ORIGINAL ARTICLES

Microbiological Quality Assessment of Edible Ice in three districts of Cau Giay, Nam Tu Liem and Bac Tu Liem, Ha Noi 2018

Nguyen Phuong Thoa[1*], Nguyen Thi Huyen Trang[1], Do Thi Tuyet Chinh[1], Dang The Hung[1], Pham Minh Khanh[2], Duong Hong Quan[1]

ABSTRACT

Objective: To assess the microbiological contamination level in edible ice produced in three districts: Cau Giay, Nam Tu Liem and Bac Tu Liem according to QCVN 10:2011/BYT regulation (National Technical Regulation for Edible Ice) by identifying five microbiological indicators.

Methods: Tests were conducted for detection and enumeration of five microbiological indicators in edible ice samples collected from 28 ice manufacturing plants in three districts: Cau Giay (8 facilities), Nam Tu Liem (10 facilities) and Bac Tu Liem (10 facilities) according to QCVN 10:2011/BYT by membrane filtration technique.

Results: Contaminations were observed in a total of 27 out of 28 edible ice samples collected from ice manufacturing plants in Cau Giay, Nam Tu Liem and Bac Tu Liem districts with microbiological indicators including *P. aeruginosa* (23 samples), *Total Coliform* (22 samples), *S. feacal* (12 samples), *E. coli* (5 samples) and *Spores of sulfite-reducing anaerobes* (0 samples). Moreover, the percentage of edible ice contaminated with *E. coli, P. aeruginosa* and *S. feacal* was significantly higher among samples collected from Bac Tu Liem compared to those of Cau Giay and Nam Tu Liem.

Conclusion: The contamination rate of edible ice samples from ice manufacturing plants in three districts of Cau Giay, Nam Tu Liem and Bac Tu Liem was at a high level according to QCVN 10:2011/BYT regulation. Therefore, appropriate interventions are needed to reduce the microbiological contamination of edible ice, thus improving quality of life and health of residents in those three districts of Ha Noi.

***Keywords**: Edible ice, Total Coliform, E.coli, S. feacal , P. aeruginosa, the spores of sulfite-reducing anaerobes (clostridia).*

BACKGROUND

Water is vital to human body, accounting respectively for 75%, from 50 to 60% and from 45 to 50% of body weight in infants, male adults and female adults (1). Furthermore, water plays a key role in our bodies' metabolism, temperature regulation and excretion. Hence, monitoring the quality of oral water intake proves to be essential in public health; and using microorganism contaminated water source can cause gastrointestinal diseases, which may lead to disease outbreaks such as cholera and typhoid in communities if not promptly controlled.

***Correspondence:** Nguyen Phuong Thoa*
Email: npt1@huph.edu.vn
[1]Hanoi University of Public Health
[2]Haiphong University of Medicine and Pharmacy

Citation: Nguyen Phuong Thoa et al
Vol. 03, No. 04-2019, pp. 23-31

Ice is water in crystal form obtained when water reaches below 0°C. It is used for different purposes with the most important one for preservation of food and beverage. Moreover, edible ice is defined as ice produced from water that meets the requirements stated in QCVN 01:2009/BYT – National Technical Regulation on Drinking Water Quality, packaged and provided for direct use. According to the World Health Organization (WHO), ice which is consumed directly or in contact with to-be-consumed food should be controlled for similar quality and safety level as drinking water (2). The Centers for Disease Control and Prevention (CDC) of the United States also reported that there were 42 food poisoning outbreaks caused by contaminated ice comsumption for years between 2013 and 2014 (3), recording 1,006 poisoned, 124 hospitalized and 13 dead cases (3).

So far, the use of ice has been known as the most common method of food preservation. However, the freezing process does not kill all presented pathogens in ice (4-6). Despite reduction in number and certain damages, microorganisms could survive and recover after the ice melted. Studies have shown that the recovery ability of microorganisms after frozen storage makes ice an ideal means of spreading these microorganisms into food and beverages (4, 6).

