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Application of advance oxidation process combination with fine bubble technology on the reduction of *Escherichia coli* O157:H7 contaminated on bird eye chilli (*Capsicum frutescens* L.)

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Abstract Nowadays there are continuously increasing worldwide concerns for development of alternative microbiocidal inactivation. Advance Oxidation Processes (AOPs) are considered a highly competitive technology for the elimination of contaminated microorganism due to their high chemical stability and low biodegradability. The effectiveness of UV/O_3 in AOP system combination with Fine bubble technology (FB) for the reduction of *E. coli* O157:H7 contaminated on Bird Eye Chilli (*Capsicum frutescens*L.) was assesed. A batch-type prototype of the UV/O_3 and FB generator treatments were constructed. Consideration the antimicrobial effectiveness of individual technique, the results indicated that the population of *E. coli* O157:H7 was reduced as 96.36% within 10 minutes 9 when UV were applied. In case of the O_3 applications, the results demonstrated that O_3 provided 97.18% reduction within 10 min. The application of AOP, UV/O_3 system, it can be found that the use of AOP provided 98.62% reduction of *E. coli* O157:H7. This finding knowledge indicated that AOP with UV/O_3 system presented the more antimicrobial potential than the use of UV or O_3 alone. The studies of FB with or without AOP on the microorganism elimination were also conducted. FB alone processed contamination reduction of *E. coli* O157:H7 at 96.53% reduction. The combination with AOP demonstrated 98.05% reduction. The use in dynamic assist process, 99.96% reduction was observed. Furthermore, applying AOP with FB technology in dynamic assist process did not affect the quality of Bird Eye Chilli after washing process. It was indicated the use of UV/O_3 as AOP combination with FB technology in dynamic assist process could be applied for microbial contamination reduction on fresh produces.

Keywords: Advance oxidation process, Ultraviolet, Ozone, Fine bubble technology, *Escherichia coli* O157:H7, *Capsicum frutescens* L.

Introduction

The enlarged accepting of the nutritional values of fresh vegetables let those to be widely consumed around the world (Gomez and Ricketts, 2013; Southon, 2000; Wargovich, 2000). Several researches have been

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reported about the benefits of vegetables such as part of a low fat and high fiber diet that probably help reducing blood pressure, diabetes, cancer and manage weight (Gomez and Ricketts, 2013). However, raw fresh vegetables like other food have been reported in contact with harmful microorganisms during harvesting, transportation and storage (Burnett and Beuchat, 2001; Guo *et al*., 2002; Solomon *et al*., 2002; Wachtel *et al*., 2002; Nipa *et al*., 2011; Rahman and Noor, 2012; Ahmed *et al*., 2014; Feroz *et al*., 2014; Noor *et al*., 2014; Chowdhury *et al*., 2016; Alam *et al*., 2015; Noor *et al*., 2015). Additionally, contamination and growth of microorganisms resulted to the limitation of safety and shelf life of vegetables (King and Bolin, 1989; Robbs *et al*., 1996; Uyttendaele *et al*., 2009; Fatema *et al*., 2013) Chilli (*Capsicum* sp.), especially Bird Eye Chilli (*Capsicum frutescens* L.), was recognized as one of the most consumed Chilli in Thailand is Brid Eye Chilli, (*Capsicum frutescens* L.). Conversely, those were often contaminated by microorganisms on the surface and can cause food poisoning illness (Joseph *et al*., 1997).

Postharvest handling such as washing or spraying with sanitizer is most important procedure in view of the fact that removed, eliminated or reduced the surface microbiological contaminant (Pasit *et al*., 2018). Consequently, several chemical compounds were used to eliminate microorganisms on vegetables. However, the complete removal of microorganisms is not achieved (Zhang and Farber, 1996; Niemira, 2007). Additionally, their antimicrobial seemly lost when those were exposed to air, light, metals, and organic substances (Abadias *et al*., 2008). The requirements to improve both quality and safety of those fresh produces have driven the innovative processes to accomplish all desirable qualities**.** An application of non-thermal disinfection technology by combining ozone and ultraviolet-C (UV-C), so called Advance Oxidation Process (AOP), was developed and shown effective in wash water for the fresh-cut vegetable industry (Selma *et al*., 2008).

