Research Article

Decreasing Microbial Contamination Logs Of Ozonated Ice Used For Meat Transport In An Icebox

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Abstract:

Meat is a very perishable food product, and improper temperature control during transportation may cause rapid growth of microbes, which poses a health hazard to the population. Ice usage is not a new concept in the transportation process, but other types of ice with antimicrobial qualities can provide a better degree of preservation.

The research was carried out at the Meat Health Laboratory, Public Health branch, College of Veterinary Medicine, University of Baghdad, for a period of 2 months. The sheep meat samples were kept in an icebox under three conditions: no ice, tap water ice, and ozonated ice for 3, 5, and 8 h. Microbial contamination was determined by determining the total aerobic bacteria count and total coliform count.

Meat transported without ice exhibited the highest bacterial counts across all time periods. Tap water ice reduced bacterial growth compared with no ice, whereas ozonated ice produced the greatest reduction in both total aerobic and coliform bacterial counts. This effect was consistent throughout the experiment and across all transportation times.

The use of ozonated ice during meat transportation significantly improves microbiological quality compared with tap water ice or no ice. Therefore, ozonated ice is a promising and effective method for enhancing meat safety and preservation during short-term transportation.

Keywords: Red Meat, Ozonated Ice, Tap Water Ice, Total Aerobic Bacterial Count, Total Coliform.

1. Introduction:

Poor storage conditions for red meat make it perishable. Therefore, meat should be stored under proper conditions and subjected to preservation techniques that prevent spoilage or alter its quality [1][2].These changes are physical and chemical and are caused by bacterial growth. The period during which meat is treated to preserve quality during freezing and cooling [3] [4][5]. Treating meat with chemicals such as salts and preservatives such as nitrates and sodium ascorbate, sometimes smoking it with substances that kill bacteria, and exposing it to irradiation, microwave, and ozone cooking [6][7]. It is better to use it for sterilizing water because it oxidizes easily and does not affect water quality or odor [8] [9]. Ice is also preferred for bulk storage and food transportation because it lowers product temperature, which slows microbiological and biochemical degradation [10] [11][12] .

Commercial poultry processors package and ship raw poultry meat using dry ice [13] [14] [15] [16]. Ice has a greater cooling effect than normal ice, thus demonstrating a bacteriostatic effect on fresh meat microorganisms [17] [18] [19][20].Another antimicrobial intervention developed by [21] is the use of ice in food packaging and transportation. ABI is an ozone-based process that preserves the increased cooling capacity of dry ice and the antimicrobial effect of ozone to inhibit pathogen growth and extend shelf life [22] [23]. Nevertheless, ice, whether alone or in combination with the ozone layer, poses certain risks, including the possibility of explosion, suffocation, and contact risks during transportation [24][25] [26] . Handling is an art that requires advanced skills, which could be a limitation. Moreover dry ice fails to improve parameters of meat quality that are important to both processors and consumers, including raw meat yield, color, texture, and cooking yield during storage and transportation[27][28][29][30][31].During meat processing, antimicrobial treatment during slaughter and deboning may fail to control pathogens and spoilage organisms during subsequent transportation and storage [32] [33][34]. Ice was studied to determine its effect on the food safety, shelf life, and quality of refrigerated raw meat [35] [36][37] [38].

This study aimed to determine whether ice cubes made with ozone-treated water could preserve red meat during transportation by reducing total aerobic bacteria, a criterion widely used as a standard and health indicator of meat cleanliness and quality. Contrast between ice cubes made from ozone water and non-ozone water in reducing bacteria in red meat. Ozone ice cubes could replace ordinary ice cubes in ice production.

2. Materials and working methods:

2.1 Ethical Approvall

This study was approved by the Scientific Committee of the Public Health Branch, College of Veterinary Medicine (Approval No.442). S.M.10-3-2024).

2.2 How to take samples:

A total of 30 fresh meat samples were collected from local slaughterhouses in Baghdad and transported aseptically to the Meat Health Laboratory, College of Veterinary Medicine, University of Baghdad. Approximately 250-g weight was used for each sample, divided into 3 parts. Such portions were exposed to three treatments: transportation without ice, transportation with ice cubes made from tap water, and transportation with ozone ice cubes. The portions were stored in sterile polyethylene bags in an insulated icebox for 3, 5, or 8 h. The study was conducted over a recommended two-month period to test consistency over time, with five repeats at a time and a transport period.

