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Effect of different concentrations of ozone on physiological changes associated to gas exchange, fruit ripening, fruit surface quality and defence-related enzymes levels in papaya fruit during ambient storage

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ABSTRACT

Papaya fruit (*Carica papaya* L.) were exposed continuously to ozone fumigation (0, 1.5, 2.5, 3.5 and 5.0 $\mu L\,L^{-1}$) for 96 h prior to ambient storage ($25 \pm 3\,^{\circ}C$ and $70 \pm 5\%$ RH) for an additional 10 days. The rate of ethylene formation and changes in the activities of the plant defence enzymes, phenylalanine ammonia-lyase (PAL) (EC 4.3.1.5), peroxidase (POD) (EC 1.11.1.7) and polyphenol oxidase (PPO) (EC 1.14.18.1), were measured in ozone treated and untreated papaya fruit after 0, 2, 4, 6, 8, 10, 12 or 14 days. The fruit treated with ozone at concentrations lower than 5 ppm had lower respiration rate and delayed ripening compared to the control. However the fruit that had received high ozone concentrations (>3.5 $\mu L\,L^{-1}$) produced more ethylene and caused injury to fruit tissue. Enzyme activities were higher in ozone treated papaya fruit than in untreated fruit throughout the storage period. The greatest changes in enzyme activities were obtained with the highest ozone dose (5 $\mu L\,L^{-1}$).

1. Introduction

Papaya (*Carica papaya* L.), belonging to the family *Caricaceae*, is rapidly becoming an important fruit internationally, both as fresh and processed products (Paull et al., 1997). The fruit is climacteric and susceptible to postharvest losses due to ethylene-induced ripening. Currently, there is a high level of interest in postharvest applications of ozone for decay control and as a potential sanitizer against human pathogens. Ozone has been affirmed 'Generally Recognized As Safe' (GRAS) status as a food processing aid and is compliant with the Environmental Protection Agency Disinfection by Products Rule (US-FDA, 1997).

Several researchers have shown that treatment with ozone extended the storage life of fresh commodities, such as broccoli, cucumbers, apples, oranges, pears and strawberries, by reducing microbial populations and oxidation of ethylene (Beuchat, 1998; Skog and Chu, 2001). It was known that increased ethylene concentration was responsible for fruit ripening. Therefore, ozone has the potential to delay fruit ripening by oxidizing the ethylene. However, some physiological responses (change in rate of ethylene production, change in rate of respiration, change in tissue and organelle permeability and change in colour) observed and reported after ozone treatment have been previously reported as ethylene responses (Pratt and Goeschl, 1969), and they may be a result of induced ethylene from oxidant injury. Ozone exposure induces the emission of ethylene in various plant species (Sandermann, 1996). The ethylene emission was positively correlated with ozone sensitivity, indicating that ethylene may appear to be a stress response of the plant to ozone.

Studies on antimicrobial capacity of ozone (Beuchat, 1998; Aguayo et al., 2006) and the effects of ozone on product quality on fresh-cut lettuce (Rico et al., 2006), fresh-cut celery (Zhang et al., 2005), fresh-cut potato strip (Beltrán et al., 2005), pistachio (Akbas and Ozdemir, 2006) and shredded packaged iceberg lettuce (Baur et al., 2004) have been widely reported. According to Skog and Chu (2001), ozone at 0.4 $mL\,L^{-1}$ was effective in removing

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ethylene from the atmosphere in an apple and pear storage room, from 1.5 to 2 mL L^{-1} to a non-detectable level. In addition to ozone, other postharvest treatments including cold storage, controlled and modified atmospheres, irradiation (gamma and UV), high-field electric pulses and microwaves, can maintain or increase phenolic content of fresh produce (Tomás-Barberán and Espín, 2001; Ali et al., 2014). However, little information is currently available about ozone-induced oxidation of ethylene, fruit quality and changes in the activities of the phenolic enzymes, phenylalanine ammonia-lyase (PAL), peroxidase (POD) and polyphenol oxidase (PPO), in papaya fruit. These enzymes are very important in plant disease resistance. They are involved in the formation of lignin and phytoalexins, which are related to plant disease development (Graham and Graham, 1996). This paper reports the effect of ozone exposure on peel colour, respiration rate, ethylene production and PAL, PPO and POD activities in papaya fruit stored at ambient temperature.

2. Materials and methods

2.1. Plant material

Mature green papaya cv 'Sekaki' of colour index 2 (green with trace of yellow) were obtained from a local fruit wholesaler at Pasar Borong Selangor, Malaysia on the day of harvest. Fruit of uniform size (800–1200 g), shape and maturity and free from any indication of mechanical injury, or insect or pathogenic infection were selected for the experiment. Fruit were washed with clean distilled water and air-dried at ambient temperature (25–28 °C) before exposure to ozone.

