j.Egypt.net.med.Assoc 76, no 1. 117 – 130 (2016)

EFFECT OF OZONE TREATED MILK ON THE MICROBIOLOGICAL QUALITY AND SHELF-LIFE OF YOGURT

By

Hanan A. El-Dahshan[1], Gihan M. O. Mohammed [2] and Taghreed A. Hafez[1]

[1]Food Hygiene Department and Bacteriology Department[2] Port-Said branch، Animal Health Research Institute

ABSTRACT

The current study was designed with three replications to study the effects of ozone gas on the microbial load of yogurt prepared from ozone treated milk at 0, 40, 60 and 70 ppm concentrations for 10 minutes and stored at refrigerator (5±1˚c). *Staph. aureus*, coliforms and mold counts of the samples were studied at the 1st, 2nd, 3rd , 5th and 7th day of storage. The results showed that Ozone gas decreased microbial load of yogurt samples, the lowest and the highest counts of microbial load were detected in samples treated with concentration of 70 ppm of Ozone gas and control respectively. Results showed that Ozone gas increased the shelf - life of yogurt samples vs. the control samples. In addition, the sensory analysis revealed that zone gas at the concentrations of 40, 60 and 70 ppm had no adverse effects on the yogurt sensory quality.

INTRODUCTION

Yogurt is one of the most popular fermented dairy products widely consumed all over the world. It is obtained by lactic acid fermentation of milk by the action of a starter culture containing *Streptococcus thermophilus* and *Lactobacillus delbruckii* spp. *bulgaricus*. The role of these two genera in yogurt manufacture can be summarized as milk acidification and synthesis of aromatic compounds **(Serra *et al.*, 2003)**. Yogurt has been known for its nutraceutical, therapeutic, and probiotic effects such as digestion enhancement, immune system boosting, anti-carcinogenic activity and reduction of serum cholesterol **(Penna *et al.*, 2007)**. Yogurt can be easily subjected to microbial contamination either from the use of poor quality milk or gained during yogurt preparation, handling and storage. Mesophilic aerobic microorganisms and coliforms are commonly found at high counts in raw milk **(Moraes *et al.*, 2009**) and are indicators of the milking and storage quality **(Oliveir *et al.*, 2011**). Some of these microorganisms are pathogens such as *Staph. aureus*, *Salmonella***;** but, the majority of

them are just spoilage microorganisms. They are able to produce enzymes that seriously compromise the milk quality during its storage and reduces the quality of dairy products causing economic losses to the food industry because spoilage of food cause change in odor, taste, color and texture of the product which render it undesirable or unsafe to human consumptions **(Pedras *et al*., 2012)**. Staph. Food-borne disease (SFD) is one of the most common food-borne diseases worldwide resulting from the contamination of food by preformed *Staph. aureus* enterotoxins. SFD causes an estimated 241,000 illnesses per year in the United States **(Scallan, 2011)**. However, the true incidence of *Staph.aureus* food-borne disease (SFD) could be a lot higher as sporadic food-borne disease caused by *Staph.aureus* is not reportable in the United States **(Bennett *et al.,* 2013)**. FBD impose a great economic burden, accounting for $50–$80 billion annually in "health care costs, lost productivity, and diminished quality of life" in the United States **(Scharff, 2012 and Byrd-Bredbenner *et al.,* 2013)** Presence of Coliforms in dairy products is an indication of fecal contamination when the hygiene is poor **(Thatcher and Clark, 1978)**. Some members of Coliforms are responsible for the development of objectionable taints in milk and its products rendering them of inferior quality or even unmarketable **(Yabaya and Idris, 2012)**. Yeasts are a major cause of spoilage of yogurt and fermented milks in which the low pH provides a selective environment for their growth **(Fleet, 1990 and Nwagu and Amadi, 2010).** Presence of yeasts and molds in milk and dairy products are undesirable even when found in few numbers as they result in objectionable changes that render the products of inferior quality **(Abdel Hameed, 2011)**. Mycotoxigenic fungi are able to grow at refrigeration temperature and produce their mycotoxins, which threaten human health **(Potter and Hotchkiss,1995)**. The most used method for controlling microbial spoilage in milk is thermal treatment by either pasteurization or Ultra Heat Treatment (UHT). Although pasteurization and ultra-heat treatment (UHT) of milk destroy all pathogenic microorganisms but the nutritional value of some water-soluble vitamins as folic acid, C and B_{12} are lost. In addition, the protein is adversely affected by high heat treatment due to denaturation of β-lactoglobulin in which can vary from as low as 35% in direct plants to close to 100% in indirect plants **(Rosenberg 2011 and Al-Kanhal *et al.* 2011**). Some flavor changes also occur due to UHT process as sulfurous or cooked flavor **(Mehta, 1980**). Therefore, the food researchers are looking for newly methods to create food without damaging important active biological and healthy components.

