

FRUIT RIPENING AND QUALITY OF 'KENSINGTON PRIDE' MANGOES FOLLOWING THE CONTROLLED ATMOSPHERE STORAGE

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Abstract

Hard mature green 'Kensington Pride' mangoes were stored in normal air (NA) or in controlled atmosphere (CA) chambers with combinations of 3% oxygen (O₂) and three concentrations of carbon dioxide (CO₂) i.e. CA1: 3%, CA2: 4%, or CA3: 5% at 13°C for 4 weeks followed by ripening at 21°C. The effects of CA on fruit quality during ripening were investigated. At fully ripe stage, fruits stored in CA were firmer and greener with concomitant lower reduction of chlorophylls compare to those stored in NA. All CA-stored fruit retained its skin carotenoids during ripening. The reduction of sucrose and glucose concentrations were measured in all CA-stored fruit, and the same condition were also measured for citric acid and succinic acid content during ripening. However, concentration of glucose, citric acid and succinic acid in CA-stored fruit were higher than NA-stored fruit. Pulp carotenoids and ascorbic acid in all CA-stored fruit were higher compare to NA-stored fruit at the ripe stage. The data suggest that CA storage could improve the ripe quality of 'Kensington Pride' mango to some extend.

Key words: 'Kensington Pride', CA-stored mango, sucrose, glucose, citric acid, malic acid, ascorbic acid, carotenoids.

Introduction

The mango fruit is commercially harvested at the mature green stage. It is a climacteric fruit and ripens rapidly within 3 to 9 days after harvest (Gomez-Lim, 1997). The short postharvest life limits its distribution to various distant export markets. Controlled atmosphere (CA) storage combined with optimum temperature is widely used in extending the shelf life and maintaining the quality of fruit.

The air composition during CA storage of mango is considered to be the critical factor affecting its storage life and quality. CA has been reported to extend storage

life and maintain fruit quality in different mango cultivars such as 'Irwin' (Maekawa, 1990), 'Tommy Atkins' (Abdulah and Basiouny, 2000; Bender et al., 2000a; Lizana and Ochagavia, 1996), 'Haden' (Bender et al., 2000b), 'Keitt' (Gonzalez-Aguilar et al., 1997; Yahia and Hernandez, 1993), 'Kent' (Bender et al., 2000b; Lizana and Ochagavia, 1996), 'Rad' (Noomhorm and Tiasuwan, 1995), R2E2 (Lalel et al., 2006), 'Kensington Pride' (Dang et al., 2008; Lalel et al., 2001; Lalel et al., 2003; McLauchlan and Barker, 1992), and 'Palmer' (De Almeida Teixeira and Durigan, 2011). Storage atmosphere composition comprising of 3%

oxygen and 6% carbon dioxide has been commercially applied for exporting 'Kensington Pride' mango from Australia to United Kingdom by sea freight (Anonymous, 2002; Lalel, 2002). However, tissue injury, fruit softening, poor colour, and off-flavour developments in ripe mango fruit are listed as problems that arise following CA storage (Anonymous, 2002; Beaudry, 1999; Bender et al., 2000a; Bender et al., 2000b; Dang et al., 2008; Lalel et al., 2006). Most of the research work has been reported on the effects of CA storage on extending storage life and quality of mango fruit. No research work has been reported on the effects of the gas composition in CA storage on fruit firmness, colour changes, and nutritive value of 'Kensington Pride' during ripening, which prompted this investigation.

Materials and methods

Fruit and experimental conditions

Hard mature green 'Kensington Pride' mangoes were sourced from a commercial orchard located at Chittering (long. 116°5'E, lat. 31°25'S), Western Australia. Uniformly size and maturity fruit, free from visual symptoms of any diseases or blemishes were stored in normal atmosphere (NA) as control and in 90-litre chambers of controlled atmosphere (CA) containing 3% O₂ and 4% CO₂ (CA1), 3% O₂ and 5% CO₂ (CA2), and 3% O₂ and 6% CO₂ (CA3) at 13 ± 0.5°C and 85 ± 3% RH. Concentration of O₂ and CO₂ in the CA chambers were adjusted with N₂. The CA storage was a continuous gas flow with opened ended system, and the conditions were maintained and monitored by a Gas Analyser (ADC 7000 series, Analytical Development Company Ltd., Hoddesdon, Herts, UK). A single chamber containing 10 fruit was treated as one treatment unit and replicated three times. The fruit were removed after 4 weeks of storage and allowed to ripen under ambient conditions at 21 ± 1°C to eating soft stage.