Fine Bubbles (FB) technology was first introduced in 2005. The different research applications of this technology have been investigated for many fields (Tsuge, 2014). The diameter of FB is the range of 10–50 μm (Parmar and Majumder, 2013; Takahashi, 2005). FB have been used to clean the dirty adhering substances (Akuzawa *et al*., 2010, Ushida *et al*., 2013, Iijima and Moriyasu, 2007), to reduce frictional resistance (Sanders *et al*., 2006), for long-term storage of fish (Wang *et al*., 2008), and to inactivate norovirus (Kozima *et al*., 2006).

Thus, in this study the efficacy of AOP in combination with FB Technology on the reduction of *Escherichia coli* O157:H7 Contaminated on Bird Eye Chilli (*Capsicum frutescens* L.) was investigated.

Materials and Methods

Bird Eye Chilli sample preparation

Fresh bird eye Chilli (*Capsicum frutescens* L.) was used as models. The fruits were purchased from the wholesale fresh market in Pathum-Thani, Thailand. Visibly damaged and wilted portions were discarded. Uniform fruits were sorted in terms of size and maturity. The samples were washed with tap water to reduce the soil and debris before being drained and left in a biological safety cabinet class II (AstecMicroflow, Bioquell, UK), followed by packing in polyethylene (PE) plastic bags and storing at 12 ± 2 ℃. Fruit were subjected to treatments on the day of preparation.

Bacterial culture and inoculation preparation

Pathogenic strains of *E. coli* O157:H7 were the tested organism. Culture was obtained from the Department of Medical Science, Ministry of Public Health, Thailand. *E. coli* O157:H7 were activated in 50 mL of tryptic soy broth (Difco, USA.) then incubated at 37 ℃ for 18 h to obtain the working inoculum with the final bacterial concentration at 7.0–8.0 Log_{10} CFU/mL. The artificial contamination was done on prepared bird eye Chilli. The initial population on the artificially contaminated was 6.0–7.0 Log_{10} CFU/g (before washing).

Advance Oxidation Process and Fine Bubble generator

Advance oxidation process system in this study was UV-O_3 system. The UV chamber consisted of 4 short wave UVR lamps producing 280 nm of UV radiation. This chamber was connected with pair type O_3 generator which produced O_3 via corona discharge reaction. Oxygen was passed through the ozone generator at 0.03, 0.06, 0.12 and 0.24 L/min. Steering impeller was installed in order to be a dynamic system. FB was generated by cavitation method. The schematic illustration of AOP and FBsystem was presented in Figure 1.

Effect of UV-C on the reduction of E. coli O157:H7 contaminated on Bird Eye Chilli

The 10 g of *ca*. 6.0 Log_{10} CFU/g artificially contaminated Bird Eye Chilli samples was placed in sterile mesh sachet. The UV-C radiation process was performed via distilled water at the intensity of UV-C were 0.72, 1.44, 2.16 and 2.88 kJ/m^3. The samples were irradiated at 0, 10, 20, 30, and 40 min. The fruits were taken at the desired time of interval and placed in 90 mL of phosphate buffer. Ten fold's serial dilution was completed. The

number of E. coli O157:H7 was enumerated on Thin Layer MacConkey agar (Difco, USA.) and incubated at 37°C for 24 h. The colonies were counted and reported.

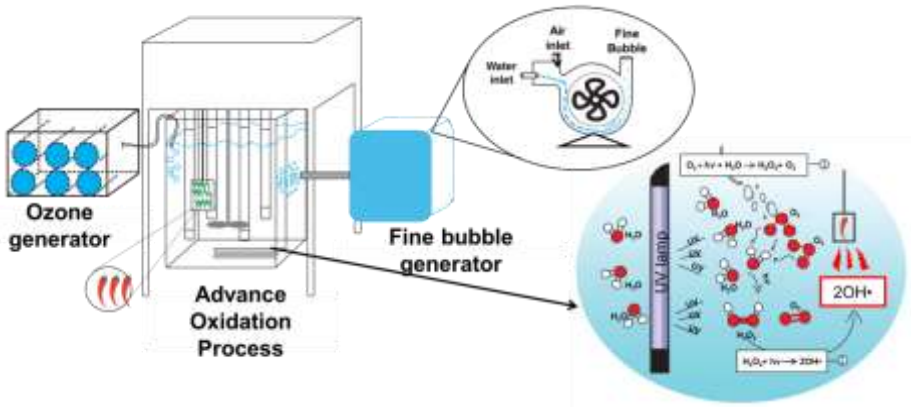

Figure 1. Schematic illustration of AOP system in combination with FB technology

Effect of O_3 on the reduction of E. coli O157:H7 contaminated on Bird Eye Chilli