Therefore, there is an urgent need to develop novel processing concepts that provide effective public health protection with minimal treatments to retain nutrients and flavor characteristics of the product. Ozone has the potential to fill a substantial gap in today's technologies that are used to ensure food safety because ozone is a powerful antimicrobial substance. Its bactericidal effects have been documented on a wide variety of organisms, including Gram positive and Gram-negative bacteria as well as spores and vegetative cells; it leaves no residues since it decomposes quickly **(Graham, 1997; Guzel-Seydim, *et al.,* 2004 and Anon, 2005)**. Another advantage of using ozone in the food industry is that it can be used in its gaseous or aqueous state. There are suggested applications of ozone in the food industry such as food surface hygiene, sanitation of food plant equipment, reuse of wastewater. Treating meat, chickens, cheese, fruits and vegetables with ozone has been found to increase shelf life of such products **(Khadre *et al.,* 2001; Guzel-Seydim *et al.,* 2004 and El- Dahshan *et al.*, 2013)**. In this review, use of ozone in food industry was discussed. Multifunctionality of ozone application makes ozone a promising agent in food industry **(Guzel-Seydim, 1996; Majchrowicz, 1998 and Dosti, 1998).** Finally, the **(FDA, 2001a and USDA/FSIS, 2001)** formally approved the use of ozone as an antimicrobial agent for the treatment, storage and processing of foods in gas and aqueous phases. The present study aimed to study the effect of using ozone treated milk on the microbiological quality and shelf life of yogurt.

MATERIAL AND METHODS

Sampling:

A total of 4 kg of fresh raw milk were collected from dairy farm in Port-Said city, Egypt. Each 1Kg of milk was bottled in a separate clean and sterile capped bottle.

Ozone application:

The samples were divided into 4 groups. The 1st was control non treated group. The 2nd, 3rd and 4th groups were treated with ozone gas at 40, 60 and 70 ppm for 10 min. Ozone gas was generated from a cold plasma ozone generator (MA 5001 Model), Germany, using oxygen with a working voltage of 220 volt, work at ambient temperature, located in Dr. Adel Abd-Elrahman clinic, Helioplis, Egypt. After ozone application, the milk samples were transported to the laboratory in icebox.

Yogurt preparation according to **(Walstra *et al.,* 2006).**The milk was heated to 42°C in a clean and sterile stainless steel container to bring the yogurt to the ideal growth temperature

for the starter culture and the starter cultures were mixed into the milk in a concentration of 2%. The milk was distributed into clean plastic containers and held at 42°C until a pH 4.5 and yogurt consistency was reached. The yogurt was covered by plastic and cooled at refrigerator.

Storage Condition:

After yogurt preparation, the four groups subsequently stored in refrigerator maintained at 5± 1˚c.

Microbiological analysis:

Control and ozonated groups were subjected to microbiological examination for *Staph. Aureus* count (FDA 2001b), total coliforms count (FDA 2002) and total mould count (FDA 2001c),

Preparation of homogenate according to **(APHA 2004):**

10g of each sample was homogenate with nine times its weight of buffered peptone water in a sterile stomacher bag to give a 1 in 10 (10^{-1}) suspension. Then decimal dilutions of 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, and 10^{-6} were prepared.

Enumeration and Identification of *Staph. aureus* according to (FDA 2001 b)

For each dilution to be plated, 1ml sample suspension was aseptically transferred to three plates of Baird-Parker agar, (0.4 ml, 0.3 ml, and 0.3 ml). Inoculum was spread over surface of agar plate, using sterile bent glass streaking rod. Plates were placed upright in incubator for about 1 h. then were inverted and incubated for 45- 48 h at 35°C. Colonies of *Staph. aureus* are circular, smooth, convex, moist, 2-3 mm in diameter on uncrowded plates, gray to jet-black, frequently with light-colored (off-white) margin, surrounded by opaque zone and frequently with an outer clear zone; colonies have buttery to gummy consistency when touched with inoculating needle. Colonies which were Gram′s positive cocci arranged in clusters resembling to bunch of grapes, coagulase positive, catalase positive, Thermonuclease positive, Lysostaphin sensitve, Anaerobic utilized of glucose and mannitol fermenter were represented the *Staph.aureus* count.

