=hours of storage)

Parameters	(Log CFU/g)					
	0d	2h	1d	2d	3d	4d
Lactic Acid Bacteria	4.81±0.13^{aA}	4.10±0.14	5.08±0.11	5.22±0.16	5.75±0.08^{b}	5.80±0.17^{B}
Enterobacteriaceae	3.18±0.39^{aA}	3.23±1.13	3.25±0.56	4.41±1.57	4.51±0.40^{b}	4.87±0.17^{B}
Yeasts	3.15±0.10^{aA}	3.06±0.32	3.04±0.37	3.49±0.14	4.35±0.24^{b}	4.66±0.25^{B}
Moulds	2.74±0.24	2.00**	2.70**	<2.00	2.70**	<2.00
B. cereus	<2.00	<2.00	<2.00	<2.00	2.00*	<2.00
Spores of *B. cereus*	<2.00	<2.00	<2.00	<2.00	<2.00	<2.00
Coagulase Positive Staphylococci	<2.00	<2.00	<2.00	<2.00	<2.00	<2.00*
Spores of Sulphite reducing Clostridia	<1.00	<1.00	<1.00	<1.00	<1.00	<1.00
Listeria monocytogenes	Absent in 25 g	Absent in 25 g	Absent in 25 g	Absent in 25 g	Absent in 25 g	Absent in 25 g

*: only one of the three samples was above the detection limit (2 Log CFU/g).
**: two of the three samples were above the detection limit (2 Log CFU/g).
a, b=P≤0.05; A, B=P≤0.001.

Yeasts were searched as they can be considered important spoilage organisms for sushi, that is characterized by high moisture content, and due to their widespread diffusion in different substrates. The conditions applied during the storage caused only a moderate increase in the counts (without reaching 5 Log CFU/g). The presence of moulds was sporadic and only low counts were detected, presumably due to a carryover from the raw matters; no growth was observed until the end of the trial.

B. cereus was always below the detection limit at all the sampling times, except for one sample where a value of 2 Log CFU/g was detected: this could be considered a strong evidence of high quality raw materials, good hygiene practices in rice cooking and chilling, and a very good hygiene level especially during the final assembly of all the prepared ingredients. The thermal abuse temperature chosen (12°C) should not be generally sufficient to allow an efficient growth of *B. cereus*, also if the possibility of the presence of psychrotrophic strains must be considered [10].

Coagulase positive Staphylococci never overcame the detection limit of 2 Log CFU/g, highlighting a satisfactory situation and adequate handling and temperature control and the absence of toxin-linked risk. In other situation different results were obtained: Liang et al. [21] found 30% of samples from take-away sushi from Hong Kong with borderline values (counts between 1.3 and 4 Log CFU/g); also Muscolino et al. [25] classified 28.95% and 15.79% of the sushi samples analysed as borderline or unsatisfactory, according to the microbiological guidelines from UK and Hong Kong.

Based on the data, sulphite-reducing Clostridia didn't represent critical contaminants for this typology of product, as they were not detected in any of the samples; the substrate characteristics and the storage conditions seem not to be prone to clostridia growth.

Considering the potential presence of *Listeria monocytogenes* in this ready to eat food, sushi is considered as a substrate that "does not support the growth of *L. monocytogenes*" by the EU Regulation 2073/2005 as the shelf-life assigned is less than 5 days. Thus, the microorganism must be below 100 cfu/g for the entire shelf-life assigned by the producer. In this study, the presence of the pathogen was never detected, neither with the quantitative method (Limit of Detection of 10 CFU/g), nor with the qualitative one (LOD of 1 CFU in 25 g), indicating the careful attention by the operators to avoid the contamination from environmental sources. However, it has to be noted that a sporadic contamination of the product can occur, due to the wide diffusion of *L. monocytogenes* in some of the ingredients used, as vegetables or smoked salmon.

The data obtained by this study, mimicking a predictable purchase during the warm season, suggest that a use-by-date of 2 days could be adequate to assure a sufficient microbiological and sensorial quality of the product. From the third day, in fact, the general aspect of sushi samples suffered of a decline, that was associated with high total viable counts, mainly composed by *Pseudomonas* spp. Thanks to the natural initial contamination of some ingredients by *Pseudomonas* spp. and to the unavoidable development of these psychrotrophic microorganisms in the usual market conditions, a reduction of "use-by" date (from 3 to 2 days) can be suggested, especially in particular months/seasons of the year.

4. Conclusions

Sushi is a product sensible to microbiological deterioration, due to the variety of the ingredients, and to the application of several critical (manual) operations during the preparation and assembly; the typologies analysed in this trial (rolls and nigiri) were chosen exactly for the high diversity of raw matters used. In addition, the possibility of poor temperature control during production and exposition on the refrigerated counters of the supermarket has to be carefully considered, especially during the warm season.

In this sense, it is really important that each actor of the chain feels empowered and responsible: the food operators should be aware of the critical role of the good manufacturing practises applied during sushi preparation; the producer should be responsible not only of the quality of the raw materials but also of the management of process hygiene (equipment, etc.) and flow (time/temperatures during or post-production) and of training and supervision of the food handlers. Keeping in mind the results obtained from this study, the producer should be aware also that in specific seasons, it could be forward-looking and preventive to reduce the use by date assigned, thus considering a thermal abuse scenario, to warrant the hygienic quality of the product. Also the supermarkets should assure the maintenance of the proper refrigeration temperature during the exposition. Finally, the last actor is the consumer, who should be conscious of the particular perishability of sushi, thus assuming the need for

consuming the product only if stored appropriately at refrigeration temperatures and before the expiry date.

Statement of Competing Interests

The authors have no competing interests.

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