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Hygienic quality of stuffed Mediterranean mussels (*Mytilus galloprovincialis*) sold by street vendors in İzmir, Turkey

Hygienische Qualität von gefüllten mediterranen Muscheln (Mytilus galloprovincialis) von Straßenverkäufern in Izmir, Türkei

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Summary

Stuffed mussels are a popular foodstuff in the Mediterranean that is sold on a ready-to-eat base by street vendors. The present paper sought to determine the microbiological quality of this product. For that, edible parts of mussels (valves and stuffing removed, n = 100) from different city districts of İzmir were analysed with regard to average total mesophilic aerobic bacteria (TMAB), coliform bacteria, faecal coliform bacteria, *Staphylococcus aureus*, *Bacillus cereus* and *Vibrio* spp. counts, as well as pH. Results were compared with the recommendations of the Turkish Food Codex. While all samples were negative for salmonellae and *E. coli*, several other criteria as recommended by the Turkish food safety authorities were not met, e. g. elevated TMBA or the presence of *Vibrio* spp. in 13 % of samples. Comparing the results with the literature it became clear that street-sold stuffed mussels can pose a threat to human health in the Mediterranean, and that means should be taken (including enhanced control and monitoring schemes) to ensure hygienic processing and marketing of this foodstuff.

Keywords: Stuffed mussels, food safety, microbiology, consumer health

Zusammenfassung

Gefüllte Muscheln sind ein beliebtes Nahrungsmittel im Mittelmeerraum, die verzehrfertig von Straßenverkäufern angeboten werden. Die vorliegende Arbeit hinterfragt die mikrobiologische Qualität dieser Produkte. Hierfür wurden die essbaren Teile der Muscheln (n = 100) aus verschiedenen Stadtteilen von İzmir im Hinblick auf die aerobe mesophile Gesamtkeimzahl (GKZ), koliforme Bakterien, fäkalkoliforme Bakterien, *Staphylococcus aureus*, *Bacillus cereus* und *Vibrio* spp. analysiert, sowie der pH-Wert bestimmt. Die Ergebnisse wurden mit den Empfehlungen des türkischen Lebensmittelkodex verglichen. Während alle Proben Salmonellen und *E. coli* negativ waren, wurden verschiedene andere Kriterien, die von den türkischen Lebensmittelsicherheitsbehörden empfohlen werden nicht erfüllt, z. B. die erhöhte GKZ oder das Vorhandensein von *Vibrio* spp. in 13 % der Proben. Vergleicht man die Ergebnisse mit der Literatur wird deutlich, dass gefüllte Muscheln aus Straßenverkäufen eine Bedrohung für die menschliche Gesundheit im Mittelmeerraum darstellen können, und dass Mittel ergriffen werden sollten (einschließlich verstärkte Kontrolle und Überwachungssysteme), um hygienische Verarbeitung und Vermarktung dieses Nahrungsmittels zu gewährleisten.

Schlüsselwörter: Gefüllte Muscheln, Lebensmittelsicherheit, Mikrobiologie, Verbraucherschutz

Introduction

Although sold by street vendors under poor hygienic conditions, stuffed mussels is one of the most frequently-consumed fast foods in the Mediterranean, particularly in Turkey and Middle Eastern countries. The Mediterranean mussel, *Mytilus galloprovincialis* (known as “black mussel” in Turkey and other Mediterranean countries), is a member of the family *Mytilidae* and inhabits mainly the Mediterranean Sea, Black Sea, Marmara Sea, Atlantic shores and in Bosphorus (Binsi et al., 2007; Erkan, 2005). This species is important in terms of human nutrition, being a source of high quality and cheap protein (Choo and Ng; 1990; Fuentes et al., 2009). Orban et al (2002) reported that fat and cholesterol content of mussels was quite low and that 42 to 45 % of their fatty acid composition related to unsaturated fatty acids. They are also important sources of vitamins A, B1, C, D and E and trace elements like Fe and Ca (Cheong and Lee, 1984). Because of the intake of high amount of sea water in their body for their survival (filter feeder), they can be contaminated by the microorganisms and toxic compounds found in water (Ripabelli et al., 1999). Additionally, because of high glycogen, free amino acid contents, high water activity (0.95 aw) and pH values (6.7–7.1), they act as an ideal medium for the growth of microorganisms. Mussels are relevant in terms of food safety and consumer health since they may lead to food intoxications (Jay, 1996; Murphree and Tamplin, 1991). For stuffed mussels as sold in the Mediterranean, this is due to poor hygienic conditions manufacturing and trading practices under.

