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EFFECTS OF GASEOUS OZONE EXPOSURE TIME ON BACTERIAL COUNTS IN RED MEAT

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Abstract

This study was carried out at the college of veterinary medicine, University of Baghdad, For three months, The aim of the present study was to investigate effect of Exposure time of ozone (10, 15and 20) minutes on the beef meat and sheep meat to reduced limited of bacteria on meat, the data obtained revealed the following results: were significant differences ($P< 0.05$) between the means of total bacterial count before exposure of ozone were limited of ranges (5.30 - 5.51) log cfu/g meat and total coliform were ranges (2.2 -3.4) log cfu/ g meat and *psychrophilic* bacterial counts were ranged from 3.1cfu/g meat to 3.3 log cfu/g meat and were significant differences($P< 0.05$) of exposure of ozone were significant difference ($p<0.05$) between the data count, with ozone exposure 10 minutes were reduced total of bacterial count (2- 4.8)cfu/g. Total coliform 1.2 to 1.9 log cfu/g meat and 1.8 to 1.9 log cfu/g to *psychrophilic* bacterial counts and with ozone exposure 15 minutes were decreased the average of total of bacterial count for above 1.2 log, coliform and *psychrophilic* bacterial to zero log cfu/g. and with ozone exposure 20 minutes were decrease the average of total of bacterial count coliform and *psychrophilic* bacterial to zero log cfu/g.

Key words: Ozone, Red meat, Beef meat, Sheep meat, Total bacterial count, Total coliform, Psychrophilic.

Introduction

The red meat and meat products are highly perishable foods and subjected to spoilage speedily, therefore must be stored in the cooling at 4°C inside the refrigerator to increase their keeping quality and protect it from any spoilage (Kondraratowiez *et al*., 2006). Standard limited of bacteria count 510/g, Coli form 50×10^2cfu/g, psychrophilic count 10^3 cfu/g (ICOSQC, 1992; USDA, 2003; CFIS, 2004). There were many methods for reduced microorganisms freezing and thawing methods (Ismail *et al*., 2016), thawing freezing cycle (Al-obaidi, 2016) radiation and ozonation (Mahapatra *et al*., 2005).

Ozone has a longer half-life in the gaseous state than in aqueous solution therefore used remove residual pesticides and microorganisms such as total bacterial counts total coliform from food products (Chin and Berube, 2005: Midgley and Small, 2006).

Ozone directly in food products involving fish, red meat and chicken meat and its usage in the food industry (Mielcke and Ried, 2004) experimentally extending the shelf life of perishable foods by reducing microbial activity (Rice *et al*., 2001; Strasser *et al*., 2002). Manousaridis *et al*., (2005). Aerobic plate count (APC) (0.7-2.1 log cycle reduction), *Pseudomonas* spp. (0.5-1.1 log cycle reduction) and producing bacteria (1.1-2.5 log cycle reduction. The application of gaseous ozone minimized or prevented growth of microorganisms on the meat surface treated with gaseous ozone for 10 min at 22-25°C and 15 psi decreased bacteria population by more than 5 log units (Mitsuda *et al*., 1990; Ölmez and Akbas, 2009; Perry *et al*., 2011; Twegh *et al*., 2020).

Different concentrations of ozone at 1.2 and 3 mg for 30s. After ozone treatment as 1.2 mg of breast with skins on and skinless breast, while after ozone treatment as 3 mg for 30s a complete kill (1.2 and 2.5 Log10) was seen for reductions for all other organisms, including 4.3 Log10 reduction in the total viable count (99.995% reduction) Ozone is also a very rapid sterilizing agent: it has been found to work hundreds to thousands of times faster than chlorine in a disinfectant role (Guzel-Seydim *et al*., 2004; Potts *et al*., 2011). The effects of gaseous ozone treatment on microbial counts and shelf life of chilled boneless chicken breasts as well as the reduced the levels of aerobic plate count, coliforms reached 7.8×105, 5.5×103 (EL dahshan *et al*., 2013; Muhlisin *et al*., 2015). Kanaan, (2017) showed that the bacterial counts after carcass treatments for 60 minutes were lower than the bacterial counts after carcass treatments for 30 minutes were

