Tropical Biomedicine 32(3): 471–477 (2015)

Presence of faecal coliforms and selected heavy metals in ice cubes from food outlets in Taman Universiti, Johor Bahru, Malaysia

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Received 17 July 2014; received in revised form 6 January 2015; accepted 9 January 2015

Abstract. Consumption of iced beverages is common in Malaysia although specific research focusing on its safety parameters such as presence of faecal coliforms and heavy metal elements remains scarce. A study conducted in Kelantan indicated that faecal coliforms were detected in the majority of the ice cube samples analyzed, largely attributable to improper handling. Hence, it was found pertinent to conduct similar study in other parts of the country such as Johor Bahru if the similar pattern prevailed. Therefore, this present cross-sectional study which randomly sampled ice cubes from 30 permanent food outlets in Taman Universiti, Johor Bahru for detecting contamination by faecal coliforms and selected heavy metal elements (lead, copper, manganese and zinc) acquires significance. Faecal coliforms were detected in 11 (36.67%) of the samples, ranging between 1 CFU/100 mL to > 50 CFU/100 mL; two of the samples were grossly contaminated (>50 CFU/100 mL). Interestingly, while positive detection of lead was observed in 29 of the 30 ice cube samples (mean: 0.511±0.105 ppm; range: 0.489-0.674 ppm), copper, manganese and zinc were not detected. In addition, analysis on commercially bottled mineral water as well as in tap water samples did not detect such contaminations. Therefore, it appears that (1) contamination of faecal coliforms in ice cubes in food outlets in Malaysia may not be sporadic in pattern but rather prevalent and (2) the source of water used for manufacturing the ice cubes that contained significant amount of lead would suggest that (3) it was neither originated from the treated tap water supply nor bottled mineral water or (4) perhaps contaminated during manufacturing process. Further studies exploring the source of water used for manufacturing these ice cubes as well as the handling process among food operators deserve consideration.

INTRODUCTION

Studies have indicated that contamination of ice cubes by pathogenic microorganisms such as faecal coliforms particularly, *Escherichia coli* may lead to considerable health problems (Wilson *et al*., 1997; Graman *et al*., 1997; Nichols *et al*., 2000; Falcao *et al*., 2004; Lateef *et al*., 2006) such as food poisoning (Wilson *et al*., 1997; Falcao *et al*., 2004) as well as nosocomial infection (Graman *et al*., 1997). Despite such importance, ice cube analysis has not been incorporated as one of the parameters in the hygiene and safety inspection of water and foods by the local authority (Zaliha & Abdullah, 2004). Additionally, the specific national microbiological and chemical guidelines for analyzing ice cubes for human consumption remains lacking in many countries (Lateef *et al*., 2006) including Malaysia (Noor Izani *et al*., 2012). Interestingly, a study conducted in Kelantan revealed that contamination of faecal coliforms ranging between 1 CFU/100mL to >50 CFU/100mL in ice cubes was detected in 53% of food outlets included in that study, even from a food outlet accredited by the District Health Office as 'grade A' in its hygienic standard and microbiological quality of food score (Noor Izani *et al*., 2012). Since specific studies focusing on the presence of faecal coliforms in ice cubes remain scarce in Malaysia, while the only study available in this aspect conducted in Kota Bharu, Kelantan (Noor Izani *et al.*, 2012) revealed an alarming result, it was found pertinent to conduct similar studies in other parts of Malaysia to investigate if the same pattern of faecal contamination prevailed. Since ice cubes for food outlets in Malaysia are commonly supplied by small-scale local vendors, the source of water used for manufacturing those ice cubes remains unclear (Noor Izani *et al.*, 2012). While water sources may be contaminated by faecal coliforms and chemicals such as heavy metals due to industrial seepage as well as geographical rock formation (Al-Mezori & Hawrami, 2013) and the fact that regulation by the relevant authorities on ice cube manufacturers remains scarce; the possibility that such contaminated water sources are used for producing ice cubes could not be ruled out. Due to such ambiguity, this present study included for the first time, the detection of commonly found heavy metals in untreated water sources (lead, copper, manganese and zinc) in the ice cube samples.