2.3 Sample preparation:

The meat was cut into three parts, and transportation was done thrice with each of the first sections (3, 5, and 8 h) without ice in an ice box (Figure 1a, b). The second section was dipped in ice tap water, and the third section was dipped in a bag containing ice ozone. Then, bacteriological tests were conducted.

a **b**

Figure (1); ice box (a. b.)

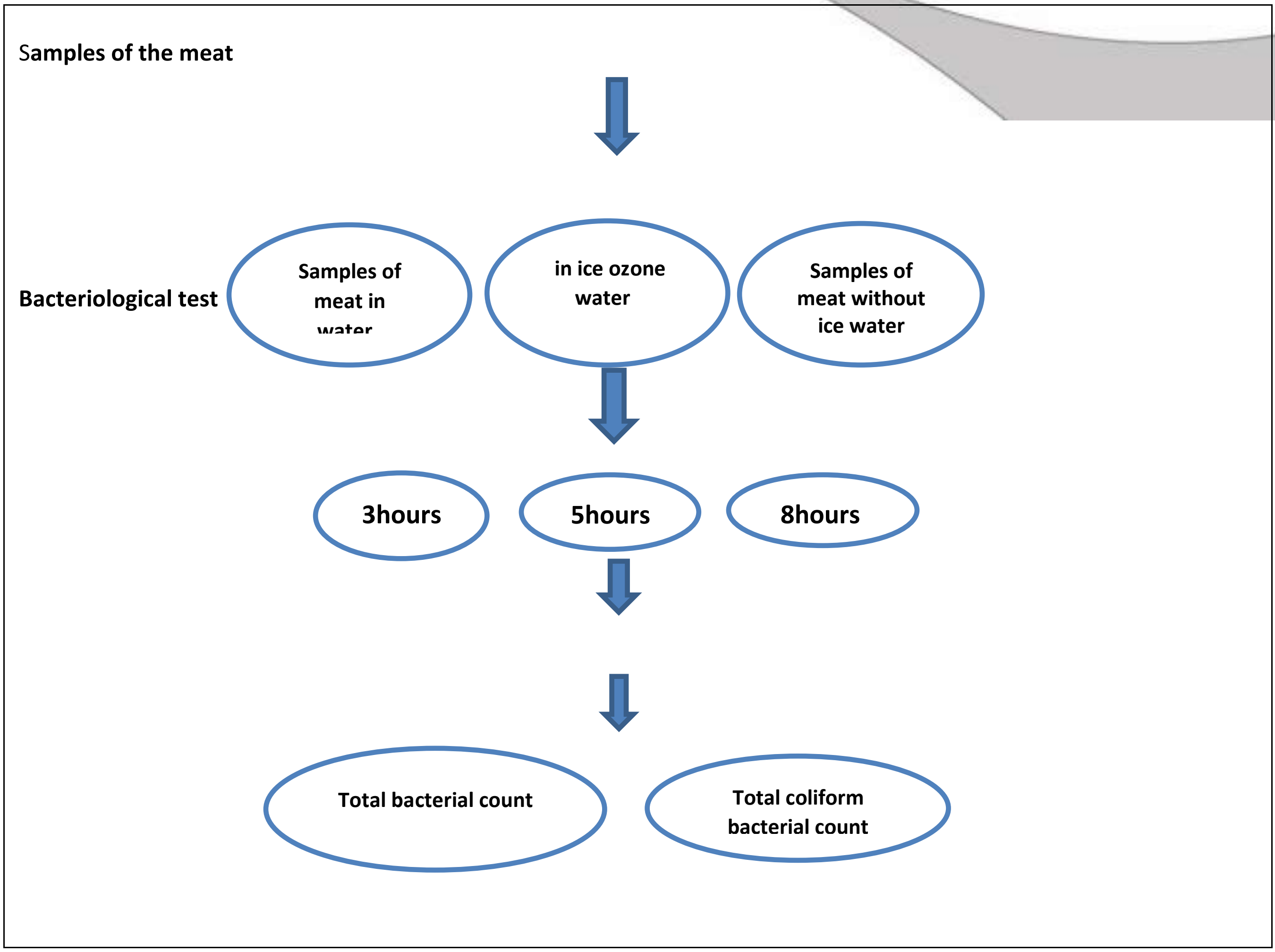


Figure 2: Experimental design of the system

2.4 Bacteriological examination of the specimens

After preparing the samples (dilution bottles and dilutions containing 1% peptone water) and the culture medium nutrient agar, the bottles and culture media were sterilized in an autoclave for 15 min. The sample is removed from the autoclave, poured into sterile dishes, and allowed to solidify. Then, we proceed with the bacteriological examination [39].

