Microbiological Quality of Packaged Ice from Various Sources in Georgia

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Running Title: Microbiological Quality of Packaged Ice from Various Sources in Georgia

Keywords: Ice, *Salmonella*, vending machines, manufactured ice

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ABSTRACT

This study determined the microbiological and chemical quality of ice produced and bagged on-premises in retail establishments and in free-standing self-service ice vending machines in the state of Georgia and compared the results with that from ice produced by manufacturing companies monitored by the International Packaged Ice Association (IPIA). Two hundred and fifty bags of packaged ice samples were obtained from retail locations and self-service ice vending machines, along with 25 bags of packaged manufactured ice. Ice samples were melted within 24 h of collection and HPC SimPlates® were used to detect heterotrophic bacteria present. Colisure® and Enterolert® assays were used to enumerate coliforms, non-pathogenic *E. coli* and enterococci. Membrane filtration coupled with enrichment was used to detect *Salmonella* and *Listeria monocytogenes*. Confirmation tests were done for presumptive positive pathogens. None of the manufactured ice had unacceptable microbial levels. Six percent of the ice samples bagged at retail sites and from ice vending machines contained unsatisfactory levels of heterotrophs compared to the limits set by the IPIA (≥500 MPN/100ml). Thirty-seven percent of these samples contained an unsatisfactory level of coliforms (≥1.0 MPN/100 ml), 1% contained nonpathogenic *E. coli* and 13% contained enterococci (≥1.0 MPN/100 ml). One sample tested positive for the presence of *Salmonella* and another positive for *Enterobacter agglomerans*. Ninety-five (38%) samples of packaged ice from retail establishments and vending machines had pH levels outside the acceptable range which can affect product flavor. Turbidity of 3 samples exceeded acceptable level. No samples had unacceptable nitrate levels. Manufactured ice had better microbiological and chemical quality than ice packaged on the premises of retail locations and from self-serve ice vending machines.

Ice is water frozen into the solid state when temperatures drop below 0°C. It has many purposes dating back centuries ago when people used ice for food preservation. Ice is often consumed on its own or mixed with beverages and allowed to melt to cool drinks for refreshment. According to the International Packaged Ice Association (IPIA), it is estimated that a total of 2 billion bags of ice are sold from all retail, wholesale, and vending producers and from those sales, 800 million bags are attributed to retail producers and 200 million bags are from vending machine sales *(15)*. The U.S. Food and Drug Administration (FDA) estimated that each American buys approximately 4 bags of packaged ice every year, with about 80% of the bags purchased during the summer months *(6)*. Overall, the ice industry has increased the production in the past century. There are close to 700 commercial ice-making companies in the United States with approximately 500 of these represented by the IPIA *(3, 6)*.

Ice is defined as a food by the FDA, which regulates packaged ice for interstate commerce *(1, 6)*. The Association of Food and Drug Officials (AFDO) have published Good Manufacturing Practices (GMPs) in order to regulate the sanitary manufacturing of packaged ice *(1)*. The GMP regulations state that ice manufacturers must produce, hold, and transport ice in a clean and sanitary condition, monitor the cleanliness, and hygiene of employees, use properly cleaned and maintained equipment, and use water that is safe and from a reliable source *(1, 6)*. All ice manufacturing companies are required to follow these basic GMPs, but are enforced differently from state to state. There are little to no specific packaged ice processing regulations at the state and federal level *(19)*. The IPIA has published the Packaged Ice Quality Control Standards (PIQCS) manual which is based on GMPs but is tailored specifically to packaged ice *(14)*. The establishment of the PIQCS/PIQCS-Plus Program within the manufacturing company is required in order to gain membership in the IPIA *(14)*. Although the development and implementation of a Hazard Analysis and Critical Control Point (HACCP) plan is not required by the FDA, the implementation of a HACCP and HACCP pre-requisites are required by IPIA *(14)*.

The World Health Organization (WHO) has stated that ice to be consumed or come into direct contact with food that is to be consumed is expected to be at the same quality and safety level as drinking water *(24)*. The Centers for Disease Control and Prevention (CDC) has reported foodborne illness outbreaks over the past several decades where the cause of the illness was contaminated ice *(2)*. The CDC reports there are over 50,000 cases per year of reported foodborne illnesses where the origin is unknown, but ice is not one of the first food products investigated, if at all, as the source of the illnesses, even though it is a widely used commodity in retail establishments *(2)*.