Measurement of fruit firmness

Firmness of the whole fruit after CA storage and ripening was measured on two opposite peeled surfaces in the equatorial region by electronic pressure tester (model EPT-1 pressure tester, Lake City Technical Products Inc., Kelowna, B, Canada). The apparatus was fitted with an 11 mm-diameter plunger. The firmness was expressed in Newton.

Fruit colour assessment

Spectrophotometer and visual measurements were used in assessing the fruit skin colour. Individual fruit from each replication was measured everyday during ripening. ColorFlex 45°/0° spectrophotometer (Hunter Associates Laboratories, Inc., Reston, Virginia, U.S.A) was used to assess the skin colour at the equatorial region of the fruit. The chromaticity of the fruit skin were assessed in L*, a*, and b* values, followed by calculations for the chroma (C*) and hue angle (hue°). The visual evaluation was conducted by scoring the percentage of yellowness developed on fruit skin as described by (Shorter and Joyce, 1998) and (Lalel, 2002). The value was scored from 1 to 5 as follow: 1 = 100% green, 2 = 75% green, 3 = 50% green/yellow, 4 = 75% yellow, and 5 = 100% yellow.

Skin pigment analysis

Skin pigments were determined according to Lichtenthaler (1987) with some modifications. The chlorophylls (a, b, and total) and carotenoids from the extract were estimated by measuring the absorbance at 663.2, 646.8, 470, and 750 nm, respectively using spectrophotometer (6405 UV-VIS, Jenway Ltd., Essex, U.K.), and calculated in µg·g⁻¹ FW skin. All the extraction procedure was performed in the dark room.

Determination of sugars and organic acids

High performance liquid chromatography (HPLC Waters, Milford, MA, USA) was used in sugar and organic acid determination. The analysis of sugar content was performed using sugar

standard (sucrose, glucose, or fructose) and samples injected through the fast carbohydrate column (100 mm x 7.8 mm) as the stationary phase with water as the mobile phase. The flow rate was maintained at $1.2 \text{ mL}\cdot\text{min}^{-1}$ and the elution was completed at 10 min. The absorbance of the effluent was recorded at 410 nm (Waters 2414 Refractive Index Detector), and the individual sugar concentration was calculated base on the standard curve ($R^2 = 0.9999$ to 1.0000). Standards of citric, malic, succinic were used in the determination of organic acids composition. Both standards and samples were injected through the Aminex® HPX-87H ion exclusion column (300 mm x 7.8 mm) preceded by a Cation-H Bio Rad Micro-Guard column (30 x 4.6 mm) as the stationary phases with water and sulphuric acid (0.5 mM) as the mobile phases. The flow rate was $0.3 \text{ mL}\cdot\text{min}^{-1}$ (50% water and 50% sulphuric acid) and the elution time was 20 min. The absorbance of the effluent was recorded at 210 nm (Waters 2487 Dual Wave length Absorbance Detector), and the individual acid concentration was calculated base on the standard curve ($R^2 = 0.9999$). The sugar and acid concentration was expressed in $\mu\text{g}\cdot\text{g}^{-1}\text{FW}$ pulp.

