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Using electrolyzed oxidizing water combined with an ultrasonic wave on the postharvest diseases control of pineapple fruit cv. 'Phu Lae'

S. Khayankarn [a], J. Uthaibutra [a,b], S. Setha [c], K. Whangchai [a,b,*]

[a] Postharvest Technology Research Institute, Postharvest Technology Innovation Center, Chiang Mai University, Chiang Mai 50200, Thailand
[b] Department of Biology, Faculty of Science, Chiang Mai University, Chiang Mai 50200, Thailand
[c] School of Agro Industry, Mae Fah Luang University, Chiang Rai 57100, Thailand

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ABSTRACT

The effects of ultrasound (US) and electrolyzed oxidizing (EO) water on postharvest decay of pineapple cv. Phu Lae were investigated using *Fusarium* sp. isolated from pineapple fruits. The effect of EO water and US irradiation on *in vitro* growth inhibition of *Fusarium* sp. was studied. Spore suspensions were treated EO water with free chlorine at 100, 200 and 300 ppm and different frequencies of 108, 400, 700 KHz and 1 MHz US irradiation for 0, 10, 30 and 60 min and incubated at 27 °C for 48 h The study showed that all treatments of EO water totally inhibited the spore germination of the fungus. Additionally, US irradiation of 1 MHz for 60 min was the most effective to suppress the spore germination when compared with the control. When the fruits inoculated with *Fusarium* sp. were washed in EO water at 100 ppm and US irradiation or combination of US and EO water significantly inhibited the decay incidence and prolonged the shelf life of the pineapple for 20 days. Treatments had no effect on fruit quality (weight loss percentage, total soluble solids, titratable acidity, pH, and ascorbic acid). The potential for EO water in combination with US in pineapple handling systems is high, due to marked synergistic effects against fungal decay of decrowned pineapple fruit during storage.

1. Introduction

Pineapple (*Ananas comosus* (L.) Merr) is an economically important fruit in Thailand. Domestically, consumption is in the form of fresh fruit, rather than canned fruit. In 2010, only 5% of the pineapple produced (3294 metric tons) was exported as fresh fruit to England, Canada, Iran, Singapore, Ireland, Japan, and other countries, but the value of the exported fruit was about 75 million Baht (Office of Agricultural Economics, 2011). The postharvest loss of pineapple fruit remains a substantial problem for foreign markets. Fungal decay is the main cause of pineapple postharvest loss. Fungal decay depends on weather conditions and the postharvest handling system. The high incidence of postharvest disease in pineapple is primarily due to the fungus *Ceratocystis paradoxa* and *Fusarium* sp. The fungi preferentially penetrate wounds caused by de-crowning that occurs in the postharvest handling systems prior to export (Ballester et al., 2006). Postharvest disease control methods of fresh fruit vary and depend on the requirements of target markets. Washing pineapples with clean water is recommended in many countries (Kader, 2009; Paull, 1992; Department Of Agriculture Kuala Lumpur Malaysia, 2004). In Thailand, harvested pineapples are distributed to markets without washing. Therefore, washing pineapple with electrolyzed water and ultrasound, as a physical disease control method, offers an attractive alternative to the use of fungicides.

EO water is generated by the electrolysis of sodium chloride solution in an electrolysis chamber where the anode and cathode are separated by nonselective membranes. Water collected at the anode has unique oxidation properties due to its content of hypochlorous acid (HOCl) and low pH (Buck et al., 2002). EO water has been shown to have powerful bactericidal effects and has applications in a wide range of fields; including aquaculture (Whangchai et al., 2003), agricultural and industrial food processes (Kim et al., 2000), as well as postharvest disease control (Al-Haq et al., 2002; Venkitanarayanan et al., 1999; Hong et al., 1998). Buck et al. (2002) reported that electrolyzed water used to treat 22 fungal species, significantly reduced growth of the thin-walled species (*e.g.,* *Botrytis* and *Monilinia*) within 30 s. Additionally, it significantly reduced growth of the thicker-walled, pigmented fungi (*Curvularia* and *Helminthosporium*) within

* Corresponding author. Postharvest Technology Research Institute, Postharvest Technology Innovation Center, Chiang Mai University, Chiang Mai 50200, Thailand. Tel.: +66 53 943346; fax: +66 53 892259.
E-mail addresses: kanda@chiangmai.ac.th, kanda.w@cmu.ac.th (K. Whangchai).

