CHAPTER 4

REDUCTION OF CHLORPYRIFOS IN FRESH BIRD CHILLI USING ULTRASONICATION AND OZONATION

4.1 Introduction

Chlorpyrifos is widely used as crop protection specifically in chilli production by to control insect pests. However, the Thai Agricultural Commodity and Food Standard (2006) reported that imported countries detected chlorpyrifos in chilli over the maximum residue limits, MRLs (0.5 mg/l). The present strategies to reduce residual pesticides include washing with water, potassium permanganate and detergents, but these methods cause water pollution and high cost with limited effectiveness. Nowadays, everyone is looking for clean technology, which is technology without requiring chemical inputs. Ultrasonication and ozonation are among the alternative methods to degrade toxic pesticides.

Ultrasonication technology has been reported to be effective in reducing a variety of organic and inorganic contaminants in water (Hoffmann *et al*., 1996; Weavers *et al*., 1998). Ozonation was a promising method for the reduction of sulfite residues in the pericarp and aril of fresh longan (Whangchai *et al*., 2004a). Furthermore, the effect on aflatoxin reduction was reported by Inan *et al*. (2007) who found that after 60 min of ozone exposure, the aflatoxin B_1 content was reduced in flaked and chopped red peppers (*Capsicum annuum*) by 80 and 93 % at the rate of 33 and 66 mg/l, respectively.

Thus, this study was conducted to determine the capability of ultrasonication and ozonation as alternative methods for washing to reduce if not destroy the chemical structure of chlorpyrifos in bird chilli. Also changes in the quality of treated chilli fruits were investigated.

4.2 Materials and Methods

Ultrasonication

Ultrasonic devices with input power of 3 W at varying frequencies of 108, 400, 700 kHz and 1 MHz (as Chapter 3). An ultrasonic device size 44.5 × 51.5 × 35 cm equipped with eight transducers and ultrasonic frequency at 1 MHz 24 W was used in large-scale application (Figure 4.1). The ultrasonic device also made by Honda Electronics Company (Toyohashi, Aichi, Japan).

Chilli sample

Bird chilli was grown in an orchard in Chiang Mai, Thailand. Chlorpyrifos was applied throughout the growing season (2 ml/l every 14 days was followed the Department of Agriculture) and the chilli was harvested 1 day after the final application to ensure the maximum amount of chlorpyrifos residue on the fruits. Green chilli fruits were harvested to study in this experiment about 60 - 90 days after cultivation.

Figure 4.1 Ultrasonic device at 1 MHz for chilli washing.

4.2.1 Chlorpyrifos degradation in fresh bird chilli after washing using ultrasonication and ozonation

Chilli fruit 10 g were immersed in 50 ml distilled water and subjected to washing using ultrasonic reactor with different frequencies (108, 400, 700 kHz and 1 MHz), ozone and their combination as described previously in chapter 3, while washing using only distilled water was employed as the control. For large-scale ultrasonic reactor with 1 MHz frequency, about 10 kg of chilli samples and 50 l distilled water for washing were utilized. Chilli samples (5 g for small ultrasonic reactor and 25 g for the large-scale ultrasonic device) were extracted after washing every 10 min. All treatments were conducted in three replicates.

Residues of chlorpyrifos in bird chilli fruits were extracted following the method described by Steinwandter (1985). Chilli fruit samples (25 g) of were extracted with 50 ml of acetone and 40 ml of methylene chloride and homogenized at 13,000 rpm for 1 min, and then dried using rotary vacuum evaporator at 340 mbar. The samples were analyzed using GC-FPD with acetone (HPLC grade) as the final solvent. Degradation percentage of chlorpyrifos residue in stored chilli was monitored every week for 4 weeks after storage at 13 °C and 95 % relative humidity (RH).

4.2.2 Effects of ultrasonication and ozonation on postharvest qualities of bird chilli

About 10 g of washed chilli (distilled water, ultrasonication, ozonation and the combination) were put on the foam tray, polyethylene plastic wrap on top and stored in refrigerator at 13 °C and 95 % RH for 4 weeks (Figure 4.2).

Quality of chilli; weight loss, disease incidence percentage, color of peel, total sensory quality evaluation and chlorpyrifos residue on chilli were monitored every week for 4 weeks during storage period. All treatments were conducted in three replicates.

1) Weight loss (%)

Weight loss percentage was determined by weighing the whole fruits packed in foam tray before and during storage every week.

$$\text{Weight loss (\%)} = \frac{\text{Fruit weight before storage - Fruit weight after storage}}{\text{Fruit weight before storage}} \times 100$$

2) Disease incidence percentage

Anthracnose disease incidence was evaluated (5 scientists) by diseased expressing it as a percentage of the total fruit package in 3 trays. The fruits were scored on a 1 - 5 scale severity for disease incidence as follows;

1 = no symptom

2 = 1 - 25 % symptom area

3 = 26 - 50 % symptom area

4 = 51 - 75 % symptom area

5 = more than 75 % symptom area

3) Color changes of peel

The exocarp colors of chilli fruits in all treatments were measured using colorimeter (MINISCAN XE PLUS, Hunter Associates Laboratory, Inc., USA). The chromaticity of each treatment was measured as L* (the lightness factor value), a* (the chromaticity coordinates; hue) and b* (the chromaticity coordinates; chroma) values. The L* value measured the darkness or brightness of exocarp color which had value 0 to 100. A low L* value corresponded to a low brightness and a higher L* value meant a brighter fruit. The a* value measured the greenness and redness on a scale of -60 to +60. A minus a* value meant a green color and a positive value of a* meant red color. The b* value measured the blueness and yellowness on a scale of -60 to +60. A minus b* value meant a blue color and a positive value of b* meant yellow color. The results were expressed as a mean value from three replications of the 6 measured samples.

