Effect of Hot Water, Chlorine and Ozone Microbubbles Treatment on Melon

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***Abstract*—In this study the efficacy of traditional treatments as hot water, chlorine alone or in combination with microbubbles on melon was investigated. Different treatments were used like hot water (55 °C, 2 and 5 min); chlorine (150 ppm, 2 and 5 min); ozone microbubbles (150 ppm, 2 and 5 min); hot water microbubbles (55 °C, 2 and 5 min); chlorine microbubbles (150 ppm, 2 and 5 min). Microbial populations were evaluated at days 0 and 4 of the shelf-life. After washing, ozone microbubbles for 5 min and hot water microbubbles for 2 or 5 min were the most effective treatments causing 2.3, 2 and 1.7 log reductions, in the number of mesophilic aerobes, respectively. Throughout 4 days of shelf-life, hot water microbubbles for 2 or 5 min and ozone microbubbles for 5 min were more effective in retarding the microbial growth than the other treatments. The results showed that microbubbles could be a promising method for controlling the microbial quality of melon or other produces with the necessity of further elucidations.**

***Index Terms*—chlorine, melon, ozone, microbubbles, microorganism, washing**

I. Introduction

Melon is a ground crop and therefore microorganisms present on melon surface easily develops during transport and storage [1]. In addition, microorganisms might adhere on melon rind due to its rough net structure [2]. These characteristics cause problem during the washing process in reducing microbial population on melon.

Chlorine is one of the most effective disinfectant agents and commonly used in food industry, particularly for fresh cut salads and vegetables. However, the limitation of chlorine relates to environmental and health risk due to formation of trihalomethane [3].

The efficacy of hot water treatment in postharvest disease reduction has been reported for numerous fruits and vegetables [4], [5]. This kind of washing method has a lot of benefits: economic cost, non-chemical, short duration treatment and ease of monitoring. However, heat damage could be detected on treated fruits at water temperature above 60 °C [6].

Recently, ozone is widely utilized for postharvest management of fruits and vegetables in gaseous form or aqueous solution, particularly for pre-storage disinfection [7]-[9]. In addition, ozone driven oxidations do not produce by-products harming human health compared to the conventional disinfectant chlorine [10]. Besides, use of ozone microbubbles (MBs) is also one of the promising techniques in water disinfection due to generating free radicals [11]. $^{\bullet}$OH radicals produced by collapsing MBs could improve disinfection ability [12]. There has been a report indicating that ozone MBs have good efficacy in controlling microbial populations [13].

This work was aimed to evaluate the efficacy of traditional sanitizers and microbubbles treatment in reducing the number of microorganisms on melon rind surface.

II. Materials and Methods

A. Materials

Fresh samples of ‘Donatello’ melons (*Cucumis melo.* var. *reticulates* L Naud. ‘Donatello’) were harvested at 3/4 slip stage from an experienced grower in July 2015, Hungary. Fruits were transported to the Faculty of Food Science of the university in Budapest, Hungary.

The 150 ppm chlorine solutions at pH = 6.5 – 7 were prepared from active water provided from the Fishmarket Kft. (Budaörs, Hungary). Free chlorine concentration was measured with chlorine test kit (Hanna Instrument, free

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and total chlorine meter HI96711, free chlorine reagent HI93701-0, Romania).

B. Treatments

Eleven treatments were conducted as follows: dip in tap water, hot water at 55 ℃ for 2 and 5 min, 150 ppm chlorine solution for 2 and 5 min, 150 ppm ozone in the form of microbubbles (MBs) for 2 and 5 min at 16 ℃ (Table I.). The combination between hot water or chlorinated water and MBs was also tested. Fruits were immersed in hot water at 55 ℃ or 150 ppm chlorinated solution containing MBs for 2 and 5 min, respectively (Fig. 1). All samples were air-dried after treatment and then kept at 20 ℃ for 4 days of shelf-life.