This present cross-sectional study was aimed at investigating the presence of faecal coliforms and selected heavy metal elements viz. lead, copper, manganese and zinc in the commercially available ice cubes, which were randomly sampled from 30 permanent food outlets in Taman Universiti, Johor Bahru. The data gathered in this study would prove useful for providing, for the first time, information on status of contamination of ice cubes by faecal coliforms as well as selected heavy metal elements in Johor that may elucidate any potential health implication following the ingestion of ice cubes *via* iced-beverages within this region.

MATERIALS AND METHODS

Experimental design

This cross sectional research was conducted in Taman Universiti, Johor Bahru during April 2014. All the permanent food outlets (47) within Taman Universiti commercial area were mapped and labeled, from which a total of 30 permanent food outlets were randomly chosen. For the purpose of standardization, temporary food premises such as night market and stalls as well as those permanent food outlets that did not sell iced beverages were not included in this present research. A sample of ice cubes (at least 0.5 liter) obtained aseptically from each food outlet was examined for the presence of faecal coliforms as well as selected heavy metal elements viz. lead, copper, zinc and manganese. In addition, samples of tap water within the Johor Bahru Campus of Universiti Teknologi Malaysia as well as commercially bottled mineral water were included as Control 1 and Control 2, respectively. All the samples were placed in a sterile cold box, transported immediately to the laboratory and analyzed within 3-4 hours after collection. Prior to analysis, the samples were left at room temperature (about 25°C) to melt. In the laboratory, each sample was divided into two parts that consisted of 0.3 and 0.2 L portions for microbiological and chemical analyses, respectively and the analyses were conducted in triplicates.

Faecal Coliforms Count

Being a selective and differential medium, MacConkey agar inhibits the growth of gram positive microorganisms, while it allows the growth of the hardier gram negative rods. In this research, the MacConkey agar was prepared in a biosafety laminar flow cabinet following the prescribed instruction provided by the manufacturer. MacConkey powder (51.5 g) was suspended in distilled water (1 L), mixed thoroughly and stirred using a glass rod. The mixture was gradually heated and boiled for dissolving the powder followed by autoclaving at 121°C for 15 minutes and upon completion, the solution was poured into a sterile plate.

Once the ice cubes melted, a volume (100 mL) of sample was mixed thoroughly by inverting the container for several times followed by filtration through a sterile cellulose acetate membrane filter (pore size: 0.45 µm; diameter: 47 mm) attached to a vacuum pump. Replicating the above procedure, three replicates were used for each sample. Then, the cellulose acetate membrane was removed from the chamber using a sterile forcep and placed in the central part of the MacConkey agar followed by incubation at 44.5°C for 24 hours. Being lactose fermenters, the thermotolerant faecal coliforms (*E. coli*) formed observable red or pink colonies surrounded by precipitated bile. Once the incubation process was completed, the cellulose acetate membrane was investigated for the presence of small and smooth convex red/pinkish lactose fermenting colonies, which were 1-3 mm in diameter (Cheesbrough, 2006) and the number of such colonies was recorded. Following the suggestion made by Cheesbrough (2006), whenever the number of colonies observed exceeded that of 50, such confluent growth was reported as 'numerous to count' i.e. >50 CFU/100 mL. For verifying the presence of faecal coliforms over other types of possible enteric pathogens the Eosin-Methylene Blue (EMB) agar and IMViC test were utilized following the procedure prescribed by Cheesbrough (2006).

Heavy Metals Analysis

Stock solutions (1000 ppm) of lead, copper, zinc, and manganese were prepared from the commercial standard solutions of $Pb(NO_3)_2$, $CuSO_4.5H_2O$, $ZnSO_4.7H_2O$ and $MnSO_4.H_2O$, respectively using distilled water. A series of 5 standards solutions (0.05, 0.25, 0.5, 1.0, 1.5 ppm) for each of the analyte viz. lead, copper, zinc, and manganese were prepared by serial dilutions using distilled water, while in blanks distilled water alone was used. Analysis of these heavy metal elements in ice cube samples were conducted using Atomic Absorption Spectrometer (AAS) (Perkin Elmer, USA).

