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# Antioxidant phytochemical and fruit quality changes in mango (Mangifera indica L.) following hot water immersion and controlled atmosphere storage

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#### Abstract

Tropical fruits such as mangoes destined for import into the United States are commonly required to have a thermal treatment against invasive pests, which could be combined with controlled atmosphere (CA) storage to prolong shelf life and preserve fruit quality. Changes in antioxidant phytochemicals and resultant quality during storage and ripening were investigated in fresh mangoes, as influenced by application of CA in combination with a hot water immersion quarantine treatment (46  $^{\circ}$ C for 75 min). Mature-green mangoes with or without a hot water treatment, were held in air,  $3\%$  O<sub>2</sub> + 97% N<sub>2</sub>, or  $3\%$  O<sub>2</sub> + 10% CO<sub>2</sub> + 87% N<sub>2</sub> and evaluated for external quality and phytochemical differences after storage for 2 weeks at 10  $\degree$ C and after subsequent ripening in air at 25  $\degree$ C. Visible appearance of anthracnose during ripening was effectively inhibited by the hot water treatments combined with CA. Concentrations of gallic acid and numerous hydrolysable tannins and their resultant antioxidant capacity were unaffected by the hot water treatment, while total polyphenolics naturally decreased throughout fruit ripening, regardless of hot water treatment or storage atmosphere. However, the overall decline in polyphenolic concentration was inhibited by the CA treatments, as a result of delayed ripening. Quality parameters such as flesh colour and titratable acidity provided supporting evidence that the CA conditions helped to delay fruit ripening. 2007 Elsevier Ltd. All rights reserved.

Keywords: Mango; Polyphenolics; Antioxidant capacity; Phytonutrients; CA storage; Hot water treatment

#### 1. Introduction

Mango (Mangifera indica. L) is a popular tropical fruit all over the world due to its bright colour, characteristic taste, and nutritional value. Mango imports to the United States (US) and Europe have grown steadily in response to increased demand and affordable prices and the US alone, between 1996 and 2004, experienced a 40% increase in imports [\(Sauco, 2004](#page-7-0)). Even though several cultivars such as Tommy Atkins, Keitt, and Kent are commercially cultivated in Florida and Hawaii, this production (2800 MT)

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accounted for less than 1% of domestic consumption in 2004 [\(FAO, 2004\)](#page-7-0). The majority of fruit consumed in the US (278,422 MT) is imported from Mexico (about 70% in 2000), followed by Brazil, Ecuador, Peru, Haiti, and Guatemala [\(Sauco, 2004\)](#page-7-0). Mangoes destined for import into the US may be infested with various species of fruit flies, such that fruit must be subjected to a thermal quarantine treatment involving complete hot water immersion for a specified time and temperature [\(USDA-APHIS, 2002\)](#page-7-0). Controlled atmosphere (CA) storage is used to maintain mango fruit quality, slow fungal decay development, and to extend postharvest life during transportation and storage [\(Bender, Brecht, Sargent, & Huber, 2000; Lalel, Singh,](#page-7-0) [& Tan, 2005; Noomhorm & Tiasuwan, 1995\)](#page-7-0). However, the effects of hot water treatment in combination with

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CA storage and the resultant change in quality and antioxidant polyphenolics have not been evaluated in mango and may prove to be an effective combination to maintain fruit quality and inhibit ripening prior to distribution.

When consumed regularly, mango can be a valuable dietary source of many phytochemical compounds, which also impart a characteristic colour and flavour to the fruit [\(Grundhofer, Niemetz, Schilling, & Gross, 2001; Haard](#page-7-0) [& Chism, 1996](#page-7-0)). Among these compounds, polyphenolics are widely distributed secondary metabolites that serve as the predominant antioxidant compounds present. Several studies have reported polyphenolic compounds in mango flesh and peel, including various flavonoids, xanthones, phenolic acids, and gallotannins ([Berardini, Knodler,](#page-7-0) [Schieber, & Carle, 2005a; Berardini et al., 2005b; Schieber,](#page-7-0) [Ullrich, & Carle, 2000](#page-7-0)). Among these compounds, gallic acid and hydrolysable tannins, which are most likely gallotannins, constitute the major antioxidant polyphenolics found in mango.