4) Total sensory quality evaluation

Chilli fruits after treated using ultrasonication, ozonation and the combination were determined for total sensory quality evaluation every week, continuous monitoring for 4 weeks, during storage. All the samples were preference tested (15 replicates). The tasters expressed their preferences in appearance, color, odor, and acceptability on the samples by using Hedonic scales, where 1 = extremely dislike and 9 = extremely like, and then the sensory quality scores were calculated.

5) Chlorpyrifos residue on bird chilli during storage

Bird chilli samples washed with different treatments were stored in refrigerator at 13 °C and 95 % RH. During storage chlorpyrifos residue from chilli samples were extracted every week based on the methods mention in 4.2.1. Then, quantitative determination of chlorpyrifos was done by GC-FPD.

Statistical analysis

All experiments were evaluated with a regression procedure using the SPSS version 17, while the differences among various treatments by Duncan's New Multiple Range test. The significant difference at $p < 0.05$ was assigned by statistical method.

Figure 4.2 Packed bird chilli stored in refrigerator at 13 °C and 95 % RH.

4.3 Results and discussion

4.3.1 Chlorpyrifos degradation in fresh bird chilli after washing using ultrasonication and ozonation

Bird chilli fruits were washed in ultrasonic reactor with different frequencies, ozone and combination in order, if any, to reduce remove residual chlorpyrifos. For ultrasonication, it was found that degradation percentage of chlorpyrifos in fresh chilli fruits increased with increasing ultrasonic time of all frequencies. Ultrasonication at 1 MHz increased chlorpyrifos degradation percentage to 62.44 % for 60 min (Figure 4.3 and Appendix; Table 9). Chlorpyrifos residue on chilli after ozonation treatment was significantly increased, compared to control, which was immersed in distilled water. The percentage of chlorpyrifos degradation on chilli after ozonation for 60 min was 52.48 % (Figure 4.4 and Appendix; Table 9). The result was suggested by Wu *et al*. (2007) which indicated that ozone water treatment could effectively reduce pesticides residue (methyl-parathion, parathion, diazinon and cypermethrin) on Pak Choi (*Brassica rapa*) surface. Ong *et al*. (1996) observed that azinphos-methyl residue on apples dipped in ozonated water were reduced by about 75 %. Similarly, Wenrong and Haiyan (2002) reported that the decomposition rate of arsenazo treated by ozone or ultrasonication was more rapid than that treated by ozone alone and the structure of arsenazo was decomposed more completely. Ikeura *et al*. (2011) found that fenitrothion in strawberries and cherry tomatoes were removed when using ozone microbubbles. In addition, both ozone gas and ozonated water could reduce pesticide residue in baby corn (*Zea mays* L.). Dipping in ozonated water for 60 minutes was more effective than exposing to ozone gas with the degradation percentage of pesticide residue at 68.35 % and 31.87 %, respectively (Whangchai *et al*., 2010b). Reduction of residual chlorpyrifos increased with the combination of ultrasonication and ozonation. The results showed that the highest percentage of chlorpyrifos removal on chilli was 76.81 % when the ultrasonic frequency was 1 MHz and ozone for 60 min. In addition, the first 10 min of all treatments significantly increased chlorpyrifos degradation on chilli when compared with control (Figure 4.5 and Appendix; Table 9). Therefore, in all methods using ultrasonication of 1 MHz, ozonation and the combination, the residue chlopyrifos was reduced with increasing contact time.

Ultrasonication of 1 MHz, ozonation and the 1 MHz/O_3 combination significantly reduced residue chlorpyrifos concentration on bird chilli, compared to the control. The minimum residual chlorpyrifos concentration was 0.26 mg/l when using 1 MHz/O_3 treatment for 60 min, compared to that using only ultrasonication, ozonation and control (Figure 4.6 and Appendix; Table 10). This tendency can be explained by the reaction of ultrasonic frequencies with ozonation producing the $^{\bullet}OH$ radical, which are highly effective for decomposition of organic molecules like the chlorpyrifos residue on the chilli surface. Gong *et al*. (2011) suggested that the ozone/ultrasound process which showed higher degradation efficiency on pesticides (diphenylamine, carbendazim and chlorothalonil) than single ozone and single ultrasound treatment was a promising method for residual pesticides degradation in apple peel.

Advanced oxidation processes (AOPs) such as ultrasound and ozone, produce oxidizing species, generally hydroxyl radicals which are very powerful and unselectively oxidize organic compounds without causing secondary wastes (Behajady *et al*., 2008). The synergistic is mainly due to excess hydroxyl radicals formed upon radiolysis of ozone by ultrasonic radicals. The degassing effect of ozone due to ultrasonic radiation was insignificant in the spared system when ozone was bubbled during sonolysis (Zhang *et al*., 2007). Yue *et al*. (2008) suggested that continuous-wave ultrasound significantly enhance the production of hydroxyl radical from radiolysis of ozone. The ultrasonication combined with ozone treatment improved detoxification of the tested chlorpyrifos solutions, suggesting a synergistic effect between ultrasonic and ozone in improving the biodegradability of pesticides in wastewater (Xiong *et al*., 2011).

