Journal of Food Science and Technology (Iran)

Homepage:www.fsct.modares.ir

Scientific Research

Assessing Ozonated Water's Ability to Reduce Malondialdehyde in Red Meat Samples from Iraq's Wasit Markets

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ARTICLE INFO

Article History:

Received: 2025/4/20
Accepted: 2025/6/3

Keywords:

ozonated water,

cattle meat,

sheep meat,

MDA,

Al- Suwaria markets

DOI: 10.22034/FSCT.22.164.103.

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ABSTRACT

Malondialdehyde (MDA), which is a secondary result of oxidation, is the major well-studied detrimental by-product of the peroxidation of polyunsaturated fatty acids. It has been used as a measurement of oxidative rancidity. The sensitive thiobarbituric acid reactive substance (TBARS) test makes it possible to determine the amount of MDA present in animal tissues. This study aimed to assess ozonated water's ability to reduce MDA in red meat samples retailed in Iraq's Wasit markets. There was a determination of MDA concentrations using the use of high-performance liquid chromatography (HPLC). An ozone generator (A2Z/AQUA-6, USA) was used to create ozone, and a CHE-Mets®-Kit (USA) was used to determine the concentration of ozone in water in terms of parts per million (ppm). Before treatment, the findings showed that all of the samples had higher MDA concentrations, with levels in frozen sheep meat (1.53-2.51 ppm) and cow meat (0.89-1.71) ppm. After being treated with ozonated water (0.5 ppm for thirty minutes), all of the samples exhibited a decrease in the measured levels of MDA (ppm). It was observed that the average levels of MDA in frozen sheep and cow meat were 1.27± 0.28 and 1.99± 0.32, respectively, before the application of treatment. However, after treatment, the average levels of MDA were found to be 1.13±0.323 for frozen sheep meat and 0.39±0.20 for frozen cow meat. There was a statistically significant difference between these two groups ($P \leq 0.05$). This decrease in MDA is very important from the point of view of public health.

1-Introduction

Meat is necessary for human nutrition since it contains protein, B vitamins, and trace minerals. Meat's n-3 PUFA and conjugated linoleic acids (CLAs) have a beneficial role in human nutrition [1]. Meat very easily gets contaminated with foodborne germs and spoiling agents. This has led food experts to seek chlorine-free meat sterilization technologies. Food specialists seeking an alternate disinfection are studying atmospheric O3 [2,3]. This gas has many food industry uses [4,5], such as inactivating bacteria and viruses. Even without hazardous residues, treated materials degrade swiftly into oxygen [6,7]. Iraqi research has shown a wide range of foodborne germs in different meat sources. These pathogens include *Acinetobacter baumannii* [8,9], *Arcobacter* spp. [10], *Echinococcus* [11], *Clostridium botulinum* [12], *Salmonella enterica* [13,14], *Staphylococcus aureus* [15-18], and *Campylobacter* spp. [19-22]. To protect customers, effective techniques to reduce disease contamination are needed immediately.

However, meat spoils quickly if not stored properly because of its high perishability. During the preparation and storage of meat, lipid oxidation takes place [23]. This process is influenced by the lipid concentration and composition of the meat. Increasing the number of lipids and the ratio of PUFAs to saturated fatty acids (SFAs) both contribute to an increase in the oxidation of lipids [23]. According to Bertolín *et al.* [24], the process of lipid oxidation is a complicated chain reaction that starts with reactive oxygen species. These reactive oxygen species undergo reactions with a wide range of biomolecules, including PUFAs, as reported by Cunha *et al.* [25]. These reactions result in the production of aldehydes, ketones, acids, alcohols, and hydrocarbons. These biomolecules cause undesirable changes in the texture, flavor, and color of the product, thereby diminishing its quality or even rendering it unsuitable for human consumption, as stated by Bertolín *et al*. [24]. Malondialdehyde (MDA) is one of the aldehydes that is produced in the greatest quantity during the process of secondary lipid oxidation. It is also the aldehyde that is most frequently used as an oxidation marker [26]. Research conducted by Reitznerová *et al*. [27] found that the presence of oxidized lipids in the diets of both people and animals led to an increase in the levels of TBARS in both plasma and tissue. There are several occasions in which MDA is the particular aldehyde that is produced in the greatest quantity as a consequence of lipid peroxidation in meals. According to Kanner [28], the compound's concentration in meat and fish products has the potential to exceed 300 µM. There is a possibility that the mutagenic and carcinogenic qualities of MDA are due to the production of adducts with nucleic acid bases. Additionally, the capacity of MDA to change and cross-link a wide range of biological macromolecules may be a contributing factor to its toxicity. Janero, [29] suggests that the covalent alteration of lipoproteins with MDA may possess a harmful function in the development of atherosclerosis. Malondialdehyde is one of the many carbonyl compounds that are created as a byproduct of secondary lipid oxidation. It has garnered a lot of interest because it has the potential to pose a threat to human health [27], as well as the fact that it has been shown to have mutagenic and carcinogenic effects [30]. For this reason, the purpose of this research was to evaluate the efficacy of ozonated water in reducing the levels of MDA that were present in samples of red meat that were obtained from Wasit markets in Iraq.