Freezing is a well-known method for food preservation and a crucial step in food processing, yet the freezing process does not destroy all pathogens present in the food *(4, 11, 17)*. While their numbers decline, some microorganisms survive freezing and although cells may be injured, the remaining microorganisms have the ability to recover their viability when ice melts *(4, 17)*. Past studies have indicated that the microorganism's capability to recover after frozen storage makes ice an ideal vehicle for transmission of pathogenic bacteria and viruses to food and beverages *(4, 17)*. Kim and Harrison demonstrated that *E. coli* O157:H7 can transfer to lettuce from melted ice made with contaminated water *(16)*. They concluded that ice is a possible route for *E. coli* O157:H7 to lettuce either by direct contact with water from melted contaminated ice or from contaminated lettuce to uncontaminated lettuce with melted ice being the vehicle of transmission *(16)*. The packaging and transportation of food products that are typically kept on ice, such as produce and fish, are shown to be at risk for contamination if the recommended precautions are not observed.

Another misconception is if pathogenic bacteria are present in ice, they can be killed when the ice is added to beverages of high alcoholic content, with high acidity, and with carbonation, such as soda *(7, 10)*. Dickens et al. tested the survival of several bacterial enteropathogens in ice of popular drinks, including cola, scotch, and tequila *(9)*. Although multiple factors could vary the outcome, such as the number and type of organisms present initially in the water and the length of time frozen, Dickens concluded that none of the organisms were completely eliminated in the test drinks when contaminated ice was added *(9)*. There are concerns about the safety of drinking water and the ice used in beverages, especially when traveling to foreign locations with a questionable water supply.

Poor quality water or lack of hygiene during the production and handling of water to produce ice can contribute to the presence of harmful microorganisms *(4, 6, 9, 10, 13, 17)*. In addition, the pH, turbidity, conductivity, alkalinity and nitrate concentration of water used for ice making can influence microbial presence and the flavor of the ice (*7*). Ice may be sold commercially that may not be closely or consistently monitored for proper sanitation and hygienic conditions. This survey determined the microbiological and chemical quality of ice produced and bagged on-premises in retail establishments and in self-service ice vending machines in the state of Georgia and compared the results with that from ice produced by manufacturing companies that are monitored by the IPIA.

MATERIALS AND METHODS

Sample Collection. A total of 250 bags of packaged ice were collected in the state of Georgia between mid-August and October 2012 from retail establishments that produced ice onpremise and self-service ice vending machines. The number of retail samples and ice vending machine samples selected was based on the population density of communities throughout the state (Table 1). One hundred and forty-nine samples were collected from retail and convenience stores, and 101 samples were collected from ice vending machines, with one bag collected per location. Samples were collected from retail establishments that were known to produce and package ice on the location's premises. The type of establishments in which these samples were bought from included gas stations, foodservice franchises, and liquor stores. The ice was collected in the bags provided at the locations and closed using their method, such as with a twist tie, metal clip, or knot. The original bags were doubled bagged with large, sterile, 5 kg bags (Whirlpak® Nasco, Fort Atkinson, WI) in case there were holes in the first packaging to prevent potential cross-contamination. Both bags were numbered with a permanent marker which corresponded with a number on a datasheet containing information and key identifying points for that particular sample, including name and type of retail establishment in which the sample was collected, the address, the type of closure of the bag, and retail labeling on the bag. Noticeable defects of the sample or packaging were recorded, and the samples were kept in the collection coolers in a walk-in refrigerator (4°C) until they were ready to be tested.

Twenty-five bags of packaged ice were collected from 2 different ice manufacturing companies near Atlanta, Georgia during January 2013. Both companies are IPIA members. Thirteen samples were collected from plant A and 12 samples from plant B. The sample bags were numbered and the location was recorded on the datasheet. The samples were kept in the collection cooler in a walk-in refrigerator (4°C) until they were ready to be tested. Table 2 shows the distribution of all 275 samples of packaged ice and the area of Georgia in which they were collected.

Sample Preparation. The ice was kept in the coolers in a 4°C refrigerator following collection and sample preparation was completed within 24 h of collecting the sample. Ice samples were removed from their original bags and separated into separate bags and containers with corresponding numbers. Approximately 1 L (or 1,000 g) of ice was separated into a large, sterile, 2.6 kg bag (Whirlpak, Nasco) for microbial analysis, and approximately 1 L (or 1,000 g) was separated into sterilized plastic bottles for chemical analysis. Excess sample was discarded. The original bags were kept for recordkeeping. The ice was allowed to melt completely at room temperature (24°C) before testing.

