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ACTIVITY OF OZONE ON MICROORGANISM COMMUNITIES ON STORED ORANGE FRUIT

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Abstract

The study was conducted to evaluate the efficiency of ozone (gas and ozonated water) in protecting orange fruits against pathogenic microorganisms contamination. Results obtained revealed that exposing stored orange fruits to ozone gas for 1.5 and 2.0 hrs caused 93.5 and 97.4% inhibition bacteria, 86.0 and 90.0% in fungi, 100 and 100% in yeast communities respectively. Treatment of orange fruits with ozonated water for 1.5 and 2.0 hrs caused 85.3 and 94.6% inhibition of fungi growth respectively. Slight decrease of bacterial was observed when using ozonated water for 2 hrs. ozonated water caused 100% inhibition of yeast growth on fruits at all 1.0, 1.5, 2.0 hrs of. It has been found that ozone gas and ozonated water provided protection period about 30 days to storage orange fruits after 2 hrs of exposition. These results indicate that using ozone may be a promising, safe, innocuous and effective in pathogens management, in the stored orange.
Keywords: ozone, microorganism, orange fruit

Introduction

The orange (*Citrus sinensis* L.) is considered as the most widespread fruit in Iraq, where from cultivation areas and production, representing 18% of total fruit tree cultivated in Iraq. The total production of orange fruits was estimated to be 72818 Ton in 2018, mainly 70% in Baghdad and Dialah (Statistic central system, 2018).

Orange fruits are exposed to decay after harvest due to infection with blue mold caused by *Penicillium degitatum* (Pers.: Fr.) Sacc and green mold caused by *P. italicum* Wehmer. Worldwide (Valencia-Chamorro *et al*., 2008).

Many fungicides including imazalil, Sodium ortho-phenyl phenate, thiabendazole, were used to control the fungi with good efficiency (Dezman *et al*., 1986; Eckert and Eaks, 1989). Due to the enormous problem created by the excessive use of fungicide to human health and ecosystem, the necessity to search for effective and innocuous alternatives, to fungicides to control pathogen became of great interest. It was found that ozone showed efficacy in prolonged storage periods for fresh including, broccoli, cucumber, apple, grape, pears, mulberry, and grapefruit (Beuchat, 1992). The exposition of stored onion to ozone caused a high reduction in molds and bacteria (Soing *et al*., 2000). The treatment of peach fruits with ozone at 0.03 ppm induced a high reduction in fungi growth and spores formation (Palou *et al*., 2001). Treatment of tomato fruits with 7ml/L^{-1} of ozone has highly reduction fungi and bacteria growth (Aguayo *et al*., 2006). Gonzales- Barrio *et al.* (2006) reported that exposition of stored grape to ozone at 3.88 and 1.67g /L^{-1} for 1,3,5 hrs. at 22c° induced accumulation of antioxidant in fruits. The study was conducted to evaluate the efficiency of ozone as gas and ozonated water to protect orange fruits from infection with fungi, bacteria, and yeasts.

Materials and Methods

Ozone applications

Samples of fruits were collected from orchards in dialah (south-est. of Baghdad) in plastic containers (1kg/container) with two opens one for ozone entering through a tube connected to ozonizer purchased from Laisem Chinese company ,the other for ozone exit.

The ozone was applicated under two forms, as gas at exposition period 0.5, 1.0, 1.5, 2.0 hrs. by ozonizer supplied 400 mg of O_3/ hr. and ozonated water supplied by tube ending with perforated stone connected to the ozonizer and dipped in the containers containing distilled water at the same periods of exposition mentioned above. Containers with non-treated orange fruits were used as control. The containers were maintained under laboratory conditions for 30 days and the contamination of fruits with microorganisms was estimated.

The treatment were distributed in complete randomized design with four replicates.

Microorganisms isolation

Tow hundreds of sterilized distilled water were added to 200g of orange fruits in a plastic container for each treatment and exposed to agitation in electric agitator for one hour. Serial dilutions of the water were prepared and cultivated on NYDA culture medium (10g dextrose, 5g yeast extract, 8g nutrient broth, 15 g agar in 1000 ml dist. Water) amended with 250 mg/L of chloramphenicol for yeast and fungi isolation, and 150mg of Cycloheximide for bacteria isolation. The medium, before solidification at 50C°, was distributed in petriplates, 9cm dim, containing 1ml of each dilution separately with three replication, and homogenized. The plates were maintained at 28C° for 3days, and the number of microorganisms groups (bacteria, fungi and yeast) colony were determined.

The percentage of each group was estimated by the following equation:

$$\% \text{ of microorganisms group colonies} = \frac{\text{No.of microorganisms group colonies}}{\text{Total number of colonies}} \times 100$$

$$\% \text{ Efficiency} = \frac{\text{No.of colonies in control} - \text{No.of colonies in treatment}}{\text{No. of colonies in control}} \times 100$$

Results and Discussion

Efficiency of ozone gas in reduction of microorganisms growth

The result (Table 1) showed that exposition of orange fruits to ozone induced high reduction in contaminated microorganisms (fungi, bacteria, yeast) growth compared with control. The more effective treatments were found with 1.5 and 2 hrs. of exposition with growth percentages 5.4 and 2.2% compared with 82.6% in control for bacteria at efficiency percentages 93.5 and 97.2% respectively.

The percentage of fungi growth were 11.7 and 8.3% at the tow exposition periods compared with 83.5% in control at efficiency of 96.0 and 90.0% respectively.