Determination of Total coliforms count according to (FDA 2002):

Two 1 mL aliquots of each dilution were transferred to petri dishes, and 10 mL Violet red bile agar (VRBA) was tempered to 48°C and poured into plates ,plates were swirl to mix, and let solidify. Another 5 mL VRBA was overlaid and let solidify. The plates were incubated at 32°C for 18-24 h. purple-red colonies that are 0.5 mm or larger in diameter and surrounded by

zone of precipitated bile acids were counted. To confirm that, the colonies were coliforms, at least 10 representative colonies were picked and transferred each to a tube of Brilliant green lactose bile (BGLB) broth. Incubated at 35°C. Examined at 24 and 48 h for gas production without pellicle.

Determination of Total mold count according to (FDA, 2001c)

Ml of each dilution was aseptically spreaded over Dichloran rose Bengal chloramphenicol (DRBC) agar plates with a sterile, bent glass rod. Then the plates were incubated in inverted position at 25°C for 5 days then the plates were counted.

Sensory analysis:

The 5 points hedonic scale conducted the sensory evaluation: one, very poor; 2, poor; 3, common; 4, good; 5, very good **(Szczesniak, 1987)**.

Statistical analysis:

All measurements were repeated three times. The results are expressed as mean values and standard deviations. The data were statistically analyzed by ANOVA and Duncan's multiple range tests. Statistical significance was accepted at a level of $P < 0.05$ **(SAS Institute, 1988)**.

RESULTS

Table (1): Effect of ozone on *microbial load* of yogurt.

storage time	*Staph.aureus* count (cfu/g)			Total coliforms (cfu/g)			Total molds count (cfu/g)		
	Control	Ozone treatment(ppm)		Control	Ozone treatment(ppm)		Control	Ozone treatment(ppm)	
	0	40	60	70	0	40	60	70	0	40	60	70
1st day	4.7×10^{2}±0.1a	<10± 0.0^{b}	<10± 0.0^{b}	<10± 0.0^{b}	2.7×10^{4}± 0.2^{a}	1.9×10^{4}±0.5^{b}	0.6× 10^{4}±0.3^{c}	1.3× 10^{3d}	3.4×10^{4}± 0.6^{a}	8.1×10^{2}± 0.2^{b}	6.7×10^{2}± 0.5^{c}	5×10^{2} ±0.2^{d}
2nd	3.8×10^{2}± 0.2^{a}	<10± 0.0^{b}	<10±0. 0^{b}	<10±0. 0^{b}	4.9×10^{3}±0 .3^{a}	3.1×10 3b	6.8×10^{2}± 0.5^{c}	3.7×10 2d	9.8×10^{4}±0 .5^{a}	9.7×10^{3}± 0.1^{b}	7.4×10^{3}± 0.2^{c}	6.6×10^{3}± 0.2^{d}
3rd	2.4×10^{2}±0.1^{a}	<10±0. 0^{b}	<10±0. 0^{b}	<10± 0.0^{b}	3.3×10^{2a}± 0.1^{a}	2.5×10 2b	9.4×10^{1} ±0.1^{c}	6.1×10 1d	5.1×10^{5}± 0.3^{a}	2.5×10^{4} ±0.4^{b}	9.8×10^{3}± 0.4^{c}	8.2×10^{3}± 0.5^{d}
5th	1.2×10^{2}±0.2^{a}	<10±0. 0^{b}	<10± 0.0^{b}	<10± 0.0^{b}	9×10^{1a}± 0.5^{a}	5.2×10 1b	<10 ±0.0^{c}	<10± 0.1^{c}	6.7×10^{6}± 0.1^{a}	9.7×10^{4}± 0.2^{b}	6.2×10^{4}± 0.5^{c}	5.1×10^{4}± 0.4^{d}
7th	0.9×10^{2}±0.2^{a}	<10± 0.0^{b}	<10± 0.0^{b}	<10± 0.0^{b}	<10±0.0^{a}	<10± 0.0^{a}	<10±0.0^{a}	<10± 0.0^{a}	8.7×10^{8}± 0.5^{a}	6.4×10^{5}± 0.5^{b}	9.4×10^{4}± 0.1^{c}	3×10^{4}± 0.1^{d}

Different superscription (a, b, c and d) within the same raw and the same feature are significantly different p≤ 0.05.

j.Egyptianmed.Assoc 76, no 1, 117 – 130(2016)

Table (2): Sensory analysis of ozonated and non- ozonated yogurt samples during chilling storage (5±1°c) period.