Microbiological Examination. The heterotrophic plate count (HPC) was enumerated using SimPlate® for HPC Multi Dose (Idexx Laboratories Inc., Westbrook, ME). One ml of the melted ice sample was slowly pipetted onto the center of the SimPlate and 9 ml of the manufacturer-provided media that was hydrated with 100 ml of sterilized deionized water in its original bottle, was pipetted onto the center of the plate, on top of the 1 ml sample. The plate was covered with its lid and gently swirled to mix and distribute the sample into all the wells. The plate was tilted forward to drain the excess liquid into the absorbent pad and inverted before it was incubated at 35°C for 48 h. After incubation, the plates were observed under a 365 nm ultraviolet (UV) light and wells that fluoresced were counted. The total number of positive wells counted and the most probable number (MPN) table that was specific to the Simplate was used to determine the MPN of HPC bacteria present in the sample.

Total coliforms and *E. coli* were enumerated using the Quanti-Tray and the Colisure® Assay test kit (Idexx Laboratories Inc.). One hundred ml of the melted ice sample was pipetted into a sterile media vessel. The contents from the provided Colisure reagent packet were added and 3-4 drops of an antifoam solution (Idexx Laboratories Inc.) was added to the vessel. Each solution was shaken until the large media particles were dispersed. The sample/reagent mixture was poured into a Quanti-Tray tray and sealed with the Quanti-Tray Sealer (Idexx Laboratories Inc.). The sealed tray was incubated with the wells lying facing down, at 35°C for 24 h. Results were read based on the color of the well and if the well fluoresced under a 365 nm UV light. If the well was yellow/gold, it was negative for both total coliforms and *E. coli*. If the well was red/magenta, it was positive for total coliforms and if it was red/magenta and fluoresced, it was positive for *E. coli*. The number of positive wells was then referenced to the MPN table specific to the Quanti-Tray to determine the MPN of total coliforms and/or *E. coli* in the sample.

Enterococci (*Enterococcus faecalis*) were enumerated using the Quanti-Tray and the Enteroelert® Assay test kit (Idexx Laboratories Inc.). One hundred ml of the melted ice sample was pipetted into a sterile media vessel. The contents from the provided Enteroelert® reagent packet were added and 3-4 drops of an antifoam solution were added to the vessel. Each solution was shaken until the large media particles were dispersed. The sample/reagent mixture was poured into a Quanti-Tray tray and sealed with the Quanti-Tray Sealer. The sealed tray was incubated with the wells lying face down at 41°C for 24-48 h. The presence of enterococci in the wells was detected by the presence of blue fluorescence under a 365 nm UV light. The number of positive wells was referenced to the MPN table specific for the Quanti-Tray to determine the MPN of enterococci in the sample.

The presence or absence of *Salmonella* and *L. monocytogenes* was determined using membrane filtration and enrichment methods. One hundred ml of the melted ice sample was filtered through a 0.45μ MicroFunnel™ Filter Funnel (Pall Life Sciences, Ann Arbor, MI). Using sterilized tweezers, the filter was separated from the funnel, placed into a stomacher bag with 100 ml of universal preenrichment broth (Becton Dickinson and Company, Sparks, MD), stomached for 1 min, and incubated at 35°C for 24 h.

For the enrichment of *Salmonella*, 0.1 ml was transferred from the sample in the preenrichment broth into a tube of Rappaport Vassiliadis (RV) broth (Becton Dickinson), and 1.0 ml was transferred into a tube of tetrathionate (TT) broth (Becton Dickinson); the tubes were incubated for 24 h at 42°C and 35°C, respectively. After incubation, portions of each broth were streaked using a sterilized loop onto separate plates of bismuth sulfite agar (BSA), xylose-lysine-desoxycholate (XLD) agar and Hektoen-Enteric (HE) agar (Becton Dickinson). The plates were incubated at 35°C for 24 h. Presumptive positive colonies were subcultured to triple sugar iron (TSI) and lysine-iron-agar (LIA) slants (Becton Dickinson) for additional characterization. For TSI and LIA slants that had positive reactions typical for *Salmonella*, an Enterobacteriaceae Micro-ID® (Thermo Fisher Scientific, Lenexa, KS) was used to confirm the identity of the *Salmonella* present.

