Communication

Application of Hydrogen Peroxide to Improve the Microbiological Stability of Food Ice Produced in Industrial Facilities

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Abstract: This work was aimed to produce an "active" food ice to preserve its microbiological safety over time. With this in mind, ice cubes were processed with the addition of H_2O_2 to water before freezing. Four food ice productions were performed at the industrial level: one control trial without the addition of H_2O_2 (0OX) and three experimental trials obtained by adding 4, 8, and 12 mg/L of H_2O_2 (4OX, 8OX, and 12OX), respectively. After production, all food ice trials were artificially contaminated with 10^2 CFU/100 mL of water-borne pathogenic bacteria (*Escherichia coli* ATCC 25922, *Enteroccus faecalis* ATCC 29212, and *Pseudomonas aeruginosa* ATCC 27853) inoculated individually. Thawed ice samples were then subjected to microbiological analyses performed by the membrane filtration method and the results indicated that only trial 12OX was able to inactivate all bacteria strains. In conclusion, the addition of 12 mg/L H_2O_2 represents an optimal cost-effective strategy to preserve the microbiological stability of food ice even when it is improperly handled after production.

Keywords: food ice production; hydrogen peroxide; hygiene; microbiological safety; water-borne bacteria

1. Introduction

In order to prevent waterborne diseases, the microbiological characteristics of drinking water are of extreme importance [1]; human life is strictly dependent on the quality of drinking water [2]. For this reason, the application of disinfection methods is necessary to reduce the microbiological risks related to drinking water [3] and its transformation products. The product obtained directly from potable water through the application of the freezing process is referred to as "food grade ice" or most commonly "food ice" [4]; this product is generally available for consumption in the form of cubes [5]. After melting, food ice becomes potable water [6] and it has to satisfy the minimum requirements on the hygiene of foodstuffs [7].

Industrial food ice is produced worldwide using ice machines and is commercialized after packing in plastic bags. Often, the quality and safety of packaged food ice does not meet the hygienic standards requested for drinking water [8] and this is imputable to improper handling during production [9], post-production contamination by the transformation environment [4], and to the packaging [10]. As a result, several microorganisms can be found in food ice, mainly intestinal bacteria [5,11,12], but also unicellular and filamentous fungi [13]. This is due to the capacity of the water microorganisms to survive the freezing process [14]. Bacteria reorganize their membrane lipids to face temperature or osmotic stresses [15,16]. Proteins placed on cytoplasm membranes are involved in various

signal transduction pathways and can modify gene expression, significantly increasing the fluidity of this structure [17–19].

In order to improve the microbiological hygiene of food ice, a wide range of methods might be used to reduce or eliminate the microbial contamination in water for human consumption, which include chemical, physical, and physicochemical methods. Among the chemical methods, water chlorination is considered as one of the most efficient tools against enteric bacteria. This method is characterized by its low cost of maintenance [20], but chlorination byproducts are toxic, mutagenic, and/or carcinogenic [21]. Regarding the other methods, ozonation generates less byproducts than chlorination, but it is more expansive than this. Hydrogen peroxide (H_2O_2) is an optimal oxidizer; it is classified as a non-specific bactericide effective against a wide range of bacteria [22] thanks also to the oxidant chemicals such as superoxide ions (O_2^-) and hydroxyl radicals (OH) that can originate [23]. H_2O_2 is characterized by a certain manipulation safety [24] and is widely applied to treat drinking water [25]; due to the low levels applied in the water industry, it generates low levels of disinfection byproducts [26]. European directives do not establish a concentration limit for H_2O_2 in drinking water, although the Italian version of the UNI EN 902 [27] states: "Chemicals used for treatment of water intended for human consumption—Hydrogen peroxide" provides a dosage up to 17 mg/L. During industrial ice cube production, the pre-treatment of water from the primary source and UV disinfection of water in the tank constitute critical control points, but after production and drying, packaging and storage represent two critical points that need to be considered for food ice microbial safety [4]. Based on the above considerations, this work was aimed to apply H_2O_2 at different doses to preserve the hygienic characteristics of food ice over time, especially during administration to the final consumers.

2. Materials and Methods

2.1. Food Ice Production

Ice cube production was carried out at an industrial facility (Ice Cube Impianti S.R.L., Termini Imerese, Italy) using drinking water from natural spring water sources of the Madonie Mountains (north Sicily). Ice making was performed by modifying the classical flowsheet of "Food Ice" making [28] using hydrogen peroxide (H_2O_2) (48% *w/w*) (Figure 1) purchased from OX-CTA (Compañia de tratamiento de aguas, S.L., Huesca, Spain).
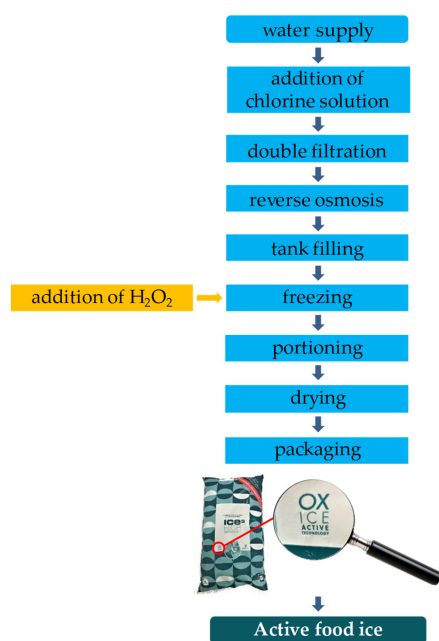

Figure 1. Flow diagrams of active food ice.

