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Cold storage temperatures and durations affect the concentrations of lupeol, mangiferin, phenolic acids and other health-promoting compounds in the pulp and peel of ripe mango fruit

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Gallic acid (PubChem CID: 370)
Chlorogenic acid (PubChem CID: 1794427)
Vanillic acid (PubChem CID: 8468)
Ferulic acid (PubChem CID: 445858)
Caffeic acid (PubChem CID: 689043)
Ascorbic acid (PubChem CID: 54670067)

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ABSTRACT

Mangoes are usually stored above 13 °C to avoid chilling injury. We investigated the effects of cold storage temperatures (5 and 13 °C) and durations (12 and 24 d) on the concentrations of lupeol, mangiferin, phenolic acids (gallic, chlorogenic, vanillic, ferulic and caffeic), ascorbic acid, carotenoids, total phenols and antioxidants in the pulp and peel of ripe 'Kensington Pride' mango fruit. Mature green mangoes were stored at 5 °C (chilling) or 13 °C (non-chilling) temperature for 12 and 24 d prior to ripening at ambient temperature (21 ± 1.5 °C). Chilling injury and concentrations of health-promoting compounds were determined at eating soft ripe stage. Chilling injury symptoms were only developed on ripe fruit following storage at 5 °C for 24 d. The concentrations of lupeol in pulp and peel, chlorogenic and caffeic acids in the pulp were significantly higher in fruit stored at 5 °C than 13 °C, whilst mangiferin, gallic, chlorogenic, vanillic, ferulic, and caffeic acids, total phenols, antioxidants and carotenoids in the peel were significantly higher when stored at 13 °C. The concentrations of lupeol and chlorogenic acid in pulp and peel and gallic acid in the pulp were significantly lower when stored for 24 d compared to 12 d, whilst vanillic acid, total phenols, total antioxidants and ascorbic acid in the pulp and caffeic acid in both pulp and peel were significantly higher when stored for 24 d. In conclusion, cold storage temperatures and duration influence the concentration of lupeol, mangiferin, phenolic acids and other health-promoting compounds in the pulp and peel of ripe mango fruit. Storage of mature green mangoes at chilling temperature (5 °C) for 12 d prior to ripening (21 ± 1.5 °C) seems to be a promising tool for maximizing the levels of lupeol in the pulp and peel of the fruit.

1. Introduction

Mango (*Mangifera indica* L.) is globally known for its appealing taste and excellent nutritional quality. Additionally, particular health-promoting compounds present in this fruit are also known for their ability to reduce the risk of chronic health issues (Masibo and He, 2008). Lupeol and mangiferin are two such compounds with a significant protective potential. Lupeol, a triterpene is one of the most important anti-carcinogenic compounds present in mango, and has been found to be capable of reducing the risk of a number of serious human diseases including cancer, cardiovascular diseases, diabetes, liver toxicity and renal diseases (Saleem, 2009; Siddique and Saleem, 2011 Siddique and Saleem, 2011). Mangiferin, a glucosyl xanthone is also known for its wide range of health protective properties such as antioxidant, anticancer, antimicrobial, cardio-protective and anti-inflammatory (Masibo and He, 2008). Moreover, a number of studies have revealed that the pulp, peel, seed and other parts of mango tree are good sources of health-promoting compounds including gallic acid, chlorogenic acid, vanillic acid among many other polyphenolic antioxidants which have a well-known potential in reducing the risk of cancer and cardiovascular diseases (Ajila et al., 2007; Masibo and He, 2008; Kim et al., 2010). Mango fruit is also rich in other dietary antioxidants, such as ascorbic acid and carotenoids which contribute to its health promoting potential (Kim et al., 2007; Ma et al., 2011).

The storage life of mango fruit is extremely limited; with fruit usually ripen in a week after harvest at mature green stage at ambient temperature (Singh et al., 2013). Therefore; the mango fruit are usually stored under low temperatures to prolong storage life (Chaplin et al.,

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1991; Medlicott et al., 1990; Talcott et al., 2005). Cold storage technology; however, cannot be exploited to its full potential in extending storage life of tropical and subtropical fruit including mango because of their susceptibility to chilling injury. Mango fruit when stored below 13 °C develop chilling injury symptoms (Chaplin et al., 1991). Previously, the impact of low-temperature storage on chilling injury and physico-chemical parameters such as colour, pulp firmness, soluble solids concentration, acidity and total and individual sugars of mango fruit have been reported (Chaplin et al., 1991; Nair and Singh, 2009; Robles-Sánchez et al., 2009; Sankat et al., 1994). Some limited and inconclusive research has been reported on the impact of cold storage and chilling injury on the concentrations of health-promoting compounds such as ascorbic acid, total antioxidants, total carotenoids and total phenols in mango fruit (Kondo et al., 2005; Nair and Singh., 2009; Robles-Sánchez et al., 2009). However, no research work has been reported on the effects of low temperature storage on the concentrations of potential anticancer compounds such as lupeol, mangiferin and phenolic acids including gallic acid, chlorogenic acid and vanillic acid in the pulp and peel of mango fruit.

Given the potential health benefits of polyphenols, there have been recent reports in the use of physical elicitors (low temperature storage, heat treatment, controlled and modified atmosphere storage) and chemical elicitors (methyl jasmonate, salicylic acid and ethylene) as an effective tool to trigger their production in fruit and vegetables (Ruiz-Garcia and Gomez-Plaza, 2013; Schreiner and Huyskens-Keil, 2006). The low temperature stress is believed to induce the biosynthesis of polyphenols via the shikimic acid pathway as a part of the plant defence mechanism (Ruiz-Garcia and Gomez-Plaza, 2013). Previously, Rivera-Pastrana et al. (2010) claimed an increased level of total antioxidants and better retention of ferulic acid and caffeic acid in the chill-sensitive fruit papaya; when stored at 5 °C. Therefore, the effect of cold storage temperatures and periods on the levels of phenolic compounds in ripe mango fruit warrants to be investigated as a potential tool to enhance its health beneficial properties.