recorded in Baghdad (4, 4 and 3 Log10 /ml). Jegadeeshwar *et al*., (2017) reported the ability of ozone to inactivate contaminant micro flora on food is variable; in some instances, however, ozone decreased food micro flora more than 5 log units. Karamah *et al*., (2019) they evaluated parameters were Escherichia coli, total aerobic of ozone exposure time (40, 80 and 120 minutes) and the replacement of ozonated water (twice, thrice and no replacement), it was able to disinfect *Escherichia coli* and TBC as much as 1.700 and 9.4×10^8 cfu/g, while on the replacement water every 40 minutes 1,700 and 1.1×10^9 cfu/g. Ayranci *et al*., (2020), reported Ozone was effective in inactivating microorganisms. Approximately 2.9, 2.3 log reductions were achieved in the counts of total aerobic bacteria. (María *et al*., 2016; Suryaningsih *et al*., 2020) used ozone immersion time (control: 5, 10, 15, 20 minutes) reduce bacterial counts total microbial decreasing in 3 log cycles, from 1.7×10^5 cfu / gram to 9.0×10^2 cfu / gram.

This study aims to limit the effective exposure time of ozone for reduction of the microbial load content of red meat.

Material and Methods

Ozone Generation

Ozone is produced from pure oxygen using an ozone generator by Kriss-Tow Multifunction Ozonizer (model GES802). This tool measures 280×205×70mm with a weight of 0.66 kg. The outlet section, is connected by a porous hose and stone made of ore sand with ozone output constructions: 500mgr /hour.

Collection of meat samples

The sample of meat was collected from different regions of Baghdad province (Al-Kargkh/ region) the beef and sheep samples of the study collect from five different regions (Abu Ghraib, Al-Mansour, Al-Khadmyaa, Allawi al Hila and Al-Durra). Number of samples 100 sample (was means 25×4). Storage the samples in the laboratory,in the freezer (-18)°cC, before beginning the bacteriological test.

Ozonation of Meat

The process of disinfecting meat with ozone is a simple process. To begin, all you need is your Aqua-6 generator, the meat you are disinfecting, water and a gallon tub.

1. Plug in your Aqua-6
2. Fill tub up with water
3. Place tube and diffuser stone into filling tub
4. Put Aqua 6 on setting 15 and let run for 30 minutes
5. Place meat/poultry into the tube after Aqua 6 finishes run time
6. Let meat/poultry soak for 30 minutes
7. Take meat/poultry out for use.

• Dilution of samples for bacteriological test:

Made the serial dilutions firstly, from an initial 10-1 to 10-6, the bottle dilutions. Content peptone water (1%) 90ml and other bottles content peptone water 9ml this bottles dilution sterilization in autoclave 121°C for 15 minutes, add 10 grams from meat samples to the first bottle dilute content 90ml peptone water to make 101 then tacked 1ml to second bottle to made 102 and then made other dilutions to 10-6.

• Bacteriological tests:

Total bacterial count or total plate count (T.P.C.) described from (A.O.A.C., 1995), after made the dilutions add 1ml from bottle dilution 101 to multiple Petri dish content media culture (nutrient agar) and 1ml from other dilutions to multiple petri dish,then incubate at 37°C for overnight (24 Hour), then count the colony of culture of bacteria ranges (30-300):-

Count of bacteria (CFU)/ gr meat = mean colony culture × dilute factor^{-1}

Total coliform count: described from (A.O.A.C., 1995), Petri dish content, media culture (VRBA) violet red bile agar and 1ml from other dilutions to the multiple petri dish, then incubate at 37°C for overnight (24 Hour), then count the colony of culture of bacteria ranges (15-150):-

Total coli form count (CFU)/ gr meat= mean colony culture × dilute factor^{-1}

Psychrophilic bacterial count (psy.c.) described from (A.O.A.C., 1995), after made the dilutions add 1ml from bottle dilution 101 to multiple Petri dish content media culture (nutrient agar) and 1ml from other dilutions to multiple petri dish, then incubate at 4°C in refrigerator for (3 day), then count the colony of culture of bacteria ranges (30-300):-

Count of bacteria (CFU)/gr meat= mean colony culture × dilute factor $^{-1}$

Statistically of analysis

The results were analyzed statistically, determining using completely randomized design (CRD). The significance of differences between groups was verified by the Duncan multiple range test; Levels of significance non-significant (ns), $p<0.05$, using the SAS program (SAS, 2012).