O_3 in aqueous phase was prepared using ozone generator described above with the different oxygen flow rate at 0.03, 0.06, 0.12 and 0.24 L/min. The exposed ozonisation processes were 0, 10, 20, 30, and 40 min. The enumeration process had been already described above.

Effect of AOP (UV-O_3) on the reduction of E. coli O157:H7 contaminated on Bird Eye Chilli

The AOP system used in this study was UV-C in combination with Ozone solution, distilled water was selected as the washing medium. 10 g of Bird Eye Chilli in mash sachet were placed under AOP chamber. UV-C irradiation and ozonisation were applied in the same time via washing medium at the time of interval as 0, 10, 20, 30, and 40 min. The enumeration process had been already described above.

Effect of AOP (UV-O_3) in combination with Fine bubble under dynamic and statics condition on the reduction of E. coli O157:H7 contaminated on Bird Eye Chilli

The AOP system was combined with FB generator. Distilled water was used as washing medium. Dynamic and static system was controlled by installed steering impeller. Prepared samples were treated under desired

condition. At the time of interval, treated Bird Eye Chilli was taken to *E. coli* O157:H7 enumeration procedure described clearly above.

Storage study

The *E. coli* O157:H7 artificially contaminated Bird Eye Chillies and the natural contaminated those were washed under AOP system in combination with FB and in assisted with dynamic condition. The washing time was 40 min. The washed Bird Eye Chilli was kept in poly ethylene bag under 4°C for 12 days. The numbers of *E. coli* O157:H7 were enumerated with the procedure described above.

Statistical analysis

The data was presented as the average of four replications ± standard deviation (SD). Statistical analysis was carried out using Analysis of Varian (ANOVA) and the difference between the means was performed with DMRT at $P < 0.05$ by using SPSS version 14.0 software.

Results

Efficiency of UV-C on the contamination reduction of E. coliO157:H7 contaminated on Bird Eye Chilli

The effectiveness of UV-C on the contamination reduction of *E. coli* O157:H7 contaminated on Bird Eye Chilli was presented in Figure 2. According to the results, it could be indicated that 71.15% of initial population was reduced after 40 min treated of UV-C at 0.72 kJ/m^2 intensity. The more increasing of UV-C intensity the more reduction was observed. The use of UV-C at 2.16 and 2.88 kJ/m^2 presented the reduction of *E. coli* O157:H7 on Bird Eye Chilli as 97.30% and 97.91%, respectively.

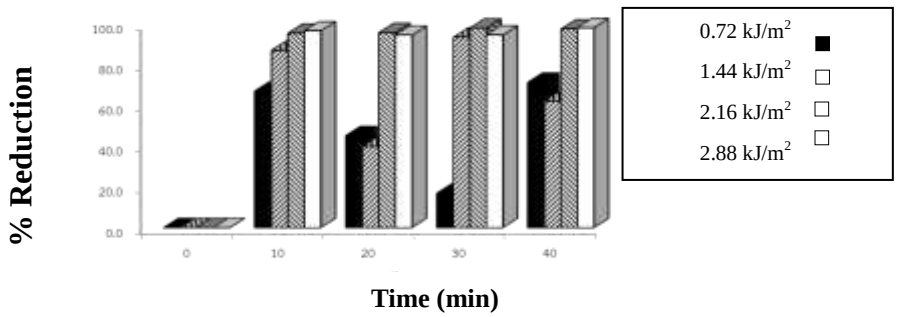

Figure 2. Reduction of *E. coli* O157:H7 contaminated on Bird Eye Chilli with different intensity of UV-C within 10, 20, 30 and 40 min

Efficiency of O_3 on the contamination reduction of E. coli O157:H7 contaminated on Bird Eye Chilli

The effectiveness of O_3 on the contamination reduction of *E. coli* O157:H7 contaminated on Bird Eye Chilli was presented in Figure 3. The results demonstrated that at the oxygen flow rate as 0.03 L/min presented the 86.51%on the reduction of *E. coli* O157:H7 contaminated on samples. In case of oxygen flow rate was increases as 0.06 L/min, the reduction was detected as 80.12%.. At the oxygen flow rated as 0.12 L/min, the reduction was 97.18%. However, the antimicrobial property of ozone was decreased at the oxygen flow rate as 0.24 L/8min.

