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RESEARCH ARTICLE

Determining the effectiveness of ozone in prolonging the preservation of soft cheese

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Abstract

This study aimed to determine the effectiveness of ozone in prolonging the preservation of soft Cheese preserved by cooling during different storage periods of up to 21 days and contaminated with the bacterial type *Escherichia coli* causing food poisoning. The study showed that the best time for exposure to ozone at a concentration of 0.5 was 30 minutes when studying chemical and microbial properties compared to pasteurized milk at 72°C for 30 seconds and ozone laboratories at different times. The Cheese was then manufactured in the laboratory by three samples, including control cheese (T1), soft cheese contaminated with *E.coli* bacteria (T2), soft cheese contaminated with *E.coli* bacteria, exposed to ozone (T3) with a test of the microbial properties of soft Cheese, and refrigerated for 21 days at 5 ºC ± 2ºC. The results showed that the total number of bacteria in the control cheese and the Cheese contaminated with food poisoning bacteria increased significantly at the probability level (p< 0.05) until the end of the storage period, but it was observed that in the T3 treatment exposed to ozone, the total number of bacteria decreased compared to the T1 and T2 samples. In contrast, the number of coliform bacteria, yeasts, and molds did not show any growth for all treatments throughout the storage periods.

Keywords: Ozone, Food poisoning bacteria, Soft cheese

Introduction

Food spoilage has been defined as a set of phenotypic and sensory changes in food or changes that make it unhealthy due to contamination with toxic chemicals or microorganisms that make it unfit for human consumption. Food decomposition and spoilage are a counter-state in its operations, with food preservation operations aimed mainly at prolonging the food preservation period for the longest without spoilage (Ghasemi-Varnamkhasti, M et al. 2018). Cheese is a milk product formed due to milk coagulation and the separation of casein protein from it. Cheese has many beneficial properties as it plays an important role in human health because of its nutritional value; in addition to its nutritional value, it has a biological value determined by the number of amino acids, vitamins, and enzymes (Bakhtiyorbekovich, Y. A et al. 2022). Cheese is an ancient food product that can be produced from different types of milk, and in general, Cheese was used as part of the natural diet of humans, as it is mainly characterized by high nutritional value and is the best source of protein, fat, vitamins and some minerals, especially calcium, and phosphorus, which are mainly required for human health and nutrition (Ting, T. et al. 2022). Despite the importance of nutritional Cheese, it is characterized by its high moisture content, which makes it a suitable medium for the growth of types of microorganisms; it is susceptible to chemical and microbial damage, so preserving this product is very important (El-Sayed SM et al 2020). One of the methods used to extend the shelf life of Cheese is the use of ozone technology, which is one of the preferred methods in dairy factories as a highly effective disinfectant due to its high efficiency and conversion to oxygen as it does not leave any unwanted residues, which is a three-atom molecule of oxygen (O3) with a high reaction that reacts quickly with the target microbes due to its large oxidative capacity and immediately decomposes into harmless diatomic oxygen molecules (O2) (Raghunathan, R. et al 2021). From the above, the study's objective was to determine the effectiveness of ozone in preserving soft Cheese manufactured in the laboratory in microbial traits after storage for 21 days in the refrigerator.

Materials and Methods

Isolate *E.coli* Bacteria

E.coli bacteria were isolated as reported in the Thongaram method (Thongaram, T. 2016) by mixing 10 g of each milk sample with 90 ml of Normal Saline solution. 100 µL of the resulting solution was taken into MacConky agar medium, distributed using a glass diffuser, and after incubation at 37°C for 48 hours. The extinct growing bacterial colonies were taken and replanted on the same medium above in a planning manner to obtain pure bacterial isolates that were diagnosed through phenotypic, microscopic, and biochemical tests appropriate to reach the bacterial species of each of them as reported by Roberts and Greenwood (Roberts, D. et al. 2003).

