Effect of Micro-bubbles Ozone for Inactivation of *Escherichia coli* O157:H7 on Fresh-cut Pineapple cv. Phu Lae

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ABSTRACT— ***This study investigated the effect of micro-bubbles ozone (MBO) for inactivation of Escherichia coli O157:H7 on fresh-cut pineapple cv. "Phu Lae". Fresh-cut pineapples were dip-inoculated with Escherichia coli O157:H7 (approximately 10^8 CFU/ml) and washed with MBO concentration of 0.14, 0.12, 0.06, and 0.03 mg O_3/L at room temperature (28±1ºC) for 5, 10, 15 and 30 min, respectively ; then stored at room temperature (28±1ºC). The results showed that the population of Escherichia coli O157:H7 on fresh-cut pineapples washed with MBO for 10 min significantly reduced after storage for 2 days (less than 1 $\log_{10}$CFU/g) when compared to all treatments and the control (distilled water). Result from scanning electron microscope (SEM) obviously shown that less Escherichia coli O157:H7 in biofilms when compared to control. Therefore, the application of micro-bubbles ozone potentially enhance the ability of pathogenic control and prolonged shelf life of fresh-cut fruits.***

Keywords— *Escherichia coli*, fresh-cut, micro-bubbles ozone, pineapple

1. INTRODUCTION

Pineapple (Ananas comosus L.) is one of tropical fruit which is commonly planted in Thailand. Value of exported fresh and frozen pineapple in January 2014 to July 2015 was about 116 million baht [1]. Recently, fresh-cut pineapple is very popular for the customers because the skin peel-off is difficult. Therefore, it is usually prepared as ready-to-eat product and much prefer by the customers in the tropical fruit market. Fresh-cut fruit in the small vender's cart and the fresh market are commonly contaminated from the micro-organism because they usually display without the refrigeration [2]. Nevertheless, the supermarket apply to low temperature about 8-10 °C for fresh-cut fruits storage [3]. The process to make fresh-cut product involves skin peeling, trimming and cutting to specific size that cause for the wounds. These wounding of the fruit tissue increases the surface browning and microbial spoilage [4]. Moreover, the effect of cutting long section per half of fresh-cut pineapple was delayed microbial growth [5]. Therefore, fresh pineapple was sanitized by washing with chlorinated solutions to reduce the number of micro-organisms. *Escherichia coli* has been considered a major micro-organisms which cause foodborne disease. The Department of agricultural Thailand regulates the amount of this bacteria in food must be less than 10 CFU/g.

Ozone is one of the sanitizing agents and it has a powerful oxidizing agent. It was confirmed in Generally Recognized As Safe (GRAS) [6]. Ozone can kill bacteria such as *E. coli*, *Samonella* spp. and other pathogens, which is free of chemical residues because it could be decomposed into oxygen with no concerns about consumption of residues ozone in the treated food product [6,7]. The application of ozone effectively improved the food safety in fresh-cut processing [8]. It has been reported that ozonated water use to wash lettuce 1.3 mM ozone at flow rate of 0.5 L/min was injected into water which reduce the total plate count about 2 log CFU/g in fresh-cut lettuce and more than 90% reduction in total plate count in Chinese cabbage [9,10]. Micro-bubbles ozone (MBO) is a technique to produce the microscopic size of ozone bubbles in water. Sizes of produced bubbles vary in the range between 50-200 µm. It should be enhance the range less than 50 µm for oxidation ability of oxidizing water [11]. The produced bubbles have a highly stability in the water [12]. These bubbles can keep the ozone for a long time, which can be utilized in efficiency

disinfection in water. Micro-bubbles will be slowly floated up to the water or any liquid surface. During the floatation, ozone will be dissolved more than normal condition. As a result, the typical property of micro-bubbles has increase in surface area, highly stability in water and MBO has a high efficiency surface cleaner. In addition, micro-bubbles were used to combine with oxygen and carbon dioxide, to enhance their dissolve and stability properties. The micro-bubbles technique reduced water pollutant, which was observed in phenol degradation (approximately 60%) [13]. As for micro-organism, ozone micro-bubbles reduced *E. coli* O157:H7 of viable cell (5.0-7.4 log) better than those of the ozonated water and resulted in decontamination on surface of leafy vegetables as well as the concentration of ozonated water at 5 ppm was controlled tomato wilting disease [14,15].