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2 min or less. Deza et al. (2003) reported that electrolyzed water, used to treat tomato peels, resulted in a decline of bacteria such as *Escherichia coli* 0157:H7, *Salmonella enteritidis* and *Listeria monocytogenes*, without any affect on the environment. Hung et al. (2010) reported that electrolyzed water treatment of strawberries and broccoli significantly reduced the *E. coli 0157:H7* counts. Paola et al. (2005) found that washing lettuce with electrolyzed water for 5 min significantly inhibited the growth of *L. monocytogenes*. Jane et al. (2008) also reported that electrolyzed water, used as a microbial decontamination agent, for structural surfaces that contain mixed populations of heterotrophic bacteria and as a rinse treatment, reduced bacterial populations on spinach and lettuce. Whangchai et al. (2009) found that electrolyzed water treatment of tangerine cv. Sai Nam Pung, at a free chlorine concentration of 215 ppm for 120 and 240 s, completely inhibited growth and development of *Penicillium digitatum*. In addition, Whangchai et al. (2010) found that washing orange with electrolyzed water with continuous ozone exposure for 2 h day^{-1} significantly controlled *P. digitatum* disease during storage.

US is a form of energy generated by sound waves of high frequencies that the human cannot be detected by ear, i.e. above 16 KHz (Jayasooriya et al., 2004). In recent years, the food industry has discovered that ultrasonic has a variety of applications in the processing (Piyasena et al., 2003). US technology has a bactericidal effect, caused by the occurrence of the cavitation phenomenon, which consists of the formation, growth and collapse of air bubbles. These bubbles generate localized mechanical and chemical energies that are capable of inactivating microorganisms (Adekunte et al., 2010; Gogate and Kabadi, 2009; Piyasena et al., 2003; Valero et al., 2007). US has been used in postharvest treatments to reduce decay, and to maintain the quality of fruits and vegetables. Yang et al. (2011) reported that using ultrasonic waves (40 KHz, 10 min) and salicylic acid (SA) (0.05 mM) on peach fruit resulted in significant control of *Penicillium expansum*, which causes blue mold. The objective of this research was to determine the effects of electrolyzed water in combined with US on the postharvest disease in pineapple.

2. Materials and methods

2.1. Fungal culture

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^{-1}.

2.2. Preparation of treatments

Electrolyzed oxidizing 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 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 ORP (oxidation–reduction potential) was measured by a pH/ORP meter. The amount of free-chlorine concentration was determined by using a DPD (N,N-diethyl-P-phenylene diamine) test (Pailin, 1967). The EO water with initial concentration of 660 ppm was diluted with distilled water to concentrations of free-chlorine at 100, 200, and 300 ppm, and used for the microbiological study, within 2 h after generation. Sterilized distilled water was used as a control for this experiment.

An ultrasonic device with an input power of 3 W 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. Spore suspension (25 ml) was sonicated in an ultrasonic reactor.

2.3. Effect of EO water and US on spore survival of *Fusarium* sp. *in vitro*

The effect of EO water on spore survival of *Fusarium* sp. was studied. Spread plate technique was applied. For EO water treatment, one ml of the spore suspension (10^5 conidia ml^{-1}) was added into 9 ml of EO water containing 100, 200 and 300 ppm free chlorine. All treatments were incubated at room temperature for 0, 10, 20 and 30 min. After treatments, each 0.1 ml of treated spore suspension was added to 0.9 ml of 0.1 N sodium thiosulfate. After well mixing, 0.1 ml of solution was spread on PDA and incubated at 27 °C for 48 h. Survival of the fungus was expressed as the mean number of colony-forming-units (CFU ml^{-1}). Each treatment consisted of 9 replicates and the experiment was repeated twice independently. The control treatment consisted of a 1 ml of spore suspension and 9 ml of distilled water.

The effect of ultrasonic wave on spore survival of *Fusarium* sp. was studied. Spore suspension (10^5 conidia ml^{-1}) was treated by adding 1 ml of spore suspension to 9 ml of sterile water. Those suspensions were subjected to ultrasonic waves at frequencies of 108, 400, 700 KHz and 1 MHz for 0, 10, 30 and 60 min. After treatments, all treated samples were counted colony-forming-units by spreading plate technique as described above.

2.4. Effect of EO water in combination with ultrasound on fruit decay and quality of pineapple after low temperature storage

Pineapple fruits (*A. comosus* cv. Phu Lae) were harvested, at the green mature stage, from a commercial orchard in Chiang Rai Province, Thailand. After harvesting, the fruits were transported immediately to the Postharvest Biology and Technology laboratory, Chiang Mai University. 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. Samples of 12 fruits were used for each replicate. The treatment which shown good results in the control of spore germination from previous experiment was used for this experiment. In the first treatment, fruits were subjected to ultrasonic waves of 3 W 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 L which was able to wash 10 kg of pineapple fruits. In the second treatment, pineapple fruits were treated with EO water with a concentration of free-chlorine at 100 ppm. In the third 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 for 10 min. After the treatments, fruit samples were placed in a basket and air-dried. Fruit were then covered with a commercial plastic bag and maintained at 13 °C for 20 days. Samples were taken initially and at 5-day intervals during storage for decay evaluation and other analysis.