Storage days	Color				Odor				Flavor			
	Control	Ozone treatment (ppm)			Control	Ozone treatment (ppm)			Control	Ozone treatment (ppm)		
	0	40	60	70	0	40	60	70	0	40	60	70
1st	4.9± 0.02^{a}	5.0± 0.01^{a}	4.85± 0.10^{a}	4.85± 0.11^{a}	5.0± 0.11^{a}	4.88± 0.12^{a}	4.82± 0.02^{a}	4.84± 0.13^{a}	5.0± 0.01^{a}	4.9± 0.11^{a}	4.9± 0.02^{a}	4.85± 0.10^{a}
2nd	4.91± 0.14^{a}	4.92± 0.03^{a}	4.9± 0.12^{a}	4.88± 0.2^{a}	4.5± 0.01^{a}	4.8± 0.13^{a}	4.79± 0.10^{a}	4.78± 0.12^{a}	4.89± 0.03^{a}	4.81± 0.32^{a}	4.84± 0.04^{a}	4.82± 0.13^{a}
3rd	4.7± 0.31^{a}	4.24± 0.12^{b}	4.31± 0.1^{b}	4.5± 0.24^{b}	3.7± 0.23*	4.14± 0.20^{b}	4.6± 0.01^{b}	4.61± 0.04^{b}	4.3± 0.60^{a}	4.5± 0.01^{b}	4.5± 0.14^{b}	4.6± 0.03^{b}
5th	3.1± 0.5^{a}	4.51± 0.04^{b}	4.5± 0.2^{b}	4.44± 0.11^{b}	2.6± 0.25^{a}	4.1± 0.6^{b}	4.0± 0.03^{b}	4.2± 0.01^{b}	3.5± 0.111^{a}	4.0± 0.31^{b}	4.0± 0.23^{b}	4.1± 0.12^{c}
7th	1± 0.15^{a}	4.1± 0.07^{b}	4.2± 0.30^{b}	4.1± 0.11^{b}	1.7± 0.30^{a}	3.5± 0.02^{b}	3.6± 0.14^{b}	3.8± 0.54^{b}	------	3.0± 0.31^{a}	3.2± 0.14^{a}	3.2± 0.16^{a}

Different superscription (a, b and c) within the same raw and the same feature are significantly different p≤ 0.05.

DISCUSSION

Table (1) provides data for the reduction of *Staph.aureus* count, total mold count and coliforms count in the three ozone treated groups. It is evident that microbial load of yogurt was affected by Ozone gas at different concentrations (p<0.05).Ozone gas had a significant decrease on *Staph.aureus* count, coliform and total mold count (Table 1). It was found that by increasing the Ozone gas concentrations from 40 ppm to 70 ppm, microbial load reduction significantly increased (p<0.05). The highest and lowest level of contamination was observed in the control and in the 70-ppm concentration of Ozone gas for 10 min respectively Table (1). **Naitoh *et al*., (1988) and Tzortzakis *et al*., (2007)** indicated that ozonation was affected on decreasing of fungal decay of strawberries, and microbial load of cereal grain powders, peas, beans, and whole spices. Scholars reported that Ozonation of onions, barley grain, black pepper, cereals, grains, peas, beans, spices, apple, orange, blackberries and grapes resulted to the reduction of microbial load **(Naitoh *et al*., 1988; Beuchat *et al*., 1992; Zhao and Cranston, 1995; Kim *et al*., 1999; Song *et al*., 2000; Khadre, 2001 and Allen *et al*., 2003)**. In our study, it was observed that, the concentrations of 70 ppm for 10 min had the highest effect on *Staph .aureus* count, coliform and total mold count in yogurt. **Han *et al*., (2002) and Proctor *et al*., (2004)** observed that decreased the count of Escherichia coli O157:H7 by increased ozone gas concentration and exposure time. Then, concentration of 70 ppm Ozone for 10 min had the lower contamination in yogurt samples was investigated. Presence of *Staph .aureus* in yogurt indicates a contamination from personnel sharing in the production and handling **Abdel Hameed and El- Malt, (2009)**. The survival of *Staph .aureus* was greatly affected by ozonation there was significant decrease in *Staph .aureus* counts between the control and ozonated groups (Table 1) similar findings were obtained by **(Kim *et al.*, 1999; Sharma and Hadson, 2008; Trinetta *et al.*, 2011 and El-Dahshan *et al.*, 2013)**. There was a decline in *Staph .aureus* counts in the control group up on storage and this was contributed to the increases in acidity, as well as by a decrease in pH value of yogurt. From the obtained results, number of coliforms in yogurt declined dramatically due to ozone treatment and was markedly different from the initial value after storage in both control and ozonated groups and disappeared from all the groups by the end of the storage period. These findings were constant with those of **Goel *et al.*, (1971)** who noticed that number of viable coliforms in yogurt declined dramatically after storage at 7.2˚ C and survival of