Particularly, Kim and Harrison's research demonstrated that *E.coli 0157:H7* can be transmitted to lettuce preserved with ice made from contaminated water, leading to the conclusion that ice is a possible pathway for spreading *E.coli 0157:H7* to clean lettuce through direct contact with or from contaminated lettuce through water from melting ice (7). Moreover, another misconception is that pathogenic bacteria in ice can be killed when contacting with highly alcoholic, acidic or carbonated drinks (7). The study of Dickens et al. examined the existence of bacterial enteropathogens in the ice of popular drinks like coca cola, scotch, and tequila. Although some of the initial factors may vary such as the number and type of microorganisms present in the water and freezing time, Dickens concluded that no microorganisms were completely removed from the drinks tested after adding contaminated ice into those drinks (8).

In Vietnam, due to tropical climate, ice is extensively used in beverage industry in form of ice cube packaged in nylon or polymer plastic bags. Edible ice is preferred for its convenience and low price. However, several studies indicate that edible ice is mostly originated from small manufacturers and does not meet the standards of food safety according to QCVN 10:2011/BYT (National Technical Regulation for Edible Ice) through assessment of five microbiological indicators including *Total Coliform*, *Escherichia coli* (*E. coli), Streptococci feacal* (*S. feacal)*, *Pseudomonas aeruginosa (P. aeruginosa)* and *Spores of sulfite-reducing anaerobes* (12). In 2015, Ha Thu Huyen published a research on edible ice quality with samples collected from 70 ice manufacturing plants in Ha Noi, in which 53 samples were contaminated (9) with at least one microbiological indicator mentioned above.

In other locations, according to reports of the Provincial Food Administration, 11/41 samples in Tay Ninh and 12/16 samples in An Giang did not meet the standards of food hygiene and safety for contamination

with at least one microbiological indicator (10, 11). Therefore, assessment and control of the edible ice quality according to QCVN 10:2011/BYT (National Technical Regulation for Edible Ice) is extremely important to improve public health.

With the desire of microbiological quality assessment of edible ice samples collected from ice manufacturing plants in Ha Noi according to QCVN 10:2011/BYT, we initially assess the risk of microbiological contamination of edible ice samples collected from 28 production facilities in three districts of Cau Giay, Nam Tu Liem and Bac Tu Liem through quantitative detection of microorganisms as prescribed.

STUDY SUBJECTS

Subjects

The edible ice samples (in form of ice cube bags) were collected from 28 ice manufacturing plants in 3 districts: Cau Giay (8 ice manufacturing plants), Nam Tu Liem (10 ice manufacturing plants) and Bac Liem (10 ice manufacturing plants).

Methods

Study duration: December 2018 to January 2019.

Study sites: Cau Giay, Nam Tu Liem and Bac Liem districts of Ha Noi City.

Study design: Edible ice samples collected from 28 ice manufacturing plants in 3 districts were assessed quantitatively to detect microbiological indicators according to QCVN 10:2011/BYT at the Department of Food and Environmental Testing, Laboratory Center, Hanoi University of Public Health.

Detection and quantification methods:

Edible ice samples were collected directly at ice manufacturing plants as intact bags and then transported to the Department of Food and Environmental Testing, Laboratory Center, Hanoi University of Public Health. In the laboratory, the edible ice samples were transferred into a neutral glass bottle with sterile screw cap. After the ice melted, homogenization and quantitative testing were performed for microbiological indicators according to QCVN 10:2011/BYT by membrane filtration method with the procedure shown in Figure 1 observing each regulation for individual microbiological indicator as follows:

- Detection and enumeration of *Coliform total, E. Coli* according to TCVN 6187-1:2009 (ISO 9308-1:2000) using membrane filter with 47 mm diameter and 0.45 µm pore size.

