



## **Assessment the availability of ozonated water on the bioavailability of deltamethrin in treated sheep's meat**

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### **Abstract**

Deltamethrin (DMT) is one of the synthetic pyrethroid compounds having wide insecticidal activity against a large number of ectoparasites and has important uses in veterinary and human health protection. In Iraq DMT has been widely used now in Baghdad and other provinces for sheep, goats and cattle dipping or spraying by the veterinarians and farmers for controlling of ectoparasites in animals and as insecticide in crop production in agricultures. The aim of this study was to determine the effects of ozonated water treatment (0.5 ppm/15 min.) on the bioavailability of DMT in treated meat. A total of 50 sheep meat samples were collected from 10 different locations of Al-Karkh /Baghdad province (Jameaa, Kadraa, Yarmouk, Huria city, Adl, Ameriya, Dora, Saideya, Gazalyia and Mansour markets) from October 2015 to March 2016. Five sheep's meat samples were purchased from different butchers per location. These samples were analyzed by using High Performance Liquid Chromatography techniques (HPLC). The residual analysis of DMT in sheep's meat samples after ozone treatment (0.5 ppm for 15 min) revealed that there was significant reduction ( $P<0.05$ ) in DMT residues (ppm), with an overall reduction of 88%. This reduction was less than or slightly above the MRLs recommended by the WHO and FAO. In conclusions, sheep's meat treated with ozonated water have the advantages in that it did not affect the color and texture characteristics of the sheep meat, can be used in mass production as a preservation technique and to eliminate or reduce pesticide residues at the same time, and can be used in any slaughter house without the need to modify the design of the buildings. Adoption of ozonated water would minimize the high risk to the consumers from the consumption of sheep's meat containing high residual concentrations of Deltamethrin in Baghdad Province

**Keywords:** DMT, Sheep, ozone, MRLs , HPLC

### **Introduction**

The United Nations Organization for Food and Agriculture (FAO) defines pesticides as any substance or mixture of substances intended for preventing, destroying or controlling any pest, including vectors of human disease or animal species , unwanted plants or animals that cause damage or otherwise interfere with the production, processing, storage, transport or marketing of food, agricultural products, wood and wood products or animal feeds or which may be administered to animals for the control of insects,

arachnids or other pests in or on their bodies (FAO, 2002 ; Salakoet *al.* ,2012).

Deltamethrin is one of the important members of pyrethroid insecticide because DMT has a broad spectrum of insecticidal activity and relatively low mammalian toxicity, this compound is widely used for field-treatment of crops and the control of endo- and ectoparasites on animals (Di Muccioet *al.*,1997). Deltamethrin has been wildly used in Iraqi provinces

for sheep, goat, buffalo and cattle dipping or spray by the veterinarians and farmers. In order to minimize the health risks from the ingestion of food contaminated with Deltamethrin, EPA and the WHO, have set maximum residue limits (MRLs) for DMT in different food commodity (FAO/WHO, 2012).

Ozone is the natural substance in the atmosphere and one of the most potent sanitizers against a wide spectrum of microorganisms (Khadreet *al.*, 2001). Ozone is a highly reactive form of oxygen where three molecules are bonded together (Smilanick, 2003). It can be used in industrial Processes: to reduce the use of chemical agents, as chlorine; to reduce the residual pesticides on food (Wu *et al.*, 2007a), Air Treatment: to purify the atmospheres contaminated with volatile organic compounds and microorganisms, Water Treatment: to reduce in great amount the chlorine use, without form chlorinated organ compound (Rice, 1997), Medicine/dentistry: like active medicine and dental surgery, presenting and displaying viral inactivation, bactericidal and fungicide effect (Bocci. 2006: Azarpazhooh and Limeback, 2008).

Ozone can oxidize many organic compounds, particularly those with phenolic rings or unsaturated bonds in their structure (Razumovski and Zaikov, 1984; Smilanick, 2003), and can therefore have a role in reducing pesticide residues in process water (Nickols and Varas, 1992).

In a study by Onget *al.* (1996) on apples, it was found that ozone with the density of 0.25 ppm may decrease the remaining amount of three Insecticides Azinphos-methyl (GuthionR), Captan (OrthocideCaptanR) and Formetanate Hydrochloride (CarzolR) on fruits up to respectively 75%, 72%, and 46%. In this study, washing by ozone had considerable effect on the remaining amount of insecticide on the surface of processed apples and water employed in washing apples.