coliforms in yogurt did not exceed 3 days of holding. The decontamination effect of ozone up on coliforms counts was clearly studied by (**Jindal *et al.* 1995; Kim *et al.* 1999; Leusink and Kraft 2008 and El-Dahshan *et al.* 2013)**. Although most coliforms do not cause any disease however there is a danger of illness in vulnerable groups of people including children and high numbers of coliforms can indicate that milk has been handled in an unsanitary manner and that condition is suitable for the presence of enteric pathogens. Coliforms typically enter the milk used in yogurt due to poor milking practices or incorrectly washed equipment. This occurs because the bacteria are contained inside the feces of cows **Biolumix, (2013)**. Presence of molds in dairy products is undesirable because it results in objectionable changes that render the products of inferior quality. Fungal spoilage of dairy foods is manifested by the presence of a wide variety of metabolic by-products, causing off-odors and flavors, in addition to visible changes in color or texture **Abdel Hameed, (2011)**. The present study cleared that mold counts was greatly decreased by ozone and these findings are similar to several studies conducted by **(Song *et al.*, 2000; Palou *et al.,* 2003; Parish *et al.,* 2003; Serra *et al.*, 2003; Di Renzo *et al.*, 2005; Selma, 2007 ; Balzam *et al.*, 2012 and El-Dahshan *et al.*, 2013).** .At the 7th day of storage the microbiological examination of yogurt samples revealed that mold counts in the control group reached 8.7×10^{8}cfu/g. Such result means that, the yogurt became unsafe for human consumption as the food is considered spoilt when the changes are detectable. Also the microbial population has reached to ca. 10^{7-9} cfu / ml, g or cm Ray and **Bhunia, (2008)**. The ozonized groups at the three concentrations were still under the dangerous level that means; ozone treatment increased the shelf life of yogurt as the shelf life of yogurt is determined by the time the product remains safe to eat and the time its sensory properties remain acceptable to consumers.

Sensory analysis:

Data obtained from the sensory analysis of control and ozone treated yogurt samples in Table (2) revealed that ozone had no negative effect on the sensory properties of the samples. Similar findings was obtained by several researchers **(Sheldon and Brown, 1986; Perez *et al.*, 1999**; **Leusink and Karft, 2000; Maris *et al.*, 2000; Song, *et al.*, 2000; Al- Haddad *et al.*, 2005; Akbas and Ozdemir, 2008; Perry and Yousef, 2011; Trinetta *et al.*, 2011 and El- Dahshan *et al.*, 2013)**. At the end of the storage period the ozone, treated yogurt samples were maintaining with acceptable sensory parameters in comparison with the control samples,

which became of poor sensory quality due to changes in its color, odor and it became unfit for human consumption due to presence of mold growths on the surface of yogurt. **(James, 2004 and Ray and Bhunia, 2008)** stated that off odor caused by the catalytic action of microbial enzymes when populations reach approximately 10^7 to 10^8 cfu/g. This result means that ozone treatment prolonged the shelf-life of yogurt samples. Similar findings were obtained by **(Jindal *et al.*, 1995, Palou *et al.*, 2003, Parish *et al.*, 2003; Di Renzo *et al.*,. 2005, Cavalcante *et al.*, 2013 and El- Dahshan *et al.*, 2013).**

CONCLUSIONS

The research showed that Ozone had a great impact on reducing the microbial load of yogurt prepared from ozone treated milk at 0, 40, 60 and 70 ppm for 10 minutes. Ozone concentration of 70 ppm for 10 min was the most effective in reducing the microbial load of yogurt samples. It is safe way to oxidize contaminants while leaving no residues and without affecting the sensory quality of food. Ozone treatment prolonged the shelf- life of yogurt by destroying the microbial populations.

REFERENCES

Abdel Hameed, K. G. and Elmalt, M. L. (2009): Public health hazard of *Staphylococcus aureus* isolated from raw milk and ice cream in Qena governorate. Assiut Vet. Med. J. 55 (121): 191-200.

Abdel Hameed, K. G. (2011): Evaluation of chemical and microbiological quality of raw goat milk in Qena province. Assiut Vet. Med. J. 57 (129): 131-144.