Table 1: Means of total aerobic bacterial count with ozone exposure time (10, 15 and 20) minutes and without ozone in beef meat with (SE±).

Samples of meat	Region	Frequency	Means of total aerobic bacterial count cfu/g meat without ozone	Means of total aerobic bacterial count cfu/g meat with ozone		
				10 min	15 min	20 min
beef	Abu Ghraib	5	4.4c±0.16	3.4c±0.21	1.47d±0.17	0
beef	Al-Durra	5	5.2a±0.16	4.5ab±0.21	3.1a±0.17	0
beef	Allawi al-hila	5	4.6bc±0.16	4.1b±0.21	2.6b±0.17	0
beef	Al-Mansour	5	4.9ab±0.16	4.2b±0.21	2c±0.17	0
beef	Khadmyaa	5	4.9ab±0.16	4.7a±0.21	2.5b±0.17	0
significant difference			P<0.05	P<0.05	P<0.05	NS

Table 2: Means of total aerobic bacterial count with ozone exposure time (10, 15 and 20) minutes and without ozone in sheep meat with (SE±).

Samples of meat	Region	Frequency	Means of total aerobic bacterial count cfu/g meat without ozone	Means of total aerobic bacterial count cfu/g meat with ozone		
				10 min	15 min	20 min
sheep	Abu Ghraib	5	4.7b±0.22	4.1b±0.14	2c±0.09	0
sheep	Al-Durra	5	5.3a±0.22	4.8a±0.14	2.7a±0.09	0
sheep	Allawi al-hila	5	4.9ab±0.22	4.5a±0.14	2c±0.09	0
sheep	Al-Mansour	5	4.9ab±0.22	4.2ab±0.14	2c±0.09	0
sheep	Khadmyaa	5	4.9ab±0.22	4.3ab±0.14	2.4b±0.09	0
significant difference			P<0.05	P<0.05	P<0.05	NS

Table 3: Means of total coliform bacterial count with ozone exposure time (10, 15 and 20) minutes and without ozone in beef meat with (SE±).

Samples of meat	Region	Frequency	Means of total aerobic bacterial count cfu/g meat without ozone	Means of total aerobic bacterial count cfu/g meat with ozone		
				10 min	15 min	20 min
beef	Abu Ghraib	5	2.7b±0.2	1.7bc±0.22	0	0
beef	Al-Durra	5	3.4a±0.2	1.4bc±0.22	0	0
beef	Allawi al-hila	5	2.7b±0.2	1.2c±0.22	0	0
beef	Al-Mansour	5	3.0ab±0.2	1.8ab±0.22	0	0
beef	Khadmyaa	5	3.0ab±0.2	1.9a±0.22	0	0
significant difference			P<0.05	P<0.05	NS	NS

Results and Discussion

The means of total aerobic bacterial count with ozone exposure time (10, 15 and 20) minutes and without ozone in beef meat showed in table 1; there were significant difference (P<0.05) in the average bacterial counts between the region without treat the ozone were ranged from 4.4 cfu/g meat in Abu ghraib to 5.2 log cfu/g meat in al-durra,this results were lower than the standard limits recorded by ICOSQ. The ozone exposure 10, 15 minutes were ranged decrease from 4.7cfu/g meat to 1.4 log cfu/g meat and with ozone exposure 20 minutes were decreased the average bacterial counts to zero log cfu/g meat.

The means of total aerobic bacterial count with ozone exposure time (10, 15 and 20) minutes and without ozone in sheep meat showed in table 2; there were significant difference (P<0.05) in the average bacterial counts between the region without treat the ozone were ranged from 4.7cfu/g.