The experimental plan included four different ice productions (OX-ICE technology): 0OX, control food ice production prepared without H_2O_2 addition; 4OX, food ice production obtained adding 4 mg/L of H_2O_2; 8OX, food ice production obtained adding 8 mg/L of H_2O_2; and 12OX, food ice production obtained adding 12 mg/L of H_2O_2. Each trial was obtained with 2000 L of water. Before production, the water was pre-treated with chlorine solution (0.2 ppm) and then first transferred to quartz sand filter, and second, in a granular activated carbon filter to grab aqueous free chlorine and reduce potential health risks [29]. After filtration, in order to remove the divalent ions [30], water underwent a softening treatment followed by reverse osmosis. The water was then added with the given concentration of H_2O_2 for each trial through a pulsed-release system (Mitho S.R.L., Brescia, Italy) and finally transferred to the ice machine Vogt Tube-ice (Louisville, KY, USA) to produce ice cubes. At the end of the process, the resulting ice was portioned, dried, and packaged. Food ice production was carried out in duplicate in two consecutive weeks.

2.2. Determination of the Concentrations of H_2O_2 in Food Ice

Residual H_2O_2 in food ice was quantified by the laboratory Ambiente S.n.c. (Accredia Lab n° 0942) located at Termini Imerese (Palermo, Italy). Analyses were carried out following the methodology UNI EN 902 [7]. Briefly, 10 mL of thawed food ice was transferred into a flat-bottomed flask to which was added with 5 mL of 9 N H_2SO_4 and 35 mL H_2O to bring the final volume to 50 mL. The flask was subjected to manual agitation and the solution was added dropwise with 0.01 N $KMnO_4$ until the appearance of a pink color. The concentration of H_2O_2 was determined according to the following equation:

$$c = (S*V*EW*1000)/Vs \tag{1}$$

where c is H_2O_2 concentration; S is the strength of $KMnO_4$; V is the volume of $KMnO_4$ used in titration; EW is the equivalent weight of H_2O_2; and Vs is the volume of food ice sample analyzed.

2.3. Inactivation Assays

In order to test the inhibitory properties of food ice containing 4, 8, and 12 mg/L of H_2O_2, three bacterial strains (*Escherichia coli*, *Enteroccus faecalis*, and *Pseudomonas aeruginosa*) belonging to the American Type Culture Collection (ATCC) were used as indicators (sensitive strains). The cultures were grown in Brain Heart Infusion (BHI) broth (Oxoid, Milan, Italy) at 37 °C per 24 h. All strains were prepared for the survival assay by centrifugation at $10,000\times$ *g* for 5 min to separate cells from supernatants, washing, and re-suspension in Ringer's solution (Sigma-Aldrich, Milan, Italy). The cells of each test bacterium were inoculated at the final concentration of 10^2 colony forming units (CFU)/100 mL, which represents the highest concentrations found in ice cubes produced at different extent [4,5], in 500 mL volume sterile bottles (LP Italiana S.p.a, Milan, Italy) containing 300 g of food ice with and without H_2O_2. All contaminated systems were left at room temperature for 90 min until complete thawing and 100 mL was 0.2 μm membrane filtered (GVS Filter Technology, Indianapolis, IN, USA). All membranes were transferred to agar media and incubated at the optimal growth conditions to allow colony development as follows: *E. coli*—Coliforms Chromogenic Medium (CHROMagar™) (Condolab, Madrid, Spain), incubated at 37 °C for 24 h, was used to enumerate *E. coli* ATCC25922; *Pseudomonas*—Agar Base (PAB) added with Cetrimide Fucidin Cephaloridine (CFC) supplement (Oxoid), incubated at 25 °C for 48 h for *P. aeruginosa* ATCC27853; kanamycin aesculin azide (KAA) agar (Biotec, Grosseto, Italy), incubated at 37 °C for 24 h for *En. faecalis* ATCC29212. Microbiological analyses were carried out in duplicate.

2.4. Statistical Analysis

Microbiological data were subjected to one-way variance analysis (ANOVA) using XL-Stat software version 7.5.2 for Excel (Addinsoft, New York, NY, USA). The Tukey's test was applied for pairwise comparison between count levels of each individual microorganism at

different H_2O_2 concentrations. Statistical significance was attributed to *p* values of $p < 0.05$ and are marked with different letters.

