International Journal of Science and Research (IJSR)
ISSN (Online): 2319-7064
Index Copernicus Value (2015): 78.96 | Impact Factor (2015): 6.391

The Efficacy of Ozonated Water on the Microbial Quality of Locally Produced Soft Cheese in Baghdad

Zinasaab Khudhir[1], Abdullah Munther Mahdi[2]

Department Public Health, College of Veterinary Medicine, University of Baghdad/Iraq

Abstract: ***The present study aimed to investigate the microbiological quality of the locally produced soft cheese made from cows' and sheep's raw milk .A total of 40 bovine and ovine locally produced soft cheese samples (20 each of ovine and bovine) were collected randomly at weekly intervals from different districts in Baghdad province . Soft cheese samples were analyzed for the total aerobic bacterial counts (TBC), coliforms counts (CPC) and yeasts and molds counts (YMPC), Data revealed that there were significant (P≤0.0001) differences in the percentages of microorganisms that affect the locally produced soft cheese quality where the highest prevalence levels of contamination were with total aerobic bacteria and Coliforms (100% for each) followed by the yeasts and molds (90%), As well as the current study was planned to investigate the effects of ozonation treatment (0.5 ppm for 10,15 min and 20 minutes) on the shelf life of bovine and ovine locally produced soft cheese samples. Exposure to the ozonated water at 0.5ppm for 20 min was sufficient to reduce the microbial counts by up to 6 log_{10} cycles in both bovine and ovine locally produced soft cheeses . There was no significant (P≥0.05) differences between the bovine and ovine soft cheese samples after using ozonated water.Locally produced bovine soft cheese samples had high microbial populations load , that exceeded the acceptable limit by the Iraqi standards. A high microbial load of the locally produced bovine and ovine soft cheese samples represent a public health hazard to the consumers and confirm the need for improving the locally produced soft cheese hygienic quality.***

1. Introduction

Locally produced soft cheese is considered as one of the most popular consumed cheese which is made without any modern technology and under unsanitary hygienic conditions by the farmers and in rural areas of Iraq and known as "Giben Arab". In general Locally produced soft cheese made from raw cows and ewes milk with very poor microbiological quality and manufactured under unsatisfactory conditions. Also, this locally product is sold uncovered and unpacked without a receptacle, thus the risk of contamination with the pathogenic microorganisms is very high. Cheese making provides a useful service for extending the shelf life of raw milk and preservation method for excessive raw milk production in the rural areas [1].The microbiological tests such as total aerobic bacterial counts ,total coliform counts and both yeasts and molds counts are used as an index of storability and sanitary conditions of the dairy products [2]. Ozonized water is highlighted by its antimicrobial characteristics and is totally degradable to oxygen, without toxic by-products [3]. Ozonized water is Generally Recognized as Safe (GRAS) [4]. The ozone could be an alternative method to reduce the bacterial load in the dairy products such as soft cheese which is difficult to be sterilized [4]. The main objectives of this study areevaluating the antibacterial effects of ozonated water process on the microbiological quality of the locally produced soft cheese and studying the effects of ozonated water treatment for extending the shelf life of the locally produced soft cheese at different storage temperatures refrigeration (4℃) and ambient (30℃) temperatures.