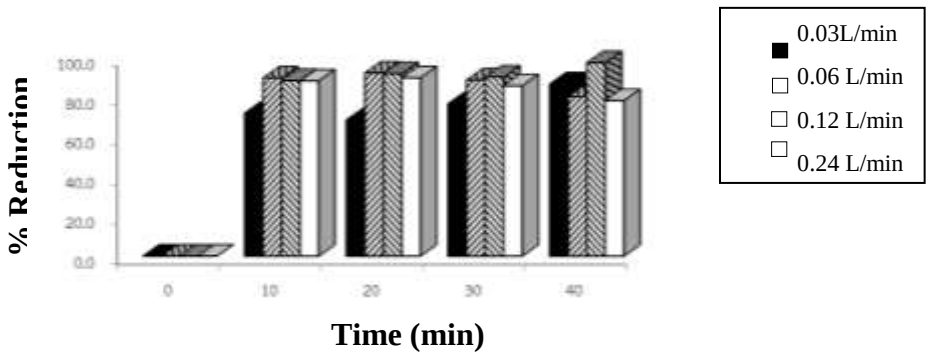

Figure 3. Reduction of *E. coli* O157:H7 contaminated on Bird Eye Chilli with different flow rate of oxygen through Ozone generator within 10, 20, 30 and 40 min

Efficiency of AOP (UV-O_3) on the contamination reduction of E. coli O157:H7 contaminated on Bird Eye Chilli

The percentage of reduction of *E. coli* O157:H7 contaminated on Bird Eye Chilli after treated with AOP (UV-O_3) system (Fig.4). The reduction was more detected when the intensity of UV-C, oxygen flow rate through Ozone generator and the exposure time was increased. It could be noticed that the combination of UV-C at 2.88 kJ/m^2 with Oxygen flow rate through the Ozone generator at 0.12 L/min demonstrated the highest antimicrobial activity. The reduction was 98.62%at 40 min of exposure time.

Efficiency of AOP (UV-O_3) in combination with Fine bubble under dynamic and statics condition on the reduction of E. coli O157:H7 contaminated on Bird Eye Chilli

The number of *E. coli* O157:H7 after wash with distilled water FB and distilled water in dynamic system is presented in Table 1. The results

indicated that the dynamic system presented the less effectiveness compare with FB. *E. coli*O157:H7 reduced as 47.52% within 40 min, while FB presented 96.53 % of reduction at the same exposure time.

The antimicrobial potential of AOP system by using UV-C at intensity of 2.88 kJ/m^2 and Ozone was produced by Oxygen at the flow rate of 0.12 L/min in combination with dynamic system and/or FB (Fig.5). The results demonstrated that 94.37% reduction was detected when AOP in combination of dynamic system was applied for 40 min., while the combination of AOP and FB presented 98.05% in reduction of *E. coli* O157:H7. In the highest efficiency, 99.96% reduction was obtained when AOP in combination of FB assisted with dynamics system was applied to the washing procedure of *E. coli* O157:H7 contaminated Bird eye Chilli.

