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Evaluation of microbiological quality of sushi sold in restaurants and supermarkets in turkey

Bestimmung der mikrobiologischen Qualität von in Restaurants und im Supermarkt erhältlichem Sushi in der Türkei

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Summary

Sushi is a popular traditional Japanese food in worldwide. This study was conducted to investigate the physical, chemical, sensory and microbiological quality of sushi sold in supermarket and restaurants. 300 sushi samples were purchased from 5 different popular restaurants (A, B, D, E, and F) and 1 supermarket (C). Four main types of sushi (California roll (CR), sesame roll (SMR), sake roll (SR), take roll (TR)) were chosen from each station. pH values of sushi rice samples were found to be all below 4.6. According to sensory overall quality of sushi samples, CR and SMR from station C had significantly the lowest values ($p<0.05$). Among the sushi samples, pathogenic bacteria *Listeria monocytogenes, Salmonella* spp. and *Staphylococcus aureus* were not detected in 25 g portion of any samples. Counts of total aerobic bacteria were high in stations A, B, D, E, and F (between 4.3–7 log CFU/g), except for station C (3.5 log CFU/g). Results for coliform counts showed similar trends as those of *E. coli* counts in samples. *E. coli* counts of all sushi types from all stations (except SMR, SR and TR from station D and SR from station E) exceeded the limit of acceptability (<100 MPN/g). Our finding may suggest a common presence of coliforms and *E. coli* in raw materials used in sushi preparation.

Keywords: Sushi, Hygienic conditions, *E. coli,* RTE sushi, quality

Zusammenfassung

Sushi als traditionelle Japanische Speise ist weltweit sehr popular. Diese Studie wurde durchgeführt um die physikalische, chemische, sensorische und mikrobiologische Qualität von im Supermarkt und Restaurants erhältlichem Sushi zu untersuchen. 300 Sushi Proben aus 5 verschiedenen beliebten Restaurants (A, B, D, E und F) und 1 Supermarkt (C) wurden gekauft. Von jeder dieser Stationen wurden vier verschiedene Sushi Sorten ausgewählt (California roll (CR), sesame roll (SMR), sake roll (SR) und take roll (TR)). Der ph-Wert jeder Sushi Probe wurde geringer als 4.6 bestimmt. Die sensorische Bewertung für die Sushi Sorten CR und SMR aus der Station C wurden mit Abstand am geringsten bewertet ($p<0.05$). Keiner der 25 g Proben waren mit den Bakterien *Listeria monocytogenes, Salmonella* spp. und *Staphylococcus aureus* infiziert. Die Anzahl der aerobien Bakterien waren hoch in den Proben der Stationen A, B, D, E und F (zwischen 4.3–7 log CFU/g), ausser für die Station C (3.5 log CFU/g). Die Kolonienzahl zeigt vergleichbare Trends wie die Anzahl der *E. coli* in den Proben. Die Anzahl der *E. coli* in allen Sushi Sorten von allen Station (ausser SMR, SR und TR aus der Station D und SR aus der Station E) überstieg die Akzeptanzgrenze von 100 MPN/g. Die Untersuchungen zeigten ein allgemeines Vorkommen von coliforms und *E. coli* im Sushi Rohmaterial.

