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Ultrasound treatment on phenolic metabolism and antioxidant capacity of fresh-cut pineapple during cold storage

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ABSTRACT

Ultrasound treatment at different power output (0, 25 and 29 W) and exposure time (10 and 15 min) was used to investigate its effect on the phenolic metabolism enzymes, total phenolic content and antioxidant capacity of fresh-cut pineapple. Following ultrasound treatment at 25 and 29 W, the activity of phenylalanine ammonia lyase (PAL) was increased significantly ($P < 0.05$) by 2.0 and 1.9-fold, when compared to control. Meanwhile, both the activity of polyphenol oxidase (PPO) and polyphenol peroxidase (POD) in fresh-cut pineapple was significantly ($P < 0.05$) lower than control upon subjected to ultrasound treatment. In the present study, induction of PAL was found to significantly ($P < 0.001$) correlate with higher total phenolic content and thus higher antioxidant capacity in fresh-cut pineapple. Results suggest that hormetic dosage of ultrasound treatment can enhance the activity of PAL and total phenolic content and hence the total antioxidant capacity to encounter with oxidative stress.

1. Introduction

Pineapple is a good source of vitamin C to prevent oxidative damage in body cells by scavenging reactive oxygen species, vitamin B to aid in digestion, vitamin A, fibre and minerals (Benitez, Soro, Achaerandio, Sepulcre, & Pujola, 2014). Considering the significant proportion of total antioxidants that can be contributed in daily diet, the consumption of pineapple is well appreciated globally. However, antioxidant constituents of pineapple are susceptible to degradation when subjected to various degree of mechanical operations prior to packaging and storage (Gil, Aguayo, & Kader, 2006). Therefore, considerable efforts have been taken in the field of food science to maintain or improve the antioxidants of fresh-cut fruits and vegetables through postharvest handling and processing.

Studies have focused on the use of edible materials such as polysaccharides, protein and lipids to form a semi permeable barrier to slow down respiration rate, ethylene production and water loss from fresh-cut pineapple (Bierhals, Chiumarelli, & Hubinger, 2011). For example, Bierhals et al. (2011) reported that the physiological changes in cassava starch coated fresh-cut pineapple was effectively slow down but the treatment was ineffective to reduce the loss of ascorbic acid following storage 12 d of storage 5 °C. Similarly, higher reduction of ascorbic acid concentration in comparison to control was observed in alginate coated fresh-cut pineapple (Benitez et al., 2014). However, the formulation of edible coating which is added with other chemical reagents such as glycerol, calcium chloride and acetic acid may affect the taste and consumer acceptance towards the end products.

Recently, the application of ultrasound has received commercial interest in food industry due to its effectiveness to maintain the quality and safety of food products by inhibiting the growth of spoilage microorganisms and inactivating several deteriorative enzymes. The inactivation of enzymes by sonication is mainly attributed by the physical and chemical effects of cavitation. High shear forces generated from the collapsed of cavitation bubbles can disrupt the hydrogen bonding and van der Waals interaction in the polypeptide chains and hence lead to the modification of secondary and tertiary structure of the protein (Kentish & Feng, 2014). In addition, sonolysis of water can generate high energy intermediates such as $O_2^{\cdot-}$ and H_2O_2 which can react with some of the amino acid residues that are involved in enzyme stability, substrate binding or enzyme catalytic activity and consequently result in the change in the biological activity (Kentish & Feng, 2014; São José et al., 2014).

Interestingly, the generation of reactive oxygen species such as $OH^{\cdot}$ and H_2O_2 may impose oxidative stress to fresh products and hence induce the antioxidant potential of fresh fruits and vegetables (São José et al., 2014). For instances, ultrasound treatment was found to increase the antioxidant capacity of mushroom

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(Lagnika, Zhang, & Mothibe, 2013) and pear juice (Zafra-Rojas et al., 2013). Therefore, present study was conducted to determine the activity of phenolic metabolism enzymes, total phenolic content and total antioxidant capacity of ultrasound treated fresh-cut pineapple during cold storage.

2. Materials and methods

2.1. Preparation of plant materials

Twenty-five pineapples (*Ananas comosus* L. cv. Josapine) were purchased from Exotic Star Sdn. Bhd in Selangor Wholesale Market, Malaysia. Fruits with uniform size (weighed about 1.3–1.5 kg) and shape and of maturity index 4 were selected and used in this experiment. The pineapples were washed under running tap water for 1 min and allowed to air dry at room temperature (25 ± 1 °C). To minimize cross contamination during processing, the blossom and stem end of pineapple were discarded. Then, pineapples were transversely cut into three rings of 2 cm thick. Each ring was further diced into 2.5 cm triangular chunks with a handheld stainless steel dicer. The weight of the pineapple cubes was 15 ± 1 g. Peeler, knife, dicer and cutting board were sterilized with 0.1% (v/v) sodium hypochlorite solution prior to use. A total of 600 pineapple chunks were obtained after 3 h of processing. All pineapple chunks were combined, mixed and randomly selected for ultrasound treatment at different power and treatment time.