2.5 Examination of the total aerobic bacteria count

Once the dilution bottles are prepared, the culture medium is poured into sterile dishes, and 1 ml of a bag with a sample of meat is transferred to the next dilution and repeated until all the dilutions of 10^{-1} to 10^{-6} are tested. Then, 1 ml of each dilution is transferred to a dish containing culture medium (NB) and incubated overnight at a temperature of 35 $^{o\,C}$ and the hearts incubated—total aerobic bacterial count (T.A.P.C.) described by [39].

Using sterile nutrient agar and incubating aerobically at 37 °C for 24 h. The colony size of the bacteria culture ranges from 30 to 300.

Count of bacteria (CFU)/gr meat = colony culture X dilute factor -1

The total coliform count (TCC) described by method [39] is similar to the total bacterial count but different from the plating method using sterile violet red bile agar (VRBA), and the colony of the culture of bacteria ranges from 50 to 150.

Coliform count (CFU)/gr meat = colony culture X dilute factor -1 and then incubated aerobically at 37°C for 24 h. Subsequently, the number of developing colonies is counted using a bacterial counting device.

2.6 Preparation of ozonated ice

To prepare the ozonated ice cubes, ozone gas was introduced into potable water using an ozone generator, and the ice cubes were then frozen. Ozonated water was poured directly into sterile ice trays, which were frozen at 20 °C. The same freezing specifications were required for the tap-water ice cubes as for potable water that had not been ozone-treated. The icebox was used to fully cover the meat samples, which underwent the same ice treatment during transportation, ensuring that they were evenly cooled. Direct comparisons of the effects of ozonated ice, tap water ice, and the absence of ice on meat transportation could be made about antimicrobial and cooling performance.

2.7 Microbiological analysis of the samples

To conduct the microbiological examination, 10 g of each meat sample was placed in 90 mL of sterile 1% peptone water and homogenized to obtain a 10^-1 dilution. Up to 10 -6 serial dilutions were made. The pour plate technique was used to count the total aerobic bacterial counts on nutrient agar; the plates were incubated at 37 °C for 24 h. The number of CFU colonies ranged from 30 to 300. The number of total coliform colonies under incubation conditions was determined using the Violet Red Bile Agar (VRBA), yielding 50-150 CFU. The final data are presented in log CFU/g of meat.

2.8. Statistical Analysis

Statistical analysis was performed to identify the effects of treatment and transportation duration on microbial counts. The meaning of variation was analyzed using SAS software [40], depending on the treatment type (ozonated ice and tap-water ice, no ice, and treatment type) and transportation time (3, 5, and 8 h). The effects incorporated in SAS were the treatment type (ozonated ice and tap-water ice, no ice, and treatment type) and the transportation time (3, 5, and 8 h). Least-squares means were used to compare the differences between treatments at each sampling period to perform statistical calculations. The results are presented as averages with a p-value of 0. Five was employed.

