CHAPTER 4

Effect of EO water and US wave on Postharvest Control and Some Biochemical Responses in Pineapple Fruit during Storage

4.1. Introduction

Thailand is a large producers and exporter of pineapples in Southeast Asia, with a yearly export value about 114 million Baht or 35.8 million US dollars (Office of Agricultural Economics, 2013). However, the pineapple trade has been limited by inconsistent quality and fungal decay, which is the main cause of pineapple postharvest loss. The high incidence of postharvest disease in pineapple is primarily due to the fungus *Fusarium* spp. The fungus penetrates through wounds, caused by de-crowning, that occur during postharvest handling. Although postharvest losses may be reduced with chemical disinfection (such as dipping in triabendazole or benomyl and methyl bromide fumigation), those treatments have toxicological risks and may cause environmental pollution. The research presented here focuses on advanced oxidation technology, EO water and US waves to control postharvest diseases of pineapple. EO water, an alternative to chlorinated water, was an idea developed in Japan (Izumi, 1999). It has been studied also for use in aquaculture (Whangchai *et al*., 2003), agricultural and food industrial processes (Kim *et al*., 2000). Several studies have demonstrated that EO water could be used as a disinfectant in food processing (Al-Haq *et al*., 2002; Venkitanarayanan *et al*., 1999; Hong *et al*., 1998). US is high frequency sound waves which humans cannot here, i.e. above 16 kHz (Jayasooriya *et al*., 2004). In recent years, US has been used for food processing in various ways (Piyasena *et al*., 2003). Yang *et al*. (2011) reported that ultrasonic waves (40 kHz, 10 min) and salicylic acid (SA) (0.05 mM) in peach fruit significantly control *Penicillium expansum* (blue mold).

Synthesis of pathogenesis-related proteins (PR) including 1,3-glucanase and chitinase, is a common defense responses in several plant species to infection (Brogue *et al*., 1991; van Loon *et al*., 2006). Plant defense-related enzymes, such as phenylalanine ammonia lyase (PAL) and peroxidase (POD) are also involved in defense against pathogenic microorganisms. PAL is a key enzyme, in the phenylpropanoid pathway that induced synthesis of various fungitoxic phytoalexins. Induction of PAL as a response to infection is associated with disease resistance (Shadle *et al*., 2003). POD is involved in cross-linking of cell wall components and the generation of antifungal metabolites (Kozlowska *et al*., 2001). POD is considered potentially important in host resistance mechanisms, as it can catalyze the last step of lignin biosynthesis (Hammerschmidt *et al*., 1982). These enzymes induce active defense mechanisms of plants to the pathogens (Sandermann, 2000). Therefore, the effects of EO water and US wave on decay control and elicitation of defense enzymes needs to be investigated. The aim of this research was therefore to evaluate the effects of EO water and US on: (1) postharvest disease in de-crowned pineapple, caused by *Fusarium* sp. and fruit quality after storage at 25 °C; and (2) the activity of key enzymes, involved in pineapple defense responses.

4.2 Materials and methods

4.2.1 Fungal

Fusarium sp. was obtained from the Department of Biology, Faculty of Sciences, Chiang Mai University, Chiang Mai, Thailand. The fungi were grown on potato dextrose agar (PDA) for 7 days at 27 ◦C. A spore suspension was prepared by flooding a 1 week old culture of *Fusarium* sp. with 10 ml sterile distilled water and transferring the spores to 100 ml of sterile distilled water in a 250 ml conical flask. The suspension was shaken for 10 min on an orbital shaker at 27 ◦C, and filtered through two layers of sterile muslin cloth. Spores (conidia) were counted with a hemacytometer and the concentration was adjusted with sterile distilled water to a final concentration of 10^5 conidia/ ml.

4.2.2 Preparation of treatments

EO water was generated by electrolysis in a cell with positively and negatively charged titanium electrodes coated with TiO_2, separated by a polypropylene membrane. The electrodes were then subjected to a direct current (DC) of 8 A and 8 V using a DC power source. A 5% NaCl solution was simultaneously introduced into the system. The pH was recorded with a pH/ion meter, and oxidation reduction potential (ORP) was measured by a pH/ORP meter. The amount of free-chlorine concentration was determined by using N, N-diethyl-P-phenylene diamine (DPD) test (Pailin, 1967). The EO water with initial concentration of 690 ppm was diluted with distilled water to concentrations of free-chlorine at 100 ppm and used for the microbiological study. Sterilized distilled water was used as a control for this experiment. An ultrasonic device with an input power of 3W and a frequency of 1 MHz was made by Honda Electronics Company (Toyohashi, Aichi, Japan). Polyethylene cylinder reactors, 10 cm in diameter, equipped with a transducer at the lower part were used.

4.2.3 Plant materials

Pineapple fruits (*Ananas comosus* cv. Phu Lae) at mature green stage without physical injuries or infections were harvested at 120 days after commercial material full bloom from a commercial orchard in Chiang Rai Province, Thailand and then transported immediately to the Postharvest Biology and Technology laboratory, Chiang Mai University within 3 h. The peduncle was cut with a knife to leave 2 cm peduncle on the fruit. The crown was trimmed to a length of 3–4 cm. Before inoculation, the de-crowned pineapple fruits were surface-sterilized by wiping with a 70% ethanol solution. Samples of 12 fruits were used for each replicate and the treatments were done by 3 replicate.

4.2.5 Effect of EO water and US wave on the control of *Fusarium* decay in inoculated pineapple during storage

The prepared spore suspension as described above. The suspension was artificially inoculated for about 0.1 ml on the center of de-crowned pineapple fruit. All fruits were incubated at room temperature for 3 h, before application of respective washing treatments. All inoculated fruits were treated with the following treatments. For US wave treatment, inoculated samples (36 fruits) were subjected to ultrasonic waves of 3 watts and at a constant frequency of 1 MHz. The experiments were carried out in an ultrasonic water bath (Honda Electronics Company, Toyohashi, Aichi, Japan) (dimensions: 44.5 × 51.5 × 35 cm). The capacity of the device was 50 liters which was able to wash 10 Kg of pineapple fruits. For the EO water treatment, inoculated samples were immersed in 50 liters with a concentration of free-chlorine at 100 ppm. In combined treatment, pineapple fruits were immersed into the ultrasonic chamber containing EO water with a free-chlorine concentration of 100 ppm and subjected to simultaneous continuous US at 1 MHz. Pineapple fruits treated with tap water were used as controls. All treatments were run at ambient temperature (28 °C) for 10 min. The treated fruits were arranged in a plastic basket and air dry. Fruits were covered with a plastic bag and separated into 2 groups: fruits were maintained at 75–80% RH and 27 ◦C for seven days and storage at 13 °C for 20 days. For room temperature storage, fruit samples were inspected daily for disease incidence through inoculated sites. For low temperature storage, samples were taking initially and 5 day intervals during storage. De-crowned pineapple fruits showing with visible mold growth were considered to be infected fruit. The severity of fruit decay was expressed as a percentage of the fruit showing fungal symptoms. Fruit disease incidence was expressed as percentage of infected fruits. The experiment was replicated three times, and each treatment in each replication was represented by 36 fruits. De-crowned pineapple fruit was removed at 0, 24, 48 or 72 h after treatments, to provide a source of infected tissue with varying stages of fungal penetration. The samples were preserved in formaldehyde-acetic acid- alcohol (FAA) solution and were examined under a scanning electron microscope (SEM) (KEYENCE VE-9800, Japan).

4.2.6 Effects of EO water and US wave on enzyme activities

4.2.6.1 Pathogenesis–related protein (PR protein)

De-crowned pineapple fruit were inoculated with *Fusarium* sp., treated with the treatments, and kept as described above. As each time of sampling during storage, healthy non-infected areas of each de-crowned pineapple fruit, 3 replicates of 3 fruits per treatment, were separated from the whole crowned fruit, cut into small pieces, frozen in liquid nitrogen, freeze-dried and stored at -21 °C for enzyme assays. Freeze-dried de-crowned samples (0.3 g) were homogenized in 10 ml of 0.2 M sodium acetate buffer, pH 5.0 and 2% polyvinylpyrrolidone at 4°C. The homogenate was centrifuged at 13,000 rpm at 4°C for 15 min and then supernatant was collected to assay as described by Romero *et al*. (2006).