In this study, it was hypothesised that the chill-storage temperature would increase the concentrations of lupeol, mangiferin and phenolic acids (chlorogenic acid, gallic acid, vanillic acid, ferulic acid and caffeic acid) and other health-promoting compounds (ascorbic acid and carotenoids) as a response to low temperature stress. To the best of our knowledge this is the first study on the effect of chilling and non-chilling low temperature storage and period on the concentrations of lupeol, mangiferin and phenolic acids (gallic acid, chlorogenic acid, vanillic acid, ferulic acid and caffeic acid) in ripe mango fruit.

2. Materials and methods

2.1. Materials

2.1.1. Fruit

Hard green mature 'Kensington Pride' mango fruit (light cream pulp, firmness: 165 ± 1 N) were harvested from a commercial orchard in Gingin (31° 27′S, 115° 55′E), Western Australia and transported within 2 h to the laboratory on the 9th of March 2015. Only mango fruit free from visual symptoms of mechanical, chemical or insect-pest injuries and symptoms of disease(s) were used in the study. The selected fruit were treated with the fungicide (Sportak (0.55 ml L^{-1}) containing prochloraz as the active ingredient (Bayer CropScience Pty Ltd., Victoria, Australia) and allowed to dry. The fruit were packed into cardboard boxes and stored at either 5 °C or 13 °C at 85% ± 0.5% relative humidity in dark for either 12 d or 24 d. After each storage period at both temperatures, the fruit were allowed to ripen at ambient temperature (21 ± 1.5 °C) until eating soft stage (depending upon peel and pulp yellow colour > 75% and/or firmness: 7.0–9.0 N). The number of days taken for the fruit to reach the eating soft stage differed depending upon the treatment (Fig. 1). Six and nine days were taken to reach this stage by the fruit stored at 5 °C for 12 d and 5 °C for 24 d respectively. The fruit stored at standard low temperature storage (13 °C) for 12 and 24 days reached the eating soft stage in 4 and 5 days respectively. The experiment followed two-factor factorial design (storage temperature and storage duration). Ten mangoes were used for each treatment unit and replicated four times.

Once mangoes reached the eating soft stage, the chilling injury symptoms were recorded. The peel and pulp samples (cut in to small pieces) of 10 mango fruit in each replication were immediately stored at −80 °C for the later determination of lupeol, mangiferin, phenolic acids, total phenols, total antioxidants, ascorbic acid and total carotenoids. The concentrations of total phenols, total antioxidants, ascorbic acid and total carotenoids were determined using thawed samples of each replication. Some representative frozen, samples were freeze dried at −50 °C and 1×10^{-1} mB vacuum pressure (Telstar Cryodos V 1.0, Terrassa, Spain), powdered and stored at − 20 °C for the later determination of the concentrations of lupeol, mangiferin and phenolic acids.

2.1.2. Chemicals

All reagents and standards of lupeol, mangiferin, phenolic acids, β-carotene, L-ascorbic acid and Trolox were purchased from Sigma Aldrich (St. Louis, MO, USA) whilst methanol, acetonitrile and n-hexane were purchased from Thermo Fisher Scientific (Thermo Fisher Scientific, Taren Point, NSW, Australia). Only HPLC grade reagents and standards were used in the study.

2.2. Chilling injury (CI)

The level of chilling injury (CI) on the ripe mango fruit was recorded using the following rating scale previously described by Zaharah and Singh (2011); 0- no damage, 1- very light damage (< 5% of the surface damaged), 2- light damage (5–10% of the surface is damaged), 3- moderate damage (11–24%) and 4- severe damage (25–50% of the surface damaged). The chilling injury index was calculated using the following formula;

$$\frac{\Sigma(\text{Injury level} \times \text{number of fruit at each level})}{\text{Total number of fruit}}$$

2.3. Determination of the concentrations of health-promoting compounds

2.3.1. Lupeol

Lupeol was extracted from the freeze dried mango pulp/peel and quantified using an Agilent HPLC system (1200 series, Agilent Technologies, Ratingen, Germany) fixed with a diode array detector (1200 Infinity, Agilent Technologies). The method was developed based on the previous reports of Ruiz-Montañez et al. (2014) and Oliveira et al. (2012) with some modifications detailed in our previous paper (Vithana et al., 2017). The amount of lupeol was quantified using a standard curve and expressed as mg kg^{-1} dry weight basis.

2.3.2. Total phenols

The total phenol concentration was estimated following the method described earlier by Robles-Sánchez et al. (2009) using Folin-Ciocalteu reagent with slight modifications which have been described in our previous paper (Vithana et al., 2017). A UV/VIS spectrophotometer (Jenway spectrophotometer Model 6405, Dunmow, Essesx, UK) was used to record the absorbance at 750 nm. A gallic acid standard curve was used to calculate the total phenol concentration and the values were expressed in g GAE kg^{-1} fresh weight basis.

2.3.3. Mangiferin and phenolic acids

Determination of the concentrations of mangiferin and phenolic acids (gallic, chlorogenic, vanillic, ferulic and caffeic) was carried out following the method previously described by Palafox-Carlos et al. (2012) using Agilent HPLC system (Agilent Technologies) equipped