Determination of pulp carotenoids and ascorbic acid

Mango pulp total carotenoids were estimated according to the method of Tomes (1963) and Lalel (2002). The absorbance was recorded at 436 nm using a spectrophotometer (6405 UV/VIS, Jenway Ltd., Essex, U.K.), calculated against standard curve of β -carotene and the concentration was expressed as $\mu\text{g}\cdot\text{g}^{-1}$ FW of pulp. The concentration of fruit pulp ascorbic acid was determined following the method of Jagota and Dani (1982), AOAC (1996), and Malik (2003). The absorbance at 760 nm was recorded by spectrophotometer (6405 UV-Vis, Jenway Ltd., Essex, U.K.). The quantification of ascorbic acid in mango

pulp was based on the standard curve of *L*-ascorbic acid and expressed as mg ascorbic acid per 100 g FW pulp.

Statistical analysis

The data were subjected to analysis of variance (ANOVA) using Genstat 9 release 9.1.0.147 (Lawes Agricultural Trust, Rothamsted Experimental Station, Rothamsted, U.K.). The effects of treatments and their interactions on parameters were assessed within ANOVA and least significant differences (Fisher's protected LSD) were calculated at $P \leq 0.05$ following a significant F test. The validity of analysis was confirmed by checking all the assumptions of analysis.

RESULTS

Fruit firmness

The ripening time significantly ($P \leq 0.05$) affected the fruit firmness irrespective of storage atmosphere. The interaction between storage atmospheres and ripening time did not significantly ($P \leq 0.05$) influence the fruit firmness (Figure 1). During ripening, the firmness of fruit from NA storage decreased by 25% within 2 days. The fruit stored in CA1 and CA2 started to loss their firmness after one day ripening period (30.12% and 23.30%, respectively). However, control fruit stored in NA were not significantly firmer than those stored in CA when ripe at day 4, and no significant changes were noticed in CA3-stored fruit during ripening.

Fruit skin colour and pigments

Fruit colour lightness (L^*), a^* , b^* , and colour saturation (C^*) significantly ($P \leq 0.05$) higher in NA-stored fruit than in CA-stored fruit started from day 0 throughout the ripening period (Figure 2). More yellow colour development was also supported by lower hue angle value exhibited in NA-stored fruit than in CA-stored fruit. During ripening, no significant colour development were noticed in L^* , a^* , b^* , C^* , and hue angle of

CA3-stored fruit. Visually, fruit stored under NA and CA conditions showed highest yellow colour development at day 4 in ripening although it was not significant ($P \leq 0.05$) between CA1- and CA2-stored fruit, and CA2- and CA3-stored fruit. No significant ($P \leq 0.05$) interaction between storage atmospheres and storage time was noticed through spectrophotometer but visual observation.

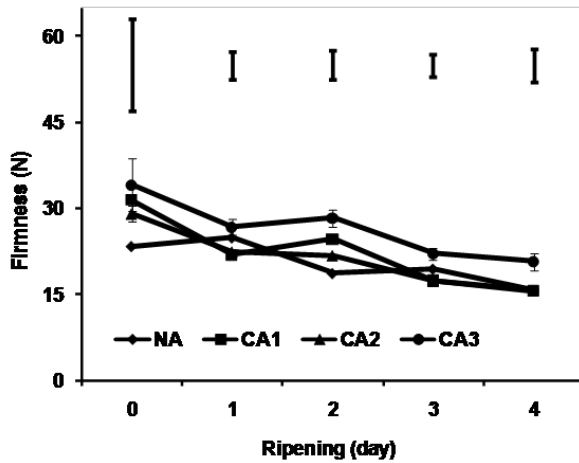


Figure 1. Effects of storage atmospheres (SA) and ripening time (RT) on firmness of ‘Kensington Pride’ mango fruit.

