

## Hygienic-sanitary evaluation of sushi and sashimi sold in Messina and Catania, Italy

Daniele Muscolino, Filippo Giarratana, Chiara Beninati, Agata Tornabene, Antonio Panebianco, Graziella Ziino

Dipartimento di Scienze Veterinarie, Università degli Studi di Messina, Italy

### Abstract

Sushi and sashimi are traditional Japanese food, mostly consisting of raw seafood alone or in combination with rice. Eating sushi and sashimi has become popular in many countries even outside Japan. This food is not free from health risks such as ingestion of pathogenic bacteria or parasite. The aim of this study was to investigate on hygienic-sanitary quality of sushi and sashimi sold in the cities of Messina and Catania, Southern Italy. Fifty samples (38 sushi and 12 sashimi) were analysed to determinate the aerobic mesophilic bacteria (AMB), psychrophilic bacteria (PB), *Enterobacteriaceae*, specific spoilage organisms (SSOs), *Pseudomonas* spp., coagulase-positive staphylococci, micrococci, *Vibrio* spp., *Bacillus cereus*, *Salmonella* spp. and *Listeria monocytogenes*. In sushi, AMB ranged from 5.00 to 8.18 log CFU/g, PB from 4.70 to 7.13 log CFU/g, *Enterobacteriaceae* from 1.41 to 6.67 log CFU/g, while SSOs and *Pseudomonas* spp. from 3.49 to 7.72 and from 3.36 to 8.00 log CFU/g, respectively. Micrococci ranged from 3.53 to 5.03 log CFU/g and coagulase positive staphylococci were found in 16 samples (2.00 to 3.60 log CFU/g). *Bacillus cereus* was found in 3 samples (1.70 to 4.00 log CFU/g), while *Vibrio* spp. was found in 15 of the sushi samples (1.70 to 3.70 log CFU/g). In sashimi, the AMB, PB and SSOs values were higher than 7.00 log CFU/g, *Pseudomonas* spp. and *Enterobacteriaceae* were from 6.00 to 8.00 log CFU/g, while *Vibrio* spp. were found in six samples with means of 2.00 log CFU/g. No *Salmonella* spp. and *Listeria monocytogenes* were detected in all sushi and sashimi samples.

### Introduction

Sushi and sashimi are traditional Japanese food, though in recent years they attracted a large number of European consumers. Sushi was originally prepared in Southeast Asia as a method to preserve fish. It was formerly used for carps, that were caught, gutted, salted and preserved between two stones, and left to fer-

ment for a period ranged from one to three years. Sushi is prepared with cold cooked rice acidified with vinegar, and it is shaped into bite-sized pieces and topped with raw or cooked fish, or formed into a roll with fish, eggs or vegetables, and wrapped in seaweed (nori) (Atanassova *et al.*, 2008).

The sashimi, instead, is characterised by the use of fish and shellfish, which are cut into thin slices and served with several sauces (*e.g.* wasabi, soy sauce or ponzu sauce), and accompanied with daikon roots (Food and Environmental Hygiene Department HKSAR, 2000).

Sushi and sashimi are ready-to-eat (RTE) food and regarded as a potentially hazardous. Frequently, indeed, foodborne diseases related to sushi e sashimi consumption have been reported (Adams *et al.*, 1994; Atanassova *et al.*, 2008; Barralet *et al.*, 2004; Food and Environmental Hygiene Department HKSAR, 2000; Masotti *et al.*, 2004; Millard and Rockliff, 2003; NSW Food Authority, 2006). Pathogenic bacteria such as *Salmonella* spp., *Staphylococcus aureus*, *Vibrio parahaemolyticus*, *Vibrio cholerae*, *Listeria monocytogenes*, *Bacillus cereus* can occur in materials used for their preparation such as fish, seafood products, vegetables and rice.

In the light of the significant increase of consumption of traditional Japanese foods in Italy and due to the attention of EC Reg. 2073/05 for RTE foods (European Commission, 2005), the aim of this study was to investigate the microbiological quality of sushi and sashimi sold in Messina and Catania (Southern Sicily, Italy).

### Materials and Methods

The study was carried out on 38 samples of sushi and 12 of sashimi collected from restaurants, sushi bar and take-away outlets of Messina and Catania. Different types of sushi were analysed: hosomaki, nigirizushi and uramaki. In addition to rice and nori seaweed, they were made with smoked fish (salmon, tuna and swordfish), canned tuna in oil, surimi, fish eggs, cream cheese, cucumbers, carrots, avocado, sesame or poppy seeds. The sashimi samples were prepared with salmon, tuna or swordfish fillets.