Meat in Abu Ghraib to 5.3 log cfu/g meat in al-Durra, this result were lower than the standard limits recorded by ICOSQ. Ozone exposure 10, 15 minutes were ranged decrease from 4.8cfu/g meat to 2 log cfu/g meat and with ozone exposure 20 minutes were decreased the average bacterial counts to zero log cfu/g meat.

The means of total coliform bacterial count with ozone exposure time (10, 15 and 20) minutes and without ozone in beef meat showed in table 3; there were significant difference (P<0.05) in the average coliform counts between the region without treat the ozone were ranged from 2.7cfu/g meat in Abu Ghraib and Allawi to 3.4 log cfu/g meat in al-Durra. While with ozone exposure 10 minutes were ranged 1.2 to 1.9 log cfu/g meat and with ozone exposure 15, 20 minutes were decreased the average of coliform counts to zero log cfu/g meat.

The means of total coliform bacterial count with ozone exposure time (10, 15 and 20) minutes and without ozone in sheep meat showed in table 4; there were significant difference (P<0.05) in the average of coliform counts between the region without treat the ozone were ranged from 2.2cfu/g meat in Abu Ghraib to 3.1 log cfu/g meat in al- Khadmyaa. While with ozone exposure 10 minutes were ranged 1. 4 to 1.9 log cfu/g meat and with ozone exposure 15, 20 minutes were decreased the average bacterial counts to zero log cfu/g meat.

The means of *psychrophilic* bacterial count with ozone exposure time (10, 15 and 20) minutes and without ozone in beef meat showed in table 5; there were no significant difference (N.S) in the average *psychrophilic*

Table 4: Means of total coliform bacterial count with ozone exposure time (10, 15 and 20) minutes and without ozone in sheep meat with (SE±).

Samples of meat	Region	Frequency	Means of total aerobic bacterial count cfu/g meat without ozone	Means of total aerobic bacterial count cfu/g meat with ozone		
				10 min	15 min	20 min
sheep	Abu Ghraib	5	2.2b±0.30	1.9a±0.19	0	0
sheep	Al-Durra	5	3.0a±0.30	1.4ab±0.19	0	0
sheep	Allawi al-hila	5	2.3b±0.30	1.4b±0.19	0	0
sheep	Al-Mansour	5	2.5ab±0.30	1.4b±0.19	0	0
sheep	Khadmyaa	5	3.1a±0.30	1.6ab±0.19	0	0
significant difference			P<0.05	P<0.05	NS	NS

Table 5: Means of psychrophilic bacterial count with ozone exposure time (10, 15 and 20) minutes and without ozone in beef meat with (SE±).

Samples of meat	Region	Frequency	Means of total aerobic bacterial count cfu/g meat without ozone	Means of total aerobic bacterial count cfu/g meat with ozone		
				10 min	15 min	20 min
beef	Abu Ghraib	5	3.2a±0.24	1.8a±0.23	0	0
beef	Al-Durra	5	3.1a±0.24	1.8a±0.23	0	0
beef	Allawi al-hila	5	3.1a±0.24	1.9a±0.23	0	0
beef	Al-Mansour	5	3.3a±0.24	1.9a±0.23	0	0
beef	Khadmyaa	5	3.3a±0.24	1.9a±0.23	0	0
significant difference			NS	NS	NS	NS

Table 6: Means of psychrophilic bacterial count with ozone exposure time (10, 15 and 20) minutes and without ozone in sheep meat with (SE±).

Samples of meat	Region	Frequency	Means of total aerobic bacterial count cfu/g meat without ozone	Means of total aerobic bacterial count cfu/g meat with ozone		
				10 min	15 min	20 min
sheep	Abu Ghraib	5	3.3ab±0.1	1.9a±0.2	0	0
sheep	Al-Durra	5	2.9c±0.1	1.7a±0.2	0	0
sheep	Allawi al-hila	5	3.5a±0.1	1.9a±0.2	0	0
sheep	Al-Mansour	5	2.9c±0.1	1.6a±0.2	0	0
sheep	Khadmyaa	5	3bc±0.1	1.9a±0.2	0	0
significant difference			P<0.05	NS	NS	NS

bacterial counts between the region without treat the ozone were ranged from 3.1cfu/g meat to 3.3 log cfu/g meat. Ozone exposure 10 minutes were ranged from 1.8 to 1.9 log cfu/g meat and with ozone exposure 15, 20 minutes were decrease the average bacterial counts to zero log cfu/g meat.