Mangoes are a climacteric fruit with a limited shelf life, the quality of the fruit rapidly decreases once fully ripe. Methods to extend fresh fruit shelf life have included reduced storage temperature, external coatings, and modified and controlled atmospheres, yet no studies have demonstrated the relationship between thermal quarantine treatment followed by CA storage on antioxidant and phytochemical concentrations.

#### 2. Materials and methods

# 2.1. Fruit preparation, postharvest treatments, and extraction

Mangoes (cv. Tommy Atkins) were kindly donated by Peter Schnebly of Fresh King. Inc., Homestead, Florida in June 2004, at the peak of the mango harvest season. Fruit (234) of uniform size (500 g), firm texture, oval shape, and green colour were pre-selected to help reduce variation among treatments. The fruit were held at  $10\,^{\circ}\mathrm{C}$  for 48 h until the hot water treatment and CA storage was applied. Untreated fruit (18) were retained as initial controls (mature-green) and USDA-APHIS guidelines were followed for hot water treatment, by immersing half of the remaining fruit in hot water  $(46.1 \degree C)$  for 75 min, with the remaining half immersed in water  $(25 \degree C)$  as a nonheated control. Following treatment, the fruit were held at 25 °C until the skin was completely dry and the core temperature returned to 25  $\rm{^{\circ}C}$  (about 60 min). Fruit from each treatment were then divided equally and transferred to CA storage chambers previously calibrated to establish the specified gas composition: CA1 (Control) at  $21\%$  O<sub>2</sub> + 79% N<sub>2</sub>, CA2 at 3% O<sub>2</sub> + 97% N<sub>2</sub>, and CA3 at 3% O<sub>2</sub> +  $10\%$  CO<sub>2</sub> + 87% N<sub>2</sub>. These conditions are in the range for gas compositions previously reported to suppress respiration rates of mango during storage [\(Kader, 2003; Lalel](#page-7-0) [et al., 2005; Nakamura, Sudhakar Rao, Shiina, & Nawa,](#page-7-0) [2003\)](#page-7-0). Gas composition was regulated and maintained by three external gas tanks (air,  $CO_2$ , and  $N_2$ ), which were mixed at constant pressure, via a gas mixing board equipped with needle valve flow meters. The fruit were stored for 2 weeks at 10  $\degree$ C with the gas mixtures monitored twice daily to ensure desired composition. After 2 weeks, half of the mangoes from each atmospheric treatment, with and without the hot water treatment, were removed and immediately prepared for analysis. The remaining fruit were transferred to a  $25^{\circ}$ C chamber and allowed to complete their ripening in air. Fruit for analysis were manually peeled and the edible flesh homogenised into puree, using a kitchen-scale food processor, prior to frozen storage. Pulp was stored at  $-20$  °C for up to 30 days prior to physicochemical analyses, whereby mango pulp was thawed and  $5 g$  incubated with  $20 \mu l$  of pectinase (Aspergillus aculeatus, 26,000 units/ml, Sigma Chemical Co., St. Louis, MO) at  $35^{\circ}$ C for  $3 h$  ([Kashyap, Vohra,](#page-7-0) [Chopra, & Tewari, 2001](#page-7-0)), to facilitate juice release and to help remove insoluble fibre. The puree was then centrifuged until a clarified supernatant was obtained for analysis.

## 2.2. Chemical analysis

Individual polyphenolics were identified and quantified by HPLC, as previously described by [Talcott, Moore,](#page-7-0) [Lounds-Singleton, and Percival \(2005\)](#page-7-0). The clarified juice was filtered through a  $0.45 \mu m$  PTFE filter (Whatman, Clifton, NJ) and directly injected into a Waters 2695 Alliance chromatography system (Waters Corp., Milford, MA). Compounds were separated on a Waters Spherisorb ODS2 column and polyphenolics detected and quantified at 280 nm, using a Waters 996 photodiode array (PDA) detector, against an external standard of gallic acid. Unknown compounds were characterised based on retention time and UV spectral similarities to authentic standards (Sigma Chemical Co.) using a Millennium 32 workstation.