Chitinase activity was assayed by incubating a standard reaction mixture containing 400 µl of diluted crude enzyme solution with 200 µL of aqueous CM-Chitin-RBV substrate and 200 µl of 0.2 M sodium acetate buffer (pH 5.0). After incubation at 40°C for 15 min, the reaction was stopped by adding 200 µl of chilled 2N HCl and cooling on ice for 10 min. The reaction mixtures were centrifuged at 7,500 rpm for 20 min to precipitate the non-degraded substrate and the optical absorption was measured at 550 nm using a visible spectrophotometer. The enzyme activity was expressed as ΔA550 min^{-1}.mg $protein^{-1}$ (Saborowski *et al*., 1993).

β-1,3-glucanase activity was determined by a colorimetric method (Silveira *et al*., 2006). The amount of reducing sugar released from glucan was measured. The standard assay contained 300 µl of the crude enzyme solution and 100 µl of 0.1% β-glucan. After incubation at 40°C for 30 min, the reaction was stopped by adding 600 µl of 1% dinitrosalicylic acid (DNS) boiling for 5 min then placed in an ice bath. The optical absorption was measured at 550 nm. The amount of reducing sugar produced was determined using a curve constructed with glucose as standard. One unit of enzyme was defined as the amount of protein necessary to produce one µmol of reducing sugar h^{-1}. β-1, 3-glucanase activity was expressed in units mg $protein^{-1}$. The protein content was

determined according to Lowry *et al.* (1951) using bovine serum albumin as the standard.

4.2.6.2 Plant defense-related enzyme

For PAL assay, freeze-dried samples of de-crowned pineapple fruit (1.0g) were homogenized with 10 ml of 150 mM Tris buffer (pH 8.5). The homogenate was centrifuged at 12,000 xg for 30 min at 4°C. The supernatant was analyzed for enzyme activity. The PAL assay system routinely consisted of 1 ml of the supernatant, 1 ml of 50 mM L-phenylalanine, and 1 ml of buffer. Controls contained buffer in place of phenylalanine. The change in absorbance at 290 nm was monitored after 1 h of the reaction at 40°C. Under these conditions, a change in absorbance of 0.01 was found to be equivalent to the production of 3.09 nmoles of cinnamic acid (Saunders and McClure, 1974). One unit of enzyme activity was defined as the production of nmol cinnamic acid per h.

For PPO and POD assay, freeze-dried samples of de-crowned pineapple fruit (0.5 g) were homogenized with 20 ml of 0.10 M sodium phosphate buffer (pH 7.0) containing 3 mM ethylenediaminetetra acetic acid (EDTA) and 0.1 g of polyvinyl pyrrolidone (PVP). The homogenate was centrifuged at 12,000 ×g for 20 min at 4 °C. The supernatant were analyzed for enzyme activities. For PPO activity assay, the reaction mixture containing 2.45 ml of substrate buffer (containing 0.2 M cathecol in 0.10 M sodium phosphate buffer) and 0.05 ml of enzyme extract. Changes in the absorbance at 420 nm were measured after 5 min at 25°C. One unit of enzyme activity was defined as the amount causing a change of 0.01 in absorbance capacity per minute (Flurkey and Jen, 1978). POD activity was determined spectrophotometrically at 470 nm according to a little modified method of Yahia *et al.* (2007). The reaction medium contained 2.855 ml sodium phosphate buffer (0.10 M, pH 7.0), 45 µL guaiacol (1%), 40 µL H_2O_2 (0.3%), and 60 µL enzyme extract. The absorbance was recorded at 470 nm and one unit of enzyme activity was defined as the amount causing a change of 0.01 in absorbance capacity per min.

The specific activity of the enzymes was expressed as units mg protein^{-1}. Soluble protein content was determined according to the method of Lowry *et al*. (1951) using bovine serum albumin (BSA) as standard.

4.3 Statistical analysis

The Statistical Package for the Social Science (SPSS version 17) software for Windows was used for the Analysis of Variance (ANOVA) and least-significant difference (LSD) at the 95 % confidence level of each variable value under completely randomized design (CRD). Each experiment had tree replicates and all experiments were run two times with similar results.

4.4 Results and discussion

4.4.1 Effect of EO water and US wave on the control of *Fusarium* decay in inoculated pineapple during storage

The observations from *in vivo* fruit trials showed differences ($P< 0.05$) in the disease inhibition between control and treated fruits (Figure 4.1, 4.2). Combined treatment of EO water with US wave showed higher percentage of disease inhibition ($P < 0.05$) than other treatments of fruits subjected to artificial inoculation with *Fusarium* sp. After treated with tap water, US wave or EO water then storage at 25 °C for 3 days, fruits had disease incidence of 81.25, 76.25 and 12.50% respectively, while the combined treatment was not found any sign of disease. Disease inhibition was 6.15% of the US wave and 84.62% by EO water and 100% for combined treatment. In addition, at 10 days after storage at 13°C, the combined treatment was able to reduce disease incidence for 45.45%, while US wave and EO water treated alone were able to reduce disease incidence for 36.41 and 27.35 % , respectively (Figure 4.2). EO water treatment induced resistance against postharvest disease cause by *Fusarium* sp. in de-crowened pineapple fruits. Similar to the previous study, we found that EO water treatment was effective in inhibiting decay incidence of pineapple fruit while the application of US wave treatment alone could not inhibit fungal decay development in pineapple (Khayankarn *et al*., 2013). However, the results of our present experiment showed that the application of

EO water combined with US wave could result in a significant enhanced control of fungal disease in de-crowned pineapple fruit. US wave destroyed microorganism by the physical forces of cavitation which is the mechanical effect responsible for the destruction of fungal cells (Piyasena *et al*., 2003). Hypochlorous acid damages the microbial cell by oxidizing nucleic acids and proteins, causing lethal damage (Acher *et al*., 1997). This implied that US wave support the sterilizing effect of the EO water. Our results agree with those McClements (1995) who suggested that inactivation of microbes using US wave is effective when used in combination with other decontamination techniques. Hung *et al*. (2010) reported that a combination of EO water and US wave resulted in a greater reduction of the bacterial contamination of broccoli. Scouten and Beuchat (2002) reported that combined treatments of chemical, heat, and ultrasound were effective at killing *Salmonella* and *E. coli* O157:H7 on alfalfa seeds. The compression pressure, generated during the use of ultrasound, may contribute to the penetration of the chemical oxidants through the cellular membrane and the cavitation process may assist in the disaggregation of the microorganisms, which culminates in an increased efficiency of the sanitization treatment (Gogate and Kabadi, 2009).

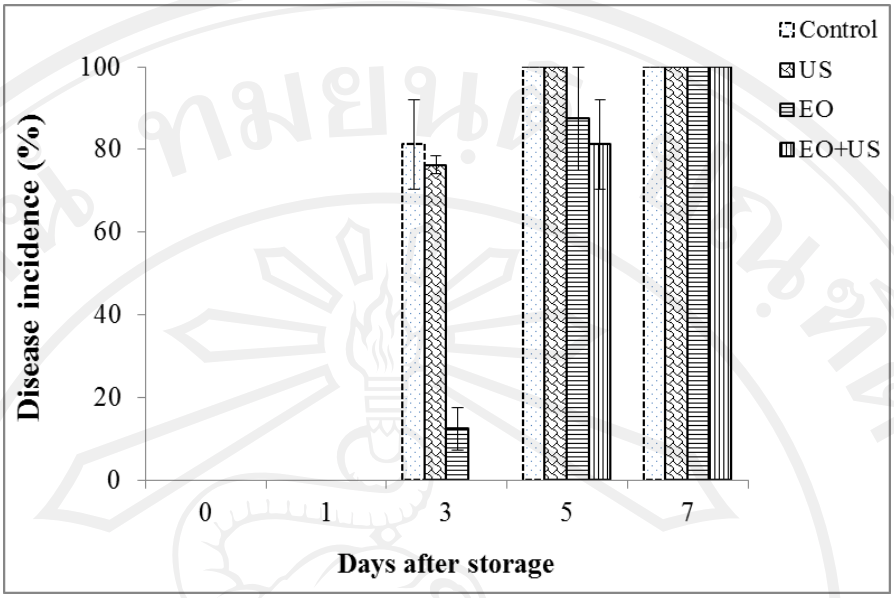

Figure 4.1 Effect of EO water, US wave and EO water combination with US wave on disease incidence (% of infected fruit) of de-crowned inoculated pineapple fruit during storage at room temperature for 7 days.