Calibration curves for lead, copper, zinc, and manganese were prepared by spiking the blank samples (distilled water) with the known amount of the standards (0.05, 0.25, 0.5, 1.0, 1.5 ppm) and a calibration curve was accepted only when (1) the regression was at least 0.995 and (2) the coefficient variation (CV%) was lesser than 20% (National Poison Centre, 2006; Man *et al.*, 2006). It has been indicated that limit of detection (LOD) is the lowest concentration of an analyte determined with signal to noise ratio of at least 3:1, while limit of quantitation (LOQ) refers to the lowest concentration of an analyte in a calibration curve with signal to noise ratio of at least 5:1 (Bramley *et al.*, 2008). In this present research, the LOD and LOQ for all of the analytes were mathematically determined from the calibration curves following the formula described by previous researcher (Sanagi *et al.*, 2009) and details on the calibration curves, LOD and LOQ are presented in Table 1. While Perkin-Elmer Intensitron® hollow cathode lamps were used for analyzing manganese, copper, and zinc, Perkin-Elmer System 2 electrodeless discharge lamps (EDL) were used for lead. The analytical condition of the AAS used for this research is presented in Table 2. Once the ice cubes were fully melted, the samples were directly introduced into the AAS for analysis and the results obtained were compared with the maximum permitted

Table 1. Calibration parameters, LOD and LOQ for heavy metal elements analyzed

Heavy Metal Elements	Range (ppm)	Calibration Curves		LOD (ppm)	LOQ (ppm)
		Coefficient of Determination (r^2)	Equation		
Lead	0.05-1.50	0.998	$y = 0.0253x - 0.0005$	0.05	0.15
Copper	0.05-1.50	0.995	$y = 0.0245x + 0.0006$	0.05	0.20
Manganese	0.05-1.50	0.996	$y = 0.0243x + 0.0001$	0.05	0.23
Zinc	0.05-1.50	0.998	$y = 0.0249x - 0.0006$	0.05	0.28

LOD: Limit of Detection
LOQ: Limit of Quantitation

Table 2. Analytical conditions for AAS

Parameters	Conditions
Technique	Flame
Fuel type	Air-acetylene
Temperature	2300ºC
Acetylene flow	4 L/minute
Wavelength (nm)	283.3 (Pb); 324.8 (Cu); 279.5 (Mn); 279.5 (Zn)
Slit (nm)	Pb: 0.5 (Pb); 0.5 (Cu); 0.2 (Mn); 1.0 (Zn)

proportion prescribed in the Twenty-Fifth Schedule [sub regulations 360B(3) and 360C(3)]) of the Food Act 1983 (Act 281) & Regulations (2013).

RESULTS

Based on the preliminary survey conducted, 47 qualified permanent food outlets were identified within the Taman Universiti commercial area and 30 of the food outlets were randomly selected. All the 30 food operators indicated that the ice cubes were not produced in-house but they were purchased from several commercial vendors. Results on faecal coliform counts and heavy metal elements in those samples are presented in Table 3. Despite the legal requirement for displaying their scoring grades on the assessment of hygienic standard of food premises and microbiological quality of food, none of them complied. Presence of faecal coliforms was detected in ice cube samples in 11 (36.67%) of the 30 food outlets, ranging between 1 CFU/100mL to >50 CFU/100mL, while such contamination was not detected in the commercially bottled mineral water as well as in tap water samples obtained within Universiti Teknologi Malaysia (Table 3). Alarmingly, ice cube samples in two food outlets (No. 9 and 21) were found as grossly polluted with faecal coliforms (>50 CFU/100 mL), in addition to the unacceptable presence of faecal coliforms in the remaining nine samples of ice cubes (Table 3). Interestingly, while lead (mean: 0.511±0.105 ppm) was found in ice cube samples obtained from 29 of the permanent food outlets (0.489 ppm to 0.674 ppm); copper, manganese and zinc were evidently non detectable in any of the samples (Table 3).