Total soluble phenolics, including contributions from Lascorbic acid, reducing sugars, and soluble proteins, were determined by the Folin-Ciocalteau assay [\(Swain & Hillis,](#page-7-0) [1959\)](#page-7-0) against an external standard of gallic acid (mg/l). The optical density (OD) values were recorded on a Molecular Devices Spectramax 96-well microplate reader (Softmax Pro, Sunnyvale, CA) at 726 nm. Hydrophilic antioxidant capacity of mango phytochemicals was measured using the oxygen radical absorbance capacity assay, run according to [Talcott and Lee \(2002\),](#page-7-0) adapted to work with a 96 well Molecular Devices fmax<sup>®</sup> fluorescent microplate reader (485 nm excitation and 538 nm emission), against a chemically-induced peroxyl radical ([Ou, Hampsch-](#page-7-0)[Woodill, & Prior, 2001](#page-7-0)). Data were expressed in umol Trolox equivalents (TE) per gram of clarified juice.

# 2.3. Quality indices analysis

External quality of mango fruit was determined on day of harvest (mature-green), after 2 weeks in  $10^{\circ}$ C storage

(mid-ripe), and after ripening in air at 25  $\rm{^{\circ}C}$ , prior to fruit deterioration (full-ripe). Due to treatment variables that influenced ripening rates, fruit treated with hot water were held for an additional 7 days in air at 25 °C following 10 °C storage, while non-heated controls were only held for 3 days. Flesh colour was determined from the mango puree and expressed as CIE colour values  $(L^*, a^*,$  and  $b^*)$ , using a Minolta Chroma Meter CR 200 Series with an 8 mm aperture (Minolta Co., Ltd., Osaka, Japan). Hue angle and chroma values were calculated from  $a^*$  and  $b^*$ , using the method described by López and Gómez (2004). Titratable acidity was measured in clarified juice by titrating against 0.1 N NaOH to pH 8.2, using phenolphthalein as an indicator, as described by [Jacobi, Macrae, and Hethe](#page-7-0)[rington \(2000\),](#page-7-0) with data expressed in citric acid equivalents.

#### 2.4. Statistical analysis

Changes in physicochemical characteristics as influenced by hot water treatment, controlled atmosphere, and stage of ripeness were analysed by analysis of variance (ANOVA) and Pearson correlations, using JMP 5 statistical software ([SAS Institute, 2002\)](#page-7-0). Mean separations were conducted using the LSD test,  $P \le 0.05$ .

## 3. Results and discussion

# 3.1. External fruit quality affected by hot water treatment and CA storage

The fruit descriptors used (mature-green, mid-ripe and full-ripe) were based on visual observations for ripening, as well as changes to titratable acidity during ripening. The acidity of mangoes will gradually decrease as a mango ripens ([Jacobi et al., 2000\)](#page-7-0) and, likewise, average titratable acidities of non-treated fruit were 1.35%, 0.86% and 0.47% at mature green, mid-ripe and full-ripe stages, respectively.

The hot water immersion treatment, required for almost all mangoes imported to the US, was effective in decreasing the severity of many common physiological skin disorders, such as darkened lenticels (small black spots) and anthracnose, a fungus that is the most common postharvest disease affecting mangoes. Non-heated fruit exhibited appreciable signs of external quality defects after 2 weeks of  $10^{\circ}$ C storage, which increased in severity as the fruit completed ripening in ambient conditions. In contrast, the hot water-treated fruit exhibited fewer external skin defects through the full ripe stage, as previously reported [\(Jacobi,](#page-7-0) [Wonga, & Giles, 1995; Jacobi & Giles, 1997\)](#page-7-0).