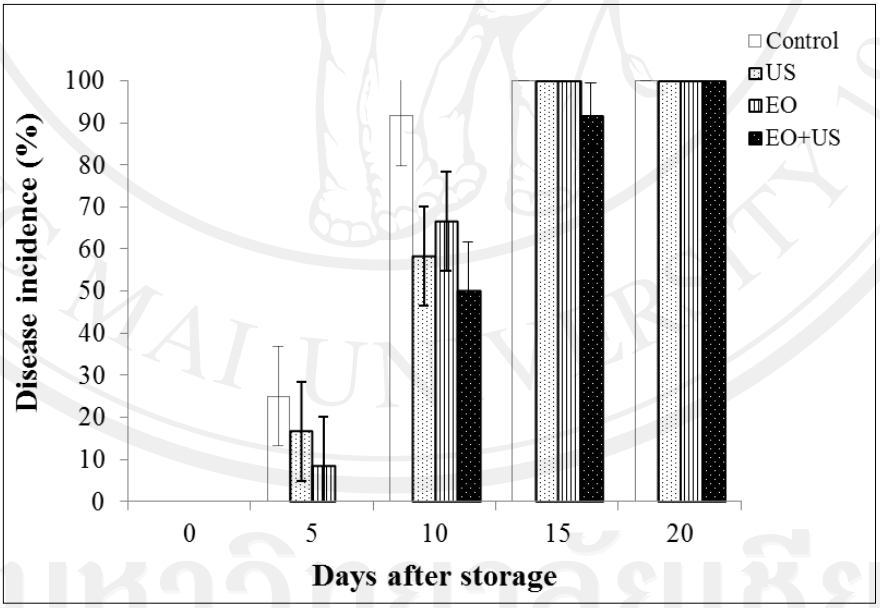

Figure 4.2 Effect of EO water, US wave and EO water combination with US wave on disease incidence (% of infected fruit) of de-crowned inoculated pineapple fruit during storage at 13 °C for 20 days.

Figure 4.3 The disease development on the 5 days of de-crowned inoculated pineapple fruits with *Fusarium* sp. those treated with EO water and US wave and then the fruit were kept at 25 ◦C.

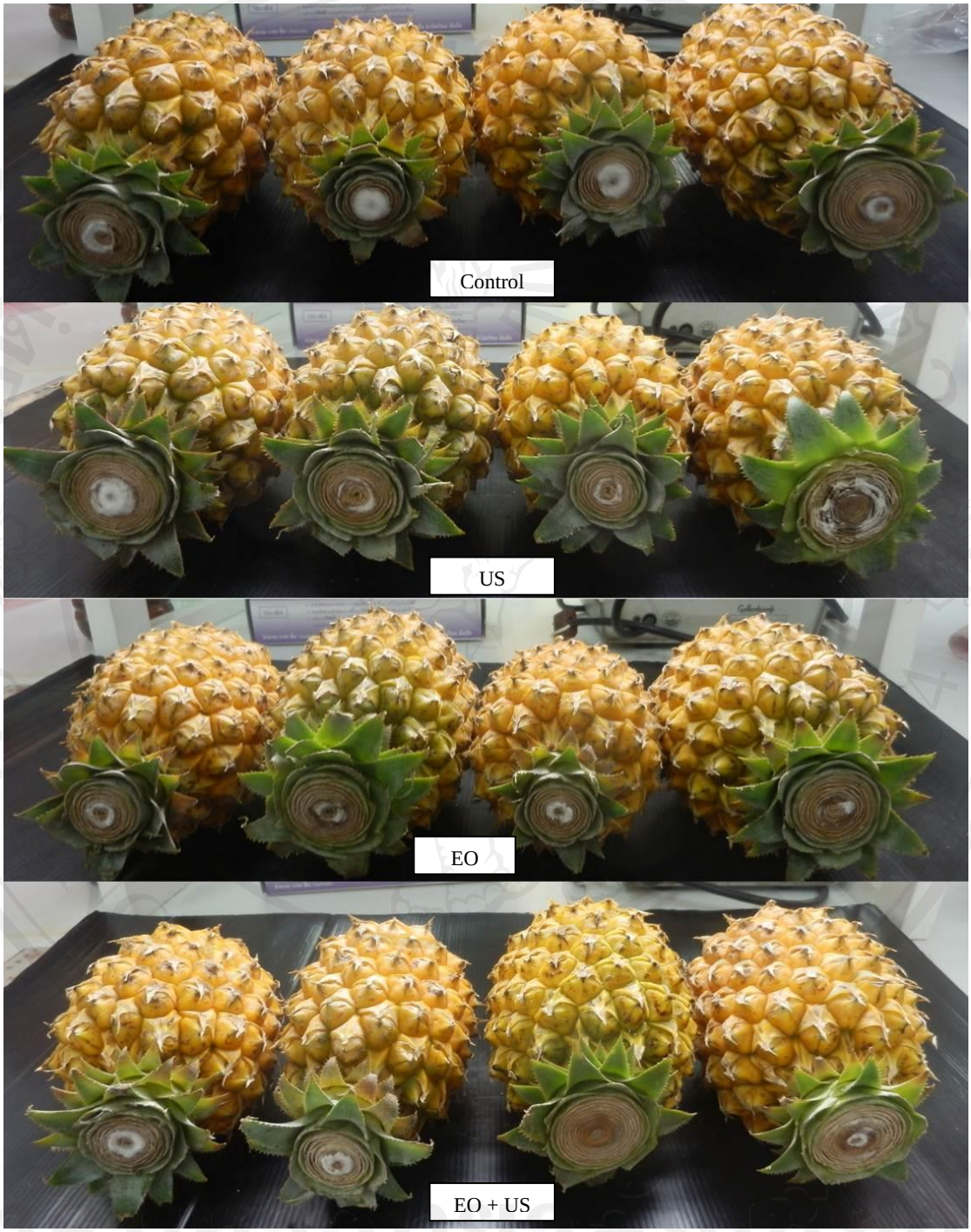

Figure 4.4 The disease development on the 10 days of de-crowned inoculated pineapple fruits with *Fusarium* sp. those treated with EO water and US wave and then the fruit were kept at 13 ◦C.

4.4.2 Effect of EO water and US on ultrastructural change of *Fusarium* sp. on de-crowned inoculated pineapple

Spore germination and mycelium growth on de-crowned pineapple fruit were investigated under SEM and shown in figure 3. These micrographs indicated the germination of *Fusarium* sp. spore on the de-crowned pineapple sections after 0, 24, 48, and 72 h incubation. After inoculation, fungal spores was attachment on de-crown pineapple (Figure 3A). Germination of fungal spores was first observed in the control group after incubation for 24 h (Figure 3B). At 48 h after incubation, the mycelium was first observed in the US wave treatment (Figure 3C). In addition, the fungal mycelium was first observed in the EO water treated group after incubated for 72 h (Figure 3D). While the combined treatment (Figure 3E) did not show any sign of disease incidence until 72 h of incubation. Combined treatment could inhibit the germination of fungal spore on the de-crowned pineapple more than individual treated of either EO water or US wave. This is due to the mechanism of US wave not only disrupt microbial cells but also facilitate the EO water decontamination process by dislodging those microorganisms, forcing them to be exposed directly to EO water disinfectants. These results agree with Brilhante São José and Dantas Vanetti (2012) who reported that the use of US wave combination with peracetic acid results in a greater reduction in the number of cells on the surface of cherry tomatoes, including *Salmonella*. Recently, Sagong *et al*., (2013) also reported that the combination of US wave and tween 20 was the most effective treatment for reducing levels of *Bacillus cereus* spore on lettuce and carrot. Therefore, a possible explanation of these combination treatments could be produced greater additional reduction because US wave provides the physical effect for detaching spores from fresh product surfaces and the accretion of the sanitizer enhances this mechanism.