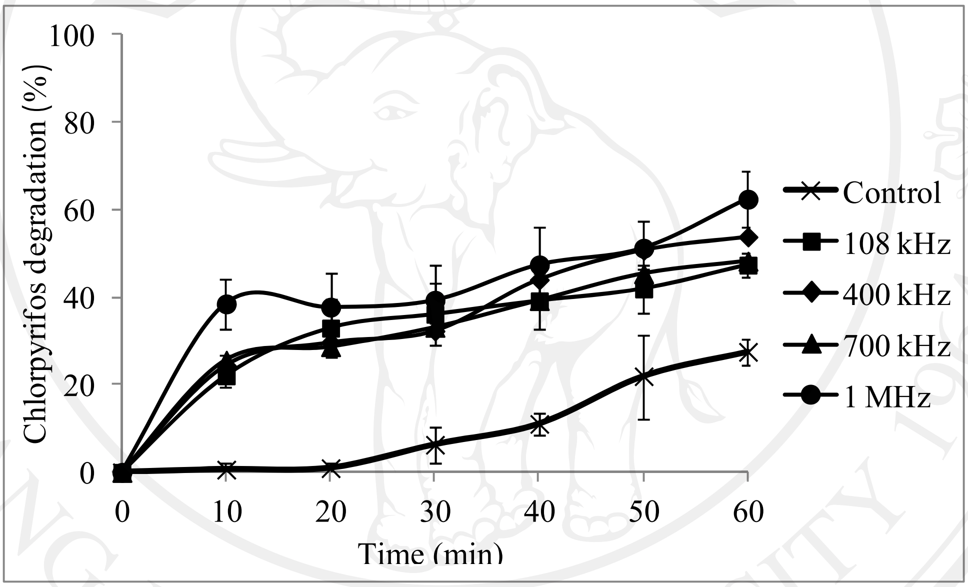

Figure 4.3 Chlorpyrifos degradation in bird chilli after ultrasonication.

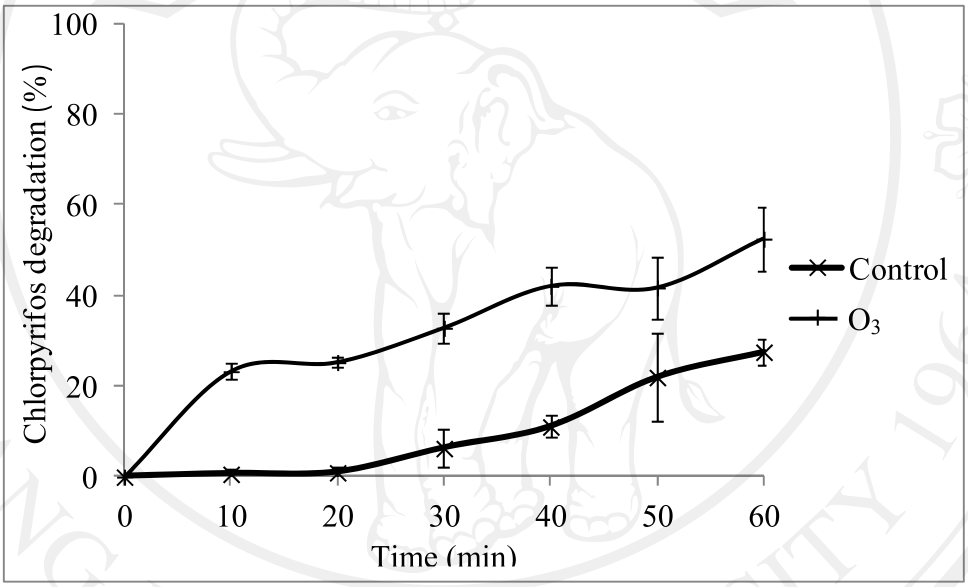

Figure 4.4 Chlorpyrifos degradation in bird chilli after ozonation.

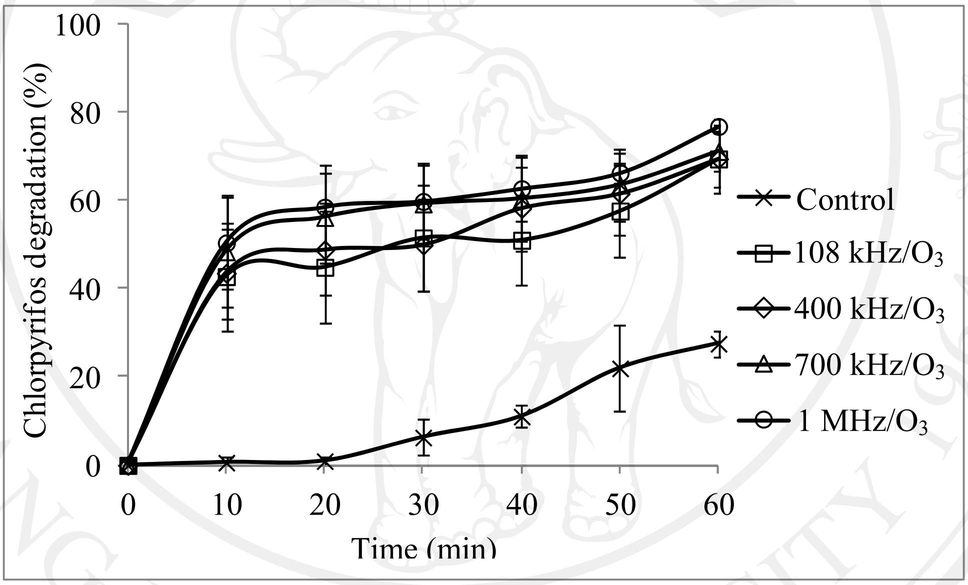

Figure 4.5 Chlorpyrifos degradation in bird chilli after ultrasonication, ozonation and the combination treatments.