2. MATERIALS AND METHODS

2.1. Samples Collection

Nineteen samples of frozen meat comprising eight samples of sheep meat and eleven samples of cow meat were retrieved from various shops in Al-Suwaria city, Wasit region of Iraq, for a total of nineteen samples of frozen meat. Each sample was placed in its sterile polypropylene container and kept in the fridge until it was time to be utilized in the lab.

2.2 Calculation of O_3 Concentration

To determine the O_3 concentration in water in ppm, we utilized Kanaan's CHE-Mets®-Kit technique [31]. Tap water was used to fill a 1.5-liter container, then sealed it with plastic. Following preparation, the aeration stone has been inserted into the container via a top hole. A five-, ten-, fifteen-, and thirty-minute exposure was chosen. This process has been repeated several times after each exposure period, each time filling up the container with fresh water from the tap and starting again. Five drops of A-7400 activator were initially placed into the empty sample cup. Subsequently, a tip of the CHE-Met ampoule has been put into the cup and fractured it to allow the aqueous O_3 to fill the container. The ampoule then inverted many times and observed a color change after one minute. While awaiting the high-range comparator to align with the ideal color, the ampoule was situated between the color standards. The concentration peaked at 0.5 ppm in water at the fifteen and thirty-minute intervals, out of the four measurements taken.

2.3. Preparation of Samples

It is necessary for us to take each sample and split it in half. For further processing, one half was placed in a deep freezer and maintained at -18 degrees Celsius, while the other half was allowed to thaw in a refrigerator at a temperature of four degrees Celsius for a period of one night. The HPLC analysis was carried out on a 100-gram chunk of the second half (lean and fat) that had been sliced with a ratio of 80% lean to 20% fat. A determination of the MDA concentration in parts per million was made by carrying out this procedure. For the first half, we thawed it in the refrigerator at 4 degrees Celsius for a whole night if the test was positive. The next step was to mince and cut one hundred grams of it, both lean and fat. An aeration stone (diffuser), was used to infuse oxygen gas into the water and ensure that it was dispersed evenly throughout the water. For the oxygen generator, the feed gas consisted of compressed air at a rate of one liter per minute, which is equivalent to 600 milligrams per hour. To determine the impact that oxygen has on the MDA concentration (ppm), we submerged gauze-wrapped samples of minced meat in water for thirty minutes.

2.4. Analysis of MDA by HPLC method

The high-performance liquid chromatography (HPLC) analysis of the samples and the standards was carried out by the conditions that were reported by Pilz *et al.* [32], with some minor adjustments. In this experiment, a Hewlett-Packard Series 1050 liquid chromatograph was used. This particular instrument had a quaternary autosampler, a thermo scientific variable wavelength detector that functioned at 307 nm, and an integrator HP 339 6II. Nucleosil C18 reversed-phase, with a particle size of 3 µm and dimensions of 125 × 3 mm, was used as the column. Marcincak *et al*. [33] The procedure was isocratic, and the mobile phase consisted of acetonitrile, water, and acetic acid in the proportions of 39 + 61 + 0.2, volume to volume.