This study is aimed to evaluate the effects of ozonated water treatment (0.5 ppm/15 min.) on the bioavailability of DMT in treated meat.

## Materials and Methods

### 1. Collection of meat samples:

Ten different locations were selected for meat samples collection located in Baghdad Province/ AL\_Karkh which included Jameaa, Khadraa, Yarmouk, Huria

city, Adl, Ameriya, Dora, Saideya, Ghazalyia and Mansour markets. Five samples were purchased from each location from different butchers shops.

### 2. Processing of meat samples:

The average weight/sample was 300 gm (80% lean and 20% fat) from the original of 500 gm. Each sample was packed separately in a sterile polyethylene bag, and the 5 samples / location were transferred to the laboratory in an ice box. Each sample was divided into two portions of 100gm. Each sample (100 gm) was chopped according to the proportions of 80% lean and 20% fat. The first portion (100gm) was sent to the laboratory to perform HPLC analysis to determine the levels (ppm) of DMT residues and the other portion (100g each) is wrapped with aluminum foil, and kept in a deep freezer (-18°C) for further processing. If the sample was +ve from HPLC, then the second portion was subjected to ozonated water to determine the effect of ozone on the levels (ppm) of DMT residues

### 3. Preparation of DMT standards:

Stock solutions (SS) [mg/ml] were prepared from Deltamethrin standard (Fig. 3.2) in methanol Ab % and kept in freezer at -20°C. The stock standard solution was used for up to 3 months. A Suitable concentrations of working standards solutions (WSS) were prepared from the stock solutions (SS) by dilution using with methanol Ab %, immediately prior to sample preparation.

### 4. Sample preparation and separation:

One hundred grams of minced meat sample were homogenized for 5 minutes in a homogenizer and stored in a clean, sealed plastic pack at -18 °C in a deep freezer. One hundred gram of the homogenate was placed into a 250 ml polypropylene centrifuge tube with 100 ml of methanol and Vortex for 1 minute. The sample was extracted ultrasonically for 10 minutes. The sample was centrifuged at a speed of 4000 r/min for 5 minutes. Removed the supernatant and were saved in a clean conical flask (250ml) and evaporated with N<sub>2</sub> below 40 °C. The residue was reconstituted in 1ml of 5 % methanol and water. Then 20 µl were subjected to HPLC analysis.

**5. Determination of Ozone concentration (ppm) using Ozone CHE Mets® Kit (K-7404/R-7404: 0-0.6&0.6-3ppm)**

a. Five drops of A-7400 Activator were added into the empty sample cup (Fig. 1).



Fig. 1.

b. The sample cup was filled to the 25ml mark with the sample to be tested (Fig.2).

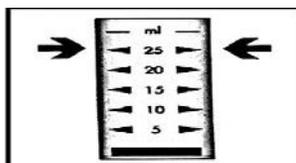


Fig. 2

c. The CHE Met ampoule tip was placed into the sample cup and, the tip of the ampoule was snapped. The ampoule was filled by the sample solution (Fig.3).

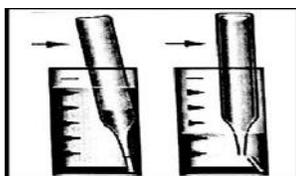


Fig. 3

- a. To mix the ampoule, the ampoule was inverted several times, allowing the bubble to travel from end to end.
- b. The ampoule was dried, and left for 1 minute for color development.
- c. The result was obtained using appropriate comparator:
  1. Low Range Comparator (Fig.4); The flat end of the ampoule was placed into the comparator.

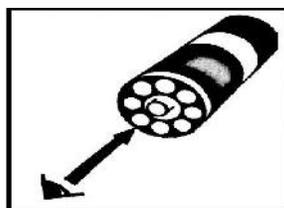


Fig. 4

2. The comparator was hold up toward a source of light, and viewed from the bottom. The Comparator was rotated until the best color match was matched.
3. High Range Comparator (Fig.5) .The ampoule was placed between the color standards until the best color was matched.