TABLE I. TREATMENT DEPICTION

Washing treatment	Treatment depiction
Control	Ctrl
Tap water	Tap water
Hot water 2 min	HW 2 m
Hot water 5 min	HW 5 m
Hot water + MBs 2 min	HW MBs 2 m
Hot water + MBs 5 min	HW MBs 5 m
Chlorinated water 2 min	Chl 2 m
Chlorinated water 5 min	Chl 5 m
Chlorine + MBs 2 min	Chl MBs 2 m
Chlorine + MBs 5 min	Chl MBs 5 m
Ozone microbubbles 2 min	Oz MBs 2 m
Ozone microbubbles 5 min	Oz MBs 5 m

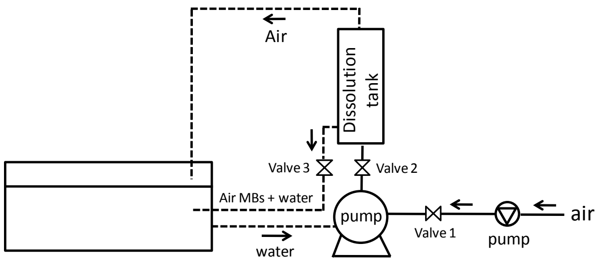

Figure 1. Schematic diagram of microbubbles generation system

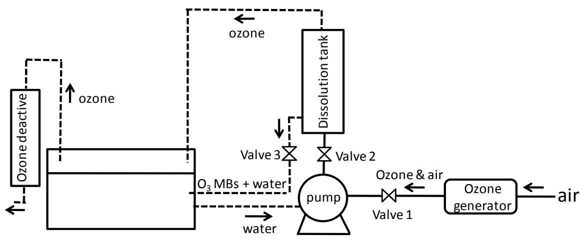

Figure 2. Schematic diagram of ozone microbubbles generation system

Ozone MBs generation system was built up for washing melon in this work as shown in Fig. 2. Seventy liters tap water (pH=7-8, t = 16 ℃) was poured into a 300 L plastic box and melons were added. Then, gaseous ozone at the concentration of 150 ppm was produced by an ozone generator (GO-R 5G, Guangzhou Ozone Environmental Technology Co., Ltd, China). The mixture of ozone and air was pumped into circulating water at a flow rate of 100 liter/min by opening valve 1. Ozone MBs were produced by gas liquid mixing pump adjusted by valve 2 (Gas liquid mixing pump Type: YL8022, model: 25GO-2SS, 1.1 KW, Guangzhou Ozone Environmental Technology Co., Ltd, China). Ozone microbubbles turn water in the box into a milky appearance. Treatment time was 2, and 5 min. Treatment conditions: temperature 16-20 ℃, pH=7-8.

C. Measurements

Number of mesophilic aerobes, disease incidence and disease severity were evaluated before treatment, after treatment and at 4th day of shelf-life.

Analysis of mesophilic aerobic bacteria: sampling was performed at sides without decay on melon rinds with a metallic ring (d=36,5 mm, A=10,41 cm^2). Gauze balls were humidified by sterile distilled water. After sampling, gauze balls were packed in sterile polyethylene bags for later analysis. Three sides on each melon surface were sampled and three fruits were used to evaluate the survival of microorganisms for each treatment. Gauze balls were put in 0.1% peptone water, then 1 milliliter of dilutions (peptone water) 10^{-1}, 10^{-2} and 10^{-3} were plated in Plate Count Agar. Mesophilic aerobes were determined after 48 h incubation on Plate Count Agar at 35 ℃.

Disease severity: decay was assessed by a scale of 1-3 based on appearance, where 1 means good, fruit without decay, 2 means fair, fruit with moderate decay; 3 means bad, fruit with severe decay. Disease severity was calculated as average score of all melons within a group [14].

Disease incidences: decay percentage was evaluated as moulds appeared on surface or stem and calculated as the number of diseased samples divided by initial number of samples multiplied by 100.