Schlüsselwörter: Sushi, hygienischer Zustand, *E. coli*, RTE Sushi, Qualität

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Introduction

Sushi consists of cold cooked rice acidified with vinegar that is shaped into bite-sized pieces and topped with raw or cooked fish, or formed into a roll with fish, egg or vegetables and wrapped in seaweed (ANON, 2008). Preparing sushi involves a great deal of handling of both raw and cooked foods (Feng, 2012). Because sushi is eaten without any further cooking and raw foods can contain bacteria. Poor handling of cooked foods can result in them becoming cross contaminated from raw foods. If not stored correctly, the number of bacteria can also grow (ANON, 2007) and they are considered as high risk food items. Pathogenic bacteria such as *Salmonella* spp., *Staphylococcus aureus, Vibrio parahaemolyticus, Vibrio cholerae, Listeria monocytogenes* and *Bacillus cereus* can occur in materials used for sushi preparation such as fish, seafood products, vegetables and rice (Muscolino et al., 2014). Sushi prepared by sushi bars had relatively high aerobic plate count levels as well as counts of *Escherichia coli* and *Staphylococcus aureus* (Leisner et al. 2014). This could be a result of processing conditions and/or cooling and hygiene conditions during storage and preparation (Atanassova et al., 2008). Based on the statistics on cases reported to the Centers for Disease Control and Prevention, between 1973 and 1987, shellfish and finfish (including some sushi favorites, such as tuna, salmon, and yellowtail) accounted for roughly 5 % of foodborne illnesses, which is essentially 3.8 million cases of food poisoning in the United States as a result of fish, including 250 deaths and 16250 hospitalizations (Feng, 2012). In Australia, from 2001 to 2007 there have been 10 outbreaks of foodborne illness associated with consumption of sushi, affecting 84 people with 7 hospitalized. This accounts for 1.4 % of all reported foodborne outbreaks, with the implicated sushi being prepared in restaurants (8/10), a commercial caterer (1/10) and a commercial manufacturer (1/10) (ANON, 2008). Hong Kong Food and Environmental Hygiene Department (FEHD) reported that from 1997 to 1999 consumption of sushi and sashimi contributed to 45/1481 (3 %) of reported food poisoning outbreaks in Hong Kong, affecting 142 people (FEHD, 2000). Because of the poor handling practices and infected food handlers, enterotoxigenic *Escherichia coli* (ETEC) was identified as the agent in a large foodborne disease outbreak in a sushi restaurant in Nevada (Jain et al. 2008)

It is generally agreed that sushi is the most popular Japanese food in the World (NG, 2001). Sushi is becoming increasingly popular in Turkey and can be purchased from the supermarkets and restaurants. This increasing popularity of sushi raises public health concerns over the consumption of sushi.

There is also an increase in the consumption of fresh RTE (ready to eat) sushi in Europe and also in Turkey. Generally fresh RTE sushi is a chilled product with a shelf life of several days and sold in the open-refrigerated display cases in supermarkets. Researches on display cabinets highlighted the importance of temperature conditions on foods (Willocx et al.1994, Cemagref and ANIA, 2004).

Although there is a growing demand for sushi, no information is available regarding the quality of sushi sold in restaurants and supermarkets in Turkey. The aim of the study was to determine the microbiological quality of freshly prepared sushi from the restaurants and chilled RTE sushi from supermarkets in Izmir, Turkey. Physical and chemical quality and sensory analysis were conducted to obtain information that can be useful to make association with data from microbiological quality analysis.

Materials and Methods

Collection of sushi

Sushi samples (n=300) were purchased from five different restaurants and one supermarket in İzmir, Turkey. The restaurants (A, B, D, E, and F) served sushi upon ordering (freshly prepared) and 1 supermarket (C) that sushi was displayed for takeaway only (where sushi were kept in the open-refrigerated display cases) were chosen. Sushi from the supermarket had been analyzed 3 days before the expiration date of the product. All the sushi samples were transported to the laboratory in a cooler box at low temperature (<5 °C) and stored at 4 °C ± 0.2, until analysis. They were analyzed within 2h of sampling. The four main types of sushi were chosen for this study. Only one supermarket in Turkey was selling RTE sushi. The supermarket was selling only two kinds of sushi. Therefore, no research data was available. The chosen sushi types were; California roll (CR), consisting of a clump of acidified rice, and raw ingredients such as cucumber, surimi, and avocado and rolled in nori sheets (dried seaweed) with an outer coating of tobiko (flying fish roe). Sesame roll (SMR), containing acidified rice, cucumber, avocado and rolled in nori sheets with an outer coating of sesame seeds. Sake roll (SR), consisting of acidified rice and salmon (raw) rolled in nori sheets. Take roll (TR), consisting of acidified rice and tuna (raw) rolled in nori sheets.

Physicochemical analysis

pH values of rice used for sushi were recorded according to ASU (1980). For determining the pH, 5 g of homogenized rice sample was diluted with 5 ml of distilled water and pH value was measured using a Hanna 211 model pH meter (Cluj-Napoca, Romania). All measurements were done in triplicate.

