

Microbiological quality of ice and ice machines used in food establishments

Hamparsun Hampikyan, Enver Baris Bingol, Omer Cetin and Hilal Colak

ABSTRACT

The ice used in the food industry has to be safe and the water used in ice production should have the quality of drinking water. The consumption of contaminated ice directly or indirectly may be a vehicle for transmission of pathogenic bacteria to humans producing outbreaks of gastrointestinal diseases. The objective of this study was to monitor the microbiological quality of ice, the water used in producing ice and the hygienic conditions of ice making machines in various food enterprises. *Escherichia coli* was detected in seven (6.7%) ice and 23 (21.9%) ice chest samples whereas *E. coli* was negative in all examined water samples. Psychrophilic bacteria were detected in 83 (79.0%) of 105 ice chest and in 68 (64.7%) of 105 ice samples, whereas Enterococci were detected only in 13 (12.4%) ice samples. Coliforms were detected in 13 (12.4%) water, 71 (67.6%) ice chest and 54 (51.4%) ice samples. In order to improve the microbiological quality of ice, the maintenance, cleaning and disinfecting of ice machines should be carried out effectively and periodically. Also, high quality water should be used for ice production.

Key words | food establishments, ice, ice machine, ice microbiology, ice quality

Hamparsun Hampikyan
Department of Gastronomy and Culinary Arts,
Beykent University, The School of Applied
Sciences,
Istanbul 34500,
Turkey

Enver Baris Bingol (corresponding author)
Omer Cetin
Hilal Colak
Department of Food Hygiene and Technology,
Faculty of Veterinary Medicine,
Istanbul University,
Istanbul 34320,
Turkey
E-mail: bingolb@istanbul.edu.tr

INTRODUCTION

Ice is used in large quantities as refreshment in alcoholic and non-alcoholic beverages, especially in spring and summer time, and is the raw material in ice slush production. Ice is also used in foods such as compote, sliced fruits, fruit salads and cacık (a Turkish appetizer which is a mixture of cucumber and yoghurt) to keep them cool. Furthermore, ice is commonly used to retain the freshness of various foods, especially fish and fishery products, which deteriorate rapidly during distribution, cold storing, display, service and whole/retail sales (Vieira *et al.* 1997; Rodriguez *et al.* 2006; Gerokomou *et al.* 2011). Because ice abates the product temperature and slows down the growth of microorganisms, it is used effectively in the poultry industry for lowering the carcass temperature and therefore, it reduces the number of carcass-associated bacteria (Berrang *et al.* 2008; Northcutt & Smith 2009). Moreover, ice is used in

the meat industry to decrease the temperature of sausage dough during meat chopping in bowl cutters. Because ice is used in large quantities in most aspects, the enterprises may use their own ice making machines for producing ice, placed in their production areas.

The water can contain harmful bacteria (*Salmonella* spp., *Escherichia coli* etc.), viruses (hepatitis A, hepatitis E etc.) and parasites (Cryptosporidium, Giardia etc.) that cause water-borne diseases and outbreaks which affect thousands of people from various regions of the world (Cetin *et al.* 2013; Frickmann *et al.* 2013; Adell *et al.* 2016; Wankar *et al.* 2016). The World Health Organization has declared that ice which is to be consumed or which is in contact directly with food is expected to be at the same quality and safety level as drinking water (WHO 1997). However, a number of bacteria can be found in water and also in ice that originate

from contaminated water. Therefore, if poor quality water is used in ice production, it is probable that the beverages and various foods that contact the contaminated ice will be contaminated with pathogenic bacteria (Falcao *et al.* 2002, 2004). Contrary to popular opinion, acidic and alcoholic beverages are not enough to inhibit the pathogenic bacteria which can be found in contaminated ice (Mako *et al.* 2014). Dickens *et al.* (1985) investigated the survival of several bacterial enteropathogens in the ice of popular drinks (cola, scotch and tequila) and concluded that none of the organisms were completely eliminated.