Akbas, M.Y., Ozdemir, M. (2008): Effect of gaseous Ozone on microbial inactivation and sensory of flaked red peppers. International J. Food Sci Technol 43:1657-1662.

Al- Haddad, K. S. H., Al- Qassemi, R. A. S. and Robinson, R. K. (2005): The use of gaseus ozone and gas packaging to control populations of *Salmonella infontis* and *Pseudomonas aeruginosa* on the skin of chicken portions. J. Food Control, 16: 405 - 410.

Al- Kanhal, H. A., Al- Othman, A. A. and Hewedi, F. M. (2001): Changes in protein nutritional quality in fresh and recombined ultra-high temperature treated milk during storage. Int. J. Food Sci. Nutr. ; 52 (6):509-14.

Allen, B., Wu, J. N. and Doan, H. (2003): Inactivation of fungi associated with barley grain by gaseous ozone. J. Environ. Sci. Health, 38: 617-630.

Anon. (2005): FAQs on ozone. Food. Rev. 32 (9): 43 - 44.

APHA (American Public Health Association) (2004): Standards Methods for Examination of Dairy aproducts. 17th Ed. American Public Health Association, Washington DC, USA.

Balzan, S., Fasolato, L., Catellani P., Sperotto D., Dall, F. and Novelli, E. (2012): Use of ozone gas to limit the growth of molds and yeasts in the room of maturation of fresh cheeses. Preliminary results. In: Proceeding of chemical reactin in food Prague, VII: 14-16.

Beuchat, L .R. (1992): Surface disinfection of raw produce. Dairy Food Environ. Sanit. 12 (1): 6-9.

Bennett, S. D., Walsh, K. A., and Gould, L. H. (2013): Foodborne disease outbreaks caused by Bacillus cereus, Clostridium perfringens, and Staphylococcus aureus-United States, 1998-2008, Clinical Infectious Diseases, 57: 425 - 433.

Biolumix, (2013): Testing for Yeast, Mold and coliform in Yogurt. https://mybiolumix. wordpress.com

Byrd-Bredbenner, C., Berning, J. , Martin-Biggers, J., and Quick, V. (2013): Food safety in home kitchens: a synthesis of the literature. International J. of Environ. Research and Public Health, 10: 4060 - 4085.

Cavalcante, D. A., Leite Júnior, B. R. C., Tribst, A. A. L. and Cristianini, M.(2013): Improvement of the raw milk microbiological quality by ozone treatment. International Food Research Journal 20(4): 2017-2021.

Di Renzo, G. C., Altieri, G. , D'Erchia, L., Lanza, G. and Strano, M.C. (2005): Effects of gaseous ozone exposure on cold stored orange fruit. In: *Acta Horticulturae*. (Mencarelli F. and Tonutti P., ed.), 1605-1610. <Go to ISI>://CABI:20063115648.

Dosti, B. (1998): Effectiveness of ozone, heat and chlorine for destroying common food spoilage bacteria in synthetic media and biofilms. Thesis. Clemson Universtiy, Clemson, SC, pp. 69.

El Dahshan, H. A., Hafez, T. A., and El Ghayaty, H A. (2013): Effect of ozone on preservation of chilled chicken. Assiut Vet. Med. J., 59 (136): 22-26.

FDA (Food and Drug Administration), (2001a): Secondary Direct Food Additives Permitted in Food for Human Consumption. *Federal Register* 66 (123):33829 -33830.

FDA (Food and Drug Administration) (2001b): Staphylococcus aureus. In: Bacteriological Analytical Manual online, Chapter 12, Bennett, R. W. and Lancette, G.A. (eds.)http://www.fda.gov/

FDA (Food and Drug Administration) (2001c): Yeasts, Molds and Mycotoxins in Bacteriological Analytical Manual online, Chapter 18, Tournas V., Stack M. E., Mislivec P. B., Koch H. A. and Bandle R. (eds.),Washington DC. http://www.fda.gov

FDA (Food and Drug Administration) (2002): Enumeration of Escherichia coli and the Coliforms. Bacteriological Analytical Manual, 8th Chapter 4. Feng P, Weagant S.D., and Grant M.A. (eds.) http://www.fda.gov

Fleet, G. H. (1990): Yeasts in dairy products. J. Applied Bacteriol. 68: 99-211.

Goel, M. C., Kulshrestha, D. C., Marth, E. H., Francis, D. W., Bradshaw, J. G. and Read, R. B. (1971): Fate of Coliforms In Yogurt, Buttermilk, Sour Cream, And Cottage Cheese During Refrigerated Storage. J. Milk Food Technol., (JMFT), 1: 2-58.