For *L. monocytogenes*, a portion of the sample in the preenrichment broth was streaked onto the selective modified Oxford agar (Becton Dickinson) using a sterilized loop and was incubated for 24 h at 35°C. Presumptive positive colonies were subcultured to a chromagar plate (Becton Dickinson) for selective enrichment, and if the plate was positive with typical *L. monocytogenes* colonies, a Micro-ID *Listeria* (Thermo Fisher Scientific) was used to confirm the *L. monocytogenes* identification.

Chemical Analysis. Chemical analysis was only done on samples collected from the retail establishments and vending machines. The Hach HQ 440d Benchtop Dual Input, Multi-Parameter Meter (Hach Company, Loveland, CO) was used to determine conductivity, pH, and the level of nitrate, using the appropriate probes, CDC40101, PHC28101, and ISENO318101, respectively. The manufacturer's instructions were followed, and the instrument was calibrated for each probe before each sample period. Turbidity was determined using the LaMotte 2020 We Turbidity meter (LaMotte Company, Chestertown, MD). The manufacturer's instructions were followed, and the instrument was calibrated before each sample period. The alkalinity of the water samples was measured using the titration method instructions published in section 2320 of *The Standard Methods for the Examination of Water and Wastewater* (8).

Statistical Analysis. Statistical analysis was completed on the results from the microbiological and chemical testing of the packaged ice. The significance and independence of variables were determined by using common statistical tests. The analysis of variance (ANOVA) was used to determine if the relationship between 2 independent variables (i.e., HPC levels and bag closures) was statistically significant. Fisher's exact test and the Chi-square test were both used to determine if the independent variables were indeed independent of each other. The likelihood ratio test and the logistic regression analysis were used to express how many times more likely the data under one variable will occur than another variable.

RESULTS AND DISCUSSION

In this study, packaged ice from multiple locations in Georgia was tested for total heterotrophic bacteria and indicator organisms, as well as *Salmonella* and *Listeria monocytogenes*. Heterotrophs and indicator organisms are used to evaluate the sanitation and hygienic conditions of the production areas, the contamination of foods, including ice, and for the possible presence of pathogens *(10, 21)*. These organisms all reflect the sanitary quality of the ice, the ice machine and scoop, the quality of the water the ice is made from, cross-contamination from food contact surfaces, and the hygiene of the staff handling the ice *(10, 19,* *21)*. The presence of *E. coli* and enterococci, such as *E. faecalis*, indicate possible fecal contamination (*10*).

The International Packaged Ice Association (IPIA) established limits for these indicator tests as a quality control measure to keep ice safe for consumers. These limits state that the heterotrophic plate count of water should not exceed 500 MPN/ml of water, total coliforms, fecal coliforms, *E. coli,* and enterococci should not be present in 100 ml of water using MPN, No pathogenic bacteria, such as *Salmonella* and *Listeria monocytogenes*, should be present.

Heterotrophic Plate Count. Heterotrophic bacteria are naturally occurring in water and the level of contamination is a common indicator of the cleanliness and quality of drinking water. Heterotrophic plate counts can also reflect the general hygiene of the production and handling of ice *(4, 13, 21)*. The presence of heterotrophic bacteria does not necessarily signify a risk for illness because low numbers may be found in treated water, but it does give a good indication of sanitary conditions during storage and handling and the efficiency of water treatments *(5, 7, 22)*. According to the WHO, the HPC value is a good indication of effective coagulation, filtration, and disinfection steps during the water treatment process *(22)*. In this survey, 178 samples (71%) of all retail and vending machine produced ice contained some level of heterotrophs, with 16 samples (6.4%) exceeding IPIA's recommended limits of less than 500 MPN/ml of water (Table 3). The majority of samples that exceeded the limits were bagged ice from retail establishments, primarily gas stations. Chi-square analysis revealed the HPC values were dependent on the type of ice, whether it is from manufacturers, retail outlets or ice vending machines ($p < 0.0001$). All of the manufactured ice had HPC within the acceptable level, with only 2 samples having detectable growth (Table 3). In comparison, there was a 3.5 times greater chance the ice purchased from a retail store will have a higher HPC value than ice from vending machines, which could be attributed to the increased handling of ice from workers in a retail store. The high levels of heterotrophs may indicate improper personnel hygienic practices of the workers at the retail establishment, cross-contamination, or poor water source. The lack of contamination in the manufactured ice indicates better hygienic control in facilities that follow the more specific guidelines (i.e., GMPs, PIQCS, etc.). Ice produced in these facilities is done with little hands-on exposure from workers and with less chance for cross-contamination from contact surfaces. The study on the quality of packaged ice collected in Iowa completed by Moyer et al. *(20)* included members and nonmembers of the IPIA in their survey and no samples collected from IPIA accredited companies exceeded the HPC limit of 500 MPN/ml, supporting the claim of higher-quality ice.