The levels of chlorophylls and carotenoids in fruit skin were significantly ($P \leq 0.05$) affected with storage atmosphere and ripening time. NA-stored fruit exhibited the highest decrease in total chlorophylls (about 45%) at first day of ripening whereas 24% loss was observed in CA3-stored fruit at the same day. CA1- and CA2-stored fruit showed decreasing chlorophylls levels (24% and 26%, respectively) after second day of ripening. Further decrease in the total chlorophylls level was recorded in CA2-stored fruit at third day of ripening but fruit from other storage treatments showed no significant decrease. Comparing the loss of total chlorophylls from the beginning towards the end of ripening period, CA3-stored fruit showed the highest reduction ($127.15 \mu\text{g}\cdot\text{g}^{-1}$ FW skin), followed by NA-stored fruit ($111.65 \mu\text{g}\cdot\text{g}^{-1}$ FW), CA2- stored fruit ($100.84 \mu\text{g}\cdot\text{g}^{-1}$ FW), then CA1-stored fruit

($71.82 \mu\text{g}\cdot\text{g}^{-1}$ FW). In spite of its highest reduction in total chlorophylls levels, CA3-stored fruit demonstrated the highest total chlorophylls level compare to other storage atmosphere treated fruit during all ripening time (Figure 3.).

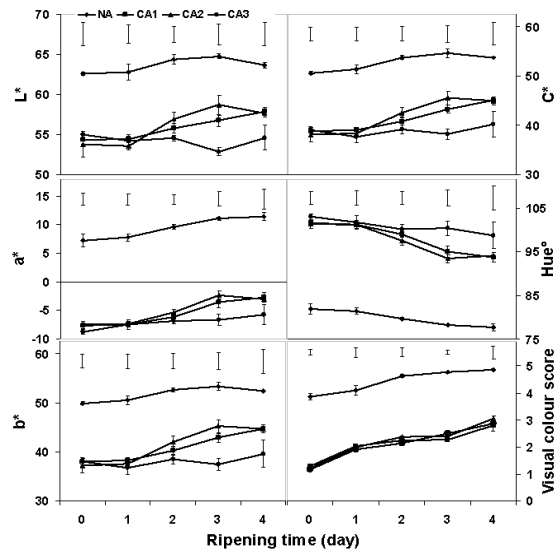


Figure 2. Effects of storage atmospheres (SA) and Ripening time (RT) on fruit skin colour during ripening.

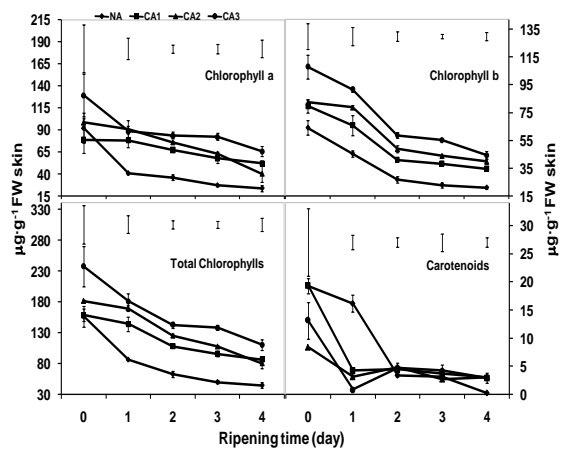


Figure 3. Effects of storage atmospheres (SA) and ripening time (RT) on skin chlorophyll and carotenoid levels in mango fruit during ripening.

The significant declining amount of carotenoids was observed in NA-stored

fruit during four days ripening period but not in CA-stored fruit. CA1- and CA3-stored fruit showed significantly decreased carotenoids levels at the first day of ripening and remained constant throughout the rest of ripening period. Carotenoids in all CA-stored fruit showed significantly higher carotenoids levels (2.97, 3.06, and 2.92 $\mu\text{g}\cdot\text{g}^{-1}$ FW in CA1-, CA2-, and CA3-stored fruit, respectively) than NA-stored fruit (0.23 $\mu\text{g}\cdot\text{g}^{-1}$ FW) at day 4, although no significant difference between them at the beginning and at day 3 and 4 of ripening time (Figure 3.). Interaction between storage atmosphere and ripening time did not significantly ($P \leq 0.05$) affect fruit skin pigments.