2. Material and Methods

Forty locally produced cow's and ewe's soft cheese blocks (20 blocks for each one) were collected in a sterile large plastic bags from different local markets in Baghdad city during the period from 1/12/ 2016 till the 1/5/ 2017. Soft cheese samples were transported to the laboratory of the department of veterinary public health in ice-cooled box. Microbiological analysis was performed on arrival of the locally produced soft cheese samples to determine the average of total aerobic bacteria , coliforms and both yeasts and molds counts per gram (cfu/ gm).For each soft cheese samples sample, tenfold serial dilutions (10^{-1}to10^{-9}) were prepared in a sterile 0.1% (wt/v) peptone water as diluent and pour plated. Eleven grams of each soft cheese sample were removed aseptically by a sterile spatula and transferred into a sterile stomacher plastic bag containing (99 ml) of sterile sodium citrate buffer (2%),the contents and then pour plated technique were used[5].Microbiological properties of locally produced soft cheese samples were determined before and after exposing to the ozonation treatment. Total aerobic bacteria were grown on plate count agar after incubation at 37°C for 24 hours. Enumeration of coliforms bacteria were carried out by using a violet red bile agar (Oxiod /UK)medium after 24 hours of aerobic incubation at 37°C. The Oxytetracycline (OGYE) agar (Oxiod /UK) was applied for the determination of yeasts and molds at 25°C for 5-7 days of incubation. The counts were expressed as a colony forming units / gram (CFU/g) of locally produced soft cheese sample ,all microbiological analysis was performed according to American Public Health Association [6, 7]. Three measurements were carried out and the average values were calculated.Before and after each ozonated water treatment, the total aerobic bacteria ,Coliforms and both yeasts and molds counts were determined by standard plating technique ,Nutrient agar was used for enumeration of total aerobic bacteria. Plates were incubated aerobically at 37°C for 48 hours [8]. Plates that had 25-250 colonies were selected for counting ,over lay violet red bile agar was used for the counting of coliforms. ,plates were incubated aerobically at 37°C for 24 hours [8] ,plates that had (25-250) colonies were selected for counting

.The apparatus which was used for ozonated treatment in the current study designed in the Veterinary Public Health / Food Hygiene department, University of Baghdad, figures 1.This apparatus was consisting from the ozone generator (A2Z) which was employed by means of a diffuser at a rate of 600 mg/h with the dose of 0.6 ppm of ozone, the range of ozone generation was at 0.05 - 3 ppm (50 - 3000 ppb) with the minimum detection limit 0.025 ppm (25 ppb)The calculation of ozone concentration (ppm/in water) by using CHE-Mets-Kit , the highest ozone concentration among the times used was obtained at both 15 and 20 minutes, which

Figure 1: Ozonation equipment

3. Results and Discussions

Results of the cultural properties of the total aerobic bacteria, coliforms, yeasts and molds counts are presented in Table 1.The quality control tests included the Standard Plate Count (SPC), Coliform plate Counts (CPC) and both yeasts and molds counts (YMPC). The nutrient agar (Oxoid) was used for the determination of the total aerobic bacteria and the plates were incubated at 37°C for 24-48 hours , while the violet red bile agar(Oxoid) was used for determination of coliforms counts and the plates were incubated aerobically at 37°C for 24hrs, typical dark red colonies were counted , each bacterial analysis were made in triplicate for all microorganisms that affect the raw milk quality, the average number of the colonies in each bacterial dilution was multiplied by the reciprocal of the dilution factor and calculated as the colony forming units (cfu) per gram of locally produced soft cheese . Yeasts and molds were counted from the locally produced soft cheese samples after 5-7 days of aerobic incubation at 25 °C on the selective Oxytetracycline GYE agar(OGYE).

Table 1: The Cultural characteristics of the microorganisms that affect the quality of the locally produced soft cheese

Microorganisms	Media	Cultural characteristics
Total aerobic bacteria	Nutrient agar	White colonies
Coliforms	Violet red bile (VRB) agar	Dark to red colonies
Yeasts and molds	Oxytetracycline (OGYE) agar	White to creamy colonies