Standard Solutions

By performing acid hydrolysis on 10 µL of 1,1,3,3-tetramethoxypropane (TMP; Sigma, Steinheim, Germany) in 10 mL of 0.1M hydrochloric acid in a boiling water bath for 5 minutes, a stock solution (SS) of MDA with a concentration of 4.37 µg/ml was generated. A rapid cooling of the solution was accomplished by using tap water. Pipetting 1.0 mL of hydrolyzed acetal into a

diluting it to a level of 0.1 M HCl, and then storing it at 4 degrees Celsius was the method that was used to make 0.01 MDA stock solution. For a period of up to one week, the stock solution was used. By diluting the MDA stock solution with water, appropriate concentrations of MDA working solutions were generated. These solutions were then used in the laboratory. Every day, fresh solutions were produced.

Cold Sample Extraction

Using certain changes, the cold extraction of samples was carried out by the methodology described by Marcincak *et al*. [33]. After weighing a ground sample that weighed 10 grams and placing it in a centrifuge tube with a capacity of 100 milliliters, three milliliters of aqueous EDTA with a concentration of 0.3% was immediately added. After the tube had been gently shaken, 5 milliliters of 0.8% BHT in hexane was added, and then the tube was gently shaken again. Before homogenization, 16 milliliters of ice-cold 5% TCA were added to the tube, and then homogenization was performed for thirty seconds at the highest possible speed. The top hexane layer was discarded after centrifugation for five minutes at a temperature of four degrees Celsius and a force of three thousand and five hundred g. The bottom layer was then filtered through Whatman filter paper No. 4 into a volumetric flask containing twenty milliliters and then diluted to volume with five percent trichloroacetic acid. An amount of 100 µL of DNPH reagent, with a concentration of 3.13 µM, was introduced into a test tube containing 2 mL of TCA extract. Marcincak *et al*. [33] The samples were combined and then incubated for thirty minutes at room temperature and in the absence of light.

Solid Phase Extraction (SPE) Purification

After being washed with 2 mL of acetonitrile and 2 mL of water, the Supelcleen LC-18 columns, which were 3 ml in volume, were activated. The samples were cleaned with two milliliters of water after being passed through the columns in a steady stream. One milliliter of acetonitrile was used to elute the MDA–DNPH combination.

HPLC Conditions

HPLC conditions is presented in Table 1

Table 1: HPLC conditions

Column	LC column Nucleosil C18 reversed-phase (3 µm, 50 × 4.6 mm); Mancherey Nagel, Düren, Germany
Flow rate	1 mL/min
Injection volume	20 µL
Column temperature	25°C
UV Detection wavelength	307 nm
Mobile phase	Acetonitrile - water - acetic acid (39: 66: 0.2, v/v/v) in an isocratic system

Calculation of MDA concentration

According to Marcincak *et al*. [33], the following equation was used in order to do the calculation of the concentration of MDA:

Concentration of MDA = Area of samples x concentration of standard

(µg/ml) Area of standard

Marcincak *et al*. (33)

2.5. Data Analyses

A statistical analysis was conducted using MedCalc Software BVBA version 23 (BE, USA). For this purpose, descriptive statistics were used, namely, the mean and standard deviation. To compare means (Mean± SD), we used a t-test with a significance threshold of 1% [34].

3. RESULTS

Our results showed that all samples of frozen sheep meat had increased MDA values of (1.53-2.51) ppm before treatment (Table 2). There was a considerable decrease in MDA (up to 1.55 ppm in sheep meat) in HPLC analyses of meat samples treated with O_3. On top of that, the MDA ranged from 1.53-2.51 before therapy, which is lower than the range of 0.78-1.62 after treatment. According to Table 2 and Figure 1, the average levels of MDA after therapy were 1.13±0.323, while the range of values before treatment was 1.99±0.32. A statistically significant change was seen between the Mean ± SD before and after treatment ($P = 0.0001$), according to our results ($P \leq 0.01$).