All samples, transported to the laboratory under refrigerated condition, were analysed for the following bacteriological determinations: aerobic mesophilic bacteria (AMB) (ISO 4833:2004; UNI, 2004), psychrophilic bacteria (PB) (ISO 17410:2001; ISO, 2001), specific spoilage organisms (SSOs) (Lyngby Iron Agar at 25°C for 5 days), *Enterobacteriaceae* (ISO 21528-2:2004; ISO, 2004), *Pseudomonas* spp. (*Pseudomonas* agar base with CFC supple-

Correspondence: Graziella Ziino, Dipartimento di Scienze Veterinarie, Università degli Studi di Messina, viale Annunziata, 98168 Messina, Italy. Tel. +39.90.3503761 - Fax: +39.90.3503937. E-mail: gziino@unime.it

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ment at 30°C for 48 h), quantitative and qualitative determination of *Vibrio* spp. (Thiosulphate Citrate Bile Salt Sucrose Agar at 37°C for 24 h), coagulase-positive staphylococci and micrococci (ISO 6888-2:1999; ISO, 1999), *Bacillus cereus* (ISO 7932:2005; UNI, 2005a), *Listeria monocytogenes* (ISO 11290-1:2005 and ISO 11290-2:2005; UNI, 2005b, 2005c) and *Salmonella* spp. (ISO 6579:2002; ISO, 2002).

Colonies of *Enterobacteriaceae*, isolated from tuna sashimi, were identified by API 20 E (bioMérieux, Marcy l'Etoile, France). Then, they were cultured on Niven medium, in order to evaluate their itidino-decarboxylase activity. The pH detection of all samples was made with pH-meter WTW pH330i.

### Results

In sushi samples, the AMB ranged from 5.00 to 8.18 log CFU/g, and PB from 4.70 to 7.13 log CFU/g (Table 1). The *Enterobacteriaceae* showed a wide range from 1.41 to 6.67 log CFU/g. The highest percentage of samples for AMB (44.74%) and PB (47.37%) were from 6.00 to 7.00 log CFU/g, while for *Enterobacteriaceae* (44.74%) from 4.00 to 5.00 log CFU/g. Specific spoilage organisms and *Pseudomonas* spp. ranged from 3.49 to 7.72 log CFU/g and from 3.26 to 8.00 log CFU/g respectively. Coagulase-positive staphylococci were isolated in 16 samples (42.11%) with values from 2.00 to 3.60 log CFU/g. Micrococci had values between 3.53 and 5.03 log CFU/g. *Bacillus cereus* was detected in 3 samples (7.89%), with

**Table 1. Microbiological results of sushi samples.**

Value (log CFU/g)	Sushi samples distribution (%)				
	AMB	PB	SSOs	<i>Pseudomonas</i> spp.	<i>Enterobacteriaceae</i>
1.00<2.00	-	-	-	-	1 (2.63)
2.00<3.00	-	-	-	-	2 (5.26)
3.00<4.00	-	-	2 (5.26)	2 (5.26)	4 (10.53)
4.00<5.00	-	2 (5.26)	1 (2.63)	5 (13.16)	17 (44.74)
5.00<6.00	13 (34.21)	11 (28.95)	16 (42.11)	13 (34.21)	9 (23.68)
6.00<7.00	17 (44.74)	18 (47.37)	11 (28.95)	13 (34.21)	5 (13.16)
7.00<8.00	7 (18.42)	7 (18.42)	8 (21.05)	3 (7.90)	-
>8.00	1 (2.63)	-	-	2 (5.26)	-

CFU, colony forming unit; AMB, aerobic mesophilic bacteria; PB, psychrophilic bacteria; SSOs, specific spoilage organisms.

**Table 2. Microbiological results of sashimi samples.**

Value (log CFU/g)	Sashimi samples distribution (%)				
	AMB	PB	SSOs	<i>Pseudomonas</i> spp.	<i>Enterobacteriaceae</i>
6.00<7.00	-	-	-	6 (50)	6 (50)
7.00<8.00	7 (58.33)	8 (66.67)	8 (66.67)	6 (50)	6 (50)
>8.00	5 (41.67)	4 (33.33)	4 (33.33)	-	-

CFU, colony forming unit; AMB, aerobic mesophilic bacteria; PB, psychrophilic bacteria; SSOs, specific spoilage organisms.

values between 1.70 and 4.00 log CFU/g and *Vibrio* spp. were observed in 15 samples (39.47%) ranging from 1.70 to 3.70 log CFU/g. In all sushi samples, no *Salmonella* spp. and *Listeria monocytogenes* were found. The pH of the rice ranged from 4.6 to 6.6 (mean 5.6), while the pH of fish from 5.06 to 8.4 (mean 6.06).