Soft cheese is one of the most assessable cheeses in the Middle Eastern countries. This type of cheese is produced either by enzymatic or acidic coagulation of fresh milk such was (0.5 ppm), the aeration stone was inserted into the plastic container , the ozonation treatment was carried out at the pH (6.5) , at both the (4°C) and the ambient temperature (30°C). The Sterilized reaction chamber consisting of rectangular plastic box with a faucet to remove the ozonated water after the treatment and before each treatmen, the plastic container was flushed with fresh distilled water and then sanitized by bubbling ozone gas for 5 minutes. .The ozonated soft cheese cubes were subjected to the ozonation process for 10, 15 and 20 min as a contact time at both therefrigeration (4°C) and the ambient temperature(30)°C. as (cow's milk). However, the use of raw milk leads to either unpredictable chemical or physical changes or possible survival of various kinds of pathogens during its manufacturing and ripening [9].Milk used for the cheese making must be of a good microbiological quality, that had total bacterial count ofless than 10^4cfu/ml and somatic cell of less than 10^5 cells/ml, in addition to the absence of pathogenic bacteria, antibiotics and inhibitors[10] .The microbiological quality of the soft cheese can be influenced by many factors, the most important of which are, the microbial quality of the raw milk, the thermal treatment of the raw milk and both the levels and types of microbial contamination that occur throughout the production and storage of the soft cheese[11].The highest significant ($P \leq 0.05$) prevalence levels of contamination with total aerobic bacteria and coliform in the locally produced bovine and ovine soft cheese samples that appeared to be 100% respectively where 40 out of 40 soft cheese samples that examined during the current study were positive for both of them, while 36 (90%) out of 40 locally produced soft cheese samples were found positive for the presence of yeasts and molds. Such high prevalence levels of contamination with these microorganisms pointed out the potential public health hazard (Table 2).

Table 2: The isolation percentage of the microorganisms that affect the microbial quality of the locally produced bovine and ovine soft cheese samples which collected from Al- Rasafa and Al- karch districts of Baghdad province

Quality control microorganisms	No. of examined	No of positive	Isolation percentage %
Total aerobic Bacteria	40	40	100% A
Coliforms	40	40	100% A
Yeasts and molds	40	36	90% B

$X^2 = 134.29$

$P \leq 0.0001$

* Different capital letters in a column revealed significant ($P < 0.0001$) differences between the isolation percentages.

The microbial quality tests as total aerobic bacterial counts and both coliforms and yeasts and molds are often used as an indicator for the hygienic quality and safety of the dairy products. It may reflect the hygienic conditions under which the dairy products are produced such as the effectiveness of heat treatment that used during the processing and sanitary conditions of equipment at commercial processing plants [12].High level of contamination with coliforms bacteria in the soft cheese is regarded as indicator for the unsanitary practices during the cheese making process. Soft cheese is characterized by many of gas holes, with spongy texture [13].The ozone concentrations output (ppm) in water, (ozonated water) that used in this study was determined by

using different exposure times as shown in Table 3. The results of 0.4, 0.5 and 0.5 ppm for the times of exposure were 10, 15 and 20 minutes respectively. The median value of 0.5 ppm for 15 and 20 minutes was chosen to minimize any adverse effect that may occur due to the ozonationtreatment.

Table 3: The concentrations of ozonated water (ppm) at different times of exposure

Time of exposure (minutes)	Ozonated water (ppm)
10	0.4
15	0.5
20	0.5

The mean log values of total aerobic bacterial counts (log cfu/gm) of bovine and ovine soft cheese samples that were subjected to the ozonated water (0.5ppm) for 10, 15 and 20 minutes after 24 hours of storage at either ambient (30°C) or refrigeration(4°C) temperatures are shown in Table 4. Ozonation of the locally produced soft cheese samples for different exposure times had a significant (P≤0.05) influence on the inactivation degree of total aerobic bacteria. Exposure the bovine and ovine locally produced soft cheese samples with initial starting number 9.46 log cfu/gm and 9.42 log cfu/gm of total aerobic bacteria respectively to the ozone treatment for 10 and 15 minutes caused a significant (p≤0.05) reduction in their counts .Exposing of locally produced bovine and ovine soft cheese samples to the ozonation treatment for 20 minutes greatly decreased the total bacterial counts of soft cheese samples after 24 hours of storage at ambient (30°C) to 2.79 log cfu/ml and 2.50 log cfu/ml and at refrigeration (4°C) temperature to 2.76 log cfu/ml and 2.75 log cfu/ml respectively.ozone is highly oxidative compound with a broad antimicrobial spectrum, able to inactivate the bacterial cells, yeasts and molds, also can kill the insects and degrade the mycotoxins[3]In the current study results indicated that both of ozonated water and refrigeration storage temperature acted synergistically in the reduction of microbial counts that affect the quality of bovine and ovine locally produced soft chees (total bacterial counts, coliforms and yeasts and molds counts) as shown in (Table 4).