This study aimed to determine the effect of micro-bubbles ozone (MBO) for inactivation of Escherichia coli O157:H7 on fresh-cut pineapple cv. "Phu Lae".

2. MATERIALS AND METHODS

2.1 Measurement of diameter and dissolved ozone in micro-bubbles ozone water

Micro-bubbles Ozone (MBO) produced using ozone generator (Ozonizer, Model SO5AE) and micro-bubbles water generator (Model 15KED02S, NIKUNI CO., LTD., Japan) at flow rate of 7L/min with an internal pressure of 0.25 MPa for distilled water. The MBO water circulated in the micro-bubbles ozone bath (Figure 1). The concentration of dissolved ozone in water measured using Indigo colorimetric method [16]. The diameter was measured by particle-size analyzer (Mastersizer, Malvern Instruments Ltd., England) for 5, 10, 15 and 30 min of MBO exposure time.

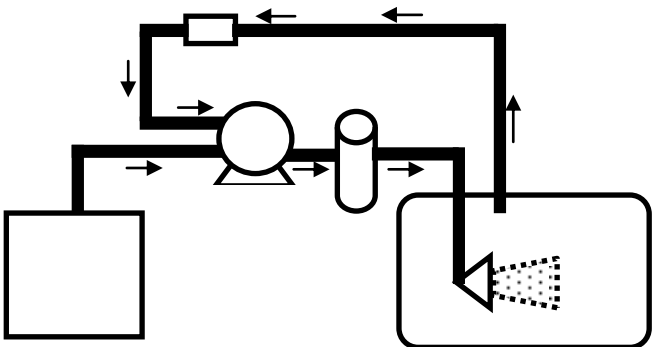

Figure 1 Schematic diagram of circulated in the micro-bubbles ozone bath

2.2 Preparation of plant material

Pineapple (*Ananas comosus* cv. Phu Lae) at harvesting stage brought from the plantation at Chiang Rai Province, Thailand and delivered to the Postharvesrt Physiology Laboratory at Chiang Mai University. The fruits were sorted for the free of wound from harvesting and puncture from insects and washed in water chlorination (100 ppm) for 5 min. The whole fruit peeled skin-off with sterile knife and cut to long section per half of fruit which divided for treatments; a control (non-inoculated) and dip-inoculated with *E. coli* O157:H7. The fresh-cut pineapples were stored at room temperature for 10 min before MBO treatments at the difference times. Four pieces of fresh-cut pineapple from each treatment were kept in plastic (PET) disposable fresh fruit box and then stored at room temperature (28±1ºC).

2.3 Preparation of E. coli O157:H7 Suspension and inoculation bacterial on fresh-cut pineapples

E. coli O157:H7 DMST 12743 was obtained from the Department of Medical Science, Bangkok, Thailand. Cell suspension of *E. coli* O157:H7 prepared from the stock culture, that inoculated into trypticase soy broth (TSB) and incubated at 37°C. The bacterial growth was observed using spectrophotometer at 600 nm every 2 hr for 24 hr. Then, Cell suspension was selected approximately 10^8 CFU/ml. Fresh-cut pineapples were dip-inoculated with *E. coli* O157:H7 and incubated for 10 min. After that, Fresh-cut pineapples were washed using MBO for 5, 10, 15 and 30 min, respectively; then stored at room temperature (28±1ºC). The microbiological analysis by inhibition of treated bacterial dilutions were evaluated using spread plate technique on trypticase soy agar (TSA), which incubated at 37°C for 24 hr.The colony of *E. coli* O157:H7 was presented as the mean number of colony forming units per grams ($\log_{10}$CFU/g).

2.4 Quality change for post- harvesting of fresh-cut fruits during storage

Fresh-cut pineapples were measured color with a colorimeter (Minolta CR-200, JAPAN). L* value and b* value were recorded for the data analysis. Firmness was measured after samples were treated using MBO. The rupture maximum force (Newton) the slices was measured using firmness tester (Force guage:FG500, Daiichi meter). Ascorbic content was determined using the 2, 6-dichoroindolephenol titrimetric method. Ascorbic acid reduced indicator dye, 2, 6-

dichloroindophenol, to a colorless solution. At the end point, excess unreduced dye was rose pink in acid solution. The results were presented as the mean value from 3 replications of each treatment.