In sashimi samples, the AMB, PB and SSOs were higher than 7.00 log CFU/g (Table 2). The *Enterobacteriaceae* and *Pseudomonas* spp. were from 6.00 to 8.00 log CFU/g. *Vibrio* spp. were observed in 6 samples (50%) ( $\geq 2.00$  log CFU/g), while in all 12 samples (100%) no *Salmonella* spp. and *Listeria monocytogenes* were found.

Twenty-five colonies of *Enterobacteriaceae*, isolated from tuna sashimi, were identified as *Citrobacter freundii*, *Klebsiella oxytoxa*, *Serratia liquefaciens*, *Rahnella aquatilis* and *Raoultella ornithinolytica*. All these strains showed istidino-decarboxylase activity on Niven medium. The pH of sashimi samples examined ranged from 5.86 to 8.4 (mean 6.98).

## Discussion

The microbiological limits for RTE foods such as sushi and sashimi are defined by EC Reg. 2073/2005 (European Commission, 2005). This regulation takes in account two food safety criteria: *Salmonella* spp. (absence in 25 g of sample) and *Listeria monocytogenes*. Sushi and sashimi characterised by shelf-life less than five days are RTE foods unable to support

**Table 3. Distribution of results in accordance with international microbiological standards for sushi.**

Parameters	Acceptable (%)	Borderline (%)	Unsatisfactory (%)
Aerobic mesophilic bacteria	13 (34.21)	17 (44.74)	8 (21.05)
<i>Enterobacteriaceae</i>	1 (2.63)	6 (15.79)	31 (81.58)
<i>Bacillus cereus</i>	35 (92.10)	3 (7.90)	-
Coagulase-positive staphylococci	21 (55.26)	11 (28.95)	6 (15.79)
<i>Salmonella</i> spp.	38 (100)	-	-
<i>Listeria monocytogenes</i>	38 (100)	-	-
<i>Vibrio parahaemolyticus</i>	38 (100)	-	-

the growth of *L. monocytogenes*. For this reason, *L. monocytogenes* must be less than 100 CFU/g during the shelf-life of these products.

All samples examined in this study were in accordance with the microbiological criteria of EC Reg. 2073/2005 (European Commission, 2005). On the basis of standards from Gilbert *et al.* (2000), Hong Kong (Food and Environmental Hygiene Department HKSAR, 2000, 2007) and Food Standards Australia (Food Standards Australia New Zealand, 2001; ANZFA, 2001), 6 sushi samples (21.05%) examined were considered unsatisfactory for AMB criteria (Table 3). Thirty-one samples (81.58%) were unsatisfactory for *Enterobacteriaceae* and 6 (15.79%) for coagulase-positive staphylococci. Seventeen samples resulted borderline for AMB (44.74%), 6 for *Enterobacteriaceae* (15.79%), 3 for *B. cereus* (7.90%) and 11 for coagulase-positive staphylococci (28.95%), as showed in Table 3.

High bacterial charges as well as potentially pathogen microorganisms were observed in

sushi and sashimi examined as previously reported by several authors (Adams *et al.*, 1994; Atanassova *et al.*, 2008; Barralet *et al.*, 2004; Millard and Rockliff, 2003). In this regard, Atanassova *et al.* (2008) described average AMB values of 6.3 log CFU/g in fresh sushi samples. *Salmonella* spp. and *L. monocytogenes* were found in 1.6 and 1.2% of samples respectively, while *Staphylococcus aureus* showed a charge of 2.2 to 4.7 log CFU/g (Atanassova *et al.*, 2008). The microbiological status of sushi and sashimi reflects the microbiology of materials used for their preparation. The detection of *Vibrio* spp. is indeed related to the fish and shellfish products used (Giuffrida and Panebianco, 2008), while *B. cereus* has been reported in plant foods (especially rice) (Eglezos *et al.*, 2010). *Salmonella* spp. and *L. monocytogenes* can occur in vegetables and dairy products (cheese), while the finding of *S. aureus* is an evidence of human contact during the preparation of food (Nogara *et al.*, 2004).

## Conclusions

This study confirms that the production of sushi and sashimi of good quality obviously depends on the choice of raw materials, as also reported by Atanassova *et al.* (2008) and Millard and Rockliff (2003). A good rice acidification (pH of the rice must be less than 4.6) and the maintaining of cold chain during preparation and storage are also essential to obtain products of good microbiological status. Finally, a proper training of personnel who manipulates this easily perishable food is desirable.

Further bio-molecular investigations will be necessary to confirm the istidino-decarboxylase activity of *Enterobacteriaceae* strains isolated from tuna sashimi as suggested by Mancusi *et al.* (2013). The presence of histamine-producing bacteria on sashimi could also represent a potential toxicological hazard.

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