Sugars and organic acids composition

Sugars and organic acids compositions during ripening of CA-stored fruit were presented in figure 4 and 5, respectively. The sucrose level in mango pulp was not significantly ($P \leq 0.05$) affected by storage atmosphere and ripening time. However, sucrose level reduced significantly at the end of ripening period in all storage treatments. CA1-stored fruit lost about 29%, followed by NA-stored fruit (28%), CA3-stored fruit (26%), and the lowest loss was exhibited in CA2-stored fruit (16%) of its sucrose level when fully ripe at day 4. Statistically, no significant differences of sucrose content between NA-stored fruit and CA2-stored fruit, and among CA-stored fruit at ripe stage.

Different storage atmospheres significantly ($P \leq 0.05$) affected glucose level. However, ripening time did not significantly affect its level (Figure 4). Glucose in NA-stored fruit was not detected after 4 weeks storage and following 3 days in ripening period which finally increased significantly at day 4. CA1- and CA2-stored fruit exhibited non-significant changes during ripening time, whilst CA3-stored fruit showed significant change at day 3 and remained stable to day 4 of ripening period. Comparing the glucose levels in different storage atmospheres treated fruit at day 4, no

significant differences among NA-stored, CA1-, and CA2-stored fruit; and among all CA-stored fruit

Storage atmospheres and ripening time significantly ($P \leq 0.05$) affected fructose level in KP mango fruit. Fruit from NA, CA1 and CA3 storage did not show significant difference in fructose levels at the end of storage (Figure 4). NA-stored and CA2 stored fruit also showed no significant difference in their fructose level before ripening period. The fruit stored in different atmospheres did not exhibit significant difference of fructose content at day 1 to day 3 during ripening. However, CA3-stored fruit showed significantly lower fructose content (34.65 $\mu\text{g}\cdot\text{mg}^{-1}$ FW pulp) than NA-, CA1-, and CA2-stored fruit (38.75, 39.69, and 42.30 $\mu\text{g}\cdot\text{mg}^{-1}$ FW pulp, respectively). The interaction between storage atmosphere and ripening time did not significantly ($P \leq 0.05$) affect fructose level in mango fruit pulp.

Different storage atmospheres and ripening time significantly ($P \leq 0.05$) affected citric and succinic acids levels in fruit pulp (Figure 5). Citric acid level in NA- and all CA-stored fruit decreased significantly as the ripening started at the first day. The level of citric acid declined continuously in NA-stored fruit until the third day of ripening and remained unchanged to day 4. CA1-stored fruit also showed significant decrease in citric acid level on day 2 but stable on day 3 before decreasing again on day 4. CA2- and CA3-stored fruit did not show significant loss in citric acid levels after second day but on the fourth day of ripening period. More than 50% loss was noticed in NA-, CA1- and CA2-stored fruit, and only 37% was noticed in CA3-stored fruit at the end of ripening time. At the ripe stage, the citric acid level in all CA-stored fruit was significantly higher than NA-stored fruit.

No significant effects of storage atmospheres and storage time on malic acid levels in mango fruit pulp. However, the interaction between storage atmospheres and storage time significantly

($P \leq 0.05$) affected malic acid levels. The fruit stored in CA1 exhibited significant change in malic acid level at 4 days ripening whilst CA3-stored fruit started at day 2 and remained unchanged throughout the observation. At the end of ripening period, malic acid level in NA-stored fruit was $4.03 \mu\text{g}\cdot\text{mg}^{-1}$ which was not significantly different from that in CA1-stored fruit ($3.74 \mu\text{g}\cdot\text{mg}^{-1}$). All CA-stored fruit exhibited similar amount of malic acid at the ripe stage.

All storage atmosphere treatments showed significant ($P \leq 0.05$) decrease in succinic acid level during ripening. CA3-stored fruit underwent the highest loss (52.7%), followed by NA-stored fruit (44%), CA1-stored fruit (39%), and then CA2-stored fruit (21%). The succinic acid level in fully ripe CA1- and CA3- stored fruit was not significantly different.