Besides the use of contaminated water in ice production, hygiene deficiencies in ice producing and handling processes, inadequate knowledge of staff about cleaning, the design and the cleanliness of ice machines are important factors in acquiring contaminated ice. This case adds a special interest to periodic maintenance and hygienic design of these machines and periodic training of relevant staff (Wilson *et al.* 1997; Hertin 2005; Chavasit *et al.* 2011).

The consumption of contaminated ice directly or indirectly may be a vehicle for transmission of pathogenic bacteria to humans producing outbreaks of gastrointestinal diseases. Outbreaks of gastroenteritis according to the consumption of contaminated ice have been reported in different regions of the world by a number of researchers and the Centers for Disease Control and Prevention (Anonymous 1990; Cannon *et al.* 1991; Quick *et al.* 1992; Pawsey & Howard 2001; Pedalino *et al.* 2003). In recent years, Young-Eun *et al.* (2015) isolated Enterotoxigenic *E. coli* ST/LT from seven of 28 people (26 baseball club students, two food handlers) and the researchers concluded that the contaminated ice cubes and ice making machines and eating ice cubes from the machines were the major risk factors for the mentioned outbreak.

The studies showed that *E. coli*, coliforms and other various bacteria can be found in ice and since the freezing process cannot eliminate these microorganisms, many of them may survive in ice. Therefore, when the ice thaws in beverages or foods, microorganisms may be able to recover their viability and if the ice is produced from contaminated water, there may be a chance of causing infection in consumers (FEHD 2005; Mako *et al.* 2014).

The relationship between contaminated ice and human diseases emphasizes the importance of studies to gain

knowledge about the hygienic condition of ice machines and ice used in the food sector. In Turkey, data on the microbiological quality of ice used in the food industry are very limited and studies about the hygienic conditions of ice making machines are not available. The objective of this first comprehensive study is to monitor the microbiological quality of ice, the water used in producing ice and the hygienic conditions of ice making machines in various food service enterprises.

METHODS

Samples

The water, ice and surface of ice machine samples were collected from 75 restaurants/fast food restaurants, 20 bars and 10 fish markets located in different regions of Istanbul. All the establishments were independent in operation. The bars were snack bars and the fish markets were public retail markets.

Water samples

A total of 105 water samples from the water inlet of ice making machines were collected from 75 restaurants/fast food restaurants, 20 bars and 10 fish markets aseptically into 1,000 mL sterile bottles.

Ice samples

Ice samples weighing 1,000 g were obtained directly from 105 ice making machines (75 restaurants/fast food restaurants, 20 bars and 10 fish markets) and sealed in sterile polyethylene bags. Water and ice samples were transported to the laboratory under cold conditions (<4 °C) as quickly as possible.

Surface samples

A total of 105 surface samples were collected from 75 restaurants/fast food restaurants, 20 bars and 10 fish markets. For surface sampling, a 10 × 10 cm aluminium template was used to swab 100 cm² areas on three sites of the ice chests

(left, right and bottom parts) yielding a total of 300 cm² area. For template sterilization, first it was dipped into 70% ethanol and then inflamed before each sampling process. Sterile cotton swabs (Cultiplast, Tampone Swab, Milano-Italy) were moistened in 10 mL of sterile saline peptone water (OXOID CM0009) and the chest surface swabbed in horizontal and vertical directions using the template. The swabs were plated immediately after the surface sampling process on-site to the relevant growth media.

Microbiological analysis

Ice and water samples

Ice samples were stored at 4 °C until completely thawed and then analysed for *E. coli*, coliform, enterococci, psychrophilic and Total Aerobic Mesophilic Bacteria (TAMB), and water samples (from the water inlet of the ice machine) were analysed for *E. coli*, coliform, enterococci and TAMB.

