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Influence of gum arabic coating enriched with calcium chloride on physiological, biochemical and quality responses of mango (*Mangifera indica* L.) fruit stored under low temperature stress

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ABSTRACT

Effects of gum arabic (GA) 10% and calcium chloride (CA) 3% on the physiological, biochemical and quality responses of mango (*Mangifera indica* L. cv. Choke Anan) fruit were investigated. Fruit were stored at 6 °C and 90% relative humidity for 28 d and then transferred to 25 °C for an additional 5 d shelf life. Significant ($P \leq 0.05$) differences were observed in fruit treated with CA 3% and GA 10% as compared to the control. The combined treatment of CA 3% and GA 10%, significantly alleviated chilling injury, malondialdehyde (MDA) content and electrolyte leakage than the control fruit. This treatment reduced the increase in hydrogen peroxide (H_2O_2) content, superoxide anion ($O_2^{\bullet -}$) production rate and enhanced DPPH radical scavenging activity. Furthermore, GA 10% alone or in combination with CA 3% effectively inhibited the loss of total phenolic content and ascorbic acid. The result of transmission electron microscopy confirmed that treated fruit maintained cell membrane integrity. These results suggest that application of GA 10% coating combined with CA 3% might be enhanced low temperature tolerance by improving the antioxidant defense system and reducing oxidative damage of mango fruit.

1. Introduction

Mango (*Mangifera indica* L.) is a popular and economically important tropical fruit throughout the world. Mango is a climacteric fruit and ripens very fast in harsh climatic conditions. The best quality is retained at the lowest possible storage temperature tolerated by the product. Low temperature is useful for maintaining quality and extending the shelf life of mango fruit (Nunes et al., 2007). However, mangoes are extremely sensitive to chilling injury (CI), when the fruit are exposed to temperatures below 13 °C, which affects the postharvest quality of fruit during transportation and marketing (Phakawatmongkol et al., 2004). Chilling injury symptoms in mango fruit appear as sunken lesions or surface pitting, browning, greyish scald like discoloration of the skin, poor aroma and flavor, uneven ripening and increased susceptibility to fungal decay (Nunes et al., 2007). Several techniques have been used to reduce CI in mango fruit, such as plant growth regulators (Wang et al., 2008), cold adaptation (Zhao et al., 2006), oxalic or salicylic acid (Ding et al., 2007), controlled and modified atmosphere (Pesis et al., 2000) and heat treatment (Nair et al., 2001).

It is well known that low temperature stress induces production of reactive oxygen species (ROS), which cause damage to membrane structure and as a result CI in mango fruit (Ding et al., 2007). Effective destruction of ROS could give protection from CI to fruit by inducing the antioxidant properties in the fruit tissues (Hodges et al., 2004). Reactive oxygen species comprise superoxide ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2) and hydroxyl radicals ($OH^{\bullet}$). During oxidative stress, overproduced ROS react with various molecules which results in protein degradation, membrane lipid peroxidation, and DNA damage, which leads to cellular damage or cell death (Blokhina et al., 2003). To combat oxidative stress and scavenge the chilling induced ROS, plants have developed two antioxidant enzymatic and non-enzymatic defense mechanisms comprised of the enzymes catalase (CAT), glutathione reductase (GR), ascorbate peroxidase (APX), superoxide dismutase (SOD), and non-enzymatic compounds such as glutathione (GSH), ascorbate (AsA), phenolics and vitamin E (Apel and Hirt, 2004;

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Gill and Tuteja, 2010). When this defense system fails to scavenge the excessive production of ROS, oxidative damage occurs.

Therefore, appropriate postharvest technologies combine with chilling temperature are needed to preserve mango fruit quality during storage. Gum arabic is a polysaccharide natural secretion from *Acacia species* and used in industries for its film forming, emulsification, and encapsulation properties (Motlagh et al., 2006). Gum arabic coatings effectively maintained antioxidant and total phenolic contents in tomato fruit (Ali et al., 2013) and in papaya (Addai et al., 2013). Gum arabic treatment reduced browning, loss of ascorbic acid and total phenolic contents of tomato slices (Eltoum and Babiker, 2014). Calcium chloride has been extensively used in the fruit and vegetable sector for whole and fresh-cut commodities as a preservative and firming agent. It has been observed that calcium is associated with fruit firmness, stress tolerance, ripening and senescence (Martin-Diana et al., 2007). Physiological disorders can be caused by low storage temperature related to calcium content. Calcium chloride dip treatment reduced flesh browning of peach fruit (Manganaris et al., 2007), and chilling injury of lemon during low temperature stress (Safizadeh et al., 2007). Calcium chloride combined with chitosan maintained a high level of vitamin C and reduced sensitivity to CI of peach fruit during refrigerated storage (Ruoyi et al., 2005). Thus, this study was designed with the following objectives; (1) to evaluate the effect of gum arabic and calcium chloride treatments on alleviation of oxidative damage, and (2) to determine the physiological, biochemical and quality responses of mango fruit stored at sub-optimal temperature.