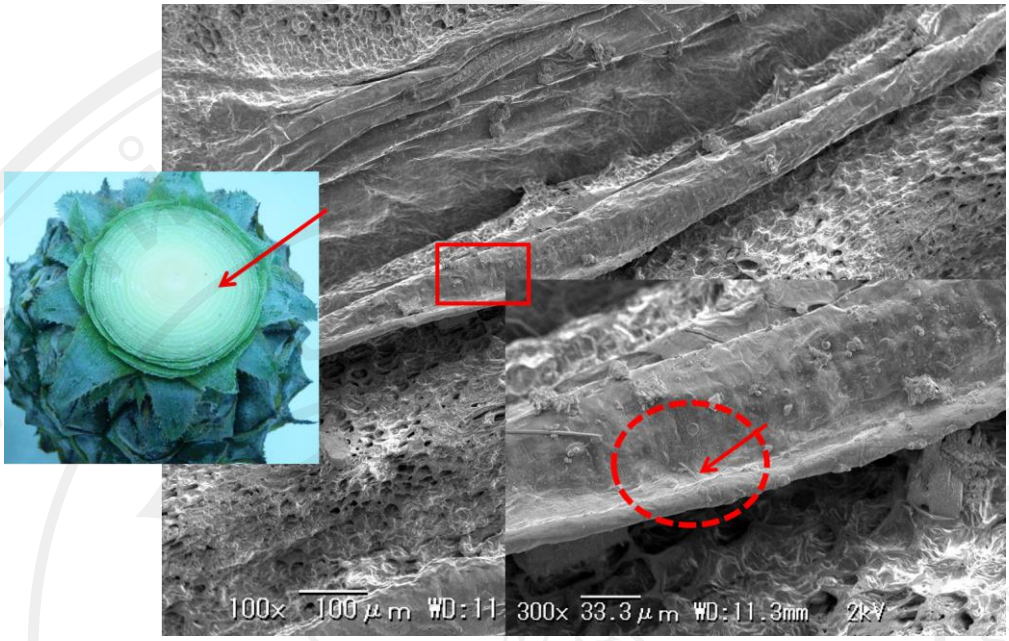

Figure 4.5 Scanning electron microscopic photographs of *Fusarium* sp. spores on de-crowned pineapple fruit peel after artificially inoculation and left at 25°C for 3 hr before treatments (arrows indicated the fungal spores).

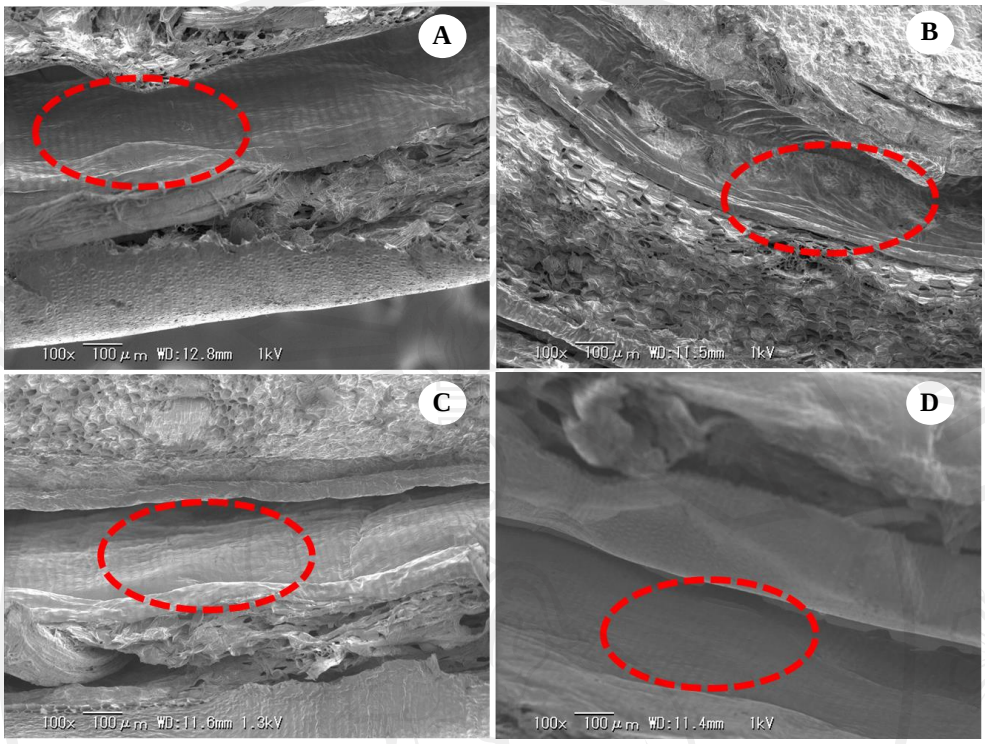

Figure 4.6 Scanning electron microscopic photographs of *Fusarium* sp. on de-crowned pineapple fruit, 12 hr after treated with distilled water (A), 1 MHz of US wave (B), 100 ppm of EO water (C) and EO water combination with US wave(D)

Note: The crown were cut, inoculated with $1x10^5$ spores/ml of *Fusarium* sp. before treatments and kept at 25°C.

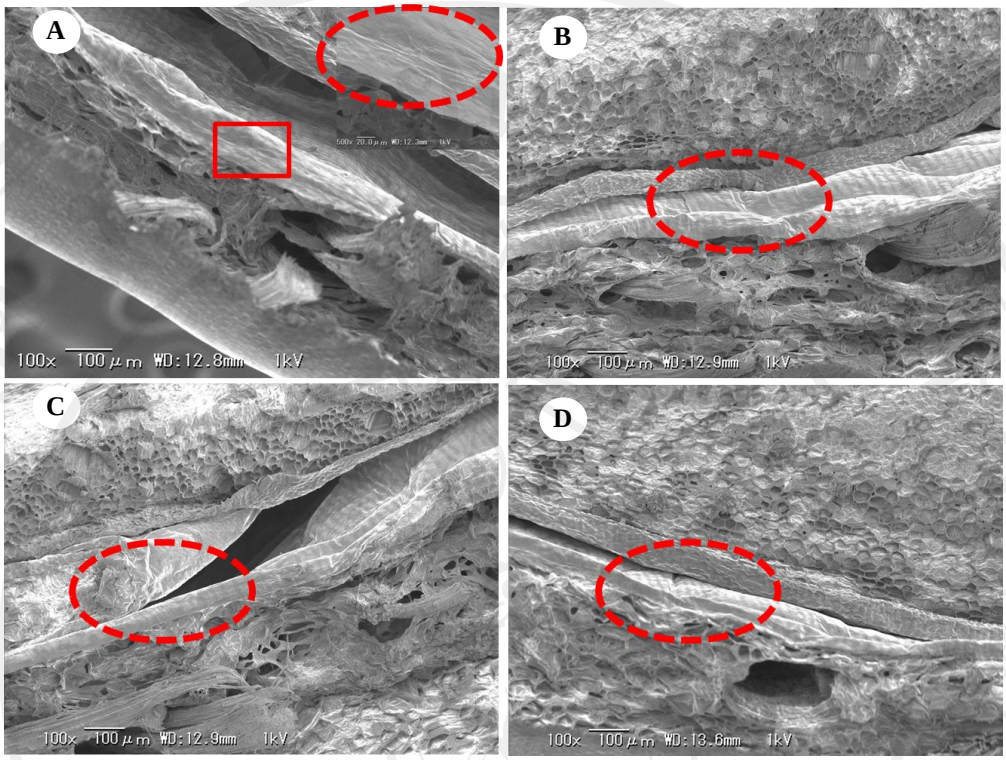

Figure 4.7 Scanning electron microscopic photographs of *Fusarium* sp. on de-crowned pineapple fruit, 24 hr after treated with distilled water (A), 1 MHz of US wave (B), 100 ppm of EO water (C) and EO water combination with US wave (D)

Note: The crown were cut, inoculated with $1x10^5$ spores/ml of *Fusarium* sp. before treatments and kept at 25°C.