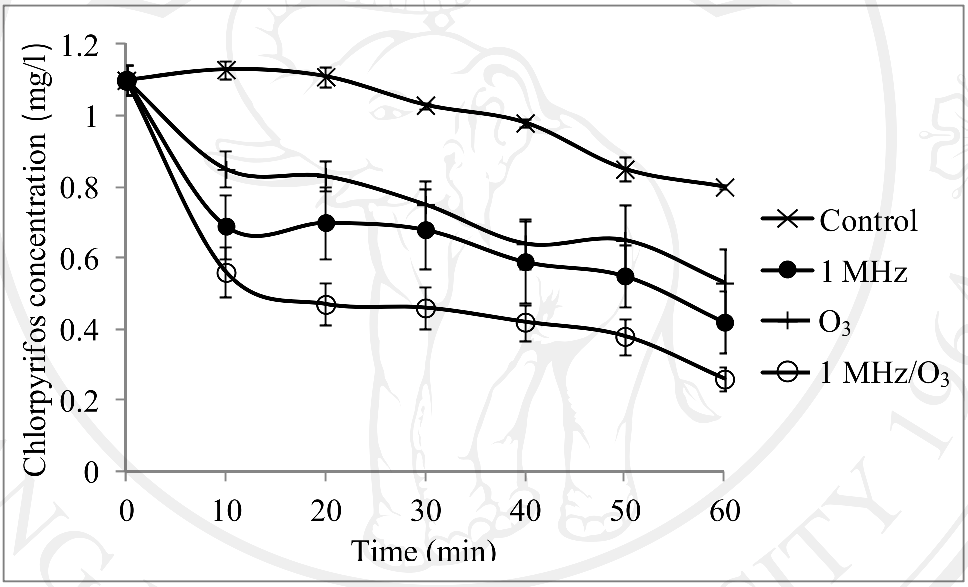

Figure 4.6 Chlorpyrifos concentration in bird chilli after ultrasonication (1 MHz) combined with ozone treatment.

4.3.2 Effects of ultrasonication and ozonation on postharvest qualities of bird chilli

1) Weight loss (%)

The weight loss of chilli after washing with ultrasonication at 1 MHz, ozonation and their combination increased with increasing contact time. The increase however in all treatments was not significantly different compared with the control (Figure 4.7). The percent weight loss after the first week of storage was in the range 3.19 - 3.43 %, and continually increased to 4 weeks of storage time (15.74 - 17.92 %) (Appendix; Table 11). The ozonation treatment had no effect to weight loss of the chilli fruits. Similarly, Whangchai *et al*. (2010a) reported that the potential of ozone had no effect on the quality changes of tangerine cv. 'Sai Nam Pung' fruits such as percent weight loss and peel color. Fruit water loss is the result of fruit respiration and diffusion through the fruit cuticle. Water loss by diffusion controlled by the water potential gradient from inside to outside the fruit and by the resistances to diffusion (Lownds *et al*., 1994). Usually, weight loss was 5 - 10 % of agricultural products, that wilt (Peleg, 1985). Chilli plugging lenticels with applied wax might be considered as a means of modifying or utilizing, tissue structure to limit water loss. The degree to which tissue can lose water prior to wilting depends on the initial level of hydration and on the elasticity of the walls of the cells comprising the tissue (Wills *et al*., 1998). Likewise, it is very likely that natural cracking of fruit peel facilitates rapid moisture loss and cause surface browning during harvest and storage. The surface cracking also impaired the physiological function of the cuticle and increased water perimeability, which may cause water soaking at the inner side of the peel (Medeira *et al*., 1999).

2) Disease incidence percentage

All treated chilli samples showed initial disease incidence after 3 weeks during storage at 13 °C and 95 % RH, including the control (Figure 4.8). However, all techniques including ultrasonication at 1 MHz, ozonation and the combined techniques significantly reduce disease incidence score in chilli, compared to the control after storage for 4 weeks (Appendix; Table 12). It is possibly because the ultrasonication and ozonation oxidized the membrane of some microorganism on surface of chilli. It could be explain that by the oxidation treatments induced some reactive oxidation species (ROS) in chilli fruits, to defense microbial more than the control treatment. According by Boonkorn *et al.* (2012), the activities of superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APX) were increased after ozone fumigation, might be mainly involved in protecting the tangerine fruit tissues from phytotoxicity caused by the oxidizing effect of ozone. In addition, the results revealed that salicylic acid (SA) combined with ultrasound treatment was more effective in inhibiting blue mold in peach fruit decay during storage than the SA treatment alone. It may be caused that the combined treatment increased activities of defense enzymes such as chitinase, β-1,3-glucanase, phenylalanine ammonia-lyase (PAL), polyphenol oxidase (PPO) and peroxidase (POD), which were associated with higher disease resistance induced by the combined treatment (Yang *et al*., 2011).

3) Color changes of peel

Results showed that using ultrasonication and ozonation did significantly change the color of chillies throughout fruit storage. The L* and a* values of all treatments tended to continually increase and there was no significant difference throughout the storage time (Figures 4.9 and 4.10). The a* values measured were minus values meaning the chilli caper green color was maintained despite the treatment and storage. On other hand, b* values tended to increase slightly until 4 weeks of storage time. The changes of peel color were observed as affected by long-term-storage durations. The chillies peel color slightly became dark when the storage duration increased. But the increase was not significantly different compared to control (Figure 4.11 and Appendix; Tables 13 - 15). Probably, all of the treatments degraded chlorpyrifos and had no effect on peel color of chilli. As Whangchai *et al.* (2011), the skin color of lychee of the ozone treatments (ozone water and gas) was not significant changed when compared with the control. Moreover, using ozone gas and ozonated water reduced chlorpyrifos in baby corn, and both treatments were not significantly different in losing weight and color change during storage at 10 °C, compared to water washing (Whangchai *et al*., 2010b).