Total Coliforms and *E. coli*. Coliforms are indicator organisms and are used to evaluate the hygienic conditions, the possible fecal contamination, and potential presence of pathogens *(4, 10)*. A total of 93 samples (37.2%) from retail establishment locations and in vending machines exceeded the recommended limits of total coliforms (less than 1 MPN/100 mL), with the majority from gas stations (Table 3). Two samples bought from gas stations also had nonpathogenic *E. coli* present. The logistic regression analysis showed the odds of a retail bag of ice having an unacceptable level of total coliforms are 1.87 times more than those of a bag from an ice vending machine and both are more likely to contain coliforms than manufactured ice. Additional chi-square analyses demonstrated that there was a statistically significant difference between manufactured and vending ice (Fisher's Exact test P-value = 0.0009) and also between manufactured and retail ice (Fisher's Exact test P-value< 0.0001) with respect to coliform counts. In comparison to past studies done by Schmidt et al., Gerokomou et al, and Moyer et al., the percentage of unacceptable levels of total coliforms and *E. coli* were slightly higher in this study *(13, 20, 23)*. Although the distribution of sample collection varied some among these studies, the results are significant enough to indicate sanitation problems. These unacceptable samples could indicate a contaminated water source, un-sanitized scoops or utensils, unsanitary packaging process or unhygienic staff handling *(13, 19, 20)*. Unless self-service ice vending machines are maintained properly and the presence of insects and animals controlled, there could be a greater chance for contamination. No coliforms or *E. coli* were detected in the ice from manufacturing plants (Table 3), which indicates good sanitary, hygienic practices may have been in place. The IPIA members from the study done by Moyer et al. *(20)* also showed no positive results during their survey.

Enterococci. Enterococci bacteria, commonly *Enterococcus faecalis*, can typically be found in human and animal intestines and can also be an indicator of poor sanitary and hygienic conditions during the production of ice *(18)*. Just as with the total coliforms and nonpathogenic *E. coli*, the presence of enterococci does not necessarily signify that illness will occur; however, it may indicate the presence of fecal pathogens that could cause nausea, vomiting, abdominal pain, and diarrhea *(18)*. No samples from the ice manufacturing plants tested positive for enterococci. In contrast, 32 samples (12.8%) from retail establishments and ice vending machines contained unacceptable levels of enterococci, exceeding the limit of 1.0 MPN/100 mL. Positive samples were found in both self-service ice vending machines and retail locations, in particular gas stations (Table 3). Based on the likelihood ratio test, the odds of a retail bag of ice having an unacceptable level of enterococci are 3.3 times more likely than the samples from the vending machines, and both are more likely to contain enterococci than manufactured ice. These results could be contributed to the process involved in the production of ice at the different types of locations. Ice from the vending machines and manufacturing plants is less likely to be handled by employees and come into contact with contaminants. Again, these results indicate that manufactured ice may be produced under more sanitary and controlled conditions than the packaged ice sold at retail establishments and self-service vending machines.

***Salmonella* and Other Organisms.** Regardless of source, no samples tested positive for *L. monocytogenes*. *Salmonella* was not detected in the manufactured ice, but one sample from a retail establishment, a foodservice franchise, tested positive for the presence of *Salmonella.* Since only the presence of this organism was confirmed and the number of cells is unknown, it was not possible to determine whether or not the consumption of this contaminated ice would have caused illness. However, the mere presence still raises concern about the conditions of the location where this sample was purchased. The presence of *Salmonella* demonstrates a more serious level of contamination and the need for attention and intensive cleaning.

Enterobacter agglomerans was also detected in a sample collected from a self-service ice vending machine. It is a common *Enterobacter* species with an unknown infectious dose and is found in the stool of healthy humans *(12)*. This microorganism can cause acute gastroenteritis with symptoms including vomiting, nausea, abdominal pain, and diarrhea *(12)*. The prevalence of these microorganisms is relatively unknown at the CDC because the symptoms are sometimes mild and go without complaint *(12)*. The presence of *E. agglomerans* is a little more significant than the presence of the indicator organisms, such as coliforms or heterotrophic bacteria, because it has been linked to foodborne illness.