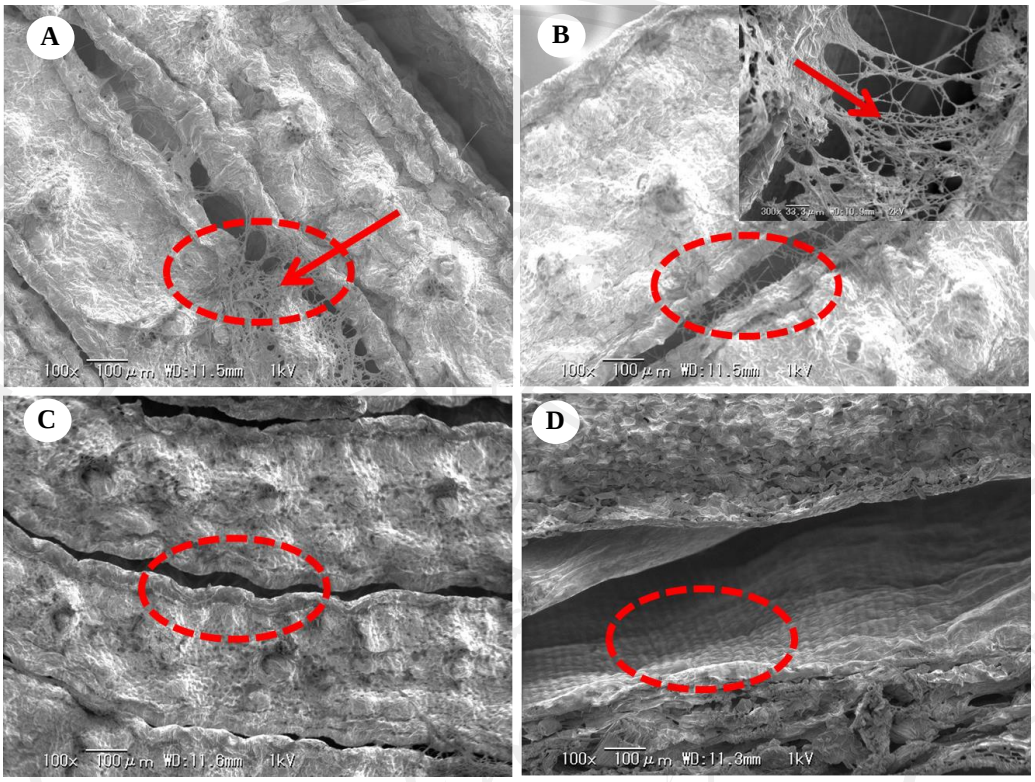

Figure 4.8 Scanning electron microscopic photographs of *Fusarium* sp. on de-crowned pineapple fruit, 48 hr after treated with distilled water (A), 1 MHz of US wave (B), 100 ppm of EO water (C) and EO water combination with US wave (D)

Note: The crown were cut, inoculated with $1x10^5$ spores/ml of *Fusarium* sp. before treatments and kept at 25°C. Arrow indicated that the fungal mycelium.

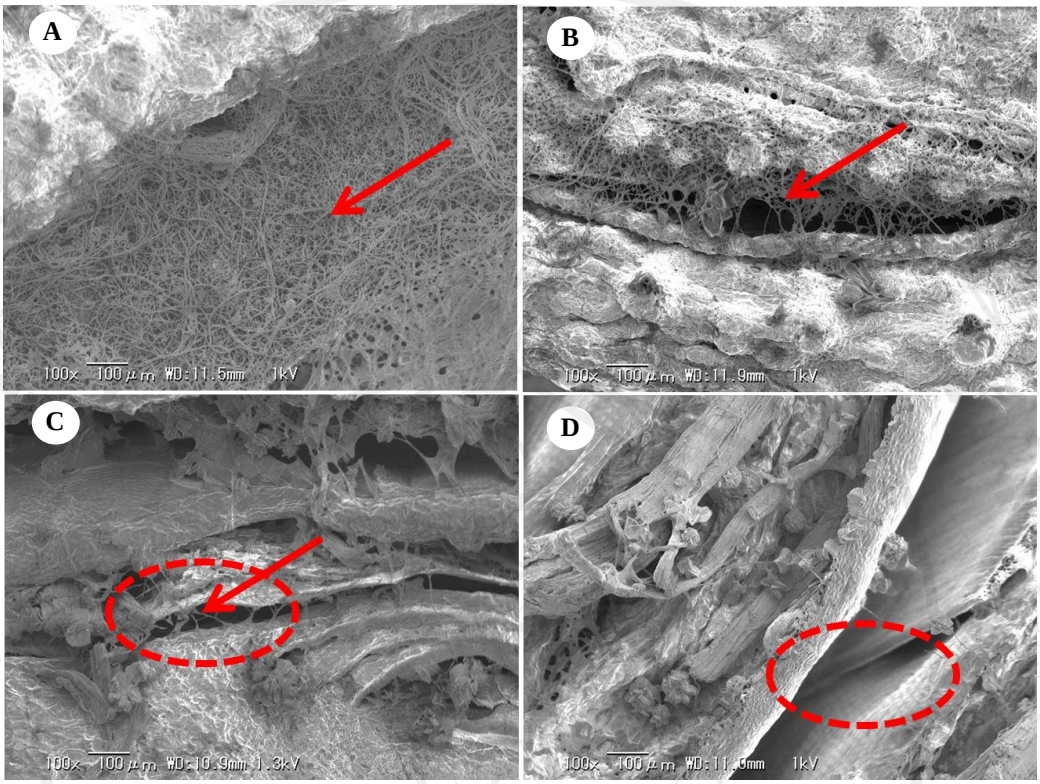

Figure 4.9 Scanning electron microscopic photographs of *Fusarium* sp. on de-crowned pineapple fruit, 72 hr after treated with distilled water (A), 1 MHz of US wave (B), 100 ppm of EO water (C) and EO water combination with US wave (D)

Note: The crown were cut, inoculated with $1x10^5$ spores/ml of *Fusarium* sp. before treatments and kept at 25°C. Arrow indicated that the fungal mycelium.

4.4.3 Effect of EO water and US wave on pathogenesis–related protein (PR protein)

Combined treatments induced the activities of chitinase, β -1,3- glucanase in de-crowned pineapple fruit stored at 25 and 13 °C (Figure 4.10, 4.11). Chitinase activity in both temperatures showed exactly the same trend, the activity increased gradually after 3 days of storage at 25°C and 10 days at 13°C, and decreased afterwards. The level of enzyme activity assayed at 2 and 5 days intervals after time 0 was significantly higher than those in US wave treated and control fruit at the same time. The levels of β-1,3-glucanase were about 1.27, 1.41 and 1.26-fold higher than that in the control, US wave and EO water treatment at the 1 day of 25°C storage time points, respectively (Figure 4.11A). The β - 1, 3-glucanase activity in de-crowned pineapple fruit storage at 13 °C maintained a relatively low level during 5 days of storage (Figure 4.11B). The activity increased afterwards and peaked at 10 days in all treated fruit, combined treatments shown highest level than other treatments. The level were approximately 1.58, 1.57 and 1.25-fold higher than that in control, US wave and EO water treated fruit at the same time.