Regarding processing, mussels are harvested from the sea, boiled for a first time and their shells cleaned. Then, mussels are stuffed with boiled rice and spices, their shells then closed again by wrapping with cord and then boiled a second time (Ateş et al., 2011). Stuffed mussels are generally sold under poor hygienic conditions on small and open pushcarts. These ready-to-eat foods are served in streets at ambient temperatures.

There are many points which can lead to contaminations during this manufacturing, i. e. microbiological risks arising from the sea from which the mussels are caught, contaminations from working staff and equipment, from raw materials (spices, rice water), and from the environment of sale and service place. Especially in mussels collected from the sewage confluence of the seas, *Salmonella* spp. and *Escherichia* (*E.*) *coli* O157:H7 are found. Also spices and rice may be important sources of *Bacillus* (*B.*) *cereus* contamination (Üzgül, 2005). Also there is an important cross-contamination risk while serving these ready-to-eat foods on pushcarts in the streets.

The constabulary of local municipality is responsible for the control of these mussel sellers and street vendors. However, effective control done by the police is very difficult. Especially at night, it is typical to see many pushcarts in the streets selling ready-to-eat foods in all Turkey.

From these points of view, a survey was performed in order to determine the microbiological attributes of stuffed mussels sold by street vendors. To the authors' knowledge, it is the first comprehensive research to provide data rela-

ted to microbiological quality of stuffed mussels sold by street vendors in different districts of İzmir, Turkey.

Materials and Methods

Stuffed mussels were purchased from street vendors in the four largest districts of İzmir (Bornova, Alsancak, Konak, and Karşıyaka). Samples from 25 different vendors in each district were collected and total of 100 different samples were analysed. With the same experimental design, research was repeated two times. Approximately twelve stuffed mussel samples (500 g) were purchased from each vendor in two replicates. Then, samples were brought in their own package (but cooled in ice-boxes) into the Microbiology Laboratory of Celal Bayar University within 30 minutes. Mussel stuffing and shells were removed aseptically. The edible parts of the stuffed mussel were used for microbiological analysis. 25 g of edible part was aseptically transferred into 225 ml of peptone water (0.1 % w/v) and further dilutions were prepared.

The Turkish Food Codex Communication for Microbiological Criteria (Anon., 2009 – Announcement No: 2001/19) details the microbiological criteria for daily ready-to-eat meat and vegetable meals. It also contains the methods to be used in the analysis, and the criteria for this foodstuff include *E. coli*, *B. cereus*, *Staphylococcus* (*S.*) *aureus* and *Salmonella* spp. (Tab. 1). Besides, total mesophilic aerobic bacteria (TMAB), total coliform (TC), total faecal coliform (TFC), *Clostridium* (*C.*) *perfringens*, and *Vibrio* spp. counts were also determined (methods according to ICMSF, 1978; Halkman, 2005; Üzgül, 2005). Finally, pH values of the samples were measured by using a pH-meter (WTW, pH3110). Results of the analyses were evaluated by using a SAS statistical analyses programme; the method was CR (Completely Randomized).

Results and Discussion

Average pH values of stuffed mussel samples are given in Table 2. As seen from the table, average pH value of

TABLE 1: Microbiological criteria for ready-to-eat meat and vegetable meals (Anon., 2009).

Microbiological criteria for ready-to-eat meat and vegetable meals	n	c	m	M
<i>Escherichia coli</i>	5	0	<10 ¹	
<i>Staphylococcus aureus</i>	5	2	10 ²	10 ³
<i>Bacillus cereus</i>	5	2	10 ²	10 ³
<i>Salmonella</i> spp.	5	0	0/25 g-mL	

n = number of sampling, c = number of allowed samples having counts between m and M values, m = upper limit for (n-c) samples, M = marks the limit beyond which the level of contamination is hazardous or unacceptable.

TABLE 2: Average pH values of stuffed mussels purchased from street vendors (n=100).

District	Min	Max	Average
Bornova	6.06	6.6	6.40b
Alsancak	6.24	7.75	6.57ab
Konak	6.27	6.88	6.48b
Karşıyaka	6.32	8.12	6.72a
Overall	6.06	8.12	6.54

Values with different letters are statistically different from each other (P<0.05).