Table 4: The means levels of the total aerobic bacterial counts (log cfu/gm) of bovine and ovine soft cheese samples that were subjected to the ozonated water (0.5ppm) for 10, 15 and 20 minutes after 24 hrs of storage at either ambient (30°C) or refrigeration(4°C)temperature

Source of cheese samples	Mean log values ±SE(log cfu/ gm)						
	Before ozonated water treatments (control)	10minutes		15minutes		20minutes	
		30°C	4°C	30°C	4°C	30°C	4 °C
Cows	9.46±0.11 Aa	6.34±0.009 Ba	6.12±0.01Ba	4.31±0.01Ca	4.19±0.01Da	2.79±0.12Ea	2.76±0.02Ea
Ewes	9.42±0.11 Aa	6.32±0.001 Ba	6.10±0.007Ba	4.30±0.01Ca	4.17±0.01Da	2.50±0.07Ea	2.75±0.02Ea
LSD	0.1636						

*Different capital letters in the row denote significant (P≤0.05) differences in the bacterial counts
*Different Small letters in a column denote significant (P≤0.05) differences between the bovine and ovine soft cheese samples.
* SE=Standard error

The mean log values of coliforms bacterial counts (log cfu/gm) of bovine and ovine soft cheese samples that were subjected to the ozonated water (0.5ppm) for 10, 15 and 20 minutes after 24 hours of storage at either ambient (30°C) or refrigeration(4°C) temperatures are shown in. (Table 5). Ozonation of the locally produced soft cheese samples for different exposure times had a significant (P≤0.05) influence on the inactivation degree of coliforms bacteria in the locally produced soft cheese samples. Exposure the bovine and ovine locally produced soft cheese samples with initial starting counts of 7.85 log cfu/gm and 7.84 log cfu/gm of coliforms bacteria respectively to the ozone treatment for 10 and 15 minutes caused a significant (p≤0.05) reduction in their counts. Exposing of the locally produced bovine and ovine soft cheese samples to the ozonation treatment for 20 minutes greatly decreased the coliforms counts of soft cheese samples after 24 hrs of storage at either ambient (30°C) to 3.08 log cfu/ml and 2.93 log cfu/ml and at refrigeration (4°C) temperature to 2.69 log cfu/ml and 2.10 log cfu/ml respectively as shown in Table 5.Wade *et al.* (14] reported the efficiency of aqueous ozone treatment in killing many kinds of bacteria such as *listeria monocytogenes*in different kinds of foods . The continuous treating of seeds with ozonated water with initial concentration (0.2 µg/ml) for 20 minutes was effective significantly for reducing *listeria monocytogenes* populations by 1.48 $\log_{10}$ CFU/g[15]Pirani[16] evaluated the effects of ozone treatment on the *Staph. aureus* and noted that one hour of ozonation treatment resulted in reduction of 3.1 $\log_{10}$ CFU/mL , also showed the need for a long contact time between the molecular ozone and the *Staph. aureus* cells.