- Detection and enumeration of *P. aeruginosa* according to TCVN 8881:2011 (ISO 16266:2010) using membrane filter with 47 mm diameter and 0.45 µm pore size.

- Detection and enumeration of *S. feacal* according to TCVN 6189-2:2009 (ISO 7899-2:2000) using membrane filter with 47 mm diameter and 0.45 µm pore size.

- Detection and enumeration of *the Spores of sulfite-reducing anaerobes (clostridia)* according to TCVN 6191-2:1996 using membrane filter with 47 mm diameter and 0.22 µm pore size.

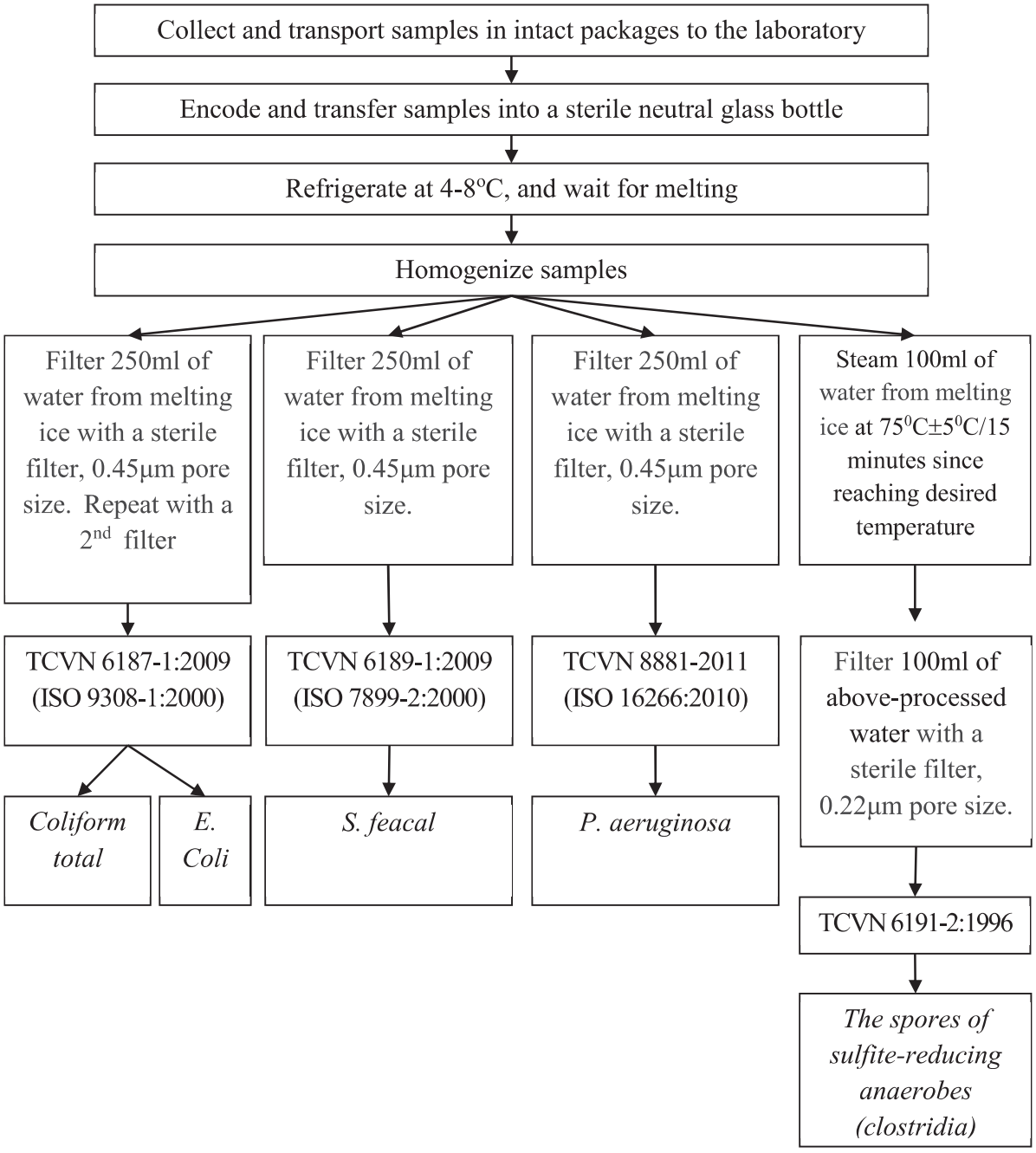

Figure 1: Method for detection and enumeration of *Total Coliform, E. coli, P. aeruginosa, S. feacal* and *Spores of sulfite-reducing anaerobes (clostridia)*

Analysis

The results of microbiological quantitative testing were analyzed, processed by Excel and use of the reference parameter table according to QCVN 10:2011/BYT for the quality of edible ice.

Limitations

The number of the edible ice samples was relatively small (28 samples from 28 production facilities) and the ice sample collection scope is not sufficient because samples were collected only from ice manufacturing plants in three districts of Cau

Giay (8 manufacturing plants), Nam Tu Liem (10 manufacturing plants) and Bac Tu Liem (10 manufacturing plants).

RESULTS

The number of edible ice samples infected with microorganisms in 3 districts of Cau Giay, Nam Tu Liem and Bac Tu Liem

The study on quality assessment of edible ice from 28 production facilities in 3 districts of Ha Noi in 2018 was conducted in order to initially assess whether quality of such edible ice meets the standards of food safety and hygiene according to QCVN 10:201/BYT by 5 microbiological indicators including *Total coliform, E. coli, S. feacal , P. aeruginosa and Spores of sulfite-reducing anaerobes*. Preliminary analysis results show that 27/28 samples were found to be contaminated with at least one microorganism type. In particular, there were 23/28, 22/28, 12/28, 5/28 and 0/28 samples contaminated with *P. aeruginosa, total coliform, S. feacal, E. coli* and the *Spores of sulfite-reducing anaerobes* (Figure 2), respectively. Moreover, compared to the prevalence of microbiological indicators in the edible ice samples collected in Cau Giay and Nam Tu Liem districts, the number of the edible ice samples collected in Bac Tu Liem district had higher contamination rates for *E. coli, P. aeruginosa and S. feacal* (Figure 2). As such, most of the samples are contaminated with at least one of microbiological indicators as specified. In particular, the highest contamination rate was found with *P. aeruginosa* and the lowest was found with the *spores of sulfite-reducing anaerobes* in those three districts.