The *E. coli*, coliform, and enterococci analyses were carried out using the membrane filtration technique signified by Sartorius Membrane Filtration Manual (Sartorius Stedim Biotech 2009) and the psychrophilic bacteria and TAMB were detected with using conventional plate count methods as proposed by Falcao et al. (2002). For *E. coli* and coliform bacteria, a 100 mL sample was filtered through a 0.45 µm sterile membrane (Sartorius Nutrient Pad Set 13906) which retained the microorganism. Then the membrane was placed in Tergitol TTC NPS (Sartorius 14056) a dehydrated media and incubated at 36 ± 2 °C for 21 ± 3 h. Yellow colonies with yellow surroundings counted as *E. coli* and red colonies with yellow dots under the membrane filter counted as coliform. For enterococci a 100 mL sample was filtered through a 0.45 µm sterile membrane (Sartorius Nutrient Pad Set 13806) and placed in Azide NPS (Sartorius 14051), a dehydrated media, and incubated at 36 ± 2 °C for 44 ± 4 h. The red, pink or reddish brown colonies with 0.5–2 mm colonies counted as enterococci. TAMB and psychrophilic bacteria were determined by plating 1 mL Plate Count Agar (OXOID CM0325) and incubating at 37 °C for 48 h and 4 °C for 10 days respectively. Serial dilutions of the samples were prepared using 0.1% sterile saline peptone water and all samples were analysed in duplicate.

Surface samples

After collecting the surface samples, the swabs were applied into related medium immediately. For coliforms, Violet Red Bile Lactose Agar (OXOID CM0107) plates were used and incubated at 36 °C for 48 h, for *E. coli* Tryptone Bile X-Glucuronide Medium (OXOID CM0945) plates were incubated at 44 °C for 24 h. For TAMB and psychrophilic bacteria, PCA (OXOID CM0325) plates were incubated at 35 °C for 48 h and 2 °C for 48 h, respectively.

RESULTS

The results of microbiological analysis of water, ice chest and ice samples are shown in Tables 1–3. TAMB were detected in 85 out of 105 (80.9%) water samples and ranged between 1.58 and 6.94 log₁₀ colony forming unit (cfu)/mL, 98 out of 105 (93.3%) ice samples ranged between 1.87 and 6.81 log₁₀ cfu/mL and all ice chest samples (100.0%) ranged between 48 and 214 cfu 300 cm². Psychrophilic bacteria were detected in 83 (79.0%) of 105 ice chest and in 68 (64.7%) of 105 ice samples. Coliforms were detected in 13 (12.4%) water, 71 (67.6%) ice chest and 54 (51.4%) ice samples. On the other hand, *E. coli* was detected in seven (6.7%) ice and 23 (21.9%) ice chest samples, whereas *E. coli* was negative in all examined water samples. Enterococci were detected only in 13 (12.4%) ice samples (Tables 1 and 2 and Figure 1). According to Turkish regulations about the water intended for human consumption, the limits for coliforms, *E. coli* and Enterococci in drinking and potable water are 0/100 mL (Anonymous 2005). Consequently, 13 water and 54 ice samples exceeded the Turkish limits in terms of coliforms, and seven and 13 ice samples were unacceptable in terms of *E. coli* and Enterococci, respectively.

DISCUSSION

The relationship between contaminated water, unhygienic ice machines and human diseases highlights the significance of studies to obtain information about the hygienic quality of ice. However, in Turkey, data on the microbiological

Table 1 | Positive counts and percentages of water, ice and ice machine samples

	Sample	N	TAMB		Psychrophilic bacteria		Coliform		E. coli		Enterococci	
			(n)	(%)	(n)	(%)	(n)	(%)	(n)	(%)	(n)	(%)
Restaurant/Fast food	Water	75	62	82.7	NA	NA	9	12.0	ND		ND	ND
	Ice chest	75	75	100	63	84.0	49	65.3	14	18.7	NA	NA
	Ice	75	71	94.7	52	69.3	36	48.0	3	4.0	6	8.0
Bar	Water	20	16	80.0	NA	NA	3	15.0	ND	ND	ND	ND
	Ice chest	20	20	100	14	18.6	14	70.0	3	15.0	NA	NA
	Ice	20	18	90.0	11	55.0	11	55.0	1	5.0	2	10.0
Fish markets	Water	10	7	70.0	NA	NA	1	10.0	ND	ND	ND	ND
	Ice chest	10	10	100	6	60.0	8	80.0	6	60.0	NA	NA
	Ice	10	9	90.0	5	50.0	7	70.0	3	30.0	5	50.0
Total	Water	105	85	80.9	NA	NA	13	12.4	ND	ND	ND	ND
	Ice chest	105	105	100	83	79.0	71	67.6	23	21.9	NA	NA
	Ice	105	98	93.3	68	64.7	54	51.4	7	6.7	13	12.4

N: Analysed sample number; n: Positive sample number; ND: Not detected; NA: Not analysed.