2.2. Ultrasound treatment and storage of fresh-cut pineapple

Ultrasonication was carried out in 240 mm × 137 mm × 100 mm (width × length × height) ultrasound water bath (Elmasonic P30, Elma Hans Schmidbauer GmbH & Co. KG, Germany) with sample to water ratio of 0.2 kg to 1 L. Based on preliminary studies (results were not shown), ultrasound treatments at 25 and 29 W and exposure time of 10 and 15 min resulted in significant reduction of spoilage microorganisms enumerated from fresh-cut pineapple. Therefore, the effect of those parameters on the phenolic metabolism enzymes, total phenolic content and antioxidant capacity of fresh-cut pineapple during 5 d of storage at 7 °C was evaluated in this study. Pineapple chunks were randomly selected, placed directly in the ultrasonic bath and treated at different power input (25 and 29 W) and exposure time (10 and 15 min) at a constant frequency of 37 kHz in this experiment. Water was replaced every 5 min with pre-cooled distilled water (dH_2O) to maintain the temperature of the ultrasound bath at 25 ± 1 °C. Fresh-cut pineapples dipped in dH_2O for 10–15 min were served as controls. Ultrasound bath surface was sterilized with 70% (v/v) ethanol before usage. After treatment, fruits were air dried at room temperature for approximately 10 min. Then, the samples were packed in a 9.0 cm × 9.0 cm × 4.0 cm (width × length × height) polystyrene containers (three pineapple cubes in each container) and stored at 7 °C and 80 ± 5% relative humidity (RH) for 5 d. Analysis of phenolic metabolism enzymes, total phenolic content and antioxidant capacity were carried out on day 0, 1, 3 and 5, respectively.

2.3. Enzymatic assays for phenolic metabolism enzymes

2.3.1. Extraction and assay of PAL, PPO and POD enzymes

Extraction and determination of PAL, PPO and POD was carried out as described by Wu, Zhang, and Adhikari (2013) with slight modifications. To measure PAL, 3 g of pineapple tissue was homogenized with 5 ml of pre-chilled 0.1 M sodium borate buffer (pH 8.8) containing 5 mM β-mercaptoethanol (Acros Organics, Geel, Belgium), 2 mM ethylene diaminetetraacetic acid (EDTA; Bio Basic Inc., Ontario, Canada) and 1% (w/v) polyvinylpolypyrrolidone (PVPP; Sigma-Aldrich, St. Louis, USA) using mortar and pestle. After incubation for 1 h at 4 °C, the homogenized sample was centrifuged at 10,000*g* for 25 min at 4 °C. 12.5 µl of supernatant was transferred and mixed 137.5 µl of 60 mM L-phenylalanine (Bio Basic Inc., Ontario, Canada) in 0.1 M sodium borate buffer (pH 8.8) in a 96-well microplate. Homogenates were incubated for 1 h at 40 °C and reaction was stopped by adding 5 µl of 6 M HCl (J.T. Baker, Pennsylvania, USA). The increase in absorbance at 290 nm due to the formation of trans-cinnamate was measured using microplate spectrophotometer (Multiskan ™GO, Thermo Fisher Scientific Inc., Wisconsin, USA). One unit of enzyme activity was defined as the amount that resulted an increase of 0.001 absorbance unit per hour and the result was expressed as unit h^{-1} mg^{-1} FW.

In PPO assay, 3 g of flesh tissue was homogenized in 8 ml of pre-cooled 50 mM sodium phosphate buffer (pH 7.8) and 0.05% (w/v) PVPP. Then, the homogenate was centrifuged at 10,000*g* for 25 min at 4 °C. Determination of PPO activity was carried out by adding 5 µl of supernatant with 145 µl of 0.1 M catechol (Acros Organics, Geel, Belgium) in 0.1 M sodium phosphate buffer (pH 6.8). The increase in absorbance at 420 nm was monitored for 4 min 25 °C using microplate spectrophotometer. One unit of PPO activity was defined as the amount of enzyme that resulted an increase of 0.01 absorbance unit per min and the result was expressed as unit min^{-1} g^{-1} of FW.

Extraction of POD was carried out by homogenizing 5 g of flesh tissue with 10 ml of 0.2 mM sodium phosphate buffer (pH 6.5). The mixture was centrifuged at 10,000×*g* for 15 min at 4 °C. POD activity was measured by adding 7.5 µl of enzyme extract and 142.5 µl of 0.1 M sodium phosphate buffer (pH 6.0) blended with 20 mM guaiacol (Acros Organics, Geel, Belgium) and 4 mM H_2O_2 (Bio Basic Inc., Ontario, Canada). The increase in absorbance at 470 nm was monitored for 4 min at 25 °C using microplate spectrophotometer. One unit of POD activity was defined as the amount of enzyme that resulted an increase of 0.001 absorbance unit per min and the result was expressed as unit min^{-1} g^{-1} of FW.

2.4. Extraction of polyphenols

The recovery of phenolic compounds from fresh-cut pineapple was carried out using extraction method as described by (Wu et al., 2013). Briefly, 1 g of flesh tissues was ground with 10 ml of 100% (v/v) methanol in a mortar and pestle. Homogenates were vortexed and centrifuged at 10,000×*g* for 15 min at 4 °C. Supernatant was filtered with Whatman paper No. 1 (150 mm diameter) and used for Folin-Ciocalteu and antioxidant capacity assays.

2.5. Total phenolic content

Total phenolic content of extract was performed using Folin-Ciocalteu assay as described by Du, Li, Ma, and Liang (2009) with some modifications. Briefly, 0.1 ml of extract was added with 6.0 ml of dH_2O and 0.5 ml of 2 N Folin-Ciocalteu reagent (Merck, Darmstadt, Germany). After incubated at room temperature for 4 min, 1.5 ml of 7% (w/v) sodium carbonate and 1.9 ml of dH_2O were added into the mixture. The solution was vortexed and incubated at 37 °C for 2 h. Blank was prepared by replacing 0.1 ml of pineapple extract with 0.1 ml of dH_2O. The absorbance value of the blue-colored complex formed was measured against a blank at 765 nm using microplate spectrophotometer. The experiment was performed similarly in the preparation of standard curve using gallic acid as a standard (100–1000 µg ml^{-1}; R^2 = 0.9986). Total phenolic content was determined against the standard curve and expressed as mg of gallic acid equivalents (GAE) 100 g^{-1} FW.