Chitinase and β-1,3-glucanase have been intensively studied and identified as the PR protein that functions in the plant defense response (Van Loon *et al*., 1998). PR proteins are induced not only resistant, but also in susceptible plant-pathogen interactions, as well as in plants, subjected to abiotic stress factors (Van Loon, 1985). The result of our experiments shown that EO water combined with US wave treated had higher activities of β-1, 3-glucanase and chitianase in de-crowned pineapple fruit. A possible explanation for this might be that EO water and US not only destroyed microbial cell but also can be caused stress to the plant likes other stress factors such as exposure to UV radiation (Nawrath *et al*., 2002), ozone (Rao and Davis 1999), salt and osmotic (Molina *et al*., 2002). In a recent study, Tomas-Callejas *et al*., 2011 reported that EO water was able to induce and increase in catalase activity throughout the shelf life of fresh-cut mizuna baby leaves. Martinez-Hernandez *et al*., 2013 demonstrated that the use of neutral electrolyzed water combined with ultraviolet light C and super atmospheric O_2 packaging could increase ascorbate peroxidase and guaiacol peroxidase activities in

fresh-cut broccoli after storage. Furthermore, Yang *et al*., 2011 also reported that salicylic acid combined with US wave treatment increased the activities of pathogenesis related protein such as chitinase, β-1, 3-glucanase, PAL, PPO, and POD on peach fruit. Therefore, the resistance of de-crowned pineapple fruit to development of fungal decay is mediated by the activation of PR protein in response to wide array of stresses including wounding and inoculating with *Fusarium* sp. and treatment with EO water and US wave, the combination of these stresses pronounce the enzyme activities.

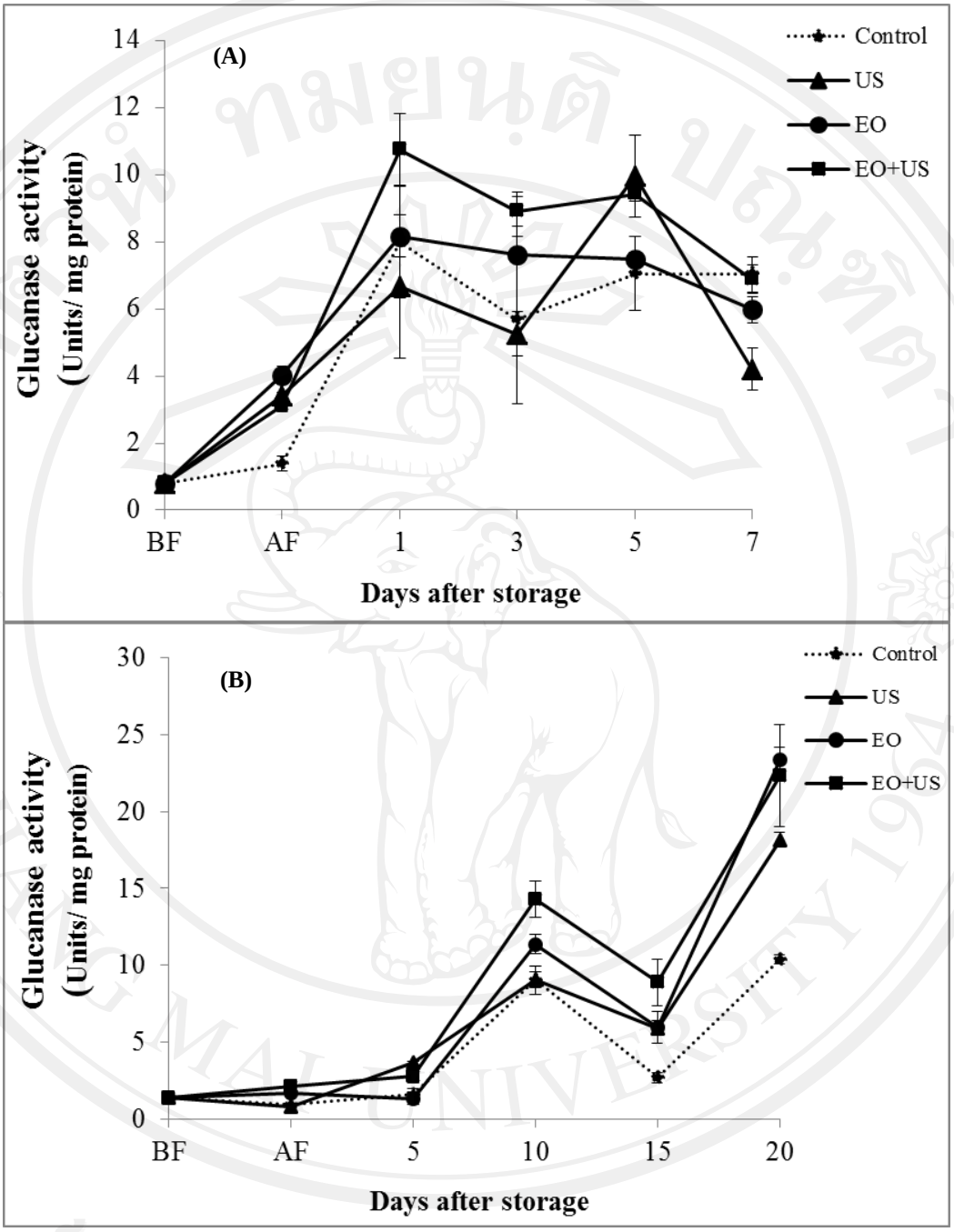

Figure 4.10 β-1,3 glucanase activity of de-crowned pineapple fruit inoculated with *Fusarium* sp. and then treated with EO water (100 ppm) and US wave (1 MHz) and EO water combined with US wave for 10 min. The fruits were kept at 25°C (A) and 13°C (B).

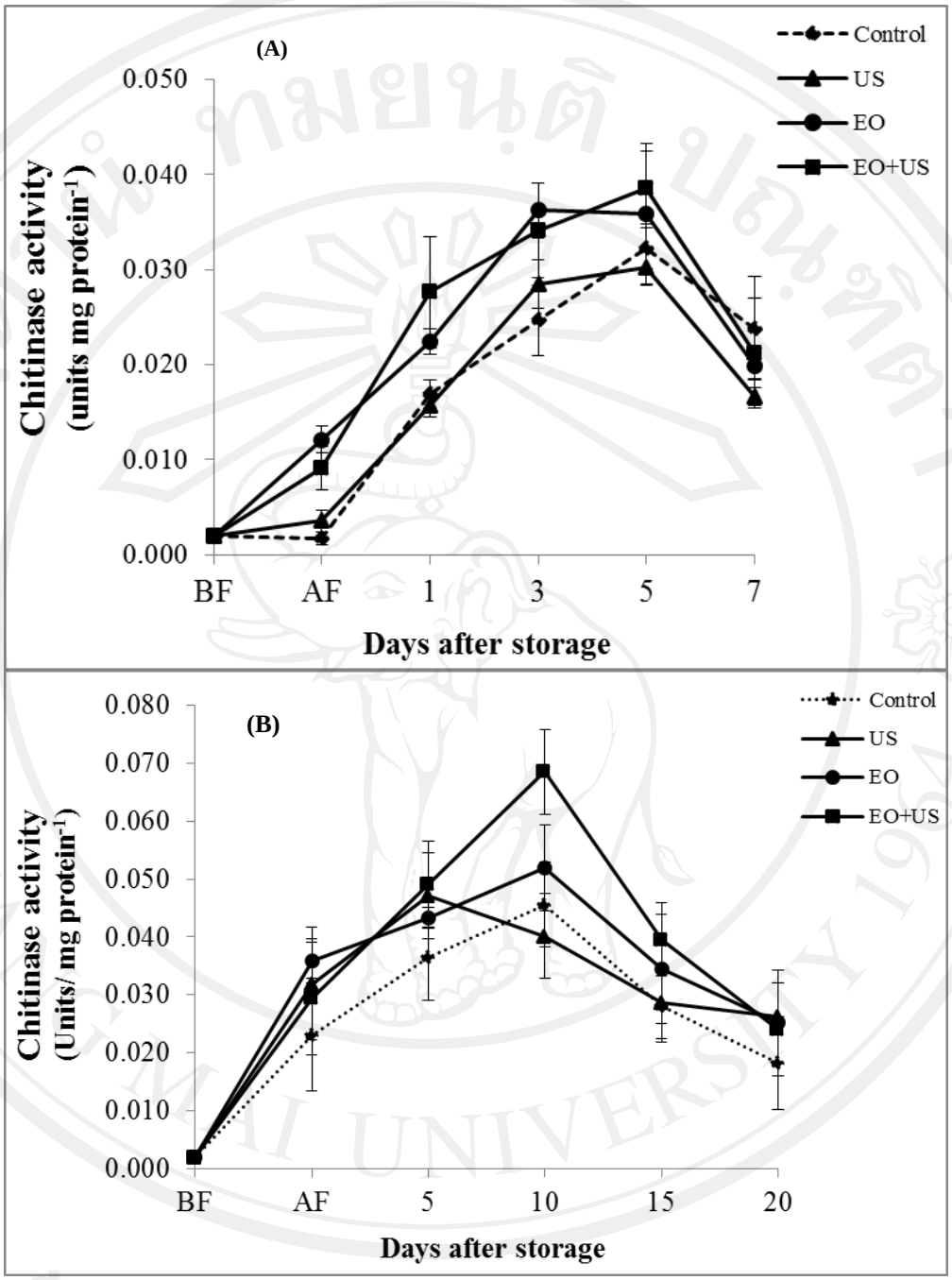

Figure 4.11 Chitinase activity of de-crowned pineapple fruit inoculated with *Fusarium* sp. and then treated with EO water (100 ppm) and US wave (1 MHz) and EO water combined with US wave for 10 min. The fruits were kept at 25°C (A) and 13°C (B).