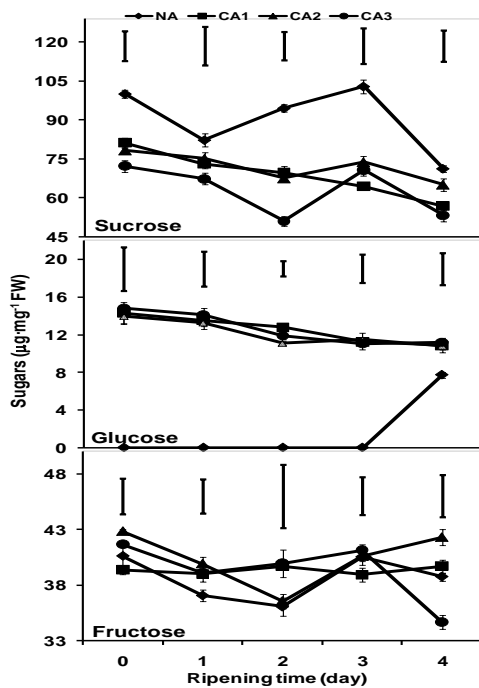


Figure 4. Effects of storage atmosphere (SA) and Ripening time (RT) on levels of different sugars in mango fruit pulp during ripening.

Carotenoids and ascorbic acid

Different storage atmospheres and storage time significantly ($P \leq 0.05$) affected

carotenoids levels in mango pulp. The interaction between storage atmospheres and storage time also significantly ($P \leq 0.05$) influenced carotenoids levels (Figure 6).

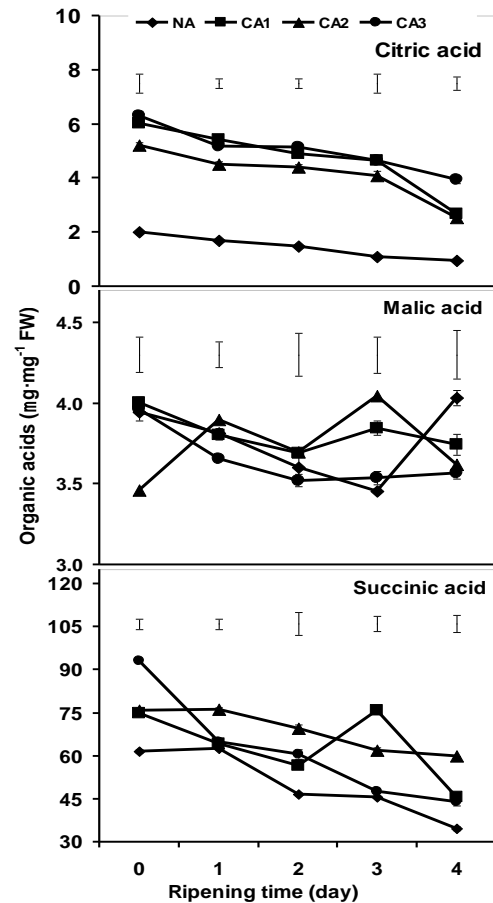


Figure 5. Effects of different storage atmospheres (SA) and ripening time (RT) on different organic acids in mango fruit pulp during ripening.

The fruit exhibited significantly increasing carotenoids levels at the first day of ripening. All fruit attained their highest carotenoids levels at day 4 although they exhibited significantly different amount. At the beginning of ripening stage, NA-stored fruit contained the highest amount of carotenoids ($53.86 \mu\text{g}\cdot\text{g}^{-1}$), followed by CA1 ($22.24 \mu\text{g}\cdot\text{g}^{-1}$), CA2 ($18.47 \mu\text{g}\cdot\text{g}^{-1}$), and then CA3-stored fruit ($13.01 \mu\text{g}\cdot\text{g}^{-1}$). However, all CA-stored fruit produced carotenoids more than 2 folds (2.2, 3.6, and 2.7 folds in CA1, CA2, and CA3 treated fruit, respectively) whilst NA-

stored fruit only gained 1.6 folds at day 4 in ripening period compare to the fruit after storage.