According to our results (Table 3), all of the samples of frozen beef showed high levels of MDA (0.89-1.71) ppm before treatment. Cattle meat samples treated with aqueous O_3 showed a decrease in MDA content of up to 1.32 ppm, according to HPLC analysis. After therapy, the range of MDA was determined to be 0.13–0.82, a greater range than before treatment, when it was 0.89–1.71. Furthermore, after therapy, the Mean ± standard deviation dropped to 0.39±0.20, a decrease from 1.27±0.28 before treatment. The results demonstrated a markedly significant alteration ($P \leq 0.01$) in the Mean ± standard deviation before and after treatment ($P < 0.0001$) (Table 3 and Fig. 2).

4. DISCUSSION

One of the key markers used to determine the quality and acceptance level of meat products is lipid oxidation [35]. The oxidative degradation of meat lipids is mostly caused by the oxidation of UFAs, especially PUFAs. Lipid oxidation results in the production of MDA after the breakdown of hydroperoxides [36]. Tables 2 and 3 shows that all of the frozen beef and sheep samples had high levels of MDA ranging from 0.89 to 1.71 ppm and 1.53 to 2.51 ppm, respectively, before treatment in this investigation. The findings showed that all meat samples had a lower percentage of MDA after being treated with ozonated water. For sheep, the percentage was between 0.78 and 1.62 ppm, and for cattle, it was between 0.13 and 0.82 ppm. The findings demonstrated that the average levels of MDA in sheep and bovine meat samples differed significantly ($P \leq 0.05$) before and after ozonated water treatment ($P = 0.0001$ and $P < 0.0001$, respectively).

A value of 0.6 mg MDA /kg of meat is regarded to be fresh meat, according to Tarladgis *et al*. [37]. According to Campo *et al*. [38), the upper limit for oxidized beef acceptability was determined to be a TBARS value of 2 mg MDA /kg.

This study's findings of increased MDA concentrations in sheep and cow meat samples may be due to the meat's extended frozen storage. There are many steps involved in getting frozen meat from abroad to the customer, including importing, clearing, distributing, and finally, retailing [39]. Lipid peroxidation and TBARS levels in food are shown to rise with time when preserved in the cold [40]. According to Wójciak *et al*. [41], the reason for the percentage drop in MDA following ozonated water treatment in this research might be because MDA reacts with other components, such as amino acids and carbohydrates, creating complexes with these substances. One possible ant oxidative mechanism of peptides in an oxidizing system is their ability

to scavenge aqueous phase radicals. This means that peptides can protect proteins from carbonylation that results from lipid oxidation by preventing the formation of reactive carbonyl species (RCS), like unsaturated aldehydes [42]. According to Hejazy et al. [36], a drop in MDA levels might be explained by an increase in SFA and a decrease in USFA. This is because USFA are more susceptible to oxidation, which means that they contribute oxygen to the double bonds in these FA. As a result, the oxidative stability of meat is enhanced.

Sheldon and Brown [43] examined the effects of ozonated processing water at concentrations of 4.0-4.5 ppm for 45 minutes at 7°C on the physical, chemical, organoleptic, and microbiological properties of poultry products. They discovered that ozone had no discernible impact on the organoleptic traits and lipid oxidation (TBA values) of treated broiler carcasses. Our results corroborate their findings. In addition, compared to water-chilled corpses, ozonated ones showed reduced levels of total bacterial aerosols in all tissues (breast, thigh, and back skin). In a different study, Graham *et al*. [44] examined the impact of ozonated water at a concentration of 2 to 4 ppm at 4°C on total bacterial aerosol (TBA) levels in chicken samples taken from a pilot chiller (the treatment group) and in fresh chicken samples from commercially processed chicken (the control group). The researchers concluded that there was no significant difference in TBA levels between the two groups. Treatment samples had lower TBA levels (0.011) than control samples (0.14), according to their findings. In a similar vein, following roasting, the TBA values of the treated and untreated samples were 0.386 and 0.421, respectively, showing no significant difference. The findings of this study do not fully align with those of Trindade *et al*. [45], who investigated the physicochemical and sensory properties of chicken carcasses exposed to ozone as a sanitizing agent in immersion chilling water for 45 minutes at 4°C with a residual level of 1.5 ppm. During fifteen days at a temperature of 2 ±1°C, the carcasses' stability was evaluated. While storing the carcasses at 2 to 1°C, they saw a little rise in the TBARS index, which averaged 0.68 mg of MDA/kg of the sample after 15 days, but no significant difference between the treated and untreated carcasses. No impact of a treatment × time interaction was also seen. The reason is, that after a few days of refrigeration, the MDA concentration rose gradually, reaching a peak after nine days. Lipid oxidation by-products were produced when the temperature was 4 °C rather than -3 °C because the lipid oxidase activity was enhanced at 4 °C and unsaturated fatty acids were consumed more quickly at 4 °C [46]. The oxidative characteristics of chicken breasts exposed to gaseous ozone at a concentration of 214 ppm O_3/m^3 while stored in the refrigerator were also examined in research by Muhlisin *et al*. [47]. For samples stored for 0–3 days, the TBARS levels varied across all groups, from 0.28–0.42 mg MDA/kg sample. The exposure of the chicken breasts to gaseous ozone during that storage time did not affect the TBARS readings ($P \geq 0.05$). They also concluded that the three-day exposure to gaseous ozone was responsible for the little rise in TBARS readings, which they ascribed to a reduction in CAT and GSH-Px activities. The method's suitability relies on the product type, processing and storage method, and the level of correlation with sensory analysis. Variations in TBARS values across studies could be explained by the instability of MDA and other reactive substances under specific conditions. Various studies have raised this