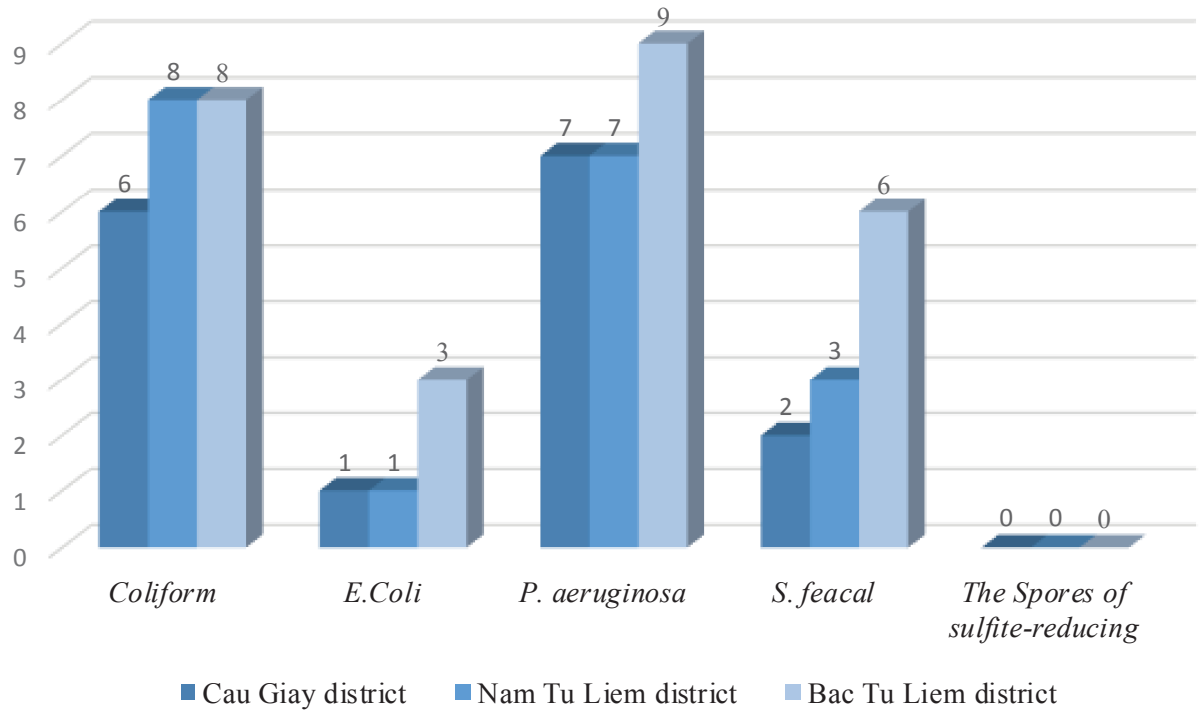

Figure 2: Number of samples contaminated with microbiological indicators by district

The level of microorganism contamination in edible ice samples collected in 3 districts: Cau Giay, Nam Tu Liem and Bac Tu Liem

To determine the level of *Total Coliform, E. coli, P. aeruginosa and S. feacal* in the collected edible ice samples, we first determined *Total Coliform* (a microbiological indicator used to assess sanitary conditions, faecal contamination and potential presence of pathogens (4,9)). The contamination of *Total Coliform* found in 6/8, 8/10 and 8/10 samples collected from in Cau Giay, Nam Tu Liem and Bac Tu Liem districts was at an average level exceeding the standard - 38 CFU/250 mL, 59.13 CFU/250 mL and 305.38 CFU/250 mL, respectively (Table 1). In particular, the average concentration of *total coliform* in samples collected in Bac Tu Liem district was much higher than that in the other two districts (Table 1).

Table 1:Average contamination level of microorganisms in edible ice samples collected in three districts of Cau Giay, Nam Tu Liem and Bac Tu Liem

No.	Microbiological	Test	Unit	Average contamination level			Upper limit
				Cau Giay district	Nam Tu Liem district	Bac Tu Liem district	
1	*Total Coliform*	TCVN 6187-1:2009	CFU/250mL	38	59.13	305.38	0
2	*E. coli*	TCVN 6187-1:2009	CFU/250mL	5	5	96	0
3	*Pseudomonas aeruginosa*	TCVN 8881:2011	CFU/250mL	92.43	18.29	5313.44	0
4	*Streptococci faecal*	TCVN 6189-2:2009	CFU/250mL	44	21.25	139.33	0
5	*The spores of sulfite-reducing anaerobes*	TCVN 6191-2:1996	CFU/50mL	Not detected	Not detected	Not detected	0

Then, we determined the contamination level of *E. coli* (a microbiological indicator used to assess the sanitary conditions and contamination of water sources for eating and daily living). Results show that average contamination level of *E. coli* found in 1/8, 1/10 and 3/10 samples collected in districts of Cau Giay, Nam Tu Liem and Bac Liem surpassed the standards - 5 CFU/250 mL, 5 CFU/250 mL and 96 CFU/250 mL, respectively (Table 1). Similar to the average *total coliform* contamination level of the edible ice samples collected in Bac Tu Liem district, the average contamination level of *E. coli* was also much higher than that in the other two districts (Table 1).