4) Total sensory quality evaluation

Total preference scores were evaluated from appearance, color, odor and acceptability evaluation of chilli samples during storage time. The chilli samples from all treatments in the first week of storage time had a high score, and after that the chilli of the control treatment got the lowest scores in all of these properties (Appendix; Table 16). Thus, the chilli after washing with 1 MHz, ozonation and the combination had significant total sensory quality scores more than the control treatment throughout storage (Figures 4.12 and 4.13). The total sensory quality evaluation was depended on many factors, one of the factors was disease of chilli fruits. The lower total sensory quality score was correlated by the results of increasing disease incidence percentage (experiment 4.3.2(2)). Chen and Zhu (2011) demonstrated that the combined treatments of chlorine dioxide (ClO_2) and ultrasound could be a promising approach to maintain postharvest storage quality of plum fruit without significant risk to consumers, and then it could be extended fruit shelf-life than the control. In this experiment, the odor of chilli as wilt and dark color in stalk of chilli samples was found after at 2 weeks of storage time, which may cause the increase of weight loss of the chilli and disease of chilli. Moreover, the disease or odor of the controlled chilli was found at 3 weeks of the storage. Accordingly by Cao *et al*. (2010a), an ultrasonic treatment (40 kHz, power 250 W) was found to be effective in inhibiting decay incidence and preserving quality in strawberries, and it was suggested from these results that such a treatment may provide an alternative for extending shelf-life and maintaining quality of strawberry fruit. In addition Cao *et al*. (2010b) found no significant effects on fruit decay and quality deterioration of strawberry fruit when using treatments with 25 and 28 kHz ultrasound. Thus, ultrasound treatment has potential to extend shelf-life and maintain quality in strawberry fruit. Using ultrasonication or ozonation combined some acids had been reported to reduce plant disease but no effect to plant quality. Yang *et al*. (2011) suggested that the combination of ultrasound and salicylic acid may have potential for commercialization for integrated management of postharvest diseases. Ozone alone or combined with oxalic acid or citric acid could be increase the efficiency of controlling disease postharvest decay in longan (Whangchai *et al*., 2006).

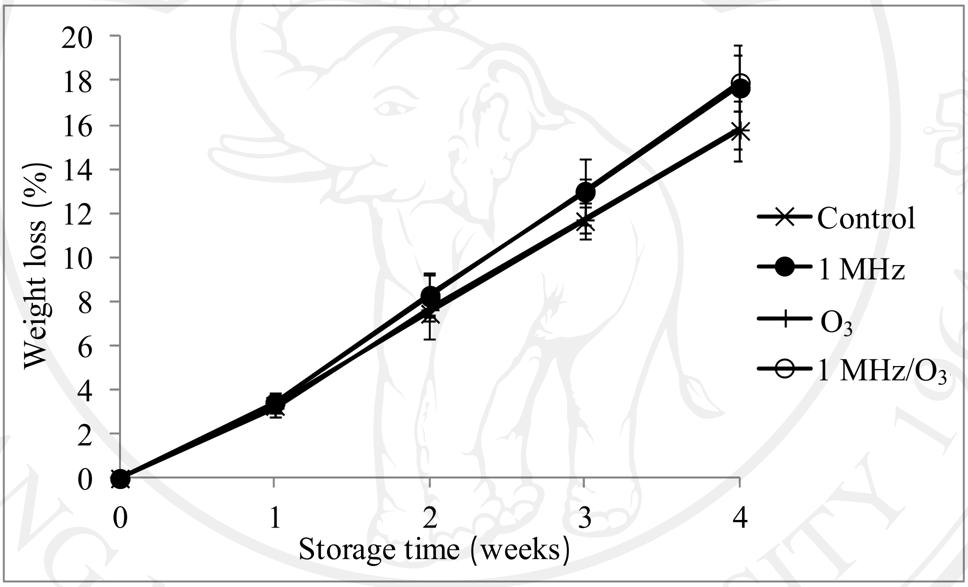

Figure 4.7 Weight loss of bird chilli fruits after washing with distilled water (Control), ultrasonication (1 MHz), ozonation (O_3) and the combination (1 MHz/O_3) for 60 min during storage at 13 ºC for 4 weeks.

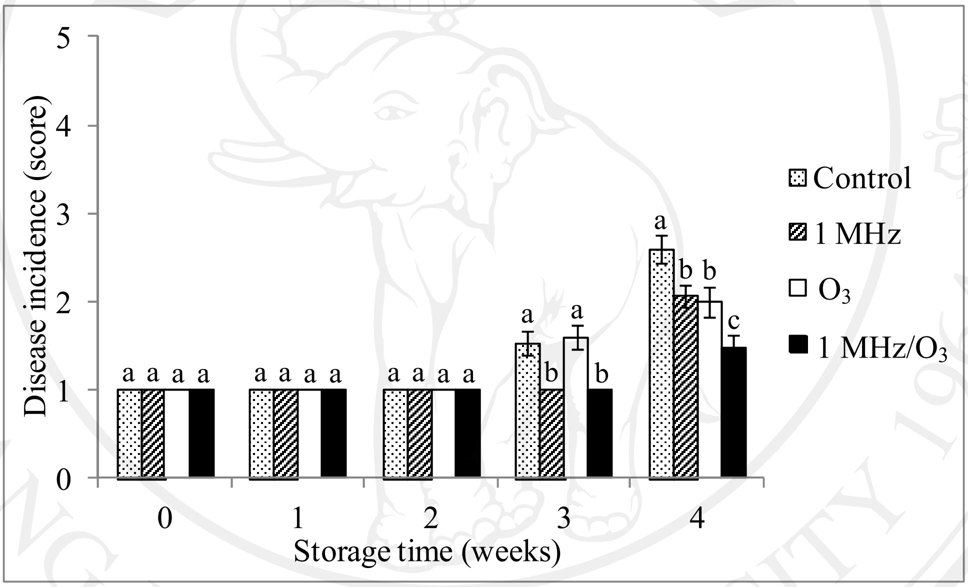

Figure 4.8 Disease incidence of bird chilli using ultrasonication, ozonation and the combination treatment, after storage at 13 °C for 4 weeks.