possibility [37, 48- 50]. According to Wang and Chen [51], catalytic ozonation has the potential to increase the proportion of hydrophilic NOM fractions, which may hydrophobically change the characteristics of big organic molecules [2]. Because of its fast inactivation process, ozone is an effective antibiotic and disinfectant. This method of disinfection is both safe and inexpensive; it eliminates pathogens, iron, and organic molecules without leaving behind any trace.

5. CONCLUSION

Our research showed that after washing the meat samples with aqueous O_3 at a concentration of 0.5 parts per million for thirty minutes the concentration of MDA in the meat samples had significantly decreased. This decrease in MDA is very important from the point of view of public health. It is impossible to overstate the significance of this reduction in carcinogenic MDA in terms of the value it has for public health. Based on these findings, O_3 treatment has the potential to serve as the foundation for a novel and risk-free method that may be used to sanitize meat and meat products before their consumption. As an antibacterial agent, treating food with O_3 has many major benefits that make it a rather useful and reasonably priced choice. Moreover, O_3 guarantees a safe and ecologically beneficial procedure as it securely reverts to oxygen and leaves no chemical residues. O_3 has other advantages as well; its great antibacterial power, which results from its better oxidation potential, helps to stop bacterial development of resistance. For this reason, the use of aqueous O_3 is highly recommended for the goal of eliminating or lowering the amounts of residual carcinogenic by-products, regardless of whether the meat is being refrigerated at the abattoir or cooked at home.

Competing Interests

The authors declare that they have no conflict of interest.

Authors' Contributions

The study design, samples collection, analysis of data, writing, and interpretation of the results was attained by MHGK. Revising and editing of the manuscript was done by FAM and SSA. All researchers have read and approved the final version of the manuscript.

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Table 1: Efficiency of ozone treatment on Malondialdehyde in sheep meat samples

Samples	**Ozone treatment/ 25 minutes**	***P value***

	Malondialdehyde (ppm) in sheep meat		
	Before	After	
Sample 1	1.53	0.94	P = 0.0001** T= -5.350
Sample 2	1.71	1.50	
Sample 3	2.10	1.40	
Sample 4	2.51	1.62	
Sample 5	2.33	0.78	
Sample 6	1.85	0.91	
Sample 7	2.01	0.89	
Sample 8	1.94	0.99	
Range	1.53-2.51	0.78-1.62	
Mean± SD	1.99± 0.32	1.13±0.323	
Highly Significant ** (P≤0.01)			

Table 2: Efficiency of ozone treatment on Malondialdehyde in cattle meat samples

Samples	**Ozone treatment/25 minutes**		***P value***
	Malondialdehyde (ppm) in cattle meat		
	Before	After	
Sample 1	1.02	0.31	P < 0.0001** T=-8.482
Sample 2	1.14	0.45	
Sample 3	1.43	0.61	
Sample 4	1.71	0.82	
Sample 5	1.56	0.34	
Sample 6	1.21	0.40	
Sample 7	0.98	0.26	
Sample 8	1.33	0.53	
Sample 9	0.89	0.15	