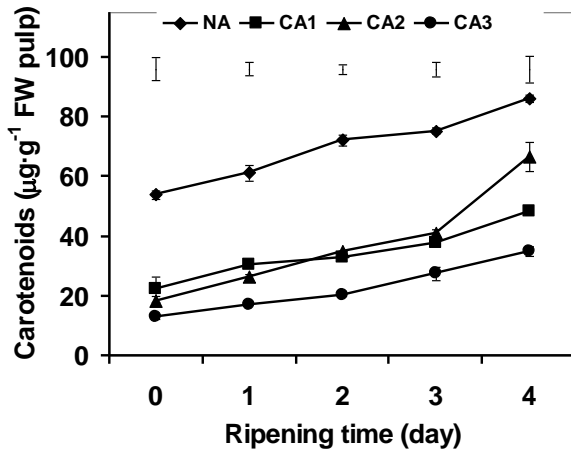


Figure 6. Effects of storage atmosphere (SA) and ripening time (RT) on carotenoid content in mango fruit pulp during ripening.

Despite the ripening time, storage atmospheres significantly ($P \leq 0.05$) influenced the ascorbic acid levels in KP mango pulp. Higher amount of ascorbic acid were recorded in all CA-stored fruit compare to NA-stored fruit during ripening period. However, CA-stored fruit gained less than 1% of ascorbic acid whereas NA-stored fruit produced about 24% of this acid during ripening period (Figure 7).

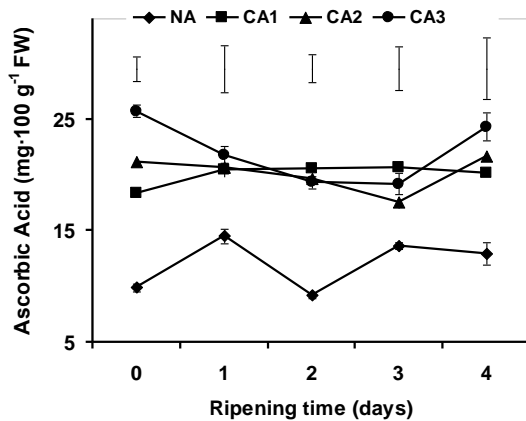


Figure 7. Effects of storage atmosphere (SA) and ripening time (RT) on ascorbic acid content in mango fruit pulp during ripening.

Discussion

Fruit from CA storage comprising of 3% O₂ and 6% CO₂ maintained its firmness better than fruit from other CA and NA conditions until day 4 during ripening.

Although higher rate of fruit softening occurred during ripening in all CA-stored fruit as compared to the NA-stored, no significant differences in ripe fruit firmness were noticed irrespective of the storage atmospheres. This results support those reported by (Yahia and Hernandez, 1993) that the fruit firmness reduced significantly during ripening of CA-stored fruit.

The CA storage has been reported to retard skin colour changes in different mango cultivars (Gonzalez-Aguilar et al., 1997; Lalel et al., 2005; McLauchlan and Barker, 1992; Noomhorm and Tiasuwan, 1995). The delay in yellow colour development during ripening of all CA-stored fruit may be resulted from the residual effect of CA. Chlorophylls degradation was recorded both in CA- and NA-stored fruit during ripening although it was less pronounced in CA-stored fruit than in fruit kept at NA. Contrarily, the carotenoids levels were decreased during ripening. These incidences indicated that the ripening process of the fruit started during storage and continued to ripening under normal air. High CO₂ (5% or 6% CO₂) in CA storage reduced the degradation of chlorophylls and maintained the carotenoids levels better than other storage atmosphere conditions during ripening (Figure 2). Storage under normal air at 20°C resulted in decreasing chlorophylls has been reported by Wang et al (2008) which in contrast to the effects of low O₂ and/or high CO₂ storage that reduced the breakdown of chlorophylls (Thompson, 1996; Lalel et al., 2005; Rao and Rao, 2008; Bender et al., 2000c). Reducing chlorophylls and carotenoids during ripening of CA stored fruit may be due to senescence process (Kays and Paull, 2004; Jha et al., 2006; Kays, 1991; Mitra and Baldwin, 1997; Thomas, 1975) that

proceeds under normal atmosphere and temperature.