Table 2 | Minimum, maximum and average microbial counts of positive water and ice samples

Sample	Establishment	TAMB (log ₁₀ cfu/mL)			Psychrophilic bacteria (log ₁₀ cfu/mL)			Coliform (log ₁₀ cfu/mL)			E. coli (log ₁₀ cfu/mL)			Enterococci (log ₁₀ cfu/mL)		
		Min	Max	Avrg	Min	Max	Avrg	Min	Max	Avrg	Min	Max	Avrg	Min	Max	Avrg
Water	Restaurant/Fast food	1.58	6.09	3.51				1.22	2.32	1.70						
	Bar	2.16	6.94	4.88		NA		1.85	3.15	2.79		ND			ND	
	Fish markets	2.62	6.33	5.10				1.95	1.95	1.95*						
Ice	Restaurant/Fast food	1.93	5.93	2.80	1.25	2.65	1.93	1.91	3.44	2.03	1.08	1.71	1.46	1.28	4.22	3.43
	Bar	2.87	6.81	5.08	1.48	4.70	3.12	2.35	4.79	3.42	1.65	1.65	1.65*	1.50	5.93	3.71
	Fish markets	3.48	6.57	5.62	1.62	3.87	3.01	2.97	5.27	4.67	1.37	2.32	1.76	1.33	4.57	3.18

NA: Not analysed; ND: Not detected (<10).

*One positive sample.

Table 3 | Minimum, maximum and average microbial counts of positive ice chest surface samples (cfu 300 cm², sum of three petri dishes)

Sample	Establishment	TAMB			Psychrophilic bacteria			Coliform			E. coli		
		Min	Max	Avrg	Min	Max	Avrg	Min	Max	Avrg	Min	Max	Avrg
Ice chest	Restaurant/Fast food	48	185	128	9	85	54	7	81	36	1	22	6
	Bar	55	166	144	17	93	63	12	69	32	1	18	8
	Fish markets	70	214	153	20	106	71	11	85	48	3	25	11

contamination of ice are quite limited. In a study conducted by Isik (2005), *E. coli* contamination was found in seven out of 70 ice samples collected from bars. The *E. coli* counts correlated well with ours.

A number of studies about the microbiological quality of ice are available in the literature worldwide. Lateef

et al. (2006) conducted research into the microbiological quality of ice used to cool drinks and foods in Ogbomoso, Nigeria. The researchers found that the ice samples collected from ice manufacturing plants and retail outlets were contaminated with various bacteria (*Pediococcus cerevisiae*, *Bacillus subtilis*, *Streptococcus pyogenes*, *Bacillus*