High significant effects of all exposition times of ozone on yeast growth were observed. The percentages of yeast colonies number were found 0.8, 0.5, 0.0, 0.0% compared with 5.1% in control at efficiency of 84.0, 90.2, 100, 100% for the exposition periods 0.5, 1.0, 1.5, 2.0 hrs. respectively.

Table 1 : Effect of ozone gas on microorganisms growth

Microorganisms	Exposition period/hr.	% of colonies number	% of Treatment efficiency
Fungi	0.5	78.7	5.9
	1.0	73.5	12.0
	1.5	11.7	86.0
	2.0	8.3	90.0
Bacteria	0.5	66.7	19.3
	1.0	88.4	0.0
	1.5	5.4	93.5
	2.0	2.2	97.4
Yeast	0.5	0.8	84.4
	1.0	0.5	90.2
	1.5	0	100
	2.0	0	100
Control	Fungi	83.5	
	Bacteria	82.6	
	yeast	5.1	
LSD (P=0.05)	22.8		

Each value is mean of 3 replicates

Efficiency of ozonated water in reduction of microorganisms growth

High reduction in fungi and yeast growth on orange fruits treated with ozonated water was contaminated (Table 2). The percentages of fungi colonies growth were found 12.3 and4.5% at 1.5 and 2.0 hrs. of exposition respectively compared with 83.5% in control at efficiency of 85.0 and 94.6% respectively.

The percentages of yeast colonies growth were 4.4, 0.0, 0.0, 0.0%at 0.5, 1.0, 1, 5, 2.0 hrs. of exposition respectively compared with 5.1% in control at efficiency of 13.7, 100, 100, 100% respectively.

No significant effect of ozonated water on bacteria growth was observed. The percentages of bacteria growth attained to 77.1, 74.2, 70.5, 58% at the four exposition periods respectively compared with 82.6% in control.

Table 2 : Effect of ozonated water gas on microorganisms growth

Microorganisms	Exposition period/hr.	% of colonies number	% of Treatment efficiency
Fungi	0.5	26.0	5.9
	1.0	20.6	12.0
	1.5	12.3	86.0
	2.0	4.5	90.0
Bacteria	0.5	77.1	6.6
	1.0	74.2	10.2
	1.5	70.5	14.6
	2.0	58.1	29.7
Yeast	0.5	4.4	84.4
	1.0	0.0	90.2
	1.5	0.0	100
	2.0	0.0	100
Control	Fungi	83.5	
	Bacteria	82.6	
	yeast	5.1	
LSD (P=0.05)	24.6		

Each value is mean of 3 replicates

Effect of ozone on *Penicillium* spp.

The exposition of stored orange fruits to ozone gas for 1.5 and 2.0 hrs. provided high protection against infection with *Penicillium* spp. reached to 30 dates, followed by exposition for 1.0 and 0.5 hrs. with protection periods 17 and 15 days respectively (Fig.1) Similar were obtained upon treatment of orange fruits with ozonated water for 0.5, 1.0, 1.5, 2.0 hrs. that gave protection period 12, 18, 28, 30 days compared with 11 days in control (Fig.1).

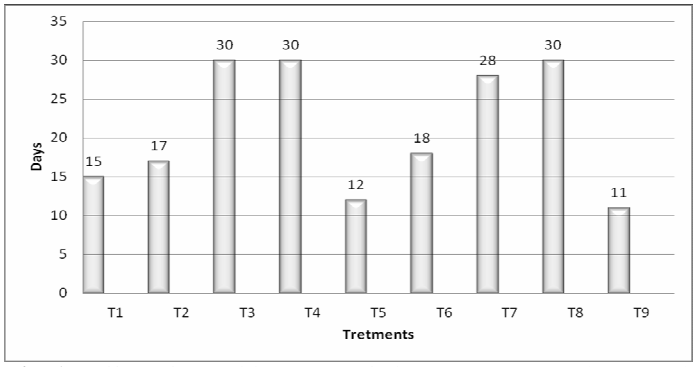

Fig. 1 : Effect of exposition orange fruits to ozone gas and ozonated water on infection with *Penicillium* spp. after 30 days of storage

T1, T2, T3, T4 = Exposition of orange fruits to ozone gas for 0.5, 1.0, 1.5, 2.0 hrs. respectively. T5, T6, T7, T8 = Treatment of orange fruits with ozonated water for 0.5, 1.0, 1.5, 2.0 hrs. respectively. T9 = control.

The results obtained in this study showed high activity of ozone as gas or ozonated water in reduction of microorganisms growth on orange fruits and prolonged the protection period of fruits against infection with *Penicillium* spp. The activity of ozone against microorganisms may be through direct effect on pathogen growth and inhibit mycelium growth and sporulation in fungi . It was reported that ozone inhibit mycelium growth and spores formation of *Penicillium italicum* and *P. digitatum* on orange fruits (Palou *et al.*, 2001).

The inhibition of pathogenic microorganisms growth by ozone through inducing the production of active compounds in the fruits acts as inhibitors to pathogens is not excluded. It

was reported that treated grapes with ozone induced accumulation of antioxidant in the fruits (Gonzales- Barrio *et al*., 2006).

The limited protection period provided by ozone against infection with *Penicillium* spp. indicated that ozone act as inhibitor without lethal effects. Gabler *et al.* (2009) reported that ozone can inhibit sporulation only on fruit surface.

Due to enormous problems caused by fungicides for human and ecosystem this research was focused on searching of safe, innocuous and effective to protect orange fruits from contamination with pathogenic microorganisms. The result of this study indicate that ozone may be promising in this direction.

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