The contamination level *of P. aeruginosa* (a microbiological indicator used to assess the

pollution of water sources for eating and daily living) for edible ice samples collected in three districts show that the average contamination level found in 7/8, 7/10 and 9/10 samples collected in districts of Cau Giay, Nam Tu Liem and Bac Tu Liem was beyond the standards - 92.43 CFU/250 mL, 18.29CFU/250 mL and 5313.44 CFU/250 mL, respectively (Table 1). Also similar to the average contamination levels of *total Coliform* and *E. coli* in samples in Bac Tu Liem district, the average contamination level of *P. aeruginosa* in Bac Tu Liem was remarkably higher compared to that in the two remaining districts (Table 1).

Finally, the average contamination level of *S. feacal* (a microbiological indicator used to assess the pollution of water sources for eating and daily living) found in 2/8, 4/10 and 6/10 samples collected in Cau Giay, Nam Tu Liem and Bac Tu Liem districts exceeded the standards - 44 CFU/250 mL, 21.25 CFU/250 mL, and 139.33 CFU/250 mL, respectively (Table 1). Similarly to above indicators, in Bac Tu Liem district, the average contamination level of *S. feacal* in edible ice was also higher than that in the other two districts (Table 1).

DISCUSSION

Viet Nam is located in the tropical monsoon climate with high average temperature, especially in the summer. Because of this, there is a high demand of edible ice in the community to use for refreshments and food preservation. Therefore, to ensure public health, the quality of edible ice must be equivalent to the quality of drinking water and meet standards of food hygiene and safety according to QCVN 10:2011/BYT regulation. However, currently there is a situation where most of the ice manufacturing plants are small in size, using outdated production lines, lack of water source quality control and hygiene during the production process, which leads to the fact that many edible ice products are at risks of contamination with microbiological indicators shown in QCVN 10:2011/BYT regulation (12). As a result, edible ice may become poisonous for consumers, affecting detrimentally public health, especially in densely populated community in Ha Noi.

Therefore, in this study, we initially assessed the quality of edible ice in samples collected from 28 production facilities in Cau Giay district (8 facilities), Nam Tu Liem (10 facilities) and Bac Tu Liem (10 facilities) in Ha Noi to assess and quantify five microbiological indicators according to QCVN 10:2011/BYT regulation. Quantitative detection analysis results have determined that 27/28 samples were contaminated with at least one microbiological indicator. Specifically, samples collected in districts of Cau Giay, Nam Tu Liem and Bac Tu Liem were contaminated with *P. aeruginosa* (23/28 samples), *Coliform* (22/28 samples), *S. feacal* (12/28 samples), E. coli (5/28 samples) but not *the spores of sulfite-reducing anaerobes* (0/28 samples). Moreover, the percentage of samples contaminated with *E. coli, P. aeruginosa* and *S. feacal* in Bac Tu Liem district was much higher than that in other two districts.

Although there have not been many studies on assessment of edible ice quality by determining the contamination through microbiological indicators according to QCVN 10:2011/BYT regulation; some

reports of the Vietnam Food Administration, Ministry of Health on reviewing and assessing edible ice quality from samples collected at manufacturing plants in Tay Ninh (2015) and An Giang (2018) show that ice samples did not meet the standards of food safety and hygiene, in which 11/41 and 12/16 samples of ice were contaminated with at least one type of microorganism (10, 11). Moreover, a study of Ha Thu Huyen (2015) published on the quality of edible ice produced from plants in Ha Noi also reported that 53/70 samples collected from 70 production facilities were contaminated with at least one microbiological indicator (12). Thus, studies on assessment of the quality of edible ice in different regions and periods have determined that edible ice samples collected from ice manufacturing plants had very high contamination levels with microbiological indicators. This raises concerns among the community who use edible ice for refreshments and food preservation and that most of these products do not meet the criteria of food safety and hygiene according to QCVN 10:2011/BYT regulation.