Statistical analysis: all data were processed by SPSS (SPSS Inc, USA) using analysis of variance (ANOVA) with the following factors: washing treatment type (11 treatments) and time (after treatment and on the 4th day of shelf-life), followed by Tukey's method with the significance level of $P< 0.05$. The results were reported with mean and standard deviation (95 % confidence interval).

III. RESULTS AND DISCUSSION

A. Population of Mesophilic Aerobes after Treatment

The efficacy of treatments in decreasing the mesophilic aerobes was shown in Fig. 3. All treatments had effect on microorganism populations but at different rates. These results indicated that ozone MBs 5 min and hot water MBs were the most effective in reducing microbial loads. Washing with ozone MBs for 5 min and hot water MBs for 2 or 5 min decreased the number of mesophilic aerobic bacteria approximately by 2.3, 2 and 1.7 log cfu/cm^2, respectively, compared to control. Although, ozone MBs treatment for 2 min was less efficient than for 5 min, however, we found lower mesophilic aerobic bacteria count than on the rest of the samples (Figure. 3). Hot water and chlorine alone or chlorine MBs could decrease mesophilic aerobes by about 1 log cfu/cm^2. Tap water was found to be less efficient than other treatments. No sign of damage on melon rind surface was detected after treatments.

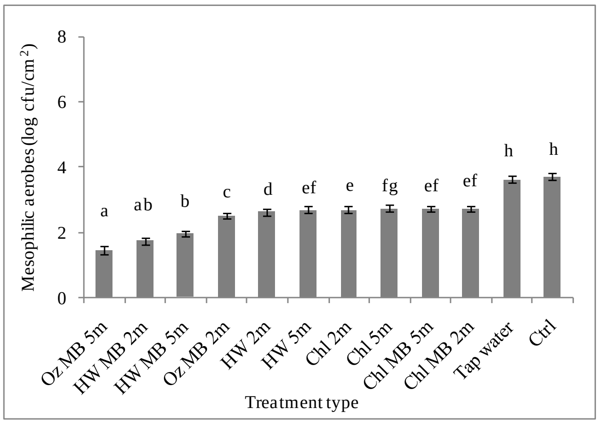

Figure 3. Population of mesophilic aerobes on melon rind surface after treatment. Values are the mean ± SD. Letter is for the comparisons of treatments (Tukey's probe, $p < 0.05$)

In this study, ozone MBs were beneficial in reducing mesophilic aerobes due to oxidative property of ozone. In addition, free radical generated by collapsing MBs improved sanitizing ability of ozone MBs [15]. Ozone attacks microbial cell surface, firstly reacts with sulfhydryl groups, peptides and proteins and then polyunsaturated fatty acids leading to leakage of cellular compositions. Ozone oxidizes the essential components of cellular microorganism causing cell death [16]. These results showed that ozone MBs for 2 min was less effective than for 5 min because treatment for 2 min may not be long enough time to disinfect melon rind surface. In our work, ozone MBs was much more effective than chlorine because ozone is the second strongest oxidant, more powerful than chlorine [17], [18]. Moreover, ozone damages most proteins inside microbial cells, whereas chlorine selectively oxidizes internal cellular enzymes [19]. Hot water MBs achieved a high reduction in mesophilic cell number than hot water alone due to generation of free radicals by collapsing MBs.

In this experiment, chlorine was less efficient than hot water. It could be explained that the contact between chlorine and microorganisms was not good enough due to roughness and waxiness of melon rind [20].

B. Population of Mesophilic Aerobes, and Decay after 4 Days of Shelf-life

There were significant differences in mesophilic aerobic population on melon surface among treatments after 4 days of shelf-life (Fig. 4). Melons treated with ozone MBs for 5 min or hot water MBs had the lowest microbial loads, followed by that of ozone MBs 2 min, chlorine alone or chlorine MBs, and hot water. The number of microorganisms on melon washed with tap water was close to that of control samples. After 4 days of shelf-life, chlorine was more effective in controlling microbial loads on melon surface than hot water treatment.