Sample Distribution of Retail-Produced and Self-Service Vending Machine Bagged Ice. Table 4 shows the geographic distribution of unacceptable samples collected. Both the Southeastern and Southern regions of the state were found to have a high likelihood of poor ice quality from retail produced and vending machine bagged ice. More than half of the samples of this type of ice, from these two regions, contained an unacceptable level of coliforms and enterococci. Results from the likelihood ratio test indicate that there are higher odds that towns in the southeast portion of the state will have an unacceptable level of HPC, total coliforms, and enterococci than the rest of the state, in this particular category of ice (retail produced and vending machine bagged ice). The levels of total coliforms were dependent on the location in which the samples were obtained ($p < 0.0001$), but the level of HPC and enterococci were not.

Influence of Type of Bag Closure on the Microbial Quality. All samples that were obtained from manufacturing companies were closed using metal clips, and as stated in the previous sections, there was little to no microbial growth present. Therefore, the statistical analysis was only done on on-site- and vending machine-produced ice. The majority of the samples from vending machines were closed with a twist tie, mainly because all ice samples from the vending machines had twist ties available at each location, with the exception of five machines/samples. Approximately 40% of samples closed with twist ties had unacceptable levels of coliforms (Table 5). Two bags were closed with tape and both were unacceptable for coliforms and one for enterococci. One sample where the bag was automatically sealed from the ice vending machine had no positive growth for any of the organisms. Although the Fisher Exact test determined that there was no significant relationship between all microbial variables and bag closures, the results suggest there is a need for more secure, sanitary methods of closing these bags, such as mechanically sealing. A more sanitary, automated method of closure for packaged ice such as that used for manufactured ice could result in a better quality bagged ice product.

Chemical Analysis. Included in the IPIA PIQCS program are safety standards for the chemical quality of the water used to produce packaged ice. In order to meet the terms for the

PIQCS accreditation, manufacturers need to follow the current standards set by the Environmental Protection Agency (EPA) and their National Primary and Secondary Drinking Water Regulations *(7, 14)*. According to the EPA secondary standards, the recommended acceptable pH level for drinking water is 6.5-8.5, less than 1.0 nephelolometric unit (NTU) for turbidity, a nitrate level less than 10 mg/L, and an alkalinity level less than 500 mg/L $CaCO_3$ *(7)*. There are no specific limits set for the conductivity levels of drinking water. The results of the chemical analysis done on the packaged ice collected from retail establishments and self-service vending machines are shown in Tables 6 and 7. Of the 250 samples, 95 (38%) samples of the packaged ice fell outside the acceptable range for pH, with 37 (39%) samples from vending samples and 58 (61%) samples from retail being unacceptable. According to the Fischer's exact test, there is a distinct relationship between the pH value and the type of ice, for ice produced at a retail establishment has a higher probability of unacceptable low pH while vending ice tends to have a higher probability of unacceptable high pH (p-value < 0.0001). More than half (50.6%) of the samples from gas stations and from the food service establishments (57.1%) were outside of the acceptable range of 6.5 to 8.5. Water with a pH that is too high or too low does not mean it is unsafe, but does have aesthetic effects on taste and odor. When a pH is too low, the water is more acidic and can become corrosive. A bitter or metallic taste can result from copper and lead being leached from the pipes carrying the water *(7)*. The level of metal in the water could also be a potential problem. A pH that is too high can produce a 'slippery' feel to the water and a soda taste to the consumer due to the high levels of alkaline minerals present *(7)*. The alkalinity level is the measure of minerals and the concentration of earth metals present, and is used to determine the efficiency of water treatments. No sample exceeded the limit set by the EPA, with the highest value measured at 127.68 mg/L $CaCO_3$ from a foodservice outlet in northern Atlanta.

Only 3 samples had turbidity levels that exceeded the recommended level of 1 NTU, 1 sample being from a vending machine and 2 samples collected from gas stations. Turbidity is the measure of the cloudiness of water and is used to show the quality of water and filtration effectiveness *(7)*. A higher turbidity level is associated with higher levels of microorganisms present in the water and there is a higher risk for potential illness if consumed *(7)*. The sample with high turbidity collected from the vending machine was also shown to have an unacceptable level of total coliforms. However, the two samples with high turbidity levels did not have significant growth of bacteria showing little correlation between turbidity level and bacterial growth in this survey. No samples were measured to have unacceptable levels of nitrates in the water. These problems can usually be fixed through proper filtration and consistent testing, which is required by the IPIA to be PIQCS accredited *(7, 14)*.