Microbiological analysis

Collected samples were analyzed for total aerobic bacterial counts (TBC), coliform bacterial counts (CBC), *Listeria monocytogenes, Salmonella* spp., *Escherichia coli* and *Staphylococcus aureus.* For TBC, 25 g of sample was weighed and mixed with 225 ml of peptone water (1:10 dilution). A sample mixture was homogenized by using a stomacher (IUL Instruments, Barcelona, Spain) for 1 min at 230 rpm. TBC were counted on plate count agar, followed by incubation for 24 to 48 h at 30 °C (Harrigan and McCance. 1976). CBC and *E. coli* detection were done by 3 tube MPN analysis using LST and BGB as in the standard methods of the US Food and Drug Administration Bacteriological Analytical Manual (FDA-BAM) (FDA, 2014). *Staphylococcus aureus* analyses were done following methods of International Commission on Microbiological Specifications for Foods (ICMFS, 1986). Dilutions of homogenized food samples (100 µl) were plated onto Baird-Parker (BP) agar (Merck, Germany). BP plates were incubated at 37 °C for 48 h. Detection of *Listeria monocytogenes* was performed following ISO11290-1. Food sample (25 g) was mixed with 225 ml of half Fraser broth (Merck, Germany), incubated at 30 °C for 24 h followed by a second enrichment for 48 h in Fraser broth at 37 °C. For each enrichment step, samples were plated on Agar Listeria according to

Ottaviani and Agosti (ALOA agar, AES Laboratoires). ALOA agar allows the differentiation of *L. monocytogenes* colonies from most other *Listeria* spp. for the detection of *Salmonella* spp., the procedures of ISO 6579 were followed. Food sample (25g) was mixed with 225 ml of Buffered Peptone Water (BPW), followed by incubation at 37 °C for 24 h. The mixture was transferred to Tetrathionate broth (Müller-Kauffmann) and Rappaport Vassiliadis soy peptone broth (Merck, Germany) for the selective enrichment steps. The enrichments were plated onto Xylose Lysine Desoxycholate (XLD) agar (Merck, Germany) and Brilliant Green (BGA) agar (Merck, Germany), followed by incubation at 37 °C for 24 h.

Sensory Analysis

Sensory analysis was conducted according to 5 point hedonic scale. Sushi samples were blind coded with 3 digit random numbers (Adenike, 2014). 5 trained panelists were asked to evaluate the overall quality with regard to appearance, odor and flavor. The participants were only allowed to drink water to rinse their mouth during the sensory analysis. The parameters were assessed using ranking scores like extremely (5), like moderately (4), neither like nor dislike (3), dislike moderately (2) and dislike extremely (1).

Statistical Analysis

Study was duplicated. The SPSS (SPSS, 16.0. Chicago, IL, USA) program was used to determine for significant differences between mean values. Differences between mean values were analyzed by one-way analysis of variance (ANOVA) followed by Tukey and Duncan tests. Statistical analyses of microbiology results were done on log- transformed data. All the results are presented as means ± SD.

Results and Discussions

Physicochemical analysis

Heat during the cooking of rice can activate certain bacterial spores that can grow and release toxins unless the rice is preserved or refrigerated. If the rice is refrigerated, it is more difficult to form sushi (AFDO, 2004) and also sushi rice is commonly stored at room temperature for the ideal warm taste. So with this aim acidified rice is commonly used in sushi and proper acidification of rice to a pH value of 4.6 or below is known to inhibit the growth of pathogenic bacteria, particularly *Bacillus cereus* (BCDDC, 2010; CFS, 2015). The pH range of plain white rice is 6.0-6.7 which falls into the range of the optimum pH of most bacteria (Forsythe, 2010). So, it is important to be sure about the acidification was done at required amount. In the present study, although rice from B and F had significantly higher pH values than the others, they were all below 4.6 (Tab. 1). Overall, rice from sushi examined in this study was acidified to the recommended level of pH 4.6 or below. Results were similar with those of Adams et al. (1994) who reported that pH values of acidified sushi rice samples ranged from 3.9 to 4.6. According to Lee and Heacock (2014), pH values of acidified sushi rice ranged from 3.71 to 4.53. On the other hand, Cadun et al. (2014), reported that the pH values of acidified sushi rice from two different restaurants ranged from 4.6 to 4.9.