Untreated or tap water washed melon had the highest disease incidence and severity than other samples (Table II). After 4 days of shelf-life, the decay percentage of samples treated with hot water MBs and ozone MBs for 5 min were approximately by one third less than other treatments. In addition, appearance of these melons was still acceptable (Table II.). Ozone MBs for 2 min was found to be less efficient than ozone MBs for 5 min and hot water MBs, but better than the rest. The disease incidence was above 50 % in case of hot water, chlorine alone or chlorine MBs treatment and the appearance of melon was almost unacceptable (Table II.).

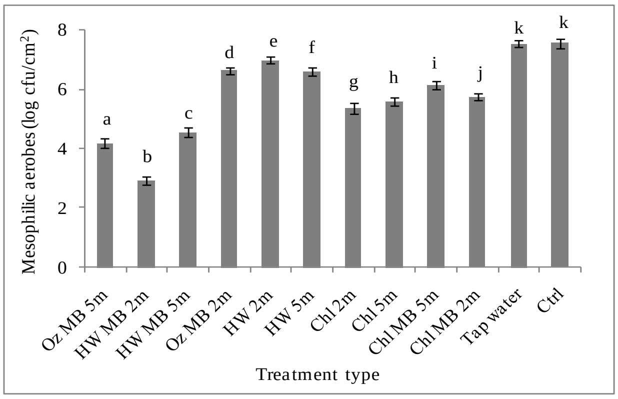

Figure 4. Population of mesophilic aerobes on melon rind surface after 4 days of shelf-life. Values are the mean ± SD. Letter is for the comparisons of treatments (Tukey's probe, $p < 0.05$)

TABLE II. DISEASE INCIDENCE AND DISEASE SEVERITY AFTER SHELF-LIFE

Washing treatment	Disease incidence (%)	Disease severity
Control	83.3 e	2.8 c
Tap water	76.7 cde	2.7 bc
Hot water 2 min	63.3 cd	2.3 a
Hot water 5 min	63.3 cd	2.3 a
Hot water + MBs 2 min	33.3 a	1.6 abc
Hot water + MBs 5 min	36.7 a	1.7 abc
Chlorinated water 2 min	60.0 bcd	2.5 abc
Chlorinated water 5 min	56.7 bc	2.3 abc
Chlorine + MBs 2 min	60.0 bcd	2.5 abc
Chlorine + MBs 5 min	66.7 cde	2.4 abc
Ozone microbubbles 2 min	43.3 ab	2.4 abc
Ozone microbubbles 5 min	36.7 a	1.8 ab

Means followed by the same letters are not significantly different at the same measurement time (Tukey's, $p < 0.05$).

Samples treated with ozone MBs for 5 min and hot water MBs had the lowest mesophilic populations and disease incidence after shelf-life due to the low level of microbial load after washing. In addition, it might be that microorganisms need time to recover after stress [21]. Chlorine controlled microbial population during shelf-life more effectively than hot water or ozone MBs 2 min due to chlorine residues on melon surface. Another reason was that washed melons might be more susceptible in poor hygiene environment [22]. It is worth to notice, that an effective sanitizing method is not able to have continuous effect during storage without good hygiene.

IV. CONCLUSION

This work showed the effect of different washing methods on decontamination of melon rind surface. Microbubbles - offering an alternative washing technique

- could be applied in postharvest management. Chlorine was found to be less efficient in reducing number of microorganisms on melon skin than hot water and ozone microbubbles. Ozone microbubbles for 5 min and hot water treatment have potential in disinfection of melon rind. However, the recontamination of washed melons easily occurred during shelf-life. Therefore, sanitizing should be used together with proper handling in order to provide produce microbially safe produce.

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