Graham, D.M. (1997): Use of ozone for processing. Food Technol., 51: 72-75.

Guzel-Seydim, Z. B. (1996): The Use of Ozonated Water as a Cleaning Agent in Dairy. Thesis. Processing. Suleyman demirel University, 32260 Cunur, Isparta, Turkey.

Guzel-Seydim, Z.B., Greene, A.K. and Seydim, A.D. (2004): Use of ozone in the food industry. Lebensm.-Wiss. u.-Technol. 37:453-460.

Han, Y., Floros, J., Linton, R., Nielsen, S. and Nelson, P. (2002): Response surface modeling for the inactivation of Escherichia coli O175:H_7 on green peppers (capsicum annum) by ozone gas treatment. J. Food Microbiol. Safety, 67: 1188-1199.

James, S. (2004): Poultry refrigeration. Chapter 4, In: Poultry meat processing and quality. 2nd edition, Edited by Mead, G.C., Publ. by Wood head Publishing Limited and CRC Press LLC.

Jindal, V., Waldroup, A. L., and Forsythe, R. H. (1995): Ozone and improvement of quality and shelf life of poultry products. J. Appl. Poult. Res. Fall., 4(3): 239-248.

Khadre, M. A., and Yousef, A. E. (2001): Sporicidal action of ozone and hydrogen peroxide: A comparative study. International Journal of Food Microbiology, 71(2-3), 131–138.

Kim, J. G., Yousef, A. E. and Dave, S. (1999): Application of ozone for enhancing the microbiological safety and quality of foods a review, J. Food Protect., 62: 1071- 1087.

Leusink, J. and Karft, G. (2000): Efficacy of gaseous ozone against generic E. coli in ground beef. Ozone Solutions Inc., http//www. Ozonesolutions.com

Leusink, J., Kraft, G. (2008): Antimicrobial Effects of Ozonated Water Against Generic *E.coli* on Swine Intestines Varying Ozone Concentrations and ExposureTimes,url:http://www.ozonesolutions.com/files/research/ecoli_swine_intestines.pdf

Majchrowicz, A. (1998): Food safety technology: a potential role for ozone? Agricultural Outlook, Economic Research Service/USDA, pp. 13–15.

Mehta, R. S. (1980): Milk processed at ultra-high- temperatures. A review,J. Food Protection, 43:212-225.

Moraes, P. M., Vicosa, G. N., Yamazi, A. K., Ortolani, M. B. and Nero, L. A. (2009): Food borne pathogens and characteristics of raw milk soft cheese produced and on retail sale in Brazil. Food borne Pathogens and Disease 6 (2): 342-346.

Naitoh, S., Okada, Y. and Sakai, T. (1988): Studies on utilization of ozone in food preservation: V. Changes in microflora of ozone treated cereals, grains, peas, beans, and spices during storage. Japan Food Sc and Technol Soci 35: 6 9 -77.

Nwagu, T. N., Amadi, E.C. (2010): Bacteria population of some commercially prepared yoghurt sold in Enugu state, Eastern Nigeria. African J. Microbiol. Res. 4 (10): 984-988.

Oliveira, C. J. B., Lopes Júnior, W.D., Queiroga, R. C. R. E., Givisiez, P. E. N., Azevedo, P. S., Pereira, W. E. and Gebreyes, W. A. (2011): Risk factors associated with selected indicators of milk quality in semiarid northeastern Brazil. Journal of Dairy Science 94 (6): 3166 -3175.

Palou, L., Smilanick, J. L., Crisosto, C. H., Mansour, M., and Plaza, P. (2003): Ozone gas penetration and control of the sporulation of *Penicillium digitatum* and *Penicillium italicum* within commercial packages of oranges during cold storage. Crop Prot. 22:1131-1134.

Parish, M.E., Beuchat, L.R., Suslow, T.V., Harris, L.J., Garret, E.H., Farber, J.N. and Busta, F.F. (2003): Methods to reduce/eliminate pathogens from fresh and fresh-cut produce. In: Kroger, M. (Ed.), Comprehensive Reviews in Food Science and Food Safety. IFT/FDA, Chicago, pp. 169-170 /http://www.cfsan.fda.gov/_comm/ift3-toc.htmlS.

Pedras, M. M., Pinho, C. R. G., Tribst, A. A. L., Franchi, M. A. and Cristianini, M. (2012): The effect of high pressure homogenization on microorganisms in milk. International Food Research Journal 19 (1): 1-5.