Sensory analysis

According to overall quality of sushi samples CR and SMR from C had significantly the lowest values ($p<0.05$) (Tab. 2). According to sensory analysis, it was concluded that RTE sushi was not preferred more when compared with ones prepared upon ordering. They were found to be disliked extremely according to appearance, odor and flavor when compared with the others. Laguerre et al. (2011) reported that the product temperature in refrigerated display cabinets is very variable and directly influences the safety and quality of food product. Although the responsible person of the supermarket (C) informed us that the temperature of the open front display cabinet was 4 °C, Laguerre et al. (2012) reported that, temperature differences of more than 5 °C were measured in the decks and temperature in one place increased toward the end of the day by 4 °C and toward the end of the week by almost 7 °C. Also the position of the food in the deck was important. On the other hand all kind of sushi samples from restaurant E had significantly higher values ($p<0.05$). California roll (consisting of a clump of acidified rice, and raw ingredients such as cucumber, surimi, and avocado and rolled in nori sheets with an outer coating of tobiko) had the highest scores from each restaurant and supermarket (A, B, C, D, E, F). California roll as made inside out (so color of nori which is usually dark, greenish couldn't be seen) and the color of outer layer tobiko was bright orange. No raw fish was used in CR which might be the reason of higher scores. Iwata et al. (2015) compared the sensory evaluation of sushi with once-frozen ingredients with those with unfrozen (fresh) ingredients and reported that freezing raw fish did not ruin sushi's taste and recommended raw fish to be frozen before consumption. Mol et al. (2014) reported that modified atmosphere packaged sushi samples taken higher sensory scores than the ones packed with air.

Microbiological analysis

Among samples tested, pathogenic bacteria *Listeria monocytogenes, Salmonella* spp. (25 g) and *Staphylococcus aureus* (CFU/g) were not detected in any samples. Counts of total aerobic bacteria were high in restaurants A, B, D,

TABLE 1: *pH values of acidified rice of sushi groups.*

Stations Analysis	1st Station (A)	2nd Station (B)	3rd Station (C)	4th Station (D)	5th Station (E)	6th Station (F)
pH	F4.21 ± 0.07^{a}	4.45 ± 0.03^{b}	4.15 ± 0.09^{a}	4.24 ± 0.04^{a}	4.23 ± 0.04^{a}	4.41 ± 0.03^{b}

Means within the same letter in the same line are not significantly different at a significance level of 0.05 (P > 0.05).

TABLE 2: *Sensory Quality of Sushi Types from Restaurants and Supermarket.*

Groups / Type of Sushi	A	B	C	D	E	F
CR	4.8±0.45^{a1}	3.6±0.55^{b1}	1.6±0.55^{c1}	4.8±0.45^{a13}	5.0±0.00^{a1}	4.2±0.45^{b1}
SMR	3.8±0.45^{a2}	3.0±0.00^{b1}	1.6±0.55^{c1}	4.0±0.00^{a2}	4.8±0.45^{d1}	3.8±0.45^{a1}
SR	4.0±0.00^{a2}	3.2±0.45^{b1}		4.2±0.45^{a23}	5.0±0.00^{c1}	4.0±0.00^{a1}
TR	4.0±0.00^{a2}	3.2±0.45^{b1}		4.2±0.45^{a23}	5.0±0.00^{c1}	

*: Means within the same letter in the same line and means within the same column with the same number are not significantly different at a significance level of 0.05. Restaurants (A, B, D, E, F), Supermarket (C). California roll (CR) rice, cucumber, surimi and avocado and rolled in nori sheets with an outer coat of tobiko; Sesame roll (SMR) rice, cucumber, avocado and rolled in nori sheets with an outer coating of sesame seeds, Sake roll (SR) rice and raw salmon rolled in nori sheets, Take roll (TR) rice and raw tuna rolled in nori sheets. Sensory scores; like extremely: 5, like moderately: 4, neither like nor dislike: 3, dislike moderately: 2, dislike extremely: 1

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TABLE 3: *Enumeration of Total Aerobic Bacteria, Coliform Bacteria and Escherichia coli counts of sushi types which were had different sushi retailers.*