TABLE 3: Average TMAB counts of stuffed mussel samples (log cfu/g) (n=100).

District	Min	Max
Bornova	<1.00	3.77
Alsancak	<1.00	4.41
Konak	<1.00	3.66
Karşıyaka	1.60	4.67
Overall	<1.00	4.67

all samples was 6.54, ranging between 6.06 and 8.12. The highest average pH value (6.72) was measured in the samples sold in Karşıyaka ($P<0.05$). As reported by ICMSF (1980), pH value of fresh bivalve aquacultures should be between 6.2 and 6.5. During spoilage, pH values decrease to 5.8. Gönül et al. (1996) reported that average pH values of stuffed mussels was 6.9, whereas according to Tatlisu (2002), pH values of stuffed mussel samples were between 5.67 and 6.43. Thus, pH values encountered in the present study frequently ranged above the recommended values, but this condition supports the observations made by other authors.

Microbiological attributes of stuffed mussel samples are provided in Tables 3 to 8. TMAB counts of the samples ranged between <1.00 and 4.67 log cfu/g (Tab. 3). Just as with pH, samples from Karşıyaka displayed higher average TMAB counts than those from other districts. Bingöl et al. (2008) reported that TMAB counts of stuffed mussels sold in markets ranged between 2 and 7.5 log cfu/g, i. e. many İzmir samples ranged below these values. According to Tatlisu (2002), the main reasons of the high TMAB count of these products are the poor hygienic conditions of manufacturing, low microbiological quality of raw material and extended holding time under inappropriate conditions prior to consumption.

TC counts ranged between 1.15 and 3.90 log cfu/g (Tab. 4). The mean TC count of the samples consumed in Karşıyaka (1.27 log cfu/g) was significantly higher ($P<0.05$) than means from other districts, with lowest value being registered from Bornova (0.34 log cfu/g). According to ICMSF (1978), the threshold for the coliform bacteria counts in aquacultured bivalves amounts to 2 log cfu/g. When this limit is taken into account (although the present samples did not originate knowingly in aquaculture), “all of the samples are in conformance in terms of coliform bacteria count” In general coliform bacteria were enumerated in 35 % of the samples between the values of 1.15 and 3.90 log cfu/g. Bingöl et al. (2008) revealed that in nearly 78 % of the stuffed

TABLE 4: Average Coliform Bacteria counts of stuffed mussel samples (log cfu/g) (n=100).

District	N_p (Min–Max)	Average _T	Average _p
Bornova	4 (1.15–2.66)	0.34b	2.10
Alsancak	9 (1.30–2.57)	0.74ab	2.07
Konak	9 (1.30–3.56)	0.76ab	2.11
Karşıyaka	13 (1.30–3.90)	1.27a	2.53
Overall	35 (1.15–3.90)	0.78	2.20

N_p = Number of Coliform positive samples; Average_T = Average count of all samples; Average_p = Average count of positive samples; Values with different letters are statistically different from each other ($P<0.05$)

ged between 4 (Bornova) and 24% (Karşıyaka and Alsancak). Mean TFC of positive samples was 1.45 log cfu/g. Stuffed mussel collected from Istanbul markets yielded faecal coliform bacteria at a comparable level (22 % of samples; Bingöl et al., 2008), and Hampikyan et al. (2008) reported a prevalence of 15 %, with, in comparison to the present results, markedly higher counts (2.34 and 4.66 log cfu/g).

As seen in Table 6, *B. cereus* was detected in 20 % of samples at amounts between 1.00 and 3.83 log cfu/g (mean 2.34 log cfu/g). This species may pose a risk for consumers because it can lead to food poisoning and gastrointestinal illness via by several protein toxins and one heat-stable peptide toxin (cereulide), causing the emetic variety of the disease (Ateş et al., 2011). According to CFSSAN (2003), *B. cereus* may cause intoxications when its amount ranges above 6 log cfu/g. The threshold for *B. cereus* in these food-stuffs is 3 log cfu/g (Anon., 2009). It was seen that *B. cereus* counts of only 2 samples were higher than this limit (2 %). As reported by Bingöl et al. (2008), *Bacillus cereus* was detected in almost 39 % of the stuffed mussel samples collected in the İstanbul region.