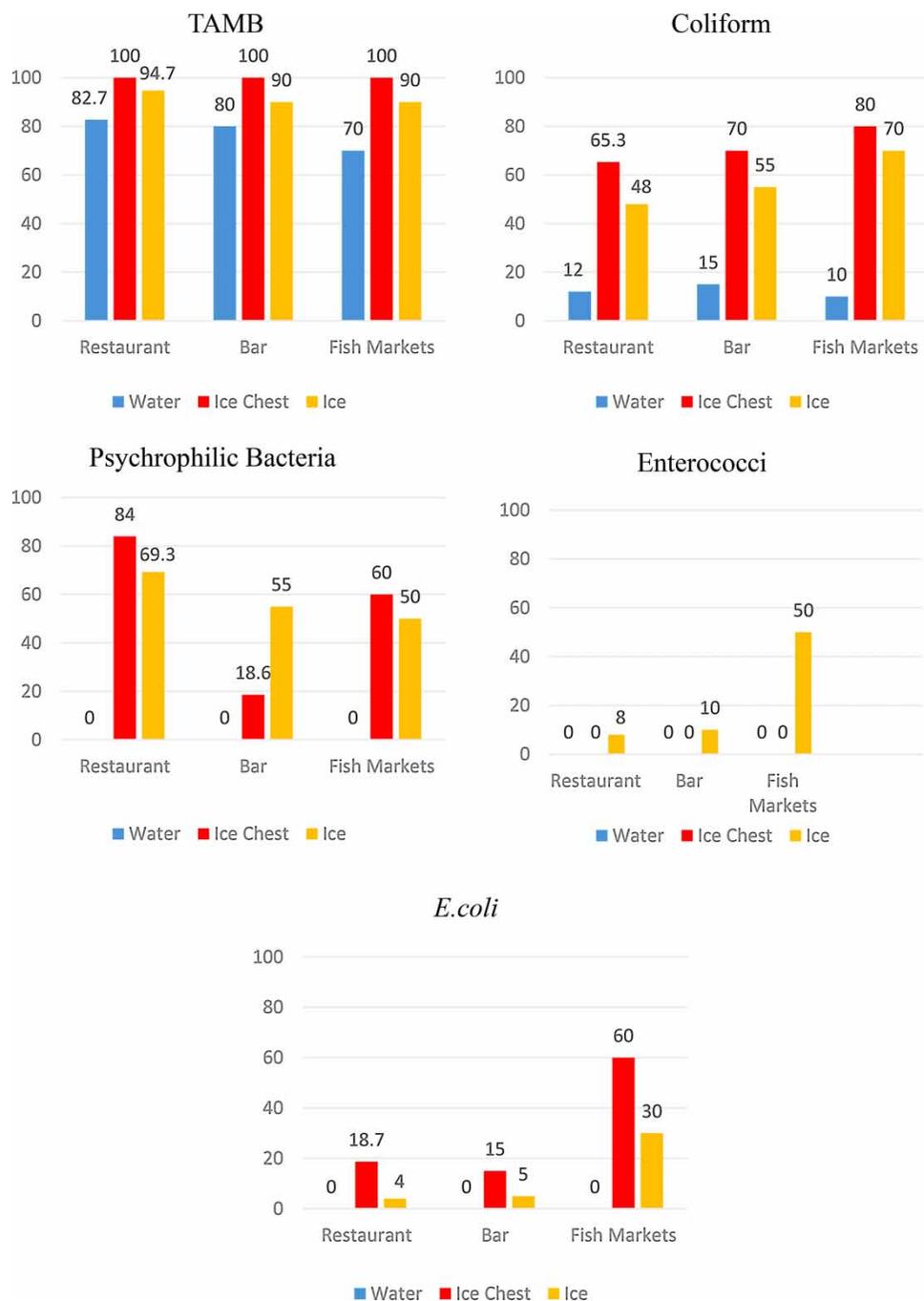


Figure 1 | The percentage of positive counts (%).

firmus, *Pseudomonas aeruginosa*, *Streptococcus equi*, *Staphylococcus epidermidis* and *Micrococcus luteus*) ranging from 1.88 to 3.20×10^{-4} cfu/mL. Chavasit et al. (2011) investigated the surfaces of ice collecting bins, manual packaging machines and the conveyor part of the ice machine and

determined the total coliform counts as 7.3, 56.9 and 7.3 MPN/10.16 cm², respectively. In another study, Gerokomou et al. (2011) reported that ice samples were contaminated with total coliforms (37%), fecal coliforms (25%) and *E. coli* (15%) ranging between 1–95, 1–100 and