4.4.4 Effect of EO water and US wave on plant defense related enzyme

Levels of PAL in the fruit rapidly increased and reached a peak on the 3 days after the treatments and storage at 25 °C, afterwards, gradually declining. The activity of PAL in combined treatment were about 1.39, 1.21 and 1.27-fold above than that in the control, US wave and EO water treatment, respectively (Figure 4.13A). At day 10 of 13°C storage, PAL activity in de-crowned pineapple fruits inoculated with *Fusarium* sp. and treated with EO water (100 ppm) combination with US wave (1 MHz) were respectively 1.8, 1.7 and 1.2 times higher than control, US wave and EO water treated groups, respectively(Figure 4.13B). Combined treatment increased POD activities in de-crowned pineapple fruit during the storage and also in an additional induction of POD enzyme after storage 3 days of storage at 25 °C and 10 days of storage at 13 °C (Figure 4.14A, B). Attending to PPO activity, it generally rose after 3 days of storage 25 °C, showing the combination the highest increases with 1.4, 0.8 and 0.9 -fold, respectively, compared to control, US wave and EO water alone on the processing day. At 15 days of storage at 13 °C, PPO activity also rose and decreased afterwards (Figure 4.15A, B).

Activation of PAL POD and PPO occurs in response to several kinds of stress. The mode of action of EO water and US wave in inducing plant pathogen resistance is not yet fully understood. However, the applied sanitizing treatments EO water has been widely reported to act as abiotic stresses in plants (Jacobo-Velázquez *et al*., 2011). Lo´pez-Galvez *et al*. (1996) reported that higher levels of PAL were induced by combining of stresses from wounding plus ethylene in lettuce midrib tissue. In the present study, we found that the activities of PAL, POD and PPO in de-crowned pineapple fruit were induced by combined treatments. Moreover, combined treatment was effective in controlling fungal decay in pineapple fruit. The control effect of combined treatment may be associated with the plant defense related enzyme in response to wide array of stresses including wounding and inoculating with *Fusarium* sp. and treatment with EO water and US wave, the combination of these stresses pronounce the enzyme activities.

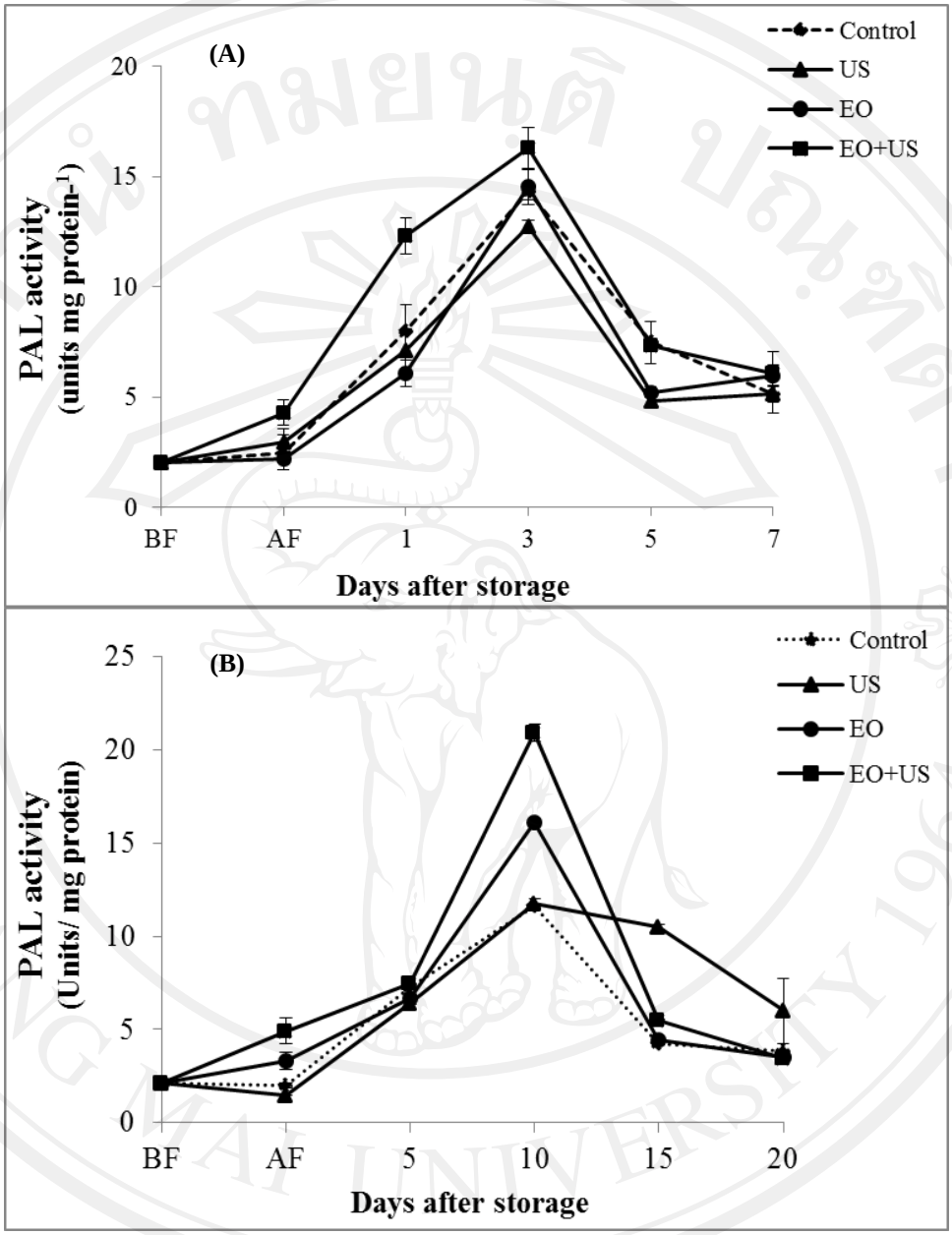

Figure 4.12 Phenylalanin ammonialyase (PAL) activity of de-crowned pineapple fruit inoculated with *Fusarium* sp. and then treated with EO water (100 ppm) and US wave (1 MHz) and EO water combined with US wave for 10 min. The fruits were kept at 25°C (A) and 13°C (B).