Penna, A.B.; Subbarao, G. and Barbosa-Cánovas, G.V. (2007): High hydrostatic pressure processing on microstructure of probiotic lowfat yogurt. Food Res. Int., 40: 510-519.

Perez A.G., Sanz, C, Rios J.J., Olias, R., Olias J.M. (1999): Effect of Ozone treatment on postharvest strawberry quality. J Agric Food Chem 47:1652-1656.

Perry, J.J., Yousef, A.E. (2011): Decontamination of Raw Foods Using Ozone-Based Sanitization Techniques. Annual. Review. Food Sci Technol 2: 281-298.

Potter, N. F. and Hotchkiss, J. H. (1995): Food Science 5th Edition, Chapman and Hall (Routledge), Florence, KY.

Proctor, A. D., Ahmedna, M., Kumar, J. V. and Goktepe, I. (2004): Degradation of aflatoxins in peanut kernels/flour by gaseous ozonation and mild heat treatment. Food Addit Contamin 21, 786-793.

Ray, B. and Bhunia, A. (2008): Fundamental food microbiology, 4th edn. CRC Press. Taylor& Francis, Boca Ratn pp: 492.

Rosenberg, M. (2011): Liquid milk products: UHT sterilized milk. In: Encyclopedia of Dairy Sciences, 2nd ed., Fuquay J.W., Fox P.F., Mcsweeney P.L.H. (Eds), vol. , San Diego, CA: Academic Press, pp.288 -296.

SAS Institute, (1988): SAS User's Guide. SAS Institue Inc., Cary, NC.

Scallan E., Hoekstra R. M. ,Angulo F. J. (2011): Food borne illness acquired in the United States -major pathogens. Emerging Infectious Diseases, vol. 17, no. 1, pp. 7-15.

Selma,M. V., Ibanez A. M., Allende A., Catwell M. and Suslow T. (2007): Effect of gaseous ozone and hot wateron microbial and sensory quality of Cantaloupe and potential transference of Escherichia coli o157:H7 during cutting. Food Microbiol, 25 (1): 162-168.

Serra, R., Abrunhosa, L., Kozakiewicz Z., Venanci A. and Lima, N. (2003): Use of ozone to reduce molds in a cheese ripening room. J. Food Prot., 66(12): 2355- 2358.

Sheldon, B. W., and Brown, A. L. (1986): Efficacy of ozone as a disinfectant for poultry carcasses and chill water. Journal of Food Science, 51(2), 305-309.

Scharff, R.L. (2012): Economic burden from health losses due to foodborne illness in the united states. Journal of Food Protection, 75 (1): 123-131.

Song, J., Fan, L., Hildebrand, P. D., and Forney, C. F. (2000): Biological effects of corona discharge on onions in a commercial storage facility. Horticulture Technology, 10(3), 608-612.

Szczesniak, A. S. (1987): Correlation sensory with instrumental texture measurements: An overview of recent developments. Journal of Texture Studies 18 (1): 1-15.

Thatcher, F. S. and Clark,D. S. (1978): Microorganisms in foods. 2nd Ed. University of Toronto Press, Toronto Buffalo, London.

Trinetta V., Vaidya, N., Linton, R., and Morgan, M. A. (2011): Comparative study on the effectiveness of chloeine dioxide gas, ozone gas and e. beam irradiation treatment for inactivation of pathogens inoculated on tomato, cantaloupe and lettuce seeds. International J. Food Microbiol, 146:203-206.

Tzortzakis, N.G., Singleton, I. and Barnes, J.D., (2007): Deplopment of low-level ozone-enrichment for the preservation of chilled fresh produce. Postharvest Biol. Technol. 43, 261-270.

USDA FSIS. (United States Department of Agriculture , The Food Safety and Inspection Service), (2001): Letter from Robert C. Post (FSIS, Washington, DC) to Mark D. Dopp (American Meat Institute, Arlington, VA).

Walstra, P.; Wouters, J.T.M.; Geurts, T.J. (2006): Fermented milks. In Dairy Science and Technology; Chapter 22, Taylor and Francis Group, LLC: Boca Raton, FL, USA, p. 551-573.

Y**abaya, A., and Idris, A. (2012):** Bacteriological quality assessment of some yoghurt brands sold in Kaduna metropolis Jorind 10 (2): 35-39.

Zhao, J., Cranston, P.M. (1995): Microbial decontamination of black pepperby ozone and the effect of the treatment on volatile oil constituents of the spice. J Sci Food Agric 68: 11-18.