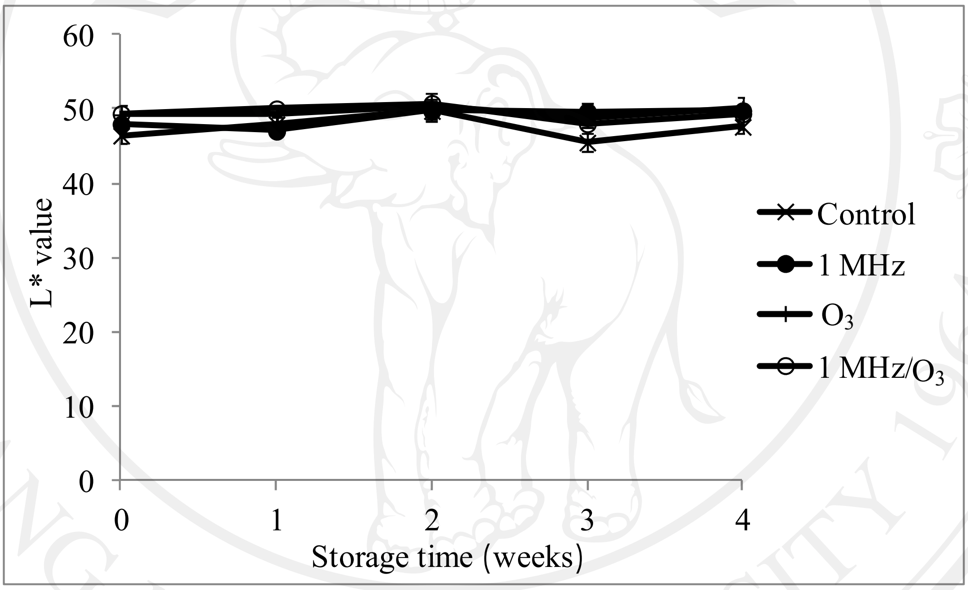

Figure 4.9 L* value of bird chilli peel color treated with ultrasonication at 1 MHz, ozonation and the combination for 60 min during storage at 13 ºC for 4 weeks.

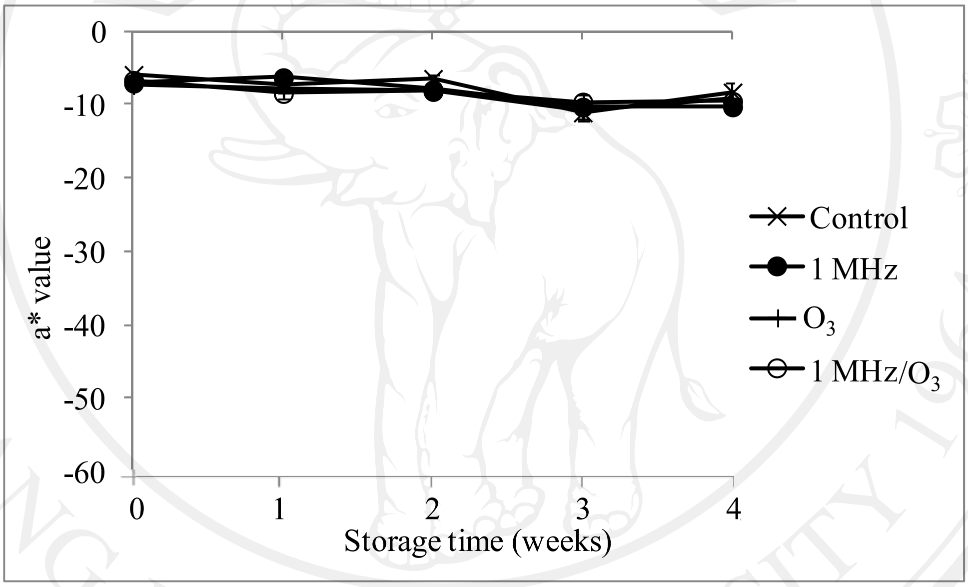

Figure 4.10 a* value of bird chilli peel color treated with ultrasonication at 1 MHz, ozonation and the combination for 60 min during storage at 13 ºC for 4 weeks.