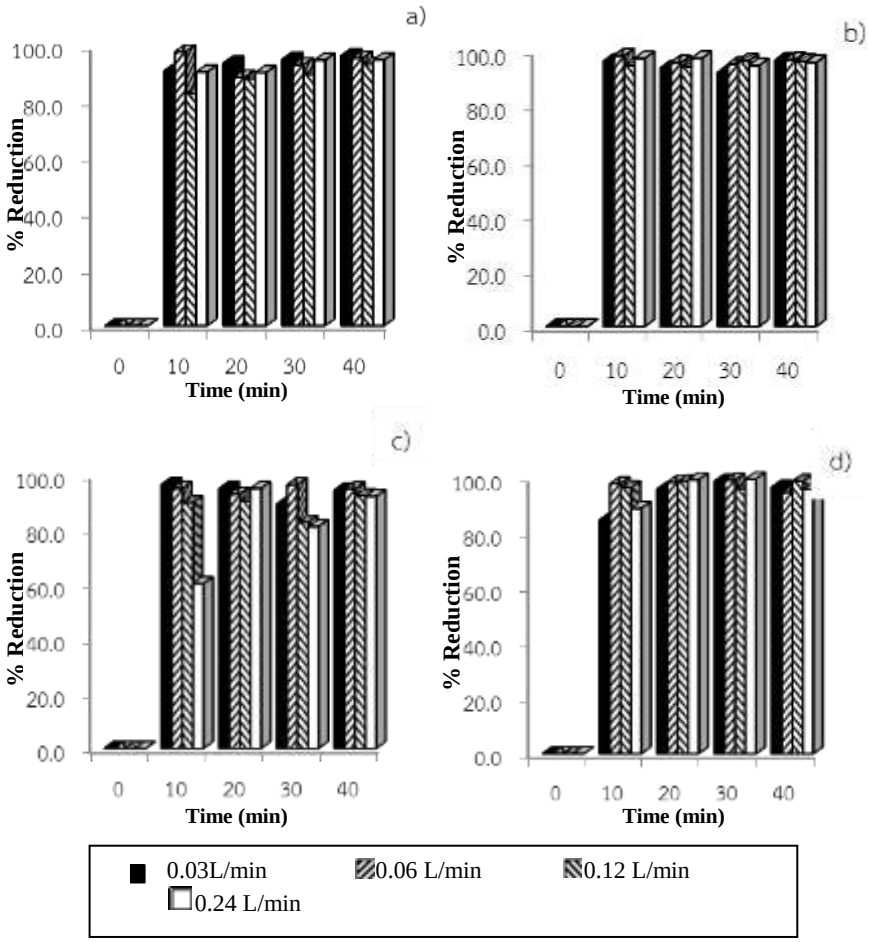

Figure 4. Reduction of *E. coli* O157:H7 contaminated on Bird Eye Chilli in AOP process (UV: a; 0.72 kJ/m^2, b; 1.44 kJ/m^2, c; 2.16 kJ/m^2 and d; 2.88 kJ/m^2)

Table 1. Reduction of *E. coli* O157:H7 contaminated on Bird Eye Chilli according to FB and dynamic system

Time (min)	% Reduction	
	FB	Dynamic system
0	0 ±0.0	0 ±0.0
10	75.45 ±4.7	36.90 ±32.6
20	86.19 ±4.2	42.45 ±26.9
30	93.24 ±6.0	39.74 ±22.7
40	96.53 ±0.2	47.52 ±38.5

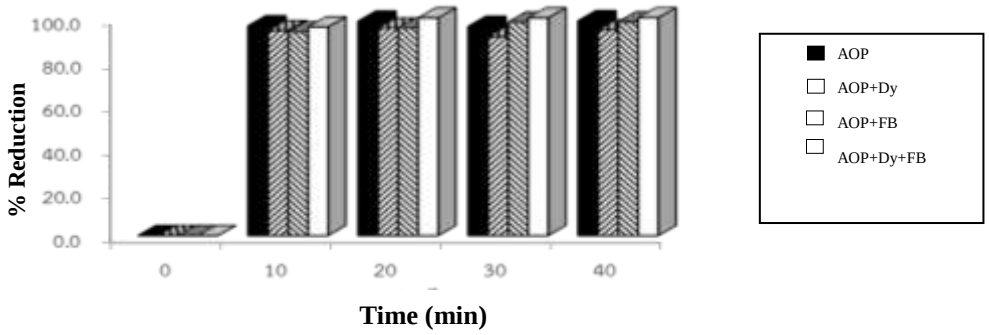

Figure5. Reduction of *E. coli* O157:H7 contaminated on Bird Eye Chilli with different system