With regard to other *Enterobacteriaceae*, all *Salmonella* spp. and *E. coli* counts ranged below the threshold of <1.0 log cfu/g. Samples analysed by other authors (Bingöl et al., 2008; Hampikyan et al., 2008) were also negative for *Salmonella*. However, Ates et al. (2011) stated that 50 % of

mussel samples, coliform bacteria were detected.

TFC were detected in 16 % of all samples in amounts varying between 1.00 and 2.45 log cfu/g (Tab. 5); depending on the district, values ran-

TABLE 5: Average Fecal Coliform Bacteria counts of stuffed mussel samples (log cfu/g) (n=100).

District	N_p (Min–Max)	Frequency (%)
Bornova	1 (1.00)	4
Alsancak	6 (1.30–1.95)	24
Konak	3 (1.48–2.23)	12
Karşıyaka	6 (1.00–2.45)	24
Overall	16 (1.00–2.45)	16

N_p = Number of Fecal Coliform positive samples

TABLE 7: Average *S. aureus* counts of stuffed mussel samples (log cfu/g) (n=100).

District	N_p (Min–Max)	Frequency (%)
Bornova	<1	0
Alsancak	3 (2.70–2.85)	12
Konak	1 (2.00)	4
Karşıyaka	<1	0
Overall	4 (2.00–2.85)	4

N_p = Number of *Staphylococcus aureus* positive samples

TABLE 6: Average *B. cereus* counts of stuffed mussel samples (log cfu/g) (n=100).

District	N_p (Min–Max)	Frequency (%)
Bornova	5 (1.00–2.83)	20
Alsancak	7 (1.00–3.83)	28
Konak	4 (2.70–3.00)	16
Karşıyaka	4 (1.00–3.70)	16
Overall	20 (1.00–3.83)	20

N_p = Number of *Bacillus cereus* positive samples

TABLE 8: Average *Vibrio* spp. counts of stuffed mussel samples (log cfu/g) (n=100)..

District	N_p (Min–Max)	Frequency (%)
Bornova	5 (2.15–3.71)	20
Alsancak	3 (1.18–7.07)	12
Konak	3 (1.09–7.07)	12
Karşıyaka	2 (1.18–1.36)	8
Overall	13 (1.09–7.07)	13

N_p = Number of *Vibrio* spp. positive samples

the stuffed mussels were unsuitable for consumption because of their high *Salmonella* count. During the same research, *E. coli* was detected in 30 % of the samples.

The presence of *S. aureus* is an important indicator for poor hygienic conditions and lack of sanitation applications (Jay, 1990). So, *S. aureus* counts of foods should be below the limit of 2 log cfu/g as stated in Turkish Food Codex Communication for ready-to-eat Foods (Anon., 2009). During the present analyses, *S. aureus* was detected in only four out of 100 samples (Tab. 7). While Karşıyaka and Bornova samples resulted negative, the mean *S. aureus* counts in the remaining districts were <1.00 log cfu/g, meaning that this criterion was met. This however contrasts to the results obtained by Bingöl et al. (2008) who encountered this pathogen in nearly 24 % of samples collected in İstanbul region, and those of Ateş et al. (2011) who reported that that 77 % of their samples were unsuitable for consumption because of their high *S. aureus* counts.

Finally, Table 8 provides the results regarding *Vibrio* spp. counts. It was detected in 13 samples out of 100, with counts between 1.09 and 7.07 log cfu/g. According to the Turkish Food Codex, *Vibrio* spp. should not be detected in ready-to-eat foods (0 cfu/25 g). So, 13 % of samples did not comply with this legal requirement. The presence of this pathogenic genus in seafood indicates an insufficient heat treatment. Since *Vibrio* spp. are inhibited after heating the seafood at 60 °C for 15 minutes, the purchased positive samples must have been submitted to an inadequate heating procedure.

Conclusion

The microbiological quality of stuffed mussels sold in streets of four larger districts in İzmir is quite poor. In many samples, several hygiene criteria as established by the national food authorities were not met. However, results from other authors showed an even more drastic situation in other areas of Turkey. These ready-to-eat foods produced and sold under poor hygienic conditions might be an important concern in terms of food safety and consumer health as they can be a major cause of food poisoning and foodborne diseases.

The results of this research showed that drastic measures should be taken to ensure that processing and marketing conditions of stuffed mussels become improved and controlled more effectively in order to make this traditional foodstuff safer.

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