2.5 Scanning electron microscopy (SEM)

The treated fresh-cut pineapples using MBO at the difference times were cut to small-sized (0.5x0.5 cm) with sterile knife. After that, Samples were fixed for scanning electron microscope by using 2.5 % glutaraldehyde for 24 hr and dehydration of serial in ethanol 50, 70, 90 % and absolute ethanol for each 3 times ethanol concentrations immersed for 10 min. After that, samples were critical point dried and sputter-coated with gold. Samples were examined under SEM (JEOL, JSM 5910LV).

2.6 Statistical analysis

All experiments were conducted with three replicated and evaluated with regression procedure using SPSS version 17. Differences among treatments determined using Duncan's Multiple Range test ($P \leq 0.05$).

3. RESULTS AND DISCUSSION

3.1 Distribution of diameter and dissolved ozone in micro-bubble ozone water

The range of the diameter of produced bubbles was 40-50 µm for exposure times and the highest value of distribution occurred in MBO at 5 min (data not shown). MBO treatments obtained the concentration of 0.14, 0.12, 0.06, and 0.03 mg O_3/L at room temperature (28±1ºC) for 5, 10, 15 and 30 min, respectively. As a results, the distribution and the concentration of dissolved ozone in MBO treatments were decreased when increased the exposure times due to the bubbles were combining together to a big-sized bubble and collapsing to water surface and micro-bubbles generated by using nozzle system had affected the bubbles grow into the larger bubbles during the rapid degradation of supersaturated liquid [17].

3.2 Effect of micro-bubbles ozone (MBO) for inactivation of Escherichia coli O157:H7 on fresh-cut pineapple

The effect of MBO for inactivation of *E. coli* O157:H7 of fresh-cut pineapples is shown in Figure 2. The result showed that all treatments were presented significant differences and the exposure time at 10 min obtained the best result in inactivation of *E. coli* O157:H7 after storage (Figure 2). As the result, the dissolved ozone in water by using micro-bubbles generator affected on bacterial membrane and disorder enzyme activity of bacteria [18]. Nevertheless, cell viability of *E. coli* O157:H7 in all treatments revealed 4.14-5.55 $\log_{10}$CFU/g after storage for 2 days (Figure 2). It might be because the ozone concentration could decompose in a few minutes to lower concentration [19] or recovery of bacterial cells. It has been shown that the treated with ozone in apple cider and orange juice resulted in reduction of *E. coli* O157:H7, and indicated the development of sublethal injury on TSA [20].

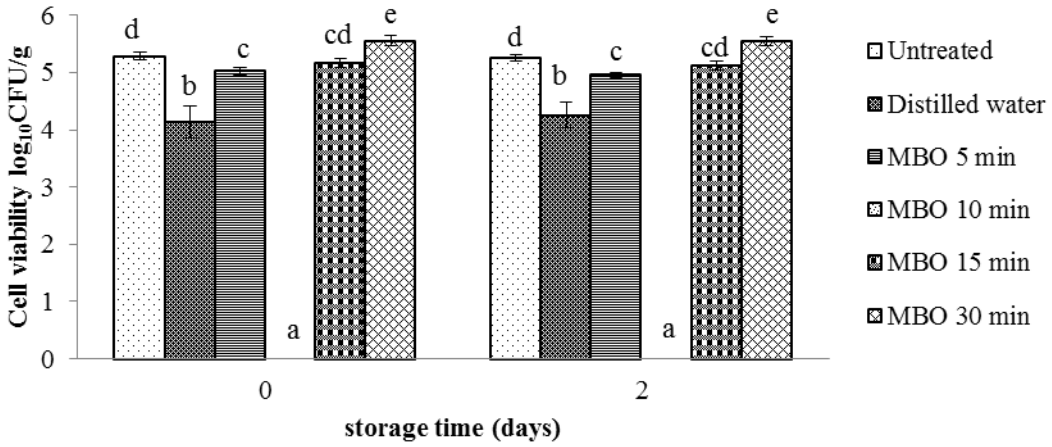

Figure 2 Effect of micro-bubbles ozone (MBO) for inactivation of *Escherichia coli* O157:H7 on fresh-cut pineapple during storage at room temperature (28±1ºC) *Error bar represented standard deviations. Values ($\log_{10}$CFU/g) with the same letter on the same color bars represented no significance differences (*P>0.05)

3.3 Quality change for post- harvesting of fresh-cut fruits during storage

The color of fresh-cut pineapple was presented L* and b* values. During the storage, L* and b* values represented brightness and yellow color, respectively. L* values at all treatments were reduced slightly and no significant differences as well as b* values did not difference and still reduced slightly after storage for 1 day. After storage for 2 days, b* values were reduced significantly in Table 1. Firmness and ascosbic acid contents at all treatments were reduced slightly during storage. After storage for 2 days, ascosbic acid contents were reduced significantly. The decay incidence was

observed that all treatments were stored for only 2 days at room temperature (28±1ºC) (data not shown). There was re-contaminated from micro-organism due to kept without the low temperature. The cut to specific size of fresh-cut pineapple gave to the wounds, which increased browning and microbial spoilage on the surface [3,4].