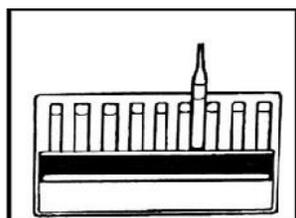


Fig. 5

**Effect of contact times of ozonated water (0.5ppm) on DMT residues in sheep meat**

**General description of the apparatuses:**

1. In this experiment a small plastic jar was used. The plastic cover has one ozone gas inlet port to inject the ozone gas into the water using aeration

2. The ozone generator was fed with 1 LPM (600mg/hr) of compressed air as a feed gas.
3. The minced meat samples were wrapped in gauze then submerged into the ozonated water.
4. The ozone water was diffused within the meat samples for different times (minutes) according to the experimental design (Fig. 6).

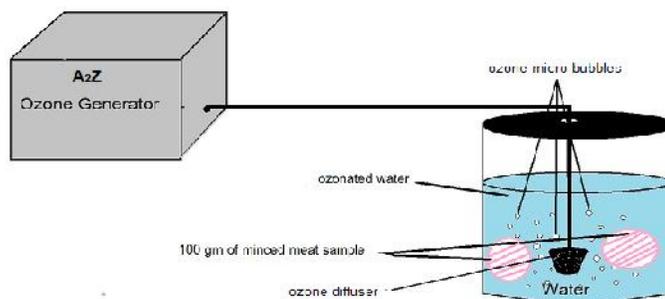


Fig.6. General description of the apparatus

**Results and Discussion**

**Determination of The effect of ozonated water (0.5 ppm/15 min.) on DMT residues (ppm) in Sheep's meat samples collected from Different Regions of Baghdad Province/ Al-Karkh.**

Twenty positive sheep's meat samples were used in this survey which were collected from 10 different regions in Baghdad province/Al-Karkh (Al-Jamia, Al-Khadra , Al-Yarmouk, Al-Hurriya, Al-Adl, Al-Mansour, Al-Ameriya, AL-Ghazaliya, Al-Doura and Al-Saideya). Two positive samples from each region were selected to determine the effect of ozonated water (0.5 ppm /15 Min.) on the levels (ppm) of DMT residues in sheep's meat samples (table 1). The treated

meat samples were kept in a cooling box and taken to the laboratory for HPLC analysis.

The HPLC residues analysis revealed reductions in the DMT residues (ppm) in sheep's meat samples after ozonated water treatment. The highest percentage decrease were recorded in Al-Doura followed by Al-Mansour, Al-Hurriya ,Al-Gazaliya, Al-Adl, Al-Ameriya ,Al-Saydiya ,Al-Khadra ,Al-Jamia, Al-Yarmouk ,100%, 98%, 97%, 96%, 95%, 93%, 88%, 82%, 79% and 17% respectively The overall reduction after treatment was 88%. The results (Table 4.7) showed that there were significant differences (P < 0.05) between the overall mean ±SD before (0.41±0.13) and after (0.05±0.06) ozone treatment ( t statistics=7.49 , degree of freedom (DF)=9 and two tail probability= 0.0000 . Fig.7) .

Table .1.The effect of Ozonated water treatment (0.5ppm/15 min.) on DMT residues (ppm) in sheep's meat samples collected from different regions of Baghdad Province/ Al –Karkh.

Parameters Regions	No of samples Tested	Ozone treatment		% Decrease
		Before	After	
Jamla	2	0.53 ± 0.01	0.11 ± 0.01	79
Khadra	2	0.56 ± 0.30	0.10 ± 0.04	82
Yarmouk	2	0.24 ± 0.13	0.20 ± 0.08	17
Hurriya	2	0.62 ± 0.04	0.02 ± 0.01	97
Adl	2	0.38 ± 0.03	0.02 ± 0.01	95
Mansour	2	0.44 ± 0.14	0.01 ± 0.01	98
Ameriya	2	0.44 ± 0.01	0.03 ± 0.01	93
Ghazaliya	2	0.23 ± 0.05	0.01 ± 0.00	96
Doura	2	0.35 ± 0.06	< LOD ± 0.00	100
Saydiyah	2	0.33 ± 0.01	0.04 ± 0.02	88
Total number of Samples tested	20			
Mean ± SD		0.41±0.13 A	0.05±0.06 B	88

< LOD = < 0.001 ppm

Ozone has been evaluated for use to break down pesticide residues on fruits and vegetables, using either wash of ozone dissolved in water or gaseous ozone (Walseet *et al.*, 2011). In this study, the aim was to evaluate the potential for using ozone to eliminate or decrease DMT residues in meat.