Milk source

Milk samples were collected from 5-year-old healthy cows in the fourth month of the milk production period in sterile and clean bottles, transported to the laboratory, and placed in the refrigerator at a temperature of 4°C. To ensure the safety of the milk and free from mastitis disease, I use the White Side Test as in AL-rawy (AL-rawy et al. 2003). When a precipitate and/or gel forms, this is evidence of udder infection.

Microbial milk tests

The total number of bacteria was estimated by the method of molded dishes according to the method Frank and Yousef. The numbers of total bacteria of the colon were estimated, by the method (Chen et al 2019) and the total number of yeasts and molds was estimated using the Frank and Yousef method (Frank, J. F et. al. 2004)

Ozone milk treatment

The milk was treated with ozone as three clean plastic containers were used, each container contained a liter and a half of raw cow's milk, and the containers were covered with plastic cover with a hole to insert the hose, which contains at the end of a porous stone to spread the gas inside the milk and then expose each sample of milk to a concentration of 0.5 ozone for times 5 minutes, 15 minutes, 30 minutes respectively and then conducting microbial tests to choose the best time for exposure.

Soft cheese manufacturing

The Cheese was manufactured and divided into three treatments (control cheese, soft Cheese contaminated with *E.coli* bacteria, soft Cheese contaminated with *E.coli* bacteria and exposed to ozone), and the Cheese was made according to the Fox et al. method (Fox, P et al. 2017). Through the following method: pasteurization of cow's milk obtained from one of the milk processors in Salah al-Din Governorate to a temperature of 63 m for 30 minutes, then cool the milk to 40 m and add rennet to it Some samples were inoculated with food poisoning bacteria (*E. coli*) and leave for 45 minutes until reaching the curd stage, then cut the curd to get rid of whey and add salt by 2.5% and put the curd in the fidgeting cloth to get rid of the largest amount of whey. The curd was packed in special molds for each sample, then the samples were marked and kept in the refrigerator for tests installed in the study.

Microbial tests of cheese

The numbers of mycobacteria, coliform bacteria, yeasts, and molds in cheese samples were estimated using the molded dish method, according to (Frank et al. 2004).

Statistical analysis

The results of the experiments were analyzed using the Linear Model General within the ready-made statistical program S.A.S. to study the effect of factors on the C.R.D. and the Duncan test was conducted to determine the significance of the differences between the averages of the factors affecting the studied traits at the level of (0.05) (Duncan, D. B. 1955)

Results and Discussion

Isolation and diagnosis of bacteria

The bacterial type *E. coli* was isolated from raw cow's milk samples left for two days after the frightening procedure were transplanted on the medium of MacConkey agar, and the diagnosis was completed after development at 37°C for 24 hours. The colonies appeared pink, and they were small and dry on the center of Akar Almakonki used the dye Cram to know the shape of the cells; the result was short bacilli negative for the dye Cram, non-forming spores and could not decompose blood, positive for the catalase test when hydrogen peroxide is added to the young colonies, and was non-producing H2S gas and negative for the oxidase test when the reagent was added. The violet color did not appear, and when conducting a mobility test showed its mobility by peripheral flagella. It showed its ability to ferment mannitol (Tab. 1).

Table 1. Results of phenotypic and biochemical diagnostic tests for *E. coli* isolate

Type of Test	Test names	Test results
Phenotypic assessments	Colonies	Colonies are small, round, and sticky, with a smooth edge
	Movement	+
	Pigmentation with gram pigment	-
	The shape of the cells under a light microscope	Its shape is sticky
Biochemical diagnostic assessments	Catalase	+
	Oxidase	-
	H2S	-
	Mannitol	+

Effect of parameters on microbial content of milk

Tab. 2 shows the effect of different treatments on the microbial content of milk. As is noted from the table that the total number of bacteria in raw milk, pasteurized milk, and milk exposed to ozone at different times (5, 15, and 30) was 4.77, 2.81, 3.59, 2.17, and 1.40, respectively. At the same time, it was found that the number of colon bacteria in raw milk was 3.12 and in milk exposed to ozone rays at a time of 5 minutes 1.08, while no growths appeared in pasteurized milk and milk exposed to ozone rays at times (15, 30). The same table also showed that the number of yeasts and molds was in raw milk 1.34 and milk exposed to ozone rays at 5 minutes 0.54, but no growths appeared in pasteurized milk and milk exposed to ozone rays at times (15, 30). The table showed that rapid pasteurization and ozone exposure coefficients decreased the total number of bacteria, coli, yeasts, and molds compared to the raw milk sample.