3. Results and Discussion

In this study, the strains *E. coli* ATCC25922, *En. faecalis* ATCC29212, and *P. aeruginosa* ATCC27853 were used as indicator strains to test the efficacy of H_2O_2 to process ice. The species *E. coli*, *En. faecalis*, and *P. aeruginosa*, generally transmitted by ice [31], represent the main pathogens used for the evaluation of the mandatory and accessory parameters provided by the Italian Legislative Decree No. 31/2001 [32] for the implementation of directive 98/83/EC. The results of survival tests, expressed as CFU/100 mL of thawed ice, are reported in Figure 2.
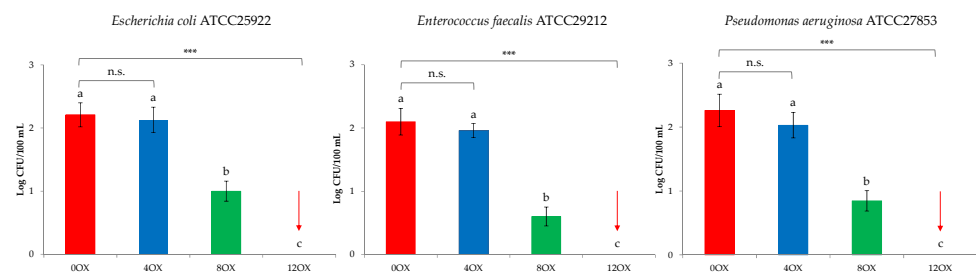

Figure 2. Counts of water-borne pathogenic bacteria in food ice activated with different concentrations of H_2O_2. *p* value: *** $p < 0.001$; n.s.—not significant. The letters a, b, and c on the histograms indicate difference between the samples analyzed according to Tukey's test.

After bacterial inoculation, the cell densities of control ice samples (0OX) were 2.21, 2.10, and 2.26 Log CFU/100 mL for *E. coli* ATCC25922, *En. faecalis* ATCC29212, and *P. aeruginosa* ATCC27853, respectively. In more detail, Figure 2 shows that the antibacterial activity of the food ice activated with different concentrations of H_2O_2 was comparable among the different strains tested. No significant differences ($p > 0.05$) were found among the levels of microbial count for the trials 0OX and 4OX between the same bacteria strains, showing that the presence of 4 mg/L of H_2O_2 in the food ice did not exert any disinfecting action. In contrast, the counts of the three bacteria decreased significantly ($p < 0.001$) with increasing H_2O_2 concentration. In particular, *E. coli* ATCC25922, *En. faecalis* ATCC29212, and *P. aeruginosa* ATCC27853 decreased consistently in the 8OX trial and were not detected in the 12OX samples, showing the bactericidal efficacy of H_2O_2 at the concentration of 12 mg/L against both Gram-positive (*En. faecalis*) and Gram-negative (*E. coli* and *P. aeruginosa*) bacteria commonly searched in water to establish its drinkability [33].

Ríos-Castillo et al. [34] demonstrated the effect of H_2O_2 at 0.5% in combination with other products (benzalkonium chloride, cationic polymer, ethoxylated fatty alcohol, and ethyl alcohol) used in food industries against *E. coli*, *Staphylococcus aureus*, *Enterococcus hirae*, and *P. aeruginosa* on stainless steel surfaces. However, the authors found differences in terms of bacterial sensitivity and evidenced a more pronounced effect of the mixtures used when the test organisms were *En. hirae* and *P. aeruginosa*. Lineback et al. [35] demonstrated that H_2O_2 at different concentrations were more than quaternary ammonium compounds effective against *S. aureus* and *P. aeruginosa* biofilms. Furthermore, Alfa et al. [36] demonstrated that a concentration of 0.5% H_2O_2 represented a highly efficient antimicrobial strategy to disinfect medical devices. Regarding food applications of H_2O_2, Arefin et al. [37] used several concentrations of this compound for raw cow's milk preservation, demonstrating that 0.14% of H_2O_2 was suitable to extend the milk shelf-life where milk cooling facilities were not available. Hence, H_2O_2 seems to be a proper compound to contain intestinal bacteria that can be found in water/food ice [5] at low cost.

4. Conclusions

This study provides, for the first time, an extended analysis on the survival of the main pathogens responsible for water-borne diseases in humans in food ice produced at industrial level with different concentrations of H_2O_2. The results of this study showed that the major bacterial species that compromise the drinkability of water such as *E. coli*, *En. faecalis*, and *P. aeruginosa* were inhibited by 12 mg/L of H_2O_2 added to food ice during production. These results represent a final solution to preserve the microbiological stability of food ice, even when it is improperly handled after production. As a matter of fact, unlike all other food products, food ice is exposed to a high risk of unintentional contaminations, especially in the HORECA (hotel, restaurants and catering) channel where this product is commonly used. Thus, OX-ICE represents a useful technology to process food ice. However, further studies are necessary to evaluate oxidizability of water from thawed ice added with H_2O_2.

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