Table 5: The means of the Coliforms counts (log cfu/gm) of bovine and ovine soft cheese samples that were subjected to the ozonated water (0.5ppm) for 10,15 and 20 minutes after 24 hrs of storage at either ambient (30°C) or refrigeration(4°C)temperature

Source of cheese samples	Mean log values ±SE(log cfu/ gm)						
	Before ozonated water treatments (control)	10minutes		15minutes		20minutes	
		30°C	4°C	30°C	4°C	30°C	4 °C
Cows	7.85±0.004 Aa	6.74±0.003 Ba	5.55±0.006 Ca	4.07±0.01 Da	3.98±0.01 Ea	3.08±0.05 Fa	2.69±0.02 Ga
Ewes	7.84±0.006	6.70±0.01	5.54±0.01	4.04±0.01	3.93±0.03	2.93±0.02	2.10±0.02

	Aa	Ba	Ca	Da	Ea	Fb	Gb
LSD	0.1636						

*Different capital letters in the row denote significant (P≤0.05) differences in the bacterial counts
*Different small letters in a column denote significant (P≤0.05)differences between the bovine and ovine soft cheese samples
*SE=Standard error

The mean log values of yeasts and molds counts (log cfu/gm) of bovine and ovine soft cheese samples that were subjected to the ozonated water (0.5ppm) for 10, 15 and 20 minutes after 24 hrs of storage at either ambient (30°C) or refrigeration(4°C) temperatures are shown in. Table 6. Ozonation the locally produced soft cheese samples for different exposure times had a significant (P≤0.05) influence on the inactivation degree of yeasts and molds counts in locally produced soft cheese samples. Exposure the bovine and ovine locally soft cheese samples with initial starting counts of 6.25 log cfu/gm and 6.21 log cfu/gm of yeasts and molds respectively to the ozone treatment for 10 and 15 minutes caused a significant (p≤0.05) reduction in their counts. While complete inactivation of yeasts and molds was achieved when the locally produced bovine and ovine soft cheese samples were exposed to ozonation treatment for 20 minutes after 24 hours of storage at either ambient (30°C) or refrigeration (4℃) storage temperatures [Table 6].Yeasts and molds are widely distributed in the environment, also considered as a part of the normal flora of the food products,the presence of yeasts and molds in raw milk and dairy products is objectionable, as they grow at wide range of the environmental temperature, pH values. Molds contamination not only cause deterioration of the dairy products, but also adversely affect the health of people and animals, since they produce the toxic metabolites called mycotoxins[17]. The ozone is highly oxidative compound with a broad antimicrobial spectrum, able to inactivate the bacterial cells, yeasts and molds, also can kill the insects and degrade the mycotoxins[3].Low concentrations of ozone (0.2-0.3) ppm were observed to significantly reduce the molds on the cheese surfaces. Another study indicated that the high-ozone and low-ozone treatments also reduced the mean molds spores counts in the curing rooms by 94% and 88%, respectively [18].

Table 6: The means of the Yeasts and molds counts (log cfu/gm) of bovine and ovine soft cheese samples that were subjected to the ozonated water (0.5ppm) for 10,15 and 20 minutes after 24 hrs of storage at either ambient (30°C) or refrigeration(4°C)temperatures

Source of cheese samples	Mean log values ±SE(log cfu/ gm)						
	Before ozonated water treatments (control)	10minutes		15minutes		20minutes	
		30°C	4°C	30°C	4°C	30°C	4°C
Cows	6.25±0.01 Aa	5.11±0.01 Ba	4.48±0.14 Ca	0.12±3.15 Da	2.71±0.10 Ea	ND	ND
Ewes	6.21±0.01 Aa	5.11±0.01 Ba	4.36±0.14 Ca	2.91±0.01 Db	1.68±0.00 Eb	ND	ND
LSD	0.2032						

N.D=Non detect
*Different capital letters in the row denote significant (P≤0.05) differences in the yeasts and molds counts
*Different small letters in a column denote significant (P≤0.05) differences between the bovine and ovine soft cheese samples
*SE=Standard error