2.2. Ozone exposure

The ozone fumigation system, located in the postharvest laboratory, School of Biosciences, University of Nottingham, Malaysia campus, was comprised of chambers constructed from 5 mm thick polycarbonate (112.0 cm length × 47.0 cm width × 43.0 cm height) and equipped with four 12 V fans positioned directly below the sample platform to ensure a well-mixed atmosphere. The chambers were ventilated with HEPA particulate-filtered air. Ozone was introduced to the chamber by an ozone generator (Model MedKlinn Professional Series, MedKlinn International Sdn. Bhd., Malaysia) and the ozone concentration was controlled manually using a sensor (Eco-Sensor, Model OEM-2, MedKlinn International Sdn. Bhd., Malaysia). The ozone concentration in the chamber was recorded by an ozone analyzer (Model IN-2000 L2-LC, IN USA Incorporated, USA). The chamber was maintained at 25 ± 3 °C and 70 ± 5% RH (relative humidity), and monitored with the aid of temperature/humidity sensors (Model U14-001, HOBO LCD data logger, Onset Computer Corporation, USA). Each chamber was prepared as a replicate and contained 14 fruit in a single layer. The fruit were exposed for 96 h to 1.5, 2.5, 3.5 or 5.0 μL L^{-1} ozone with 4 replicates for each concentration. Fruit in chambers without exposure to ozone served as controls at 25 ± 3 °C and 70 ± 5% RH.

2.3. Peel colour determination

Peel colour on three points of whole fruit was determined using a Minolta CR-300 Chroma Meter (Minolta Corp., Japan) and expressed in chromaticity values of L^*. Three fruit were measured in each replication. Lightness (L^*) forms the vertical axis with values ranging from 0 = black to 100 = white.

2.4. Respiration rate determination

To determine the respiration rate, two papaya fruit in each replicate were randomly removed from treatment chambers every 2 days. A single fruit from each replication was sealed in an oblong storage container (13.5 cm diameter × 26.5 cm height). After 1 h, 1 mL of gas was withdrawn from the container headspace and analysed for CO_2 using a gas chromatograph (GC) (Claru-500, Perkin-Elmer, USA) equipped with a stainless steel column packed with Porapak R (80/100 mesh) and a thermal conductivity detector (TCD). Helium was used as the carrier gas with a flow rate of 20 mL min^{-1}. Temperatures were 60, 100 and 200 °C for the oven, injector and TCD, respectively. One millilitre of 1.0% CO_2 gas (Scotty Gases, Bellefonte, PA) was used as the external standard for calibration. Independent CO_2 samples were taken from each replication and the result expressed as means (mL kg^{-1} h^{-1}).

2.5. Ethylene determination

To determine the rate of ethylene production, the same papaya fruit in each replicate was sealed in an oblong storage container (13.5 cm diameter × 26.5 cm height). After 1 h, a 1 mL gas sample was withdrawn from the container headspace and ethylene content was quantified using a gas chromatograph (Claru-500, Perkin-Elmer, USA) equipped with a Porapak R column and a flame ionization detector (FID). Flow rates for nitrogen, hydrogen and air were 20 mL min^{-1}. Nitrogen served as the carrier gas. Temperatures were 150, 200 and 200 °C for the oven, injector and FID, respectively. One millilitre of ethylene gas (10 μL mL^{-1}) (Scotty Gases) was used as an external gas standard for calibration. Independent ethylene samples were taken from each replicate and the results expressed as means (μL kg^{-1} h^{-1}). The free headspace of each container was estimated by subtracting the fruit volume from the total volume of each sealed storage container.

2.6. Enzymatic assays

2.6.1. Crude enzyme preparation

The pulp from two papaya fruit in each replicate was randomly sampled and homogenized at 4 °C using mortar and pestle in 10 mL of 100 mM sodium phosphate buffer, pH 6.4 containing 0.2 g of polyvinylpolypyrrolidone (PVPP) (Liu et al., 2007). The fruit pulp (2 g) was sampled from the equatorial region of the whole fruit and was a pooled sample from each replicate. The homogenates were centrifuged at 12,000 rpm for 30 min at 4 °C and the supernatant was used as the crude enzyme extract for determining the activity of polyphenol oxidase and peroxidase enzymes. The crude enzyme extracts were kept on ice during the preparation.

2.6.2. Polyphenol oxidase (PPO)

Crude enzyme extract (0.1 mL) was added to 3 mL of 500 mM catechol substrate in 100 mM sodium phosphate buffer (pH 6.4). PPO (EC 1.14.18.1) activity was assayed according to the method of Liu et al. (2007) by measuring the conversion of catechol to quinine mediated by PPO. The increase in absorbance at 398 nm was automatically recorded for 3 min, using a spectrophotometer (Model: Biochrom Libra S12; Biochrom Ltd., Cambridge, UK). The PPO activity was expressed as units (U) mL^{-1} enzyme extract, where one unit of the enzyme activity is defined as the amount that causes a change of 0.001 in absorbance per minute.

2.6.3. Peroxidase (POD)

Crude enzyme extract (0.1 mL) was mixed with 2 mL of 8 mM guaiacol in 100 mM sodium phosphate buffer (pH 6.4) and incubated for 30 min at 30 °C and then 1 mL of 24 mM hydrogen peroxide was added. POD (EC 1.11.1.7) activity was assayed by measuring the conversion of guaiacol to tetraguaiacol as described by Liu et al. (2007). The increase in absorbance at 460 nm was recorded for 3 min using a spectrophotometer (Model: Biochrom Libra S12; Biochrom Ltd., Cambridge, UK). The POD activity was expressed as units (U) mL^{-1} enzyme extract, where one unit of the enzyme