CONCLUSION

Preliminary research has shown the contamination prevalence of microorganisms in edible ice samples collected from production facilities in Cau Giay, Nam Tu Liem and Bac Tu Liem districts in Ha Noi according to QCVN 10:2011/BYT regulation. Particularly in Bac Tu Liem district, the average contamination levels of *total coliform, E. coli, P. aeruginosa* and *S. feacal* in the edible ice samples were many times higher than the permitted level compared to such levels in the other two districts, which would affect the public health in the area. Therefore, it is necessary to take appropriate interventions to reduce the risk of contamination with microbiological indicator in edible ice in the area according to QCVN 10:2011/BYT regulation. In addition, large-scale assessment studies are needed to determine the contamination of microbiological indicators in edible ice samples of all manufacturing plants in Ha Noi as well as across Viet Nam.

REFERENCES

1. Ta Thanh Van. Clinical Biochemistry Textbook. Medical Publishing House. 2013.
2. World Health Organization. Guidelines for drinking-water quality: second addendum. Vol.1, Recommendation, 3rd ed. 2008.
3. Katharine M. Benedict, Hannah Reses, Marissa, et al. Surveillance for Waterborne Disease Outbreaks Associated with Drinking Water — United States, 2013–2014. MMWR Morb Mortal Wkly Rep. 2017; 66(44):1216-1221.
4. Juliana P. Falcão, Seise P. Falcão, Tânia A.T. Gomes. Ice as a vehicle for diarrheagenic *Escherichia coli*. Int J Food Microbiol. 2004; 91(1):99-103.
5. Agbaje Lateef, Julius K. Oloke, Evariste B. Gueguim Kana, Esther Pacheco. The microbiological quality of ice used to cool drinks and foods in Ogbomoso Metropolis, Southwest, Nigeria. Internet J Food Safety. 2006; 8:39-43.
6. Jin Kyung Kim, Mark A. Harrison. Transfer of Escherichia coli O157:H7 to romaine lettuce due to contact water from melting ice. J Food Prot. 2008; 71(2):252-6.
7. J.P. Falcão, A.M.G. Dias, E.F. Correa, D.P. Falcão. Microbiological quality of ice used to refrigerate foods. Food Microbiology. 2002; 19(4):269-76.
8. D. Lynn Dickens, Herbert L. DuPont, Philip C. Johnson. Survival of bacterial enteropathogens in the ice of popular drinks. JAMA. 1985; 253(21):3141-3.
9. Ha Thu Hien. Assessment of ice quality and description of factors affecting ice quality in Ha Noi, 2015. Available at: https://text.123doc.org/document/4394681-danh-gia-chat-luong-

nuoc-da-va-mo-ta-cac-yeu-to-anh-huong-toi-chat-luong-nuoc-da-tai-thanh-pho-ha-noi-nam-2015.htm.

10. Tay Ninh Provincial Food Administration, Vietnam Food Administration, Ministry of Health. Tay Ninh inspects establishments producing and trading ice and bottled drinking water. 2015. Available at: http://vfa.gov.vn/hoat-dong-chi-cuc/tay-ninh-thanh-kiem-tra-co-so-san-xuat-kinh-doanh-nuoc-da-nuoc-uong-dong-chai.htm.
11. An Giang Provincial Food Administration, Vietnam Food Administration, Ministry of Health. An Giang: Results of inspecting food safety hazards for ice cubes in the province. 2017. Available at: http://vfa.gov.vn/hoat-dong-chi-cuc/an-giang-ket-qua-giam-sat-moi-nguy-an-toan-thuc-pham-doi-voi-nuoc-da-vien-tren-dia-ban-tinh.html
12. QCVN 10:2011/BYT - Natioanl Technical Regulation for Edible Ice. Ministry of Health. 2011.