The conditions of CA storage were also effective for inhibiting anthracnose and other surface disorders. Among the treatments, the air control (CA1) developed more visible anthracnose, compared to fruit in CA2 ( $3\%$  O<sub>2</sub> + 97%) N<sub>2</sub>) and CA3 (3% O<sub>2</sub> + 10% CO<sub>2</sub> + 87% N<sub>2</sub>), after 2 weeks at 10  $\degree$ C, with appreciably less incidence observed in CA3 fruit. Elevated  $CO<sub>2</sub>$  concentrations were previously observed to slow fruit ripening and decay more than simply lowering  $O_2$  concentrations ([Tian, Jiang, Xu, & Wang,](#page-7-0) [2004](#page-7-0)). CA storage has also been shown to decrease ethylene production and may reduce fruit sensitivity to ethylene, and thus slow the ripening process ([Kader, Zagory, & Ker](#page-7-0)[bel, 1989; Kader, 2002; Mitra & Baldwin, 1997](#page-7-0)). Even after moving fruit to ambient conditions for up to 7 days, CA storage seemed to have a residual effect on the development of anthracnose in visual observations. Furthermore, the hot water treatment, in combination with the high  $CO<sub>2</sub>$ concentration (CA3), served to synergistically improve external fruit quality and extend shelf life.

## 3.2. Antioxidant and polyphenolic effects of hot water

Gallic acid was identified as the major polyphenolic present in mangoes, followed by six hydrolysable tannins (HT) that constituted about 98% of the total polyphenolics identified ([Fig. 1](#page-3-0)). Four minor compounds, p-OH-benzoic acid, m-coumaric acid, p-coumaric acid, and ferulic acid were also identified but were not evaluated in this study.

Only minor differences in concentrations of gallic acid, total HT, total soluble phenolics, and antioxidant capacity were observed due to the hot water treatment. The observed effects of hot water were in agreement with [Tal](#page-7-0)[cott et al. \(2005\),](#page-7-0) who reported that hot water immersion was not a major factor influencing gallic acid concentration in mangoes held at 20  $\mathrm{^{\circ}C}$ . It has been reported that phenolic compounds, such as gallic acid, decrease as fruit ripen ([Haard & Chism, 1996; Mitra & Baldwin, 1997\)](#page-7-0), and in this study gallic acid decreased by 22% and 26% in hot water and control fruit, respectively, during storage and ripening ([Fig. 2](#page-4-0)a). The fruit also exhibited an appreciable and consistent decrease in total polyphenolic and antioxidant capacity during ripening. On average, the sum of the six identified HTs decreased by 57% ([Fig. 2](#page-4-0)b and [Table 1\)](#page-4-0), total soluble phenolics by the Folin-Ciocalteu assay decreased by 57% ([Fig. 2](#page-4-0)c), and antioxidant capacity by 45% [\(Fig. 2d](#page-4-0)) as the fruit ripened and were not affected by hot water treatment. Results for total HT were in contrast with [Talcott et al. \(2005\),](#page-7-0) who reported an increase in HT in 'Tommy Atkins' mangoes during ripening, indicating that appreciable differences may occur among fruit under different growing conditions or harvest years. Several studies have shown that polyphenolic compounds generally decrease in climacteric fruits, such as mangoes, bananas, tomatoes, and guavas during ripening ([Haard &](#page-7-0) [Chism, 1996; Lakshminarayana, Subhadra, & Subraman](#page-7-0)[yam, 1970; Mitra & Baldwin, 1997; Selvaraj & Kumar,](#page-7-0) [1989](#page-7-0)) and, likewise, significant changes with ripening were observed in this study. Changes in total soluble phenolics were proportional to those for both gallic acid and total HT, and correlated well with antioxidant capacity  $(r = 0.98)$ . Although mangoes contain other antioxidant components, the use of a clarified juice removed the majority of carotenoids that would have made at least a small contribution to overall antioxidant capacity, and L-ascorbic

Fig. 1. Typical HPLC chromatogram of polyphenolics in mango. Peak 1 represents gallic acid and those peaks labeled '2' represent six hydrolysable tannins. The six identified hydrolysable tannins are tentatively identified in [Table 1.](#page-4-0)

acid is generally present in relatively low concentrations in mango in relation to its polyphenolic content ([Cao, Ales](#page-7-0)[sio, & Cutler, 1993, 1998; Leong & Shui, 2002](#page-7-0)).