Sample 10	1.05	0.13	
Sample 11	1.61	0.29	
Range	0.89-1.71	0.13- 0.82	
Mean± SD	1.27± 0.28	0.39± 0.20	
Highly Significant ** (P≤0.01)			

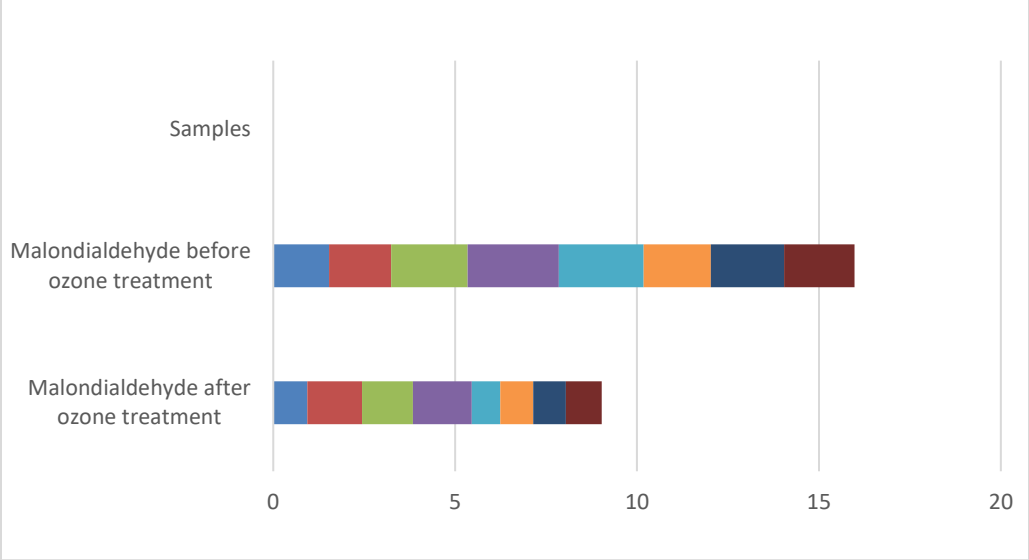

Figure 1: Percentage decrease in the level of malondialdehyde in sheep meat samples after ozone treatment

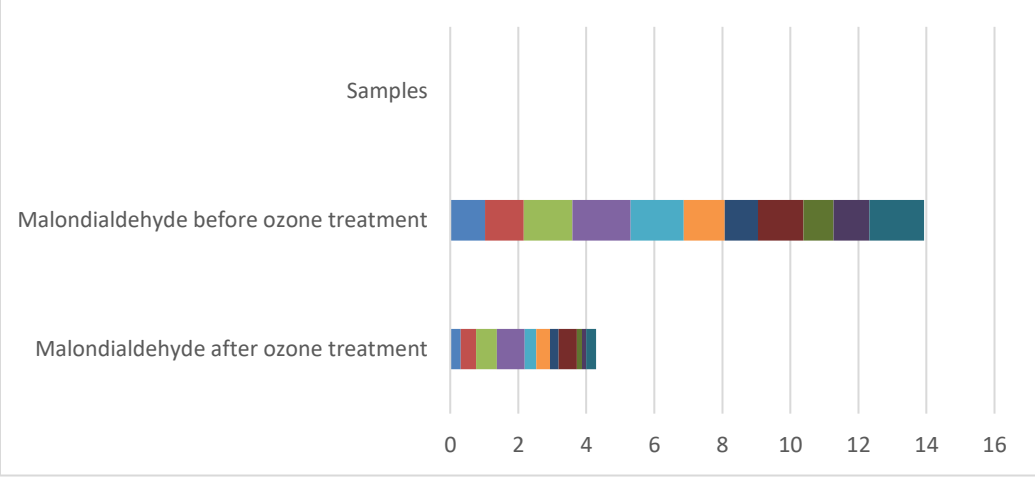

Figure 2: Percentage decrease in the level of malondialdehyde in cattle meat samples after ozone treatment