1–50 cfu/100 mL, respectively. In a similar research conducted by Vieira *et al.* (1997), the ice samples were collected from fish stalls and TAMB counts ranged between 10 and 2,700 cfu/mL and fecal coliform counts ranged between 0 and 1,100 MPN/mL. Mako *et al.* (2014) reported that 64 (43%) and 26 (17.5%) retail ice samples, 29 (28.7%) and six (5.9%) vending machine ice samples were unacceptable in terms of coliforms and enterococci, respectively. Wilson *et al.* (1997) examined 194 ice samples and found coliforms in 60 and *E. coli* in three samples. In addition to this, the authors reported TAMB counts ranged between <10 to <3 × 10³ cfu/mL. In a similar study conducted by Jang & Lee (2015), ice samples were collected from restaurants/food stores in different areas in Korea. The researchers detected total aerobic bacteria counts ranging between 1.83 and 2.31 log cfu/g, meanwhile one sample was contaminated with *E. coli* (<1 log cfu/g) and three samples with coliforms (two samples <1 log cfu/g and one sample 1–2 log cfu/g). In another study performed by Schmidt & Rodrick (1999), coliform counts exceeding the regulatory limit (<1/100 mL) were observed in 13.5% of examined ice samples. Nichols *et al.* (2000) investigated 4,346 ice samples collected from different food establishments and detected coliforms in 317, *E. coli* in 35 and enterococci in 35 samples at the levels of >10² cfu/100 mL. Similar research was conducted on commercially bagged ice by Falcao *et al.* (2002) and *E. coli* was isolated from 23 out of 60 analysed ice samples. Contrary to this, it was stated that *E. coli* was not found in unused ice by Northcutt & Smith (2009) in a poultry establishment. However, the researchers detected the mean levels of TAMB, coliforms and *Enterobacteriaceae* in ice samples as 0.3, 0.4 and 0.4 log₁₀ cfu/mL, respectively.

Data from the present study demonstrate that ice samples had undesirable hygienic conditions due to the presence of indicator microorganisms such as coliforms and *E. coli*. Possibly the water supply was not the main source of contamination and the transmission of bacteria to ice corresponds with ice making machines which can play an important role, based on irregular cleaning of the water tank and ice chest, infrequent cleaning and maintenance intervals and untrained personnel. On the other hand, environmental contaminations such as ambient air, equipment (ice clip, buckets etc.) should be considered as

important potential sources of ice contamination. Especially in restaurants, the ice is usually held in coverless ice buckets and in refrigerators and this situation is favourable for environmental contamination. Besides this, the personnel involved in ice are not trained enough in matters of personal hygiene and this situation can be evaluated as another important contamination method of ice with enteric bacteria.

CONCLUSIONS

Worldwide, ISO 22000 and hazard analysis and critical control points (HACCP) are the food safety management systems that can be used frequently and effectively by various food industries along with good manufacturing practices (GMPs) and good hygiene practices (GHPs) to enhance food and water safety and to protect public health. These systems include pre-requisites such as considering the food handling, hygiene and cleaning (the conditions of cleanliness and sanitation of the equipment and supplies), pest control, storage, water supply (ensuring the water quality) etc. (Lateef *et al.* 2006). In the light of these data, the quality of water used for different purposes in various food establishments has to be ensured by GMPs and GHPs.

In conclusion, in order to improve the microbiological quality of ice, pre-requisites should be performed. For this purpose, the maintenance, cleaning and disinfecting of ice machines should be carried out effectively and periodically. High quality water should be used for ice production; additionally the water should be sanitized by using sufficient amounts of chlorine or other proper methods such as UV and ozone treatments. Routine training should be given to relevant staff regarding hygiene matters and maintenance of ice machines. Furthermore, periodic surveys should be performed regarding the microbiological quality of ice, the hygienic conditions of ice machines and the hygiene of employees as a necessity of HACCP and ISO 22000 food safety management systems.

Nowadays, quality assurance standards and guidelines are widely applied in the food industry in many countries. For this reason the HACCP plan should be developed and applied for ice production processes. In addition to this,

microbiological standards for ice should be determined and regular inspection by proper authorities should be established for consumer's health in order to prevent the risk of exposure to pathogenic bacteria.

Several studies have focused on the microbiological quality of ice or just the water that ice was produced from. This is a comprehensive study that investigates not only the microbiological quality of ice, but also the water used in producing ice and the hygienic conditions of ice making machines in various food establishments such as restaurants/fast food restaurants, bars and fish markets. Consequently, even if the water used in ice production is appropriate for WHO's criteria, during the different steps of its production, ice can be contaminated by hazardous biological agents which can pose various risks for consumers. Taking this into consideration, the results of the current research provide significant data that demonstrate the possible pathways of ice contaminations.

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