## 3.3. Changes in polyphenolic and antioxidant content by CA storage

The two CA storage treatments were compared to the air control with the goal of determining the effects of lowered  $O_2$  and/or increased  $CO_2$  levels on ripening-related changes in mango phytochemicals. Previous studies have demonstrated that the optimum  $O_2$  and  $CO_2$  levels in low temperature storage for mango range from 3% to 5% and 5–10%, respectively, depending on the stage of ripeness [\(Kader, 2002; Mitra & Baldwin, 1997\)](#page-7-0). Due to the small chemical differences and similarities in ripening between hot water and control fruit, only non-heat treated mangoes were evaluated to determine the effects of CA storage on phytochemical content and antioxidant capacity.

The initial gallic acid concentration in mature-green fruit was 180 mg/l and, after 2 weeks at 10 °C, the concentrations did not significantly change for the two CA treatments, whereas gallic acid decreased by 27% in CA1 [\(Fig. 3](#page-5-0)a). After removal from 10 °C storage to complete ripening, gallic acid concentrations declined in CA1 and CA3, but did not change in CA2 during subsequent ambient storage. At the end of the experiment, the gallic acid concentrations in CA2 and CA3 fruit were not statistically different, but both were significantly higher in phytonutrients, than in CA1. Therefore, retention of gallic acid was enhanced by CA storage, with some residual effect observed during subsequent ripening, compared to CA1 fruit.

Total HT concentration was 170 mg/l in mature-green fruit and concentrations in CA2 and CA3 fruit did not significantly change during  $10\,^{\circ}\text{C}$  storage, whereas the concentration in CA1 decreased appreciably [\(Fig. 3b](#page-5-0)). The HT concentrations in all treatments decreased by 38–58% during the experiment, yet remained higher than CA1 fruit, due to inhibition of ripening by CA ( $P \le 0.05$ ). CA storage conditions are known to be effective for slowing respiration and ethylene production [\(Mitra & Baldwin, 1997\)](#page-7-0), and the phytochemical composition of the fruit confirm an inhibition of ripening during the 2-week CA storage. As a result, while decreases in polyphenolics were inhibited during CA storage, the polyphenolics proceeded to decrease when returned to ambient conditions consistent with CA1.

The initial concentration of total soluble phenolics in mature-green mangoes was 399 mg/l and concentrations decreased by 55%, 60% and 48% in CA1, CA2 and CA3, respectively, throughout fruit ripening [\(Fig. 3c](#page-5-0)). The effect of lowered  $O_2$  or increased  $CO_2$  resulted in a small decrease  $(5-10\%)$  in total soluble phenolics during CA storage (CA2 and CA3) compared to an 18% decrease in air (CA1). However, no treatment differences were observed in the full ripe fruit, with 46%, 55% and 46% overall decreases in concentration in CA1, CA2 and CA3, respectively, during the period in air at 25 °C following 10 °C storage, which was in accordance with the observed decreases in gallic acid and HT.

The changes in antioxidant capacity for mangoes during ripening were well correlated to total soluble phenolics  $(r = 0.98)$ , with an initial antioxidant capacity in maturegreen mangoes of 5.43  $\mu$ mol TE/g ([Fig. 3d](#page-5-0)). The antioxidant capacity of CA3 did not alter during  $10\,^{\circ}\text{C}$  storage and was significantly higher than CA1 and CA2 after 2 weeks. An overall antioxidant capacity decrease of from 37% to 43% was observed in all treatments over the course of the experiment, such that the final values for the

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Fig. 2. Changes in gallic acid (mg/l) (a), total hydrolysable tannin (mg/l GAE) (b), total soluble phenolics (mg/l GAE) (c), and antioxidant capacity (µmol  $TE/g$ ) (d) in CA1 (air control) during ripening for fruit samples with or without hot water treatment. Different letters in each graph represent significant difference during ripening. Samples were taken at the mature-green (on the day of harvest), the mid-ripe (after 2 weeks in storage at 10 °C), and the fullripe stage (prior to fruit deterioration in air at  $25^{\circ}$ C).