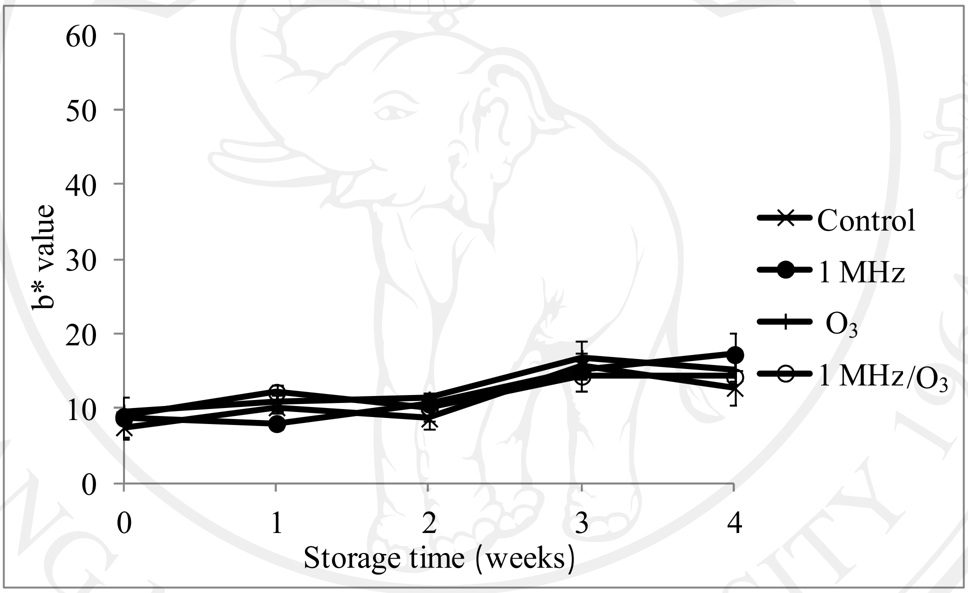

Figure 4.11 b* value of bird chilli peel color treated with ultrasonication at 1 MHz, ozonation and the combination for 60 min during storage at 13 ºC for 4 weeks.

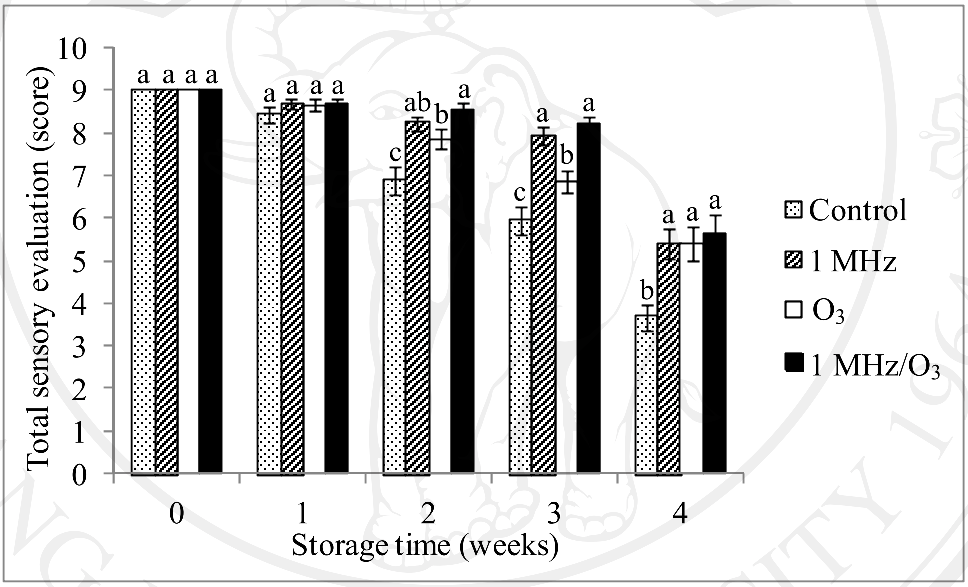

Figure 4.12 Total sensory evaluation of bird chilli treated with ultrasonication at 1 MHz, ozonation and the combination for 60 min during storage at 13 ºC for 4 weeks.