Results

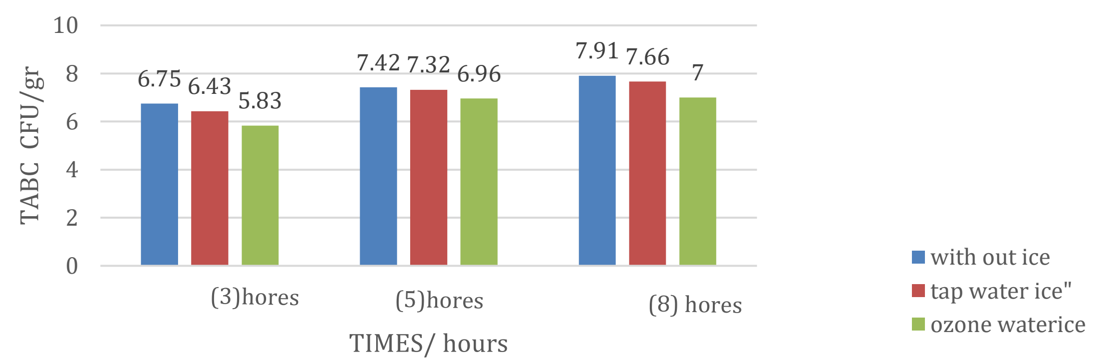
First month

Table 1 gives an informative account of the total aerobic bacteria counts in sheep samples in the first month of the experiment, with emphasis on the influence of two ice treatments: ice water and ice ozone. The variations between these treatments were statistically significant ($p< 0.05$). The most notable difference was in the samples left without any ice cubes, which recorded the bacteria levels to be more than the upper limits allowed by the Iraqi standards, as found in the Central Iraqi Quality Control System (1992). In a further study on the state of transport, it was discovered that the number of bacteria increased tremendously when the meat was stored in an ice box without ice. The growth rates of bacteria in a 3-8-hour transport period could be compared to 6.75 and then to 7.91 log CFU, which emphasizes the importance of proper cooling of food to guarantee its safety. In addition, Table 1 indicates that the count of bacteria using tap water ice cubes was also significantly lower than that using ice cubes without ice, with the counts being between 6.43 and 7.66 cfu/g of meat. Bacterial counts in sheep meat, as shown in Table 1, varied across the 3-, 5-, and 8-h intervals, with significant differences ($p = 0.05$). The bacterial counts ranged from 5.83 to 7.00 cfu/g of meat, indicating that bacterial growth was less active with increasing ozone exposure, supporting the beneficial effect of ozone in reducing bacterial counts during application.

Table 1 shows the mean of total aerobic bacteria in sheep meat samples, which were transported for 3, 5, and 8 h in an ice box.

Time (hours)	With ozonated ice	With tap water ice	Without ice	Number of samples
3 hours	5.83^{bc}	6.43^{ab}	6.75^{ab}	15
5 hours	6.96^{bb}	7.32^{aa}	7.42^{aa}	15
8 hours	7.00^{aa}	7.66^{aa}	7.91^{aa}	15
Significance	P < 0.5	P < 0.05	P < 0.05	—

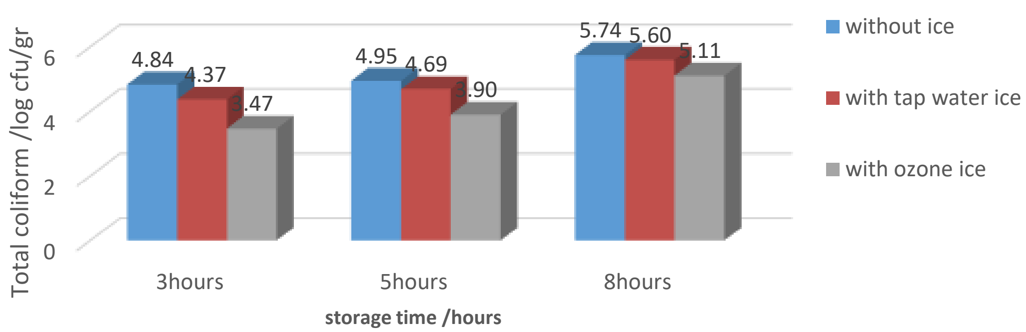
Storage periods of 3, 5, and 8 h with ice and ozone were the most suitable for reducing bacterial growth. They showed significant differences ($P<0.05$) compared with ice-cube tap water, and the rates were lower than those in samples without ice cubes.
Table 2 shows the average total coliform bacterial count in sheep meat samples during the first month of the experiment at periods 3, 5, and 8 h of adding ice cubes potable water, ice ozone cubes where the rate of bacterial growth with out of storage in ice was 4.84 cfu/gm of meat reached 5.74 cfu/g meat, rate of bacterial growth with tap water ice was 4.37 cfu/gm of meat reached 5.60 cfu/g meat and this rate of bacterial growth decreased with The optimum period 3, 5, and 8 h of ice ozone were the most successful to reduce the bacterial growth and significant differences (($P< 0.05$) were between meat with tap water ice cubes and rates were less than samples without ice cube.



figure(3): The means of total bacterial count in meat samples in time (3 , 5 , and 8) hours in ice box

Table (2): The means of total coliform bacteria counts of sheep meat samples, transportation times (3, 5, and 8) hours in an ice box.