Table 1 Changes in six identified hydrolysable tannins by hot water treatment

<b>CA</b>	<b>HWT</b>	Sampling time	Hydrolysable Tannin1	Hydrolysable Tannin2	Hydrolysable Tannin3	Hydrolysable Tannin4	Hydrolysable Tannin5	Hydrolysable Tannin6	Total Hydrolysable Tannin
CA1	No <b>HWT</b>	Mature- green	125a	1.20c	32.6 <sub>bc</sub>	6.50d	2.90 d	$3.10\text{ c}$	171a
		Mid-ripe	48.6 b	6.50 <sub>b</sub>	35.1 <sub>b</sub>	8.70 a	$3.80$ bc	5.80 <sub>b</sub>	108 <sub>b</sub>
		Full-ripe	21.7c	2.20c	32.4c	8.00 <sub>b</sub>	4.40 <sub>b</sub>	5.90 b	74.4 c
	<b>HWT</b>	Mature- green	125a	1.20c	32.6 <sub>bc</sub>	6.50d	2.90 d	$3.10\text{ c}$	171a
		Mid-ripe	47.3 <sub>b</sub>	5.90 <sub>b</sub>	34.8 bc	8.60a	$3.80$ bc	5.80 <sub>b</sub>	106 <sub>b</sub>
		Full-ripe	$28.1\text{ c}$	12.5 $a^A$	43.1 $a^A$	7.00 $c^A$	9.60 $a^A$	8.20 $a^A$	68.3 c

Only mangoes stored in CA1 (21%  $O_2$  + 79% N<sub>2</sub>) were used to determine hot water effect without interference from CA storage. Samples were taken at the mature-green (on the day of harvest), the mid-ripe (after 2 weeks in storage at 10 °C), and the full-ripe stage (prior to fruit deterioration in air at 25 °C). <sup>A</sup> Indicates a significant difference between no hot water and hot water treatment at each ripeness stage.

treatments were not remarkably different. Although antioxidant capacity is a desirable attribute for marketing the potential health benefits of many fruits, CA storage had only a minor effect on the retention of radical scavenging properties and the residual effects of the lowered  $O_2$  plus elevated  $CO<sub>2</sub>$  in CA3 on other properties did not extend to antioxidant capacity in fully ripe fruit. In studies conducted by [Sanchez-Mata, Camara, and Diez-Marques](#page-7-0)

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Fig. 3. Changes in gallic acid (mg/l) (a), total hydrolysable tannin (mg/l GAE) (b), total soluble phenolics (mg/l GAE) (c), and antioxidant capacity (µmol TE/g) (d) in non-hot water-treated mangoes. Control fruit (CA1) were held in 21% O<sub>2</sub> + 97% N<sub>2</sub> and the two CA storage treatments were 3% O<sub>2</sub> + 97% N<sub>2</sub> (CA2) and 3%  $O_2$  + 10%  $CO_2$  + 87% N<sub>2</sub> (CA3). Different letters in each graph represent significant difference during ripening. Samples were taken at the mature-green (on the day of harvest), the mid-ripe (after 2 weeks in storage at 10 °C), and the full-ripe stage (prior to fruit deterioration in air at 25 °C).

[\(2003\)](#page-7-0), and Tian et al. (2004), CA storage was more effective in preventing or reducing fruit decay, if reduced  $O_2$  and elevated  $CO<sub>2</sub>$  levels were applied together. Therefore, stable antioxidant capacity in mango during storage could be an additional benefit of CA.

## 3.4. Fruit flesh colour

The colour change of mango is a reliable parameter to determine the extent of fruit ripening [\(Ninio, Lewinsohn,](#page-7-0) [Mizrahi, & Sitrit, 2003; Schaffer & Andersen, 1994\)](#page-7-0) and was beneficial in assessing the extent of ripening in this study. Colour values are presented as lightness, hue angle, and chroma by conversion from  $a^*$  and  $b^*$  (López & Gómez, 2004). Hue angle in the case of ripening 'Tommy Atkins' mango fruit, indicates how yellow (90°) or green  $(180^{\circ})$  a fruit is, and chroma describes the vividness to dullness of the colour (Jeong, Huber, & Sargent, 2003; López & Gómez, 2004). No differences between hot water and control fruit were observed in colour values in this study, therefore only the control fruit are discussed. Differences

were not observed among storage atmosphere treatments during ripening for lightness, with L-values at 71.44, 72.93, and 73.73 after 10 °C storage, decreasing to 70.22, 71.40, and 71.87 after the next 7 days in ambient conditions for CA1, CA2 and CA3, respectively.