A total of 20 positive sheep meat samples were chosen (Table.1) for the determination of the effect of ozonated water treatment (0.5ppm/15 min.). Deltamethrin residues in these samples were above the maximum residual limits (0.062) and reduced to (<LOD - 0.20) after ozonated water treatment. The minimum decreased percentage of DMT residue after ozonated water treatment was (17%) and the highest decreased percentage was (100%). Table (1) showed that there were significant differences (P 0.05) in the mean levels of DMT residues before (0.41±0.13) and after (0.05±0.06) ozonated water treatment and the overall reduction percentage was (88%). Oxidation is the major degradation process for common pesticides, it is logical that application of common oxidants, including ozone, is expected to be promising for the removal of pesticide residues (O'Donnell *et al.*, 2012).

Kiris and Velioglu (2016) found that ozonated water treatment for 5 min reduced chlorpyrifos, -cyfluthrin, -cypermethrin and imidacloprid contents by 38%, 50%, 55% and 61% respectively in olives.

Wu *et al.* (2007b) in their study indicated that more than 60% of cypermethrin and 27-55% of diazinon, methyl parathion and parathion were removed from vegetables. Rinsing at 1.4 ppm initial dissolved ozone for 15 minutes removed 27-34% of residual pesticide. Rinsing at a higher concentration of initial dissolved ozone (2.0 ppm) increased the efficiency of pesticide removal to 30-54%. Extended rinsing at 2.0 ppm initial dissolved ozone for 30 minutes increased the pesticide removal efficiency to 45-61%.

Ozonation of fruits can be applied in the food industry for the purpose of producing healthier food for vulnerable groups of consumers, such as infants and young children. Moreover, the process can not only reduce the pesticide residue levels but also improve microbiological purity of the final product (Antoset *et al.*, 2013).

Hwang *et al.* (2001) studied the effectiveness of various wash treatments (chlorine, chlorine dioxide, hydrogen peroxy acetic acid and ozone) on the removal of themancozeb and ethylenethiourea (ETU) on and in fresh and processed apples. They observed

56-97% decreases in mancozeb residue and complete removal of ethylenethiourea after ozone (3 ppm) washing.

A study carried out in Egypt by Marzouk and Mohamed (2014) to evaluate the effect of ozonated water on reduction organophosphorus (chlorpyrifos, fenthothion, profenofos, agrothion and indoxacarb), and two carbamate insecticides (carbosulfan and methomyl) in potato, they found that ozonated water had profound effect in reducing pesticides residues in potato and the potency of reduction was increased with increasing the ozonated water concentration.

The results showed in (Table 1) could be explained by the finding reached by Lozowicka *et al.* (2016) who found that after 5 min of washing in ozone water, pesticide residues were reduced between 36.1 % for tetraconazole and 75.1 % for chlorpyrifos. This large reduction is possible because the dissolved ozone generates hydroxyl radicals that are highly effective at decomposing organic molecules such as pesticide residues (Sumikura *et al.*, 2007). Moreover, they conclude that the molecular weight of each compound could affect the percentage of reduction. Washing with ozone water was more effective in the removal of pesticides with a lower molecular mass (Lozowicka *et al.*, 2016).

In a study of Kusvuran *et al.* (2012) showed that aqueous ozone (10 mmol /mol, 5 min) was enough to oxidize pesticide residues, such as schlorothalonil, tetradifon and chloropyrifosethyl up to 92, 59.9 and 48.5% in lemons and 100, 56.6 and 40.4% in oranges, respectively. Moreover, they concluded that the increasing of applied ozone dosage did not significantly improve the removal of the pesticides.

In principles, the results of this study agreed with Kim *et al.* (2000) who treated soybeans with 0.3 ppm ozone water for 30 min during the soaking period. Residues of carbendazim, captan, diazinon, fenthim, dichlorvos and chlorpyrifos as affected by various soaking/ozonation treatments were examined. Ozone treatments destroyed more pesticide than soaking in pure water, captan being the most susceptible and chlorpyrifos least. Onget *et al.* (1996) also observed that about 53% of azinphos-methyl pesticide residue was removed with the water wash and 75% with ozone-containing water (0.25 ppm) compared to unwashed apples.

## Conclusion

ozone in aqueous phases (0.5 ppm) can be considered to be the most suitable with regard to quality and removing of residual deltamethrin from sheep's meat under home condition and mass production

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