Time (hours)	With ozonated ice	With tap water ice	Without ice	Number of samples
3 hours	3.47	4.37	4.84	15
5 hours	3.90	4.69	4.95	15
8 hours	5.11	5.60	5.74	15
Significance	$p \leq 0.5$	$p \leq 0.5$	$p \leq 0.5$	—



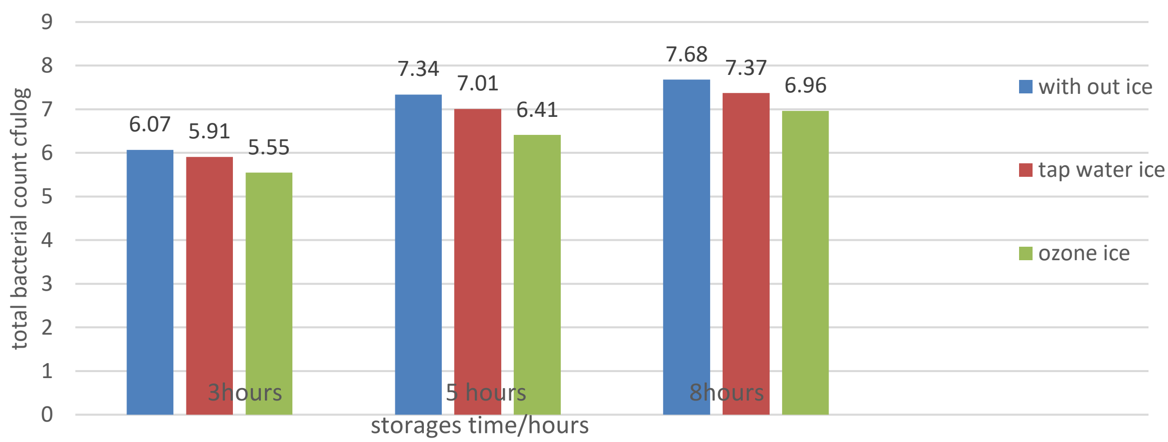
fig(4): the mean of total coliform in samples sheep meat storage time (3,5and 8)hours in ice box

Second month

Table 3 shows the total aerobic bacteria rates in the second month for sheep treated with ice-potable water, with no significant differences (P<0.05) compared with the samples without ice cubes. In these samples, bacterial counts decreased by more than a logarithm, exceeding 7 log10 cfu/g of meat. Ozone-infused ice was particularly effective at reducing bacteria because it damages cell walls through disintegration and oxidation, thereby lowering bacterial activity. This finding aligns with previous research (25, 26) and confirms that ozone effectively preserves meat by inhibiting bacterial growth.

Table (3): Means of total aerobic bacteria of sheep meat samples, transportation times (3, 5, and 8 h in the ice box, respectively)

Time (hours)	With ozonated ice	With tap water ice	Without ice	Number of samples
3 hours	5.55^{bb}	5.91^{ab}	6.07^{ab}	15
5 hours	6.41^{ab}	7.01^{aa}	7.34^{aa}	15
8 hours	6.96^{aa}	7.37^{aa}	7.68^{aa}	15
Significance	P < 0.05	P < 0.05	P < 0.05	—