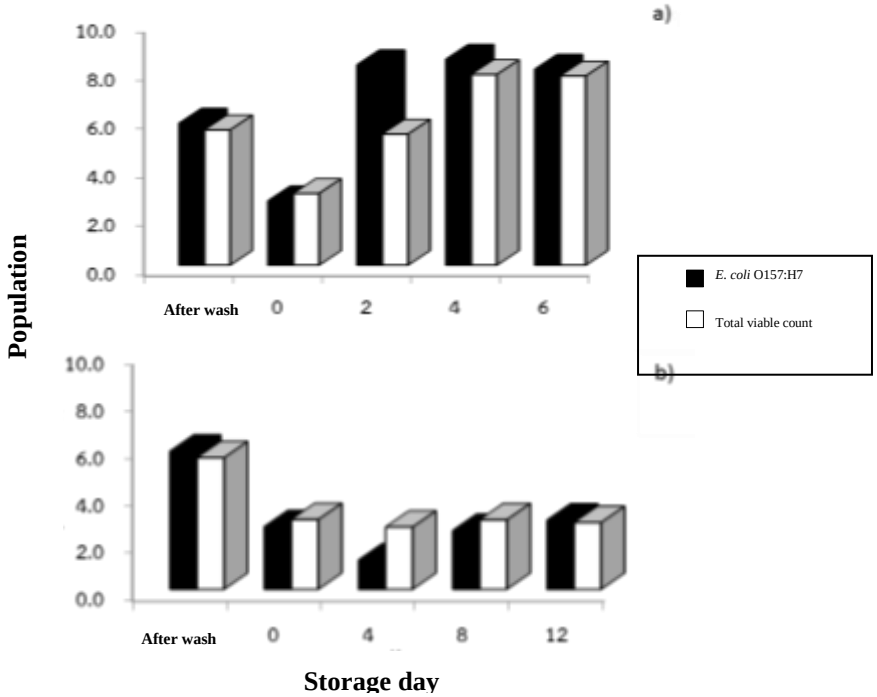

Figure 6. Population of *E. coli* O157:H7 and Total viable count contaminated on Bird Eye Chilli after washing with AOP+FB+DY stored at room temperature (a) and 4°C (b)

Storage study

The total viable count and *E. coli* O157:H7 contaminated on Bird Eye Chilli after treated with AOP in combination with FB and dynamic system assisted stored at 4°Cand room temperature (27±2°C) for 12 days (Fig.6). The results indicated that total viable count and *E. coli* O157:H7 were increased when the samples were stored at room temperature. The numbers were increased from 2.95 to 7.78 Log_{10} CFU/g and 2.65 to 8.06 Log_{10} CFU/g, respectively. For the storage at 4°C, total viable count and *E. coli* O157:H7 still maintained during the 12 days of storage.

Discussion

In general, the contamination of *E. coli* O157:H7 can be noticed in environmental sources of fresh produce farms (Morakotjinda, 2012, Ontoum, 2010). There was a report mentioned that *E. coli* O157:H7 has been frequent pathogenic bacteria identified in contaminated vegetables (Warriner *et al*., 2009). Bird Eye Chilli commonly used fresh in cuisine and washing is the only way for decontamination of fresh those. The use of UV-C demonstrated the decontamination properties against *E. coli* O157:H7 contaminated of Bird Eye Chilli. The results were in agreement with Winnie and Mark (2016) who reported that the use of UV-C demonstrated the potential to reduce numbers of *Salmonella* sp. contaminated on tomatoes. In the agreement results, the antimicrobial properties of UV-C increased when the irradiation process was increased. The use of UV-C presented the effectiveness on the decontamination of microorganism contaminated of surface of fresh produces as a whole or as fresh cut products. UV-C affects several physiological processes in plant tissues and damages microbial DNA (Lucht *et al*., 1998, Koutchma *et al*., 2009) Lado and Yousef (2002) described that UV-C light presented the ability to inhibit microbial contamination through the generation of hydroxyl radicals from water, which eradicate hydrogen atoms form DNA components. UV at 254 nm encouraged the formation of pyrimidine dimmers which modified the DNA helix and blocked microbial cell replication. The destructiveness of cell was concurred according to the un-repairable of damaged DNA. Erkan *et al*. (2001) demonstrated that the zucchini squash slices exposed to UV light for 10 and 20 min. presented the microbial reduction activity and deterioration during subsequent storage. Similar results were obtained for bell peppers (Mercier *et al*., 2001), lettuce (Yaun *et al*., 2004; Allende and Artés, 2003a; Allende and Artés, 2003b), apples (Yaun *et al*., 2004), pear (Schenk *et al*., 2008) strawberry (Darvishi *et al*., 2012; Erkan *et al*., 2008), broccoli (Civello *et al*., 2008), tomato fruits (Charles *et al*., 2008; Charles *et al*., 2009), spinach (Escalona *et al*., 2010), oyster mushrooms (Ha *et al*., 2011a; Ha *et al*, 2011b) and many other fruits and vegetables. Moreover the study