2. Materials and methods

2.1. Plant material

Mango (*Mangifera indica* L. cv. Choke Anan) fruit were obtained from a commercial orchard located in Ipoh, Perak state of Malaysia. Maturity index 2 fruit were used for this experiment, which initially had a green skin colour, light cream pulp, soluble solid concentration of 6.15% and titratable acidity of 0.98%. The fruit were transported with minimal delay after harvest and brought to the postharvest laboratory within a day. Only firm and well-developed fruit of uniform size and maturity, free from pests, diseases, injuries, bruises and blemishes were selected for the experiment. Calcium chloride and gum arabic powder of food grade were purchased from Sigma Chemical Co., USA.

2.2. Preparation of dipping solutions

Calcium chloride 3% (w/v) solution was prepared by dissolving 3 g of $CaCl_2$ in 100 mL of distilled water. The solution was agitated constantly using a magnetic stirrer (model SP 18420-26 Barnstead Thermolyne 2555 Kerper Boulevard Dubuque, USA) for 30 min, and 0.2 mL of Tween 20 (Polyoxyethylene sorbitan monoleate, Sigma–Aldrich) was added to the solution to improve wettability. Gum arabic solution 10% (w/v) was prepared by dissolving 10 g of gum arabic powder in 100 mL distilled water. The solution was heated at 40 °C for 60 min by using a hot plate with magnetic stirrer. Glycerol 1.0% was also added as a plasticizer to the coating solutions. The pH of the solutions was adjusted to 5.6 with 1 mol L^{-1} NaOH using a pH meter (GLP 21, Crison Barcelona). For the combined treatment, the calcium chloride solution was incorporated into gum arabic 10% stirred for 30 min and the pH of the solutions were adjusted to 5.6 with 1 mmol L^{-1} NaOH using a pH meter. Treatments for the experiment were: (1) distilled water as a control, (2) calcium chloride 3%, (3) gum arabic 10%, and (4) calcium chloride 3% + gum arabic 10%.

2.3. Fruit preparation and dipping treatments

Before treatments, mango fruit were washed with 0.01% sodium hypochlorite for 2 min, and then air-dried at room temperature for 1 h. The mangoes were randomly divided into four lots of 60 fruit each. All the treatments were conducted with three replicates. One of the four dipping treatments was then applied to each lot. The first lot was dipped in distilled water containing Tween-20 and was used as a control. The other three lots were dipped in the corresponding solutions. Mango fruit were dipped in the solutions for 3 min. After dipping treatments, fruit were air-dried for 1 h. All fruit were packed in plastic boxes (40 × 30 × 12 cm) covered with polyethylene plastic films 0.02 mm thickness to maintain relative humidity (RH) of about 90%. To avoid modifying the atmosphere around the fruit, five holes of 7 mm in diameter were made in the plastic film. The fruit were then stored at 6 ± 1 °C for 28 d and after that the samples were transferred to 25 ± 2 °C for an additional 5 d shelf life. The data were collected before treatment (day 0) and at 7 d intervals for 28 d during cold storage and for an additional 5 d shelf life. Twelve fruit of each treatment were sampled at 7 d interval. Chilling injury and electrolyte leakage were evaluated immediately after removing the samples from the cold storage. A mixed sample of four fruit peels from each replication was immediately crushed in liquid nitrogen and stored at −80 °C for measurements of $O_2^{\bullet -}$, H_2O_2 content, MDA content, total phenolics, ascorbic acid and DPPH radical scavenging activity.

2.4. Evaluation of chilling injury (CI) index

Chilling injury symptoms were recorded visually as described by Chidtragool et al. (2011) with slight modification. The severities of the symptom were assessed using a scale: 0 = no signs of chilling injury, 1 = 1–10%, 2 = 11–25%, 3 = 26–40%, 4 = 41–50%, 5 = >50%. CI index was calculated using the following formula:

$$\text{CI index} = \frac{\Sigma(\text{CIlevel}) \times (\text{numberoffruitattheCIlevel})}{\text{Total number of fruit in the treatment}}$$

2.5. Measurement of cell membrane permeability

Cell membrane permeability was measured according to the method of Zhao et al. (2006) with slight modification. Cylinders of mango peel tissue were excised with a cork borer (5 mm diameter) of 4 fruit from each replicate. Two pieces of 4 mm thickness were cut from each cylinder and washed three times (2–3 min) with deionized water. Twelve pieces were placed in a glass vial with 25 mL deionized water and shaken at 1.7 s^{-1} at 25 °C for 30 min. Electrolyte leakage was determined by using a conductivity meter (Radiometer analytical Ion check 65, France). The glass vial was heated at 98 °C for 15 min in boiling water. After cooling the samples, the conductivity was again measured. Membrane permeability was calculated using the following formula:

$$\text{Electrolyte leakage\%} = \frac{\text{ion leakageat25°C}}{\text{ion leakageat98°C}} \times 100$$

2.6. Measurement of membrane lipid peroxidation

The malondialdehyde (MDA) content in mango peel was determined following the method of Dhindsa et al. (1981). One gram peel tissue were homogenized in 5 mL of 10% trichloroacetic acid (TCA) and centrifuged at 12,000 × *g* for 20 min. The supernatant 2 mL was added to 2 mL of 0.67% thiobarbituric acid (TBA), heated for 20 min at 100 °C in boiling water and then directly cooled down with ice. The solution was centrifuged at 3000 × *g* for