The hue angle decreased throughout storage and corresponded to an increase in carotenoid synthesis as the fruit ripened. Carotenoids will increase in most mango varieties and is associated with the climacteric increase in respiration that is initiated by the action of ethylene [\(Saltveit, 1999\)](#page-7-0). Hue angle was significantly higher for CA2 and CA3 treatments than for CA1 after 10  $\rm{^{\circ}C}$  storage ([Fig. 4a](#page-6-0)), and provided additional evidence for delayed ripening during CA storage. The CA1 fruit were apparently full ripe after 2 weeks at  $10^{\circ}$ C since their hue angle showed no further change during the next 7 days under ambient conditions. Thus, maximal carotenoid synthesis affecting yellow colour perception occurred in CA1, while hue angle values for CA2 and CA3 remarkably declined with further ripening, to equal the CA1 fruit, indicating that CA storage had no residual effect on carotenoid synthesis.

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Fig. 4. Changes of hue angle (°) (a) and chroma (b) affected by CA storage without hot water treatment. Treatments and sampling times were as described in the [Fig. 3](#page-5-0) legend. Hue angle and chroma were defined by the coordinates  $a^*$  and  $b^*$ . Different letters in each graph represent significant difference during ripening.

Additionally as fruits such as mango ripen, a corresponding increase in chroma value often accompanies an increase in carotenoids ([Prono-Widayat, Schreiner, Huys](#page-7-0)kens-Keil, & Lüdders, 2003). In this study, values for chroma were higher in CA1 following the 2-week storage period (Fig. 4b), indicating their advanced ripeness, compared to chroma values for CA2 and CA3. When the fruit were transferred to ambient conditions to complete their ripening, significant increases were observed in CA1 and CA2, which were possibly due to additional carotenoid synthesis in CA2 and to a change in carotenoid composition in CA1, since  $\beta$ -carotene is known to predominate over xanthophylls in the latter stages of mango fruit ripening [\(Bhaskarachary, Sankar Rao, Deosthale, & Reddy,](#page-7-0) [1995](#page-7-0)). Chroma changed very little in CA3 fruit during 7 days at ambient conditions, despite its hue angle reaching the same values as the other treatments. This suggests that the elevated  $CO<sub>2</sub>$  concentration in CA3 had a residual effect on some aspect of carotenoid synthesis, which may have affected the carotenoid composition of the fruit.

## 3.5. Titratable acidity

Titratable acidity was evaluated as an additional indicator of the degree of fruit ripeness with each treatment. According to [Jacobi et al. \(2000\),](#page-7-0) and [Tovar, Garcia, and](#page-7-0) [Mata \(2001\),](#page-7-0) acidity decreases as mangoes ripen, as citric and malic acid are used as respiratory substrates. Overall titratable acidity decreased with all treatments from mature-green to full ripe stages (Fig. 5). CA storage prevented the decline in titratable acidity observed for CA1 during the initial 2-week storage at  $10^{\circ}$ C. However, when the fruit were transferred to ambient conditions, rapid decreases in titratable acidity were observed in all treatments over the next 7 days with 46%, 48%, and 47% changes observed in CA1, CA2 and CA3, respectively.



Fig. 5. Change of average titratable acidity (%) in mangoes without hot water treatment for CA1, CA2 and CA3 during mango ripening. Treatments and sampling times were as described in the [Fig. 3](#page-5-0) legend. Different letters in each graph represent significant difference during ripening.

## 4. Conclusion

The external quality of mango fruit during ripening was greatly improved by low  $O_2$  and/or high  $CO_2$  storage conditions and hot water immersion treatments as quarantine against invasive pests. Gallic acid, total HT, total soluble phenolics, and antioxidant capacity significantly decreased throughout fruit ripening from mature-green to full ripe stages, but were unaffected by the hot water treatment. CA storage delayed fruit ripening, as evidenced by physicochemical changes, and the hot water treatment plus CA storage was an effective treatment combination to extend the postharvest life of mangoes, without adversely changing the nutritional profile of the fruit.

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