Table 2. Effect of conventional pasteurization and ozone (0.5g / for different times) on the microbial content (LWT/ml) of milk.

Microbial content	Raw Milk	Quick pasteurization (72ºC/15 Min)	Ozone's timings (Minutes)		
			5	15	30
Total Content	4.77^{a}	2.81^{c}	3.59^{b}	2.17^{d}	1.40^{e}
Coliform bacteria	3.12^{a}	N.D	1.08^{b}	N.D	N.D
Yeasts and molds	1.34^{a}	N.D	0.54^{b}	N.D	N.D

The different letters in one row indicate that there are significant differences between the storage period at the level ($p<0.05$).

The high total number of microorganisms in raw milk is due to the use of contaminated raw milk, the lack of hygienic conditions when milking, causing increased contamination, as well as storage under inappropriate conditions, which helps the reproduction and growth of microorganisms already present in milk. The total number of microorganisms in milk before and after pasteurization is a key indicator of milk quality and a measure of the extent of its contamination by microorganisms. The results of this study are consistent with the findings of Mohamed and Elzubeir, who found that storing raw cow's milk in the refrigerator led to an increase in the total number of bacteria, possibly because the temperature used was suitable for the growth and activity of the bacteria. The results agreed with the findings of Al-Jubouri,, who found a significant difference between the total number of bacteria in raw milk, which was 4.77, and the total number of bacteria in pasteurized milk quickly, as it was 2.51, and attributed the reason for the decrease in the total number to the fact that pasteurizing milk with rapid pasteurization distorts enzymes in bacteria, which leads to damage to the bacterial cell shell. These results are consistent with the Aldouri findings, which found a decrease in the total number of bacteria treated with traditional pasteurization, as the number of bacteria decreased from 4.9330 to 3.521. The reason for this was attributed to the fact that the treatment of liquid milk at pasteurization temperature has a role in reducing the total number of bacteria as a result of the destruction of microbes. These results also agreed with (Younis et al.,2006) who indicated that the exposure of milk to ozone radiation at a concentration of 0.5 for different times(5, 15, 30) were less in number of total bacteria at the time of exposure 30, as it recorded 2.40 compared to the raw sample, which was 7.22. The results agreed with Todar who indicated a decrease in the number of bacteria in milk exposed to ozone, and this study is consistent with the findings of Al-Obaidi who found that exposing liquid foods to ozone rays led to a decrease in the total number of bacteria, attributing the reason to that ozone gas tears cell envelopes and kills bacteria.

As for the number of coliform bacteria, the high numbers in raw milk are attributed to the use of unpasteurized raw milk, the non-application of health conditions in milk production, and allowing coliform bacteria to grow during the period between milking, processing, and contamination. The results agreed with Elaageb, who indicated that pasteurization of raw milk led to the elimination of coliform bacteria as their presence in pasteurized milk means that the milk is not pasteurized properly or that the milk has been contaminated with fecal sources or contaminated containers. The results of the pasteurized milk samples at a temperature of (72°C) for 15 / second agreed with what was shown by the U.S. Food and Drug Administration standard, which states that the number of coliform bacteria in pasteurized milk should not exceed 10 W.T.M./ml.The results agreed with what was found by Aldouri, who stated that the total number of coliform bacteria in raw milk was 2.477, and when pasteurized with rapid pasteurization, no growth of coliform bacteria was observed. These results also agreed with (Cavalcante et al. 2005), who observed that 5-minute ozone use led to a significant reduction in the number of coliform bacteria compared to raw milk. The results also agreed with (Metwally et al. 2003), who found that treating milk with ozone gas for 10 minutes at a concentration of 0.5 led to no growth in the number of coliform bacteria and attributed the reason to this that ozone gas affects the metabolism of bacterial cells by inhibiting and blocking the work of the enzymatic control system, which leads to the elimination of bacteria.