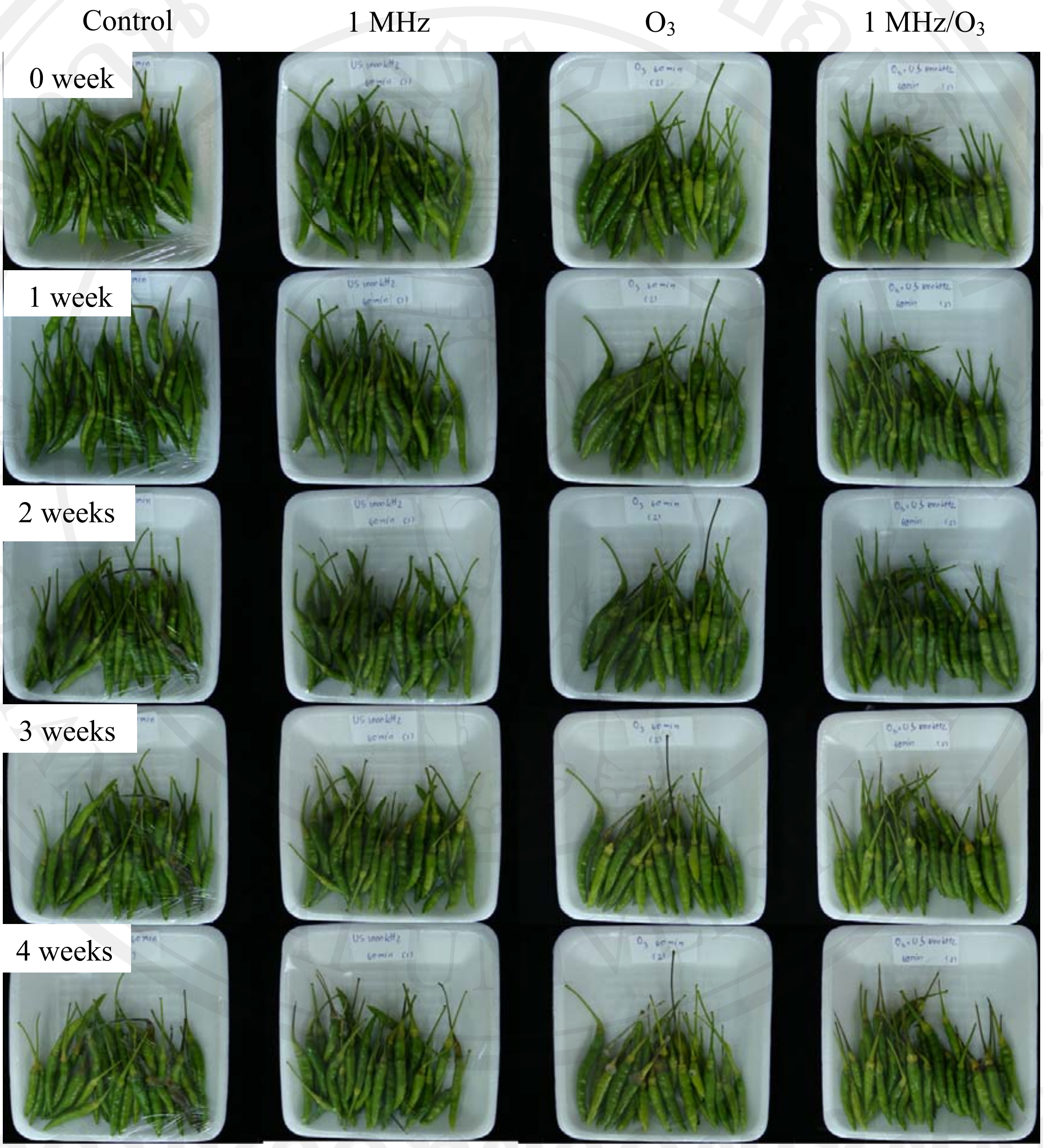

Figure 4.13 Appearance of bird chilli during storage at 13 °C for 4 weeks.

5) Chlorpyrifos residue on bird chilli during storage

Chlorpyrifos concentration residue on chilli at the initial time were positively reduced in all treatments when compared to the control (distilled water washing), especially in the combination treatment that the concentration of chlorpyrifos residue on chilli was reduced to 0.26 mg/l, which was significantly less than the control (0.80 mg/l). The application of ultrasonic combined with ozone was synergistic effect to significantly reduce residue chlorpyrifos on chilli, compared to other treatments, since initial time and during storage time. Moreover, at 4 weeks of storage period, residue chlopyrifos concentration of the combination was reduced to 0.15 mg/l, while chlorpyrifos residue of the control was 0.30 mg/l (Figure 4.14 and Appendix; Table 17). Ordinarily, chlorpyrifos residues on plant foliage dissipate with half-lives of less than 1 to 7 days. The dislodgeable residues on plant foliage dissipate even faster with half-lives of 0.1 to 3.4 days (Ware *et al*., 1993). Thus, residue chlorpyrifos concentration was reduced from initial concentration when prolong time. The synergistic effect between using advance oxidation techniques and storage long time positively reduce pesticides on plant surface. Whangchai *et al*. (2010b) observed after storage at 10 °C for 21 days that chlorpyrifos residue degradation in baby corn was increased from 12.38 % to 21.88 % and 65.81 % to 77.92 % after which exposed to ozone gas and dipping in ozonated water for 60 min, respectively.

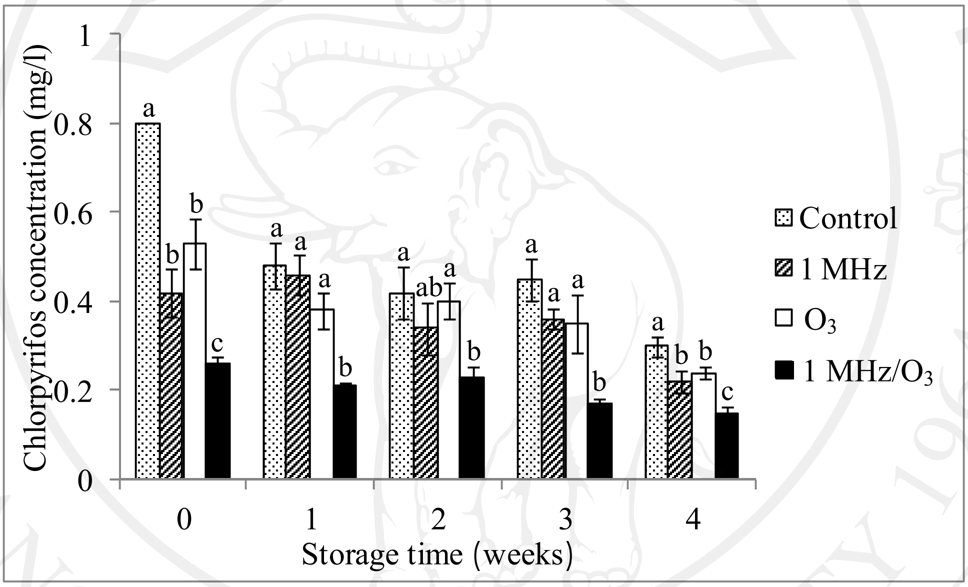

Figure 4.14 Chlorpyrifos residue in bird chilli after treated ultrasonication combination with ozone treatments for 60 min and storage at 13 ºC for 4 weeks, data are present as the mean ± S.E. (n=3).

4.4 Conclusion

Chlorpyrifos residue removal using ultrasonication and ozonation on bird chilli was positively correlated to contact time. Using 1 MHz of ultrasonication combined with ozonation for 60 min significantly reduced residual chlorpyrifos concentration on fresh bird chilli from 1 mg/l to 0.26 mg/l, compared to all treatments. Weight loss and color of chilli using ultrasonication, ozonation and the combination were not difference from washed in distilled water. Significant of percent weight loss and peel color of chilli were not observed in ozonation treatment. However, the combination of ultrasonic and ozone techniques reduced disease incidence score of chilli comparing to the control. In addition, the combination also positively increased total sensory quality evaluation of chilli after storage at 13 °C for 4 weeks. Thus, the application of ultrasonication combined with ozone had synergistic effect to significantly reduce chlorpyrifos residue on chilli, compared to other treatments interns of initial time and during storage time. Therefore, further studies to confirm toxicity of chlorpyrifos solution and wastewater of different chilli washing by ultrasonication and ozonation are also required.