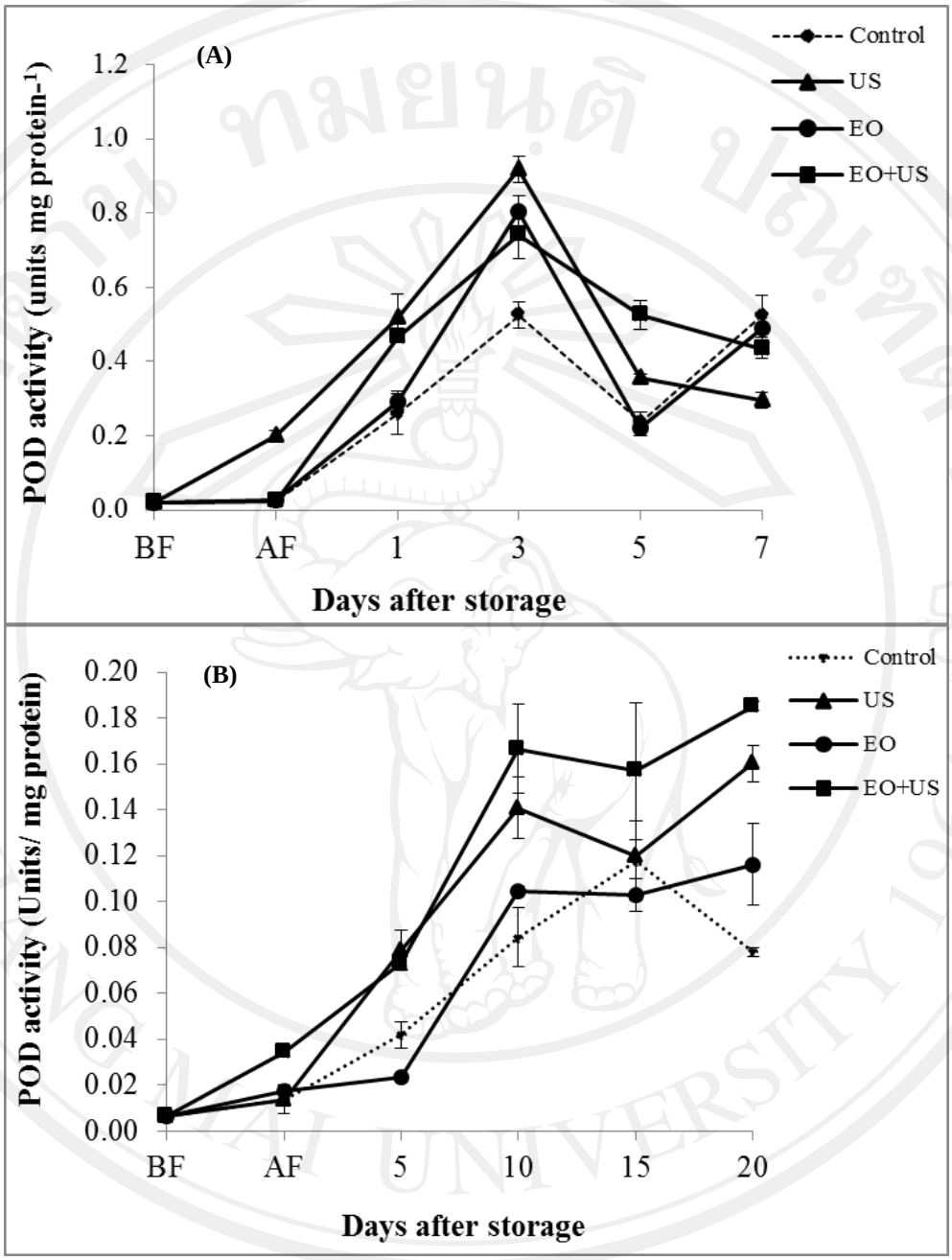

Figure 4.13 Peroxidase (POD) activities of de-crowned pineapple fruit inoculated with *Fusarium* sp. and then treated with EO water (100 ppm) and US wave (1 MHz) and EO water combined with US wave for 10 min. The fruits were kept at 25°C (A) and 13°C (B).

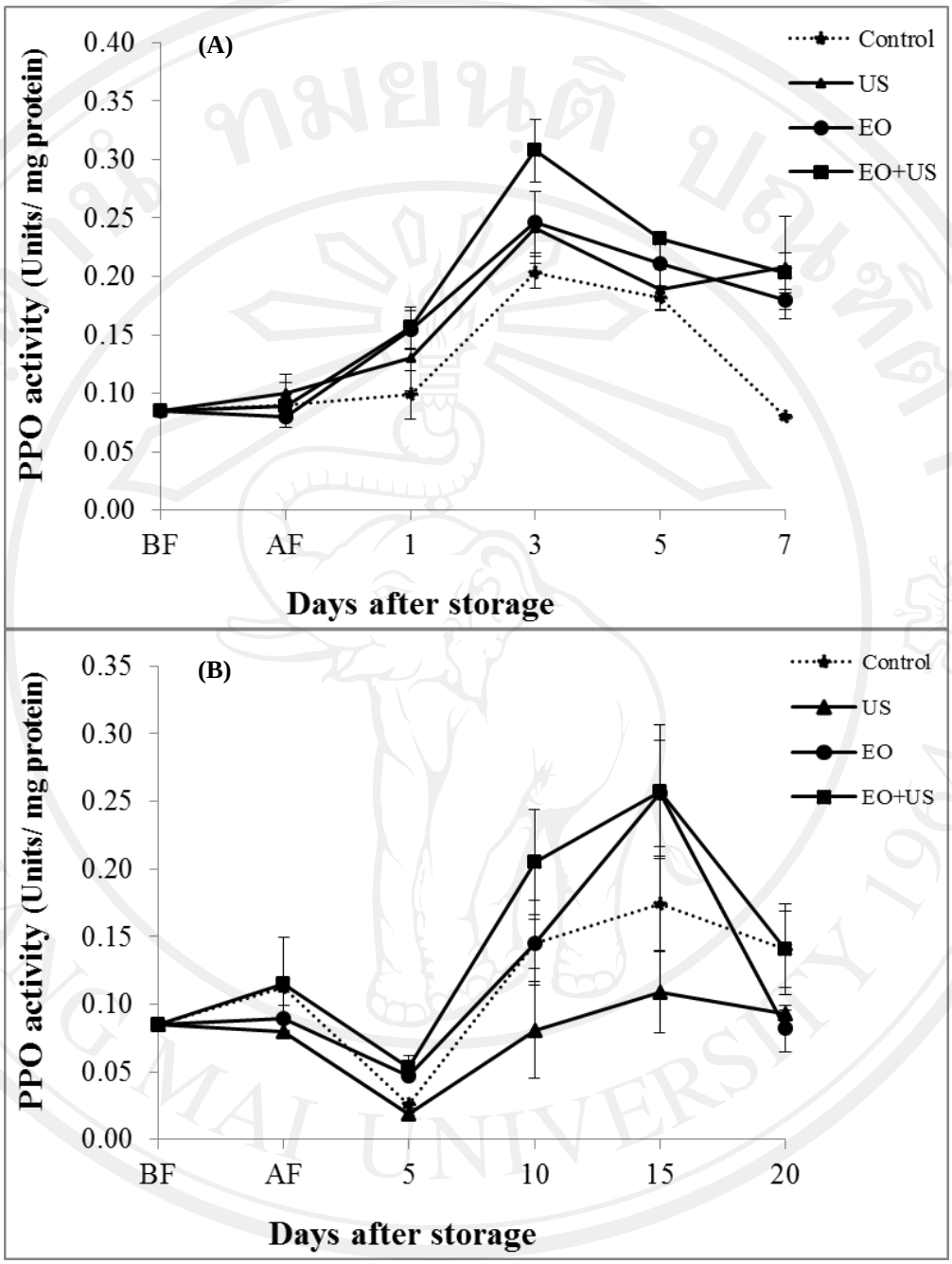

Figure 4.14 Polyphenol oxidase (PPO) activity of de-crowned pineapple fruit inoculated with *Fusarium* sp. and then treated with EO water (100 ppm) and US wave (1 MHz) and EO water combination with US wave for 10 min. The fruits were kept at 25°C (A) and 13°C (B).

4.5 Conclusion

The present study demonstrated that EO water, in combination with US wave, is more effective at controlling *Fusarium* sp. in de-crowned pineapple fruit than either treatment alone. The combination treatment resulted the highest increases of some defense responses enzyme activities such as β-1, 3-glucanase, PAL and POD. Moreover, combined treatment was effective in controlling fungal decay in pineapple fruit. The control effect of combined treatment may be associated with the plant defense related (PR) protein and plant defense related enzyme in response to wide array of stresses including wounding and inoculating with *Fusarium* sp. and treatment with EO water and US wave, the combination of these stresses pronounce the enzyme activities.