As for yeasts and molds, the results of pasteurized milk samples at a temperature of 72°C for 15 / second agreed with what was shown by the U.S. Food and Drug Administration standard, which states that the number of yeasts and molds in pasteurized milk should not exceed 10 W.T.M./ml. The results of pasteurized milk were consistent with (Hossain et al. 2009), who reported no yeasts and molds in milk after treatment with traditional pasteurization. The results agreed with Al-Obaidi, who found that treating liquid foods with ozone gas led to the complete killing of yeasts and molds and the absence of growths.

Effect of different treatments on the microbial content of soft cheese

The Total Content of Aerobic Bacteria: Tab. 3 shows the total number of bacteria in soft Cheese manufactured in a laboratory and stored for 21 days at 5 ± 2°C contaminated and uncontaminated with *E.coli* bacteria and ozone laboratories at a concentration of 0.5 g for 30 minutes. It is noted that the total numbers of bacteria in the control sample increased significantly, and their values on days 1, 7, 14, and 21 of storage 39×10^6, 58×10^6, 80×10^6, 87×10^6 respectively. In Cheese contaminated with *E. coli*, it was recorded 44×10^6, 64×10^6, 87×10^6, and 93×10^6 during the storage periods 1, 7, 14, and 21, respectively. Still, if Cheese contaminated with *E. coli* bacteria was exposed to ozone for the same storage periods, the numbers decreased to 15×10^4, 20×10^4, 23×10^4, 25×10^4 respectively, for the storage days mentioned above.

Table 3. The total number of bacteria in soft Cheese contaminated and not contaminated with *E. coli,* is treated with ozone when stored for 21 days at a temperature of 5 ± 2°C.

Type of Fresh Cheese	Duration of storage (days)			
	1	7	14	21
Normal Fresh Cheese	$39\times10^{6\ Db}$	$58\times10^{6\ Cb}$	$80\times10^{6\ Bb}$	$87\times10^{6\ Ab}$
Cheese contaminated with E. Coli	$44\times10^{6\ Da}$	$64\times10^{6\ Ca}$	$87\times10^{6\ Ba}$	$93\times10^{6\ Aa}$
Cheese contaminated with E. Coli	$15\times10^{4\ Dc}$	$20\times10^{4\ Cc}$	$23\times10^{4\ Bc}$	$25\times10^{4\ Ac}$

The different capital letters in one row indicate significant differences between the storage period at the level ($p<0.05$).

The total number of bacteria in all treatments began to increase during the storage period, and the reason is attributed to the growth and multiplication of other types of microorganisms in Cheese during storage as a result of the change in the chemical composition of soft Cheese with the progress of this period. The results agreed with what Al-Jubouri, who indicated that the number of bacteria increases with the continuation of storage periods for 21 days. They attributed this increase to the growth and activity of microorganisms with the progress of the storage period for soft Cheese and to storage conditions and environmental conditions such as heat play an important role in increasing the number of bacteria in Cheese.

The results agreed with Shati, who indicated that exposing Cheese contaminated with food-poisoning bacteria to ozone rays led to a clear decrease in the total number of bacteria, and this is an indication of the inhibitory effect of ozone, as ozone has inhibitory action against many types of organisms causing food poisoning, due to being a strong oxidizing agent that tears the bacterial cell envelope, resulting in a change in cell permeability and leakage of its contents into the surrounding environment. The results of this study are also consistent with (Novoa et al. 2004),who found that the treatment of Cheese contaminated with food poisoning bacteria with ozone radiation led to a decrease in the total number of bacteria due to the oxidative and deadly action of ozone radiation against food poisoning bacteria.