The means of *psychrophilic* bacterial count with ozone exposure time (10, 15 and 20) minutes and without ozone in sheep meat showed in table 6; there were significant difference (P<0.05) in the average *psychrophilic* bacterial counts between the region without treat the ozone were ranged from 2.9cfu/g meat to 3.5 log cfu/g meat. Ozone exposure 10 minutes were ranged from 1.6 cfu/g meat to 1.9 log cfu/g meat and with ozone exposure 15, 20 minutes were decrease the average *psychrophilic* bacterial counts to zero log cfu/g meat.

The results (Table 1) showed the means of total aerobic bacterial without ozone in beef meat there were significant difference (P<0.05) in the average bacterial counts between the region were ranged from 4.4cfu/g meat in Abu Ghraib to 5.2 log cfu/g meat in al-Durra, this results were lower than the standard limits recorded by ICOSQ, (1992). The ozone exposure 10, 15 minutes were ranged decrease from 4.7cfu/g meat to 1.4 log cfu/g meat and with ozone exposure 20 minutes were decreased the average bacterial counts to zero log cfu/g meat.

The results (Table 2) showed the means of total aerobic bacterial count with ozone exposure time (10, 15 and 20) minutes and without ozone in sheep meat showed in table 2; there were significant difference (P<0.05) in the average bacterial counts between the region without treat the ozone were ranged from 4.7cfu/g meat in Abu ghraib to 5.3 log cfu/g meat in al-durra, this results were lower than the standard limits recorded by ICOSQ. Ozone exposure 10, 15 minutes were ranged decrease from 4.8cfu/g meat to 2 log cfu/g meat and with ozone exposure 20 minutes were decreased the average bacterial counts to zero log cfu/g meat.

The results of this study (Table 1, 2) indicated that the number of surviving bacterial cells represented by cfu. From treated samples with ozonated water were less than from untreated samples. As well as, the number decreased with increasing the exposure time to ozonated water at the same concentration (0.5 ppm), possible explanation could be due to increasing the exposure time lead to longer the contact time of ozone with microorganisms, leading to lower inactivation rate this results agreement with other research (Cardenas *et al*.,

2011; Kanan, 2017; Suryaningsih *et al*., 2020) reduces bacterial counts.

The results of this study (Table 3, 4) the means of total coliform bacterial count with ozone exposure time (10, 15 and 20) minutes and without ozone in beef and sheep meat there were significant difference ($P<0.05$) in the average coliform counts between the region without treat the ozone were ranged from 2.2cfu/g meat to 3.4 log cfu/g meat. Ozone exposure 10 minutes were ranged 1.2 to 1.9 log cfu/g meat and with ozone exposure 15, 20 minutes were decrease the average of coliform counts to zero log cfu/g.

The results of this study (Table 5, 6) the means of *psychrophilic* bacterial count with ozone exposure time (10, 15 and 20) minutes and without ozone in beef and sheep meat there were no significant difference (N.S.) in the average coliform counts between the region without treat the ozone were ranged from 3.1cfu/g meat to 3.3 log cfu/g meat. Ozone exposure 10 minutes were reduced 1.8 to 1.9 log cfu/g meat and with ozone exposure 15, 20 minutes were decrease the average of coliform counts to zero log cfu/g.

The results of total coliform bacterial count and *psychrophilic* bacterial count with ozone exposure time (10, 15 and 20) minutes and without ozone in beef and sheep meat (Tables 3, 4, 5, 6) agreement with other research (Patil *et al*., 2010; Cardenas *et al*., 2011; Kanan, 2017; Suryaningsih *et al*., 2020) in that time of ozone reduced microorganisms 99.9%.

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