In conclusion, this study indicates less sanitary concerns with manufactured ice compared to that associated with ice packaged on the premises of retail locations and from self-serve ice vending machines. The manufacturing companies are required to follow the GMPs that were set up by the FDA *(1)*. Additionally, members of organizations, like the IPIA, are required to follow not only these GMPs but also the PIQCS guidelines that incorporate a HACCP program for these companies *(14)*. Congress directed the FDA to work to educate manufacturers regarding safe production of ice *(19)*. The issuance of a Food Facts sheet informing the public about existing FDA regulations that apply to ice manufacturers could also be beneficial if applied to locations who insist on making and selling their own ice. It is critical to train and educate workers at these locations about appropriate hygienic practices, the importance of regular cleaning and sanitizing, the risks of transferring contaminated water and ice, and prevention techniques they can take to avoid causing any foodborne illness. Consumers who purchase ice should also be educated about the risk they take by purchasing this product and ways they can also prevent cross-contamination in their own homes.

ACKNOWLEDGEMENTS

Funding was provided by the International Packaged Ice Association and the Georgia Experiment Stations. Statistical Analysis was provided by Dr. Kim Love-Myers and graduate consultant, Hejiao Hu of the Department of Statistics, University of Georgia.

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Table 1. Types of ice samples collected from manufacturing companies, retail establishments, and self-service vending machines.

	# of Samples	% of Total Samples	% of Total Retail
Total Samples	275	100	-
Manufactured	25	9.1	-
Vending	101	36.7	-
Retail	149	54.2	-
Gas Stations	81	29.5	54.4
Liquor Stores	19	6.9	12.8
Food Service	49	17.8	32.8

Table 2. Sample distribution of packaged ice purchased from retail establishments with on-site production of ice, self-service vending machines, and ice produced in a manufacturing plant.

Regions (cities)	# of Retail (%)	# of Vending (%)	# of Manufactured (%)	Total (%)
Northeast (Athens Commerce Gainesville)	19 (12.7)	22 (21.8)	0 (0)	41 (14.9)
East (Augusta)	7 (4.7)	12 (11.9)	0 (0)	19 (6.9)
Northwest (Atlanta Marietta Alpharetta Griffin)	33 (22.1)	15 (14.8)	25 (100)	73 (26.6)
South (Valdosta Albany Macon)	32 (21.5)	30 (29.7)	0 (0)	62 (22.5)
Southeast (Savannah)	29 (19.5)	12 (11.9)	0 (0)	41 (14.9)
West (Columbus LaGrange)	29 (19.5)	10 (9.9)	0 (0)	39 (14.2)
Total	149	101	25	275

Table 3. Frequency of acceptable and unacceptable levels of heterotrophic bacteria, coliforms, and enterococci in retail establishments and self-service vending machine produced ice based on the different retail sources.

		Heterotrophic bacteria[a]		Coliforms[b]		Enterococci[c]	
	# of Total Samples	# of Samples within Acceptable Limits (%)	# of Samples within Unacceptable Limits (%)	# of Samples within Acceptable Limits (%)	# of Samples within Unacceptable Limits (%)	# of Samples within Acceptable Limits (%)	# of Samples within Unacceptable Limits (%)
Manufactured Ice	25	25 (100.0)	0 (0.0)	25 (100.0)	0 (0.0)	25 (100.0)	0 (0.0)
Total On-site Samples	250	234 (93.6)	16 (6.4)	157 (62.8)	93 (37.2)	218 (87.2)	32 (12.8)
Vending	101	97 (96.0)	4 (4.0)	72 (71.3)	29 (28.7)	95 (94.1)	6 (5.9)
Retail	149	137 (91.9)	12 (8.1)	85 (57.0)	64 (43.0)	123 (82.5)	26 (17.5)
Gas stations	81	72 (88.8)	9 (11.1)	39 (48.1)	42 (51.9)	65 (80.2)	16 (19.8)
Liquor Stores	19	18 (94.7)	1 (5.3)	15 (78.9)	4 (21.1)	15 (78.9)	4 (21.1)
Food Service	49	47 (95.9)	2 (4.1)	31 (63.3)	18 (36.7)	43 (87.7)	6 (12.3)

[a] Acceptable level was based on the IPIA level of <500 MPN/ml of water.