of Yaun *et al*. (2004) indicated that the inoculated Red Delicious apples, leaf lettuce and tomatoes with *Salmonella* sp. or *Escherichia coli* O157:H7 obtained different reductions of microbial populations varying from 2.19 logs for tomatoes inoculated with *Salmonella* sp. to 3.3 logs for apples inoculated with *E. coli* O157:H7 at the hignest intensity of UV-C light as 24 mW/cm^2. D'hallewin *et al*. (1999) demonstrated that melon treated with UV light had lower populations of aerobic microorganism compared to control and post-cuttreated pieces.

Ozone was discovered by CF Schonbein in 1839 (Rubin, 2001). It was recommended as a disinfectant for drinking water because of its powerful ability to destroy microorganisms. The aqueous form is uncomplicated to handle and is a potent microbicidal solution suitable as a soaking solution.According to the high concentration of O_3 in aqueous solution, hydroxyl radical and active oxygen produced from the dissociation of ozone, representation of antimicrobial activity of ozone, was oxidized by ozone itself, the effectiveness of ozone depended on the critical concentration or equilibrium concentration of ozone at which the self-oxidation would not be detected. The results indicated the antimicrobial of O_3 in aqueous solution, so in agreement of the previous research of Nurul and Asgar (2014) who reported the antimicrobial of O_3 using in the production of fresh-cut paprika.

The antimicrobial potential of AOP was higher than the use of UV-C and O_3 alone at the same intensity and Oxygen flow rate. In AOP system, hydroxyl radical was generated in high amount and reacted with molecule of water resulting to the high concentration of Hydrogen peroxide that demonstrated the high oxidation potential and cause the reduction of microorganism. The recent work showed that AOP system could reduce the population of E. Coli O157:H7 contaminated on Bird Eye Chilli, the effectiveness is more than the use of UV-C or Ozone alone. As the previous study on the potential of fine bubble on the destructive of attached microorganism on the fresh produce surface, the results were in agreement with the report from previous study (Klintham *et al*., 2017).

According to report of Takahashi (2005) on the electrical properties of fine bubbles, it was designated that the fine bubbles demonstrated a negative charge on their surface. The zeta potential of fine bubbles in distilled water was -35 mV (pH 5.8) in approximately. The negative charge on the bubbles surface possibly will affect on the charge on the microbial cell wall, therefore fine bubbles expect to drive out bacterial cells attached on produce surface. The presence of the same charge between the surface of *E. coli* O157:H7 (Ukuku and Fett , 2002) and the bubbles showed a little effect on washing efficacy. The decontamination effect of fine bubble against *E. coli* O157:H7 may possibly explain by attenuation of fine bubbles greatly decreases the hydrogen bonding that result in decreased surface tension according to the studied of Himuro (2007). Fine bubbles threw in to

weakening of hydrogen bonding which may delay the attachment of microorganisms and surface of vegetables.

No studies have ever been conducted on the effect of AOP-FT on the quality of vegetables and fruits. However, it could be assumed that free radicals generated through collapsing of air in fine boubble could decompose phenol dissolved in water solution, and suggested that the same process could decompose fruit and vegetable organic compounds. The results of our study indicated that bubbling AOP does not affect the colour or physical properties of tested model food.

It is concluded AOP (UV-O_3) in combination with FB and dynamic system assist demonstrated the effectiveness on the reduction of *E. coli* O157:H7 contaminated on Bird Eye Chilli. The optimum condition of AOP system was 2.88 kJ/m^2 of UV-C and 0.12 L/min Oxygen flow rate through the ozone generator. The treated those can be stored at 4°C for at least 12 days without alteration.

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