The Total Content of Coliform Bacteria: Tab. 4 shows the total number of coliform bacteria in soft Cheese manufactured in a laboratory and stored for 21 days at 5 ± 2 °C contaminated and uncontaminated with *E.coli* bacteria and ozone laboratories at a concentration of 0.5 g for 30 minutes. It is noted from the table that there is no growth of coliform bacteria in all the studied transactions during the periods of cold storage, and this is evidence of good working conditions and indicates that ozone can provide a sterile environment for storing soft Cheese, and this falls within the limits of the Iraqi standard for the year (2000) No. (3725)|5 stipulates that the number of colon bacteria does not exceed 103 cfu/g.

Table 4. The total number of coliform bacteria in ozonated Cheese contaminated with *S. aureus* and *E. coli* when stored for 21 days at a temperature of 5 ± 2ºC.

Type of Fresh Cheese	Duration of storage (days)			
	1	7	14	21
Normal Fresh Cheese	N.D	N.D	N.D	N.D
Cheese contaminated with *E. Coli*	N.D	N.D	N.D	N.D
Cheese contaminated with *E. Coli* and Ozone parameters	N.D	N.D	N.D	N.D

The different capital letters in one row indicate significant differences between the storage period at the level ($p<0.05$).

The results agreed with what (Al-Jubouri et. al. 2011) found, which found that the number of coliform bacteria in traditional pasteurized Cheese did not show any growth because the pasteurization was done correctly. The results agreed with Shati, who indicated that there is no growth of coliform bacteria in all ozone-exposed treatments because ozone sterilization is fatal to microbial species, as it breaks down the enzymes in the cells and stops their metabolic activities, in addition to breaking down the wall of microbial cells when using ozone and thus killing microbial species.

The Total Content of Yeasts and Molds: Tab. 5 shows the total number of yeasts and molds in soft Cheese manufactured in a laboratory and stored for 21 days at 5 ± 2 °C contaminated and uncontaminated with *E.coli* bacteria and ozone laboratories at a concentration of 0.5 g for 30 minutes. It is noted from the table that there is no growth of yeasts and molds in all the studied

transactions during the cold storage periods due to good storage conditions.

Table 5. The total number of yeasts and molds in soft Cheese contaminated and uncontaminated with *E.coli* bacteria and ozone laboratories when stored for 21 days at 5 ± 2ºC.

Type of Fresh Cheese	Duration of storage (days)			
	1	7	14	21
Normal Fresh Cheese	N.D	N.D	N.D	N.D
Cheese contaminated with *E. Coli*	N.D	N.D	N.D	N.D
Cheese contaminated with *E. Coli* and Ozone parameters	N.D	N.D	N.D	N.D

The different capital letters in one row indicate significant differences between the storage period at the level ($p<0.05$).

These results agreed with what (Hassan, GM et al. 2018) found, as they found that yeasts and molds did not grow in laboratory-made Cheese from pasteurized milk on the first day of storage. The results converged with what found in the treatments of pasteurized Cheese with traditional pasteurization (Al-Badrani et al. 2004), as it was found that yeasts and molds could not grow in all cheese transactions in the first week of storage.

The results agreed with Shati, who found no growth in yeasts and molds in Cheese during cold storage periods and attributed the reason to the fact that ozone gas effectively reduces the growth of microorganisms, including yeasts and molds. The results converged with Al-Obaidi, who indicated that there was a general killing of yeasts and molds in all storage periods of 21 days because ozone sterilization had a major role in inhibiting yeasts and molds as a result of their destruction (Roushdy,M.M et al. 2011).

Conclusions

The study proved that treating raw and pasteurized cow's milk at 72°C for 15 seconds and exposure to ozone at a concentration of 0.5 for different times (5, 15, 30) minutes was the best exposure time was 30 minutes. The effectiveness of ozone inhibition of total bacteria, coliform bacteria, yeasts, and molds at a concentration of 0.5 at 30 minutes for soft Cheese also proved that exposing cheese samples containing food poisoning bacteria to ozone at a concentration of 0.5 for 30 minutes led to a significant decrease in the number of total bacteria, and this was within the limits stipulated by the Iraqi standard.

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