[b] Acceptable level was based on the IPIA level of < 1MPN/100 ml of water.

[c] Acceptable level was based on the IPIA level of <1 MPN/100 ml of water.

Table 4. Number (%) of packaged ice samples exceeding acceptable limits collected from retail establishments and self-service vending machines in different regions in Georgia.

Regions	# of Total Samples	# with unacceptable levels of HPC (%)[a]	# with unacceptable levels of Coliforms (%)[b]	# with unacceptable levels of Enterococci (%)[c]
Northeast	41	2 (12.5)	9 (9.7)	2 (6.3)
East	19	0 (0.0)	3 (3.2)	1 (3.1)
Northwest	73	3 (18.8)	8 (8.6)	7 (21.9)
South	62	0 (0.0)	29 (31.2)	10 (31.2)
Southeast	41	9 (56.2)	30 (32.2)	5 (15.6)
West	39	2 (12.5)	14 (15.1)	7 (21.9)
Total	275	16 (100.0)	93 (100.0)	32 (100.0)

[a] Acceptable heterotropic plate count (HPC) level was based on the IPIA level of <500 MPN/ml of water.

[b] Acceptable coliform level was based on the IPIA level of <1 MPN/100 ml of water.

[c] Acceptable enterococci level was based on the IPIA level of <1 MPN/100 ml of water.

Table 5. Number of packaged ice samples exceeding the acceptable limits based on the different type of bag closures provided by retail establishments and self-service vending machines for heterotrophic plate count (HPC), total coliforms and enterococci.

Types of Bag Closures	# of Total Samples	# with unacceptable levels of HPC (%)[a]	# with unacceptable levels of Coliforms (%)[b]	# with unacceptable levels of Enterococci (%)[c]
Knotted	17	1 (6.3)	4 (4.3)	2 (6.3)
Metal Clip	23	3 (18.7)	13 (14.0)	4 (12.5)
Nothing Provided	5	0 (0.0)	0 (0.0)	0 (0.0)
Thread	33	1 (6.3)	9 (9.7)	6 (18.8)
Twist Ties	169	11 (68.7)	65 (70.0)	19 (59.4)
Other[d]	3	0 (0.0)	2 (2.2)	1 (3.1)
Total	250	16 (100.0)	93 (100.0)	32 (100.0)

[a] Acceptable heterotropic plate count (HPC) level was based on the IPIA level of <500 MPN/ml of water.

[b] Acceptable coliform level was based on the IPIA level of <1 MPN/100 ml of water.

[c] Acceptable enterococci level was based on the IPIA level of <1 MPN/100 ml of water.

[d] One bag was sealed; 2 bags were taped closed.

Table 6. Summary of the pH, turbidity, conductivity, alkalinity and nitrate concentrations for ice samples from retail establishments and vending machines.

Variable	N	Mean	Std Dev	Minimum	Maximum
pH	250	7.24	1.01	4.65	9.83
Turbidity	250	0.21	0.33	-0.07	4.40
Conductivity	250	70.39	75.32	2.33	574.50
Alkalinity	250	16.94	21.65	1.64	127.68
Nitrate	250	1.15	0.63	0.02	3.69

Table 7. Chemical analysis of ice collected from retail establishments and self-service vending machines based on limits set by the Environmental Protection Agency.[a]

	pH		Turbidity		Nitrate Concentration	
	# Acceptable (%)	# Unacceptable (%)	# Acceptable (%)	# Unacceptable (%)	# Acceptable (%)	# Unacceptable (%)
Total	155 (62.0)	95 (38.0)	247 (98.8)	3 (1.2)	250 (100.0)	0 (0.0)
Vending	64 (63.4)	37 (36.6)	100 (99.0)	1 (1.0)	101 (100.0)	0 (0.0)
Retail	91 (61.1)	58 (38.9)	147 (98.7)	2 (1.3)	149 (100.0)	0 (0.0)
Gas Stations	40 (49.4)	41 (50.6)	79 (97.5)	2 (2.5)	81 (100.0)	0 (0.0)
Liquor Stores	17 (89.5)	2 (10.5)	18 (100.0)	0 (0.0)	19 (100.0)	0 (0.0)
Food Service	21 (42.8)	28 (57.2)	49 (100.0)	0 (0.0)	49 (100.0)	0 (0.0)

[a]Limits set by the EPA for pH (6.5-8.5), turbidity (<1.0 NTU), and nitrate concentrations (<10mg/L).