The fruit skin colour is the reflection of pigments (Kays and Paull, 2004). The reduced carotenoids synthesis is reflected in a paler skin colour (Chaplin et al., 1991) and lower chroma value (Brecht et al., 2003). Carotenoids synthesis as reflected in yellow colour development was almost unnoticed visually during CA storage (Figure 2). In contrary, increase in visual colour score accompanied by decreasing carotenoids was noticed in ripe fruit despite of storage atmospheres (Figure 3). CA storage can retard colour development in 'Kensington Pride' mango (McLauchlan and Barker, 1992) but promotes colour development during ripening of 'Delta R2E2' mango (Lalel, 2005). The inferior colour development was maybe due to the inhibition action of CA and low temperature on carotenoid pigments formation (de Almeida Teixeira, 2011; Singh and Singh, 2012). The retardation of 'Palmer' mango fruit colour development during CA storage has been reported by de Almeida Texeira and Durigan (2011). The increasing yellowness of visual colour may be due to the carotenoids levels in mango pulp (Figure 6) as reported by Vásques-Caicedo et al (2005).

Sucrose increased during ripening (Castrillo et al., 1992) and was reported as the main sugar in the ripe mango (Ito et al., 1997; Mitra and Baldwin, 1997) followed by fructose as the major reducing sugar (Castrillo et al., 1992; Ito et al., 1997). Huyskens-Keil et al (2006) also reported that the CA condition did not affect the carbohydrate metabolism of pepino (*Solanum muricatum* Ait.) fruit during storage.

The major sugar upon the retrieval of mango fruit from all storage conditions was sucrose, suggesting that the fruit were ripened during storage (Castrillo et al., 1992; Ito et al., 1997). Sucrose level in all fruit reduced during ripening at ambient temperature but presented as the highest contributor in total sugars, followed by

fructose and glucose (Figure 4). However, the sucrose level in fruit from CA storage was lower than those from NA storage and conversely to the level of glucose, showing the hydrolysis of sucrose to its reducing forms. Several studies on different mango cultivars revealed that different cultivars demonstrated different levels of sugars (Mitra and Baldwin, 1997) and CA conditions may have impeded the glycolytic pathway during ripening as reported by Burg (2004).

After 4 weeks of storage, fruit ripened at ambient temperature and normal atmosphere for 4 days showed decreased amount of citric and succinic acids, whereas no significant changes in malic acid regardless of the storage conditions. This may be related to the nature of the temporary inhibition effects of CA storage on the respiration and ripening process. When the fruit regained their normal metabolism, the TCA cycle process returned to normal and the enzymes involved in this process resumed their activity (Mir, 2002). Decreased in levels of citric and succinic acid during ripening of mango fruit has also been reported by Lizada (1993).

On the other hand, high rate of carotenoids synthesis but lower carotenoids level was noticed in all CA-stored fruit compare to NA stored after 4 days in ripening. Brecht et al (2003) reported that the development of the pulp colour was resumed CA-stored 'Keitt' mango ripened in air for 3 days. The carotenoids synthesis in mango pulp may have been reflected as the colour of the fruit during ripening (Vásques-Caicedo, 2005) due to the transparency of the skin when chlorophylls was degraded to pheophoride and skin carotenoids were oxidised (Kays and Paull, 2004).

CA storage affects significantly the ascorbic acid during ripening (Figure 5). All fruit from CA storage maintained high level of ascorbic acid in ripening. These results suggested that CA conditions preserved the ascorbic acid better than NA during ripening of 'Kensington Pride'

mango which were in contrast to the general trend in mango reported by Tefera et al (2007).

In conclusion, CA storage comprise of 3% O₂ and 5% CO₂ appears to be the promising composition to extend the storage- and shelf-life of 'Kensington Pride' mango fruit up to 4 weeks, while allows fruit to ripen normally with the development of yellow skin colour, increases sugars and reduces organic acids, and high level of carotenoids and ascorbic acid within 4 days.

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