References

[1] Alalade OAand Adeney JA. The effect of short term frozen storage on the chemical composition and coliform microflora of Wara cheese "Wara cheese under frozen storage" .J.Dairy Science .2006. 1(2): 126-130.
[2] Tudor DA and Board RG.Food spoilage yeasts. In: The Yeast Technology (Rose, A.H., Harrison, J.S. Eds). 2nd ed. Academic Press, London. 2010 , 5: 436- 451.
[3] Tiwari B K. Brennan C S Curran T Gallagher E Cullen P J and Donnell C P O . Application of ozone in grain processing, Journal of Cereal Science. 2010 ,51(3): 248-255.
[4] Dhillon BWiesenborn D Wolf-Hall C and MantheyF. Development and Evaluation of an Ozonated Water System for Antimicrobial Treatment of Durum Wheat. Journal of Food Science .2009, 74(7): E396-E403.
[5] Kafili TRazaviH EmamDjomeh Z Naghavi MRÁlvarez-Martín. P and Mayo., B. Microbial characterization of Iranian traditional Lighvan cheese over manufacturing and ripening via culturing and PCR-DGGE analysis: Identification and typing of dominant lactobacilli. Eur. Food Res. Technol .2009 :229(1): 83-92.
[6] APHA . Standard Methods for the Examination of Dairy Products: American Public Health Association, 16th Edition, Washington DC.2002
[7] Samelis JKakouri ARogga, KJSavvaidisI.N.andKontominas M.GNisin treatments to control *Listeria monocytogenes* post-processing contamination on Anthotyros,a traditional Greek whey cheese, stored at 4 °C in vacuum packages. Food Microbiology.2003, 20: 661–669.
[8] Mucchetti G Remagni C. MGhiglietti RLocci, FBarzaghi SFrancolino, APerrone ARubiloni, P CampoMGattik and DCarminati. Influence of cheese-making technology on composition and microbiological characteristics of Vastedda cheese. Food Control.2008, 19: 119–125.
[9] Deeb A.M.MAman, IM. and Ahmed HF . Bacteriological quality of kareish cheese and a trial to control S.aureus in cheese. Alex. J.Vet. Sci.2004, 21(2): 514- 523.

[10] Skeie S. Characteristics in milk influencing the cheese yield and cheese quality. J. Anim. Feed Sci.2007, 16 (l): 132-142.
[11] Bintsis T and Papademas P. Microbiological quality of white-brined cheeses: a review. Int. J. Dairy Technol.2002, 55 (3): 113- 120.
[12] I.CMSF. (International Commission on Microbiological Specification for Food): Microbial Ecology of Foods .1986, Vol, (1-2). University of Toronto Press, Toronto, Buffalo and London.
[13] Bintsis T . Quality of brine. In Brined cheese, Edited by Tamime, A. 2006,PP. 264-301. Blackwell Publishing .
[14] Wade WNScouten, K.M Watters M.CWick R.LDemirci AFett, WF and Beuchat. L.R. Efficacy of ozone in killing *Listeria monocytogenes* on alfalfa seeds and sprouts, and effects on the sensory quality of the sprouts. J. Food Prot.2002, 66 (1): 44-51.
[15] Cavalcante M.ALeiteJúnior BR.CTribst A.A.L. and Cristianini, M. . Improvement of the raw milk microbiological quality by ozone treatment. International Food Research .2013 .20(4): 2017-2023.
[16] Pirani S .Application of ozone in food industries.. Theis (Doctor and Animal Nutrition and Food Safety) - UniversitàdegliStudi di Milano, Milão.2011.
[17] Ismail M.A and Sabreen M.S . Associated mycobiota of some types of cheese and cooking butter. Assiut Vet. Med. Journal 2001, 44(88): 176-197.
[18] Fuhrmann H N Rupp ABüchnerA and Braun P. The effect of gaseous ozone treatment on egg components. Journal of Science and Food Agriculture .2010, 90(4): 593–598.