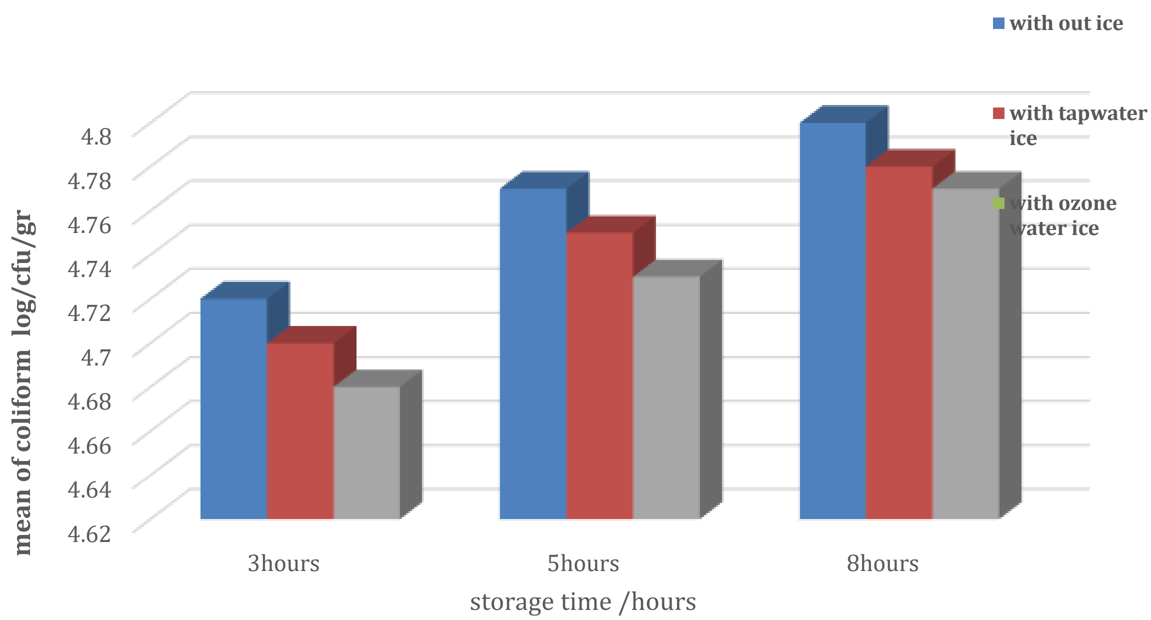


fig(5)the mean of total bacterial count in samples sheep meat in storages time (3,5and 8)hours in ice box.

Table 3 shows the mean total aerobic bacterial count in sheep meat samples at the end of the second month of the experiment in potable water ice, ozone ice cubes, and absent ice storage at 3-, 5-, and 8-h intervals. The bacterial count in the absence of ice storage was 6.07 cfu/g, which increased to 7.68 cfu/g. The average bacterial count for tap water ice at 3, 5, and 8 h were 5.91 cfu/g, which increased to 7.37 cfu/g. The total number of ozone water ice was 5.55 cfu/g, which increased to 6.96 log10 cfu/g. Ice, especially the ozone layer, reduces bacterial growth rate, thereby lowering bacterial efficacy. This is considered an important component, along with energy and oxygen, as reported in previous studies (23,24). Storage times of 3, 5, and 8 h with ice ozone were most effective in inhibiting bacterial growth, and there were significant differences (p<0.05) between meat stored in tap water ice cubes and meat not stored with ice cubes, which had lower rates.

Table (4): Total coliform bacteria count of sheep meat samples transported for 3, 5, and 8 h in an ice box

Time (hours)	With ozonated ice	With tap water ice	Without ice	Number of samples
3 hours	4.68ᵃ	4.70ᵃ	4.72ᵃ	15
5 holes	4.73ᵃ	4.75ᵃ	4.77ᵃ	15
8 holes	4.77ᵃ	4.78ᵃ	4.80ᵃ	15
Significance	N.S	N.S	N.S.	—



Fig(4):the mean of coliform count in meat samplesstorages(3,5 and 8) hours in ice box

Discussion:

The total aerobic bacterial counts were measured without ozone ice cubes during the first and two months of the experiment, and significant differences (P < 0.05) were observed. Bacterial counts increased more in samples with ice cubes than in samples without ice cubes, which does not support bacterial growth. This variation has implications for public health, particularly for the transport of meat without ice, as confirmed by researchers (26, 27, 28). Bacterial counts were continuously determined without the addition of ozone ice cubes, and the difference was significant (P < 0.05). The numbers increased significantly compared to the samples without ice, indicating the significance of ice in safe meat transport. Support for these findings is provided by several studies (36, 37, 38). Tables 3 and 4 show the data on total aerobic bacteria and total coliform bacteria in sheep meat samples.

Furthermore, the microbicides disruption pattern validates that dissolved ozone selectively targets double bonds within bacterial cellular envelopes, rendering it highly effective against both Gram-negative and Gram-positive populations in fresh meat commodities during initial handling chains [6] While ozonated ice offers a highly functional combination of low temperature and active disinfection, its long-term biocidal longevity is strictly dependent on the chemical half-life of trapped ozone crystals, which diminishes dynamically during continuous storage [24] Enteric bacteria like coliforms exhibit extreme susceptibility to initial oxidative interventions; however, under extended cold storage regimes, environmental freezing stress becomes the dominant suppressing factor, minimizing statistical variance between treated samples and controls over time [22].

Conclusions

This research demonstrates that the transportation of sheep meat without ice greatly affects microbial contamination and can pose a threat to food safety. The addition of ice in transit reduces bacterial growth, and some control of microbial development is obtained with tap-water ice. The ozonated ice was significantly superior in destroying the total aerobic and coliform bacteria compared with the tap-water ice and no-ice conditions. This effect was constant over 3, 5, and 8 h of transportation time and even in various months. Therefore, ozonated ice can be suggested as an alternative to traditional ice in short-term meat transportation to improve microbiological quality and contribute to the well-being of the population.

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Authors' contributions

Dhafir A.A. Alobaidi: Conceptualization, Methodology, Data collection, and Writing – original draft. Nadia I. Abdullaal: Data analysis and Writing – review & editing. All authors have read and agreed to the published version of the manuscript.

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