DISCUSSION

Faecal coliform count or sometimes referred to as thermotolerant coliform organisms or *E. coli* has been frequently used for monitoring faecal contamination in water due to its specificity and ease of analysis (Cheesbrough, 2006; World Health Organization, 2008). Many studies have associated the presence of faecal coliform as well as a variety of microorganisms in ice cubes with health problems (Wilson *et al.*, 1997; Falcao *et al.*, 2004) and transmission of pathogens in hospitals (Graman *et al.*, 1997), which may prove detrimental towards human health. While studies on contamination of manufactured edible ice cubes in foods and drinks are gradually gaining popularity (Nichols *et al.*, 2000; Falcao *et al.*, 2004; Lateef *et al.*, 2006), review of the literature reveals that there has been only one specific research focusing on this aspect reported in Malaysia i.e. Noor Izani *et al.* (2012). They (Noor Izani *et al.*, 2012) reported that 53% of the ice cube samples obtained from permanent food outlets in Kubang Kerian (Kelantan) were contaminated with faecal coliforms. Since it has been indicated that specific national microbiological and chemical guidelines for ice cubes for human consumption remain lacking in many countries (Lateef *et al.*, 2006) including Malaysia and the fact that ice cubes added to beverages melt and directly ingested while drinking, they too should be of the same quality as drinking water (Noor Izani *et al.*, 2012). In this context, it has been

Table 3. Presence of faecal coliforms as well as selected heavy metal elements in all the ice cube samples

Food outlets	Faecal coliform counts (CFU/100mL)	Lead	Copper	Manganese	Zinc
No. 1	ND	0.597	Not detected	Not detected	Not detected
No. 2	ND	0.523	Not detected	Not detected	Not detected
No. 3	ND	0.511	Not detected	Not detected	Not detected
No. 4	3	0.522	Not detected	Not detected	Not detected
No. 5	ND	0.489	Not detected	Not detected	Not detected
No. 6	ND	0.517	Not detected	Not detected	Not detected
No. 7	7	Not detected	Not detected	Not detected	Not detected
No. 8	ND	0.674	Not detected	Not detected	Not detected
No. 9	>50	0.569	Not detected	Not detected	Not detected
No. 10	1	0.547	Not detected	Not detected	Not detected
No. 11	ND	0.520	Not detected	Not detected	Not detected
No. 12	4	0.536	Not detected	Not detected	Not detected
No. 13	19	0.511	Not detected	Not detected	Not detected
No. 14	ND	0.522	Not detected	Not detected	Not detected
No. 15	1	0.501	Not detected	Not detected	Not detected
No. 16	ND	0.511	Not detected	Not detected	Not detected
No. 17	ND	0.552	Not detected	Not detected	Not detected
No. 18	ND	0.497	Not detected	Not detected	Not detected
No. 19	12	0.516	Not detected	Not detected	Not detected
No. 20	3	0.511	Not detected	Not detected	Not detected
No. 21	>50	0.493	Not detected	Not detected	Not detected
No. 22	ND	0.462	Not detected	Not detected	Not detected
No. 23	ND	0.531	Not detected	Not detected	Not detected
No. 24	ND	0.517	Not detected	Not detected	Not detected
No. 25	ND	0.504	Not detected	Not detected	Not detected
No. 26	7	0.619	Not detected	Not detected	Not detected
No. 27	ND	0.528	Not detected	Not detected	Not detected
No. 28	ND	0.508	Not detected	Not detected	Not detected
No. 29	ND	0.521	Not detected	Not detected	Not detected
No. 30	ND	0.523	Not detected	Not detected	Not detected

