Introduction

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 determine the aerobic mesophilic bacteria (AMB), psychrophilic bacteria (PB), Enterobacteriaceae, specific spoilage organisms (SSOs), Pseudomonas spp., coagulase-positive staphylococci, micrococci, Vibri spp., Bacillus cereus, Salmonella spp. and Listeria monocytogenes.

Results

In sushi samples, the AMB ranged from 5.00 to 6.00 log CFU/g, while SSOs and Pseudomonas spp. ranged 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.

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) (Lynch Iron Agar at 25°C for 5 days), Enterobacteriaceae (ISO 21528-2:2004; ISO, 2004), Pseudomonas spp. (Pseudomonas agar base with CPC supplement 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 20E (bioMérieux, Marcy l’Etoile, France). Then, they were cultured on Niven medium, in order to evaluate their hidrophilic-decarboxylase activity. The pH detection of all samples was made with pH-meter WTW pH330i.
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%) (>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 oxytoca*, *Serratia liquefaciens*, *Rahnella aquatilis* and *Raoxiella 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 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 et al., 1994; Millard and Rockliff, 2003). *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 histidine-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.

References

Adams AM, Leja LL, Jinneman K, Beeh J, Yuen GA, Weckel MM, 1994. Anisakid parasites, Staphylococcus aureus and Bacillus cereus in sushi and sashimi from Seattle area restaurants. J Food Protect 57:311-7.

ANZFA, 2001. Safe food Australia: a guide to the food safety standards. Commonwealth of Australia, Australia New Zealand Food Authority Publ., Canberra, Australia.

Atanassova V, Reich F, Klein G, 2008. Microbiological quality of sushi from sushi bars and retailers. J Food Protect 71:860-4.

Barralet J, Stafford R, Towner C, Smith P, 2004. Outbreak of Salmonella Singapore associated with eating sushi. Available from: http://www.health.gov.au/internet/main/publishing.nsf/Content/cda-2004-cdi2804p.htm

Eglezos S, Huang B, Dykes GA, Fegan N, 2010. The prevalence and concentration of Bacillus cereus in retail food products in Brisbane, Australia. Foodborne Pathog Dis 7:867-70.

European Commission, 2005. Regulation of 15 November 2005 on microbiological criteria for foodstuffs, 2073/2005/EC. In: Official Journal, I, 338/1, 22/12/2005.

Food and Environmental Hygiene Department HKSAR, 2000. Risk assessment studies report No. 2. Microbiological hazards evaluation. Sushi and sashimi in Hong Kong. Available from: http://www.cfs.gov.hk/english/programme/programme_haccp/files/s_s_ras2_eng.pdf

Food and Environmental Hygiene Department HKSAR, 2007. Microbiological guidelines for ready-to-eat food. Available at: http://www.cfs.gov.hk/english/whatsnew/w hatsnew_act/files/MBG1_RTEf/20food_e.pdf

Gilbert RJ, de Louvois J, Donovan T, Little C, Nye K, Kibek CD, Richards J, Roberts D, Bolton FJ, 2000. Guidelines for the microbiological quality of some ready-to-eat foods sampled at the point of sale. Available from: http://webarchive.nationalarchives.gov.uk+/+http://www.hpa.org.uk/cdhp/issuses/CDPHVol3/no3/guides_micro.pdf

Giufrida A, Panebianco A, 2008. [Igiene e tecnologie dei prodotti della pesca freschi e trasformati]. In: Colavita G, ed. [Igiene e tecnologia dei prodotti di origine animale]. [Book in Italian]. Le Point Véterinaire Italie ed., Milano, pp 274-6.

ISO, 1999. Microbiology of food and animal feeding stuffs. Horizontal method for the enumeration of coagulase-positive staphylococci (Staphylococcus aureus and other species). Part 2: technique using rabbit plasma fibrinogen agar medium. ISO Norm 6888-2:1999. International Standardization Organization ed., Geneva, Switzerland.

ISO, 2001. Microbiology of food and animal feeding stuffs. Horizontal method for the detection of Salmonella spp. ISO Norm 6579:2002. International Standardization Organization ed., Geneva, Switzerland.

ISO, 2004. Microbiology of food and animal feeding stuffs. Horizontal methods for the detection and enumeration of Enterobacteriaceae. Part 2: colony-count method. ISO Norm 21528-2:2004. International Standardization Organization ed., Geneva, Switzerland.

Mancusi R, Bini RM, Cecchini M, Delle Donne G, Rosmini R, Trevisani M, 2013. Occurrence of histamine in fish products on markets. Ital J Food Safety 3:135-9.

Masotti G, Amadai P, Lanni I, 2004. [Pesce crudo. Condizioni igieniche, operative e strutturali nei ristoranti giapponesi]. [Article in Italian]. Available from: http://www.sivemp.it/userfiles/_documents/_rivista_/26_58_62_pesce.pdf

Millard G, Rockliff S, 2003. Microbiological quality of sushi. Available from: http://www.health.act.gov.au/healtha?d=a&did=10060511&pid=1094601516

Nogara M, Rossari C, Stella S, Cozzi M, Cantoni C, 2004. [Valutazione microbiologica del pesce crudo utilizzato per la preparazione del sushi]. [Article in Italian]. Pesce 5:121.

NSW Food Authority, 2006. Temperature control of sushi. A technical report on predicting microbial growth in sushi stored at various temperature. New South Wales Food Authority ed., Silverwater, Australia.

UNI, 2004. [Microbiologia di alimenti e mangimi per animali. Metodo orizzontale per la conta di microorganismi. Tecnica della conta delle colonie a 30°C]. [Regulation in Italian]. UNI Norm 4833:2004. Italian Unification Institute ed., Milan, Italy.

UNI, 2005a. [Microbiologia di alimenti e mangimi per animali. Metodo orizzontale per la conta di Bacillus cereus presunto. Tecnica della conta delle colonie a 30°C]. [Regulation in Italian]. UNI Norm 7932:2005. Italian Unification Institute ed., Milan, Italy.

UNI, 2005b. [Microbiologia di alimenti e mangimi per animali. Metodo orizzontale per la ricerca e la conta di Listeria monocytogenes. Parte 1: metodo per la ricerca]. [Regulation in Italian]. UNI Norm 11290-1:2005. Italian Unification Institute ed., Milan, Italy.

UNI, 2005c. [Microbiologia di alimenti e mangimi per animali. Metodo orizzontale per la ricerca e la conta di Listeria monocytogenes. Parte 2: metodo per la conta]. [Regulation in Italian]. UNI Norm 11290-2:2005. Italian Unification Institute ed., Milan, Italy.