Stations	Type of Sushi	TAMBC (log CFU/g)	Coliform Bacteria Count (MPN/ml)	*Escherichia coli* (log CFU/g)
1st Station	CR	6.25±0.06^{a1}	>110	4.71±0.01^{a1}
	SSR	6.09±0.03^{a1}	>110	3.45±0.05^{b1}
	SR	4.72±0.28^{b1}	>110	3.89±0.04^{c1}
	TR	4.69±0.28^{b13}	>110	3.60±0.04^{d1}
2nd Station	CR	5.02±0.22^{a2}	>110	4.81±0.02^{a1}
	SSR	4.84±0.07^{a2}	>110	5.00±0.01^{b2}
	SR	6.43±0.08^{b2}	>110	5.01±0.02^{c2}
	TR	6.21±0.16^{b2}	>110	4.95±0.02^{c2}
3rd Station	CR	3.52±0.12^{a3}	>110	3.78±0.05^{a2}
	SSR	3.97±0.10^{a3}	>110	3.54±0.03^{b1}
	SR	–	–	–
	TR	–	–	–
4th Station	CR	6.97±0.01^{a4}	>110	3.87±0.03^{a2}
	SSR	5.10±0.12^{b4}	<0.30	1.04±0.04^{b3}
	SR	7.09±0.09^{a3}	<0.30	1.29±0.03^{c3}
	TR	5.06±0.12^{b1}	<0.30	1.03±0.02^{b3}
5th Station	CR	6.61±0.01^{a4}	>110	5.23±0.03^{a3}
	SSR	6.82±0.03^{a4}	>110	5.17±0.08^{a4}
	SR	4.56±0.18^{b1}	<0.30	2.91±0.04^{b4}
	TR	4.27±0.01^{c3}	4.30	2.02±0.03^{c4}
6th Station	CR	6.20±0.02^{a1}	>110	4.26±0.03^{a4}
	SSR	5.18±0.03^{b5}	>110	4.09±0.06^{b5}
	SR	5.68±0.01^{c4}	24.00	2.93±0.03^{c4}
	TR	–	–	–

Means within the same column (in the same station) with the same letter and means within the same coloumn (in the same type) with the same number are not significantly different at a significance level of 0.05 ($P > 0.05$).

E, F (between 4.3–7 log CFU/g), except for the supermarket C that showed lower number of total bacteria counts (3.5 log CFU/g) (Tab. 3). This may suggest that hygienic practices or initial contamination levels of food components used for sushi preparation can be different among these establishments (Taché and Carpentier, 2014). In this study, results from coliform counts showed similar trends as those from *E.coli* counts. Our finding may suggest a common presence of coliforms and *E. coli* in raw materials used in sushi preparation. A previous study showed that indicator organism coliforms and *E. coli* may be present in the environment or from human or animal faeces or through storage, processing and handling of some raw materials (Aycicek et al. 2006) used in sushi preparation. In the present study, *E. coli* counts of all sushi types from all sources (except SMR, SR and TR from D and SR from E) exceeded the limit of acceptability (<100 MPN/g) (FSANZ, 2001; CFS, 2007; FDA, 2013; CFS, 2015).

For station C, although total aerobic bacteria counts in CR showed 3.5 log CFU/g which was somewhat lower than CR from other stations. However, and *E. coli* were low, and coliforms showed to be high (>1100 MPN/g and >110 MPN/g, respectively) as observed in CR samples from other stations. On the other hand, although total aerobic bacteria counts were high, coliform and *E. coli* showed low numbers for station D. This was observed in three types of sushi rolls, SMR, SR and TR which had fewer amounts of raw vegetable ingredients than those in CR (Faour-Klingbeil, 2016). This finding might indicate that contamination in raw materials with other microorganisms might occur after washing as counts of aerobic bacteria showed to be higher than other indicator organisms. In addition, as fresh produce is known to carry high numbers of aerobic bacteria, however, post-process contamination can also lead to high total counts of aerobic bacteria. Therefore, to indicate microbiological quality of sushi, microbiological criterion based on total aerobic bacteria counts is not applicable as sushi is RTE food containing fresh produce. It would be expected that sushi would have an inherent high plate count because of the normal microbial flora present. Appropriate washing of raw materials and hygienic practices of employees in the establishments are important to reduce the incidence of bacterial contamination in sushi (Feng, 2011; Taché and Carpentier, 2014).

Conclusion

Results of the microbiology analysis showed that raw materials used in sushi preparation may represent common source of coliforms and *E. coli.* Vegetables used for sushi should be well washed. And temperature of the refrigerated storage of RTE sushi displayed on the supermarkets should be monitored and the temperature of the products should be checked.

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Conflict of interest

The authors declare that no conflict of interest exists.

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