Table 1 Quality change of fresh-cut pineapple during storage for 2 days at room temperature (28±1°C)

Treatments	Color measurement		Firmness (N)	Ascorbic content (mg/100ml)
	L*	b*		
Untreated control	32.52a	14.66ab	4.87a	5.67b
Distilled water	31.82a	14.69ab	6.25a	4.00cd
MBO 5 min	31.67a	15.23c	6.62a	5.33bc
MBO 10 min	31.78a	15.25abc	7.70a	7.00a
MBO 15 min	31.01a	13.73a	5.26a	4.67b
MBO 30 min	32.39a	13.76a	4.89a	3.33d

Data followed by the same letter within the column are not significantly different (*P>0.05)

3.4 Scanning electron microscopy (SEM)

Scanning electron microscope photographs of fresh-cut pineapples inoculated with *E. coli* O157:H7 were studied. It was found that *E. coli* O157:H7 cells attached the surface and biofilm formation still presented on fresh-cut pineapple surface tissues which unwashed using MBO after storage at room temperature (28±1°C) for 18 hr. The treated fresh-cut pineapples after washed using MBO at the difference times decreased the biofilm formation (Figure 3). Biofilm formation represented cells survival on the surface. Also, SEM photographs of lettuce inoculated with *E. coli* O157:H7 and washed with 200 ppm chlorine for 2 min shown that the bacterial cells attached in stomata and incorporated into biofilm culture [21]. Therefore, the treated fresh-cut pineapples might be penetrated the biofilm on the surface by washing with MBO and affected to inactivate cells of *E. coli* O157:H7. Moreover, ozone treatment had affected the cells structure damaging of *E. coli* after exposure for 150 min by using SEM [22] as well as ozone had the effective to damage in the cytoplasmic space and loss of intercellular contents of *E. coli* when examined with microscopic [23]. The enlargement of reaction time should be enhance the penetration of sanitizing agents and inactivation of bacterial cells.

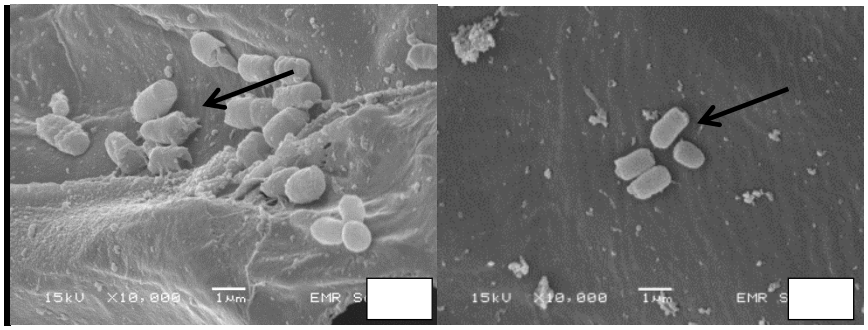

Figure 3 SEM photographs of fresh- cut pineapples were inoculated with *E. coli* O157:H7. The photographs were presented attachment and biofilm formation on the surface then storage at room temperature (28±1ºC) for 18 hr (A) and the photographs were presented the decrease in biofilm formation after washed MBO for 10 min then storage at room temperature (28±1ºC) for 12 hr (B).

4. CONCLUSIONS

The study of MBO inactivated *E. coli* O157:H7 on fresh-cut pineapple during storage at room temperature (28±1°C) was conducted. The exposure time of MBO for 10 min resulted in inactivation of *E. coli* O157:H7 which the amount of cells on surface shown less than 1 $\log_{10}$CFU/g. At the room temperature, it presented re-contamination of micro-organism. However, the fresh-cut processing must be control temperature and the application of MBO may be enhanced the disinfection on fresh-cut fruits.

5. ACKNOWLEDGEMENT

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