Faecal coliforms were not detected in all the tap water (UTM: Control 1) and commercially bottled mineral water (Control 2). Food Act 1983 (Act 281) & Regulations (2013) via its Twenty-Fifth Schedule [subregulations 360_B(3) and 360_C(3)] prescribes that faecal (thermotolerant) coliform bacteria must not be detectable in any 100 mL sample of Packaged Drinking Water and Vended Water. The same act also prescribes that the maximum permitted proportion of chemical (ppm) for Packaged Drinking Water and Vended Water for lead, copper, manganese and zinc were 0.02, 0.2, 0.02 and 0.6 ppm, respectively.

strongly suggested that faecal coliforms must not be detected in any 100 mL sample of drinking water for safe human consumption (World Health Organization, 2011; Food Act 1983 (Act 281) & Regulations, 2013). In addition to faecal coliforms, presence of heavy metal elements such as lead, copper, manganese and zinc in water has been comprehensively indicated in the literature (Khan *et al.*, 2013; Kelepertzis, 2014) although such aspect has never been explored in ice cube samples. Chronic exposure of such elements has been attributable to the occurrence of various adverse effects on human such as cancer (Zhao *et al.*, 2014), damage of the nervous system (Karalliedde & Brooke, 2012), kidney stones as well as brain problems in children (Toplan *et al.*, 2004). It is pertinent to mention here that while there is only one specific research on contamination of faecal coliforms in ice cubes that is available in Malaysia reported in Kelantan, (a northeastern state of Peninsular Malaysia); the same pattern of contamination could not be generalized throughout Malaysia. Due to the fact that specific research on the presence of heavy metal elements in ice cubes is lacking, its status of contamination as well as negative impacts on public health remain unclear. In this context, this present research that addresses both of the contamination of faecal coliforms as well as presence of heavy metal elements in ice cubes sampled from food outlets in Taman Universiti, Johor Bahru deserves consideration.

Results of this preliminary study revealed that faecal coliforms were detected in ice cube samples collected from 11 (36.67%) of the 30 food outlets, with two of the samples were found as grossly polluted by faecal coliforms (>50 CFU/100mL). Johor Bahru being a densely populated city in the southern part of Peninsular Malaysia, the observed pattern of contamination by faecal coliforms appears to be similar to that of reported by Noor Izani *et al.* (2012) in Kota Bharu, Kelantan, indicating that the pattern may not be sporadic or accidental in nature but rather prevalent throughout Malaysia and therefore further research in other parts of Malaysia proves vital. In addition, result of this study supports the notion indicated by Noor Izani *et al.* (2012) that excluding faecal coliforms in ice cubes as one of the parameters, inference drawn on the status of cleanliness of food outlets may prove misleading.

Secondly, presence of lead was detected in 29 of the ice cube samples (mean: 0.511±0.105 ppm; range: 0.489-0.674 ppm) and the concentrations exceeded that of the 0.02 ppm maximum permitted proportion for drinking water prescribed in the Twenty-Fifth Schedule [subregulations $360_B(3)$ and $360_C(3)$] of the Food Act 1983 (Act 281) & Regulations (2013). In contrast, copper, manganese and zinc were not detected in any of the samples. Despite being the first record on the presence of lead in ice cubes, this present research also indicated that the water source used for manufacturing the ice cubes in Taman Universiti, Johor Bahru may not originate from the tap water supply and/or mineral drinking water sources or perhaps contaminated during the manufacturing process; a long term potential health hazard for human as well as an avenue for further research. This is due to the fact that all the ice cube samples contained considerable amount of lead while in tap water (Control 1) and commercially bottled mineral water (Control 2) samples, lead was not detected. Considering that prolonged exposure of heavy metal elements such as lead at high concentration would cause significant deterioration of human health (Toplan *et al.*, 2004; Karalliedde & Brooke, 2012; Zhao *et al.*, 2014), concerted efforts deem pivotal in exploring the source of water used for making the ice cubes as well as enlightening the relevant authorities about this serious issue.

Acknowledgement. We thank Professor Wan Azlina Ahmad and the Biotechnology Laboratory research members of the Faculty of Science UTM for allowing the use of instruments in the laboratory. Assistance rendered by staffs at the Instrumentation Laboratory for analyzing the samples for heavy metal elements is appreciated.

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