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Ethephon Time of Immersion and Concentration on 'Carabao' Mango Postharvest Ripening

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HIGHLIGHTS

- Immersion time affects the ethephon action on the postharvest mango ripening.
- Ethephon between 1 and 2 g L^{-1} is ideal for optimizing the ripening of 'Carabao' mango.
- Postharvest ethephon improves the mango color, without affecting anthracnose occurrence.

Abstract: Mangoes are worldwide consumed fruits providing health benefits due to flavonoids content. Nevertheless, its quality may be affected along the supply chain and then ripening strategies after harvest must be optimized. There are few studies evaluating different exposure times to ethephon on mango ripening. Additionally, the use of ethephon may promote the development of anthracnose, affecting fruits quality. Typically, ethephon is used for immersion times between 2 and 5 minutes. There are no studies about immersion times of less than 1 minute for mangoes ripening and to reduce its effect on anthracnose. Hence, this report aims to evaluate 'Carabao' mango quality after treatment with two immersion times, four ethephon concentrations in four storage periods. The experiment was conducted in a completely randomized design in Viçosa, Brazil. Pulp hue angle and pulp carotenoids content were influenced by storage, immersion time and ethephon concentration. Respiratory rate, soluble solids, titrable acid and weight loss were influenced by storage while firmness and weight loss were influenced by immersion time. Ascorbic acid content and soluble solids content were impacted by ethephon concentration and immersion time. In general, the lower immersion time with 1 to 2 g L^{-1} ethephon concentrations anticipated fruits ripening. Despite the high anthracnose incidence and severity, ethephon use had no effect on increasing the disease development, showing its suitability as a postharvest ripening agent for mangoes. Therefore, the ideal conditions for mango ripening using ethephon must be further investigated to improve mango quality after harvest and throughout distribution network.

Keywords: anthracnose; 2-chloroethylphosponic acid; fruit quality, *Mangifera indica* L.; plant growth regulator.

INTRODUCTION

Mango is worldwide produced tropical fruit, where the most producers are India in Asia, Brazil in South America, Nigeria in Africa and Australia in Oceania [1]. Although a less produced variety, 'Carabao' mango is considered one of the tastiest in the world due to its sweetness and non-fibrous pulp [2] which favors its acceptance by consumer market [3]. As mango usually has a long supply chain before reaching the consumer market, different mango ripening management approaches may be necessary to ensure their quality from harvest, during transport and commercialization [4].

Hence, strategies for fruits quality maintenance are essential to enable mango distribution to consumer markets located distant from farm production. Different mango ripening management approaches may be necessary to ensure their quality from harvest, during transport and commercialization [4]. As a climacteric fruit, the anticipation or delay of mango ripening can be achieved by exogenous application of ethylene [5,6] or 1-methylcyclopropene [5], respectively. Ethephon, 2-chloroethylphosponic acid, has been employed [7] ethylene releaser [8–10] to promote fruit ripening for more than 50 years [11,12]. For mangos, ethephon have has been employed to 'Ubá' [13], 'Neelum' [8], 'Carabao' [10], 'Keitt' [9] and 'Tommy Atkins' [14] varieties, mainly on preharvest [9,13,14].

Mostly, the effect of ethephon concentrations on mango postharvest ripening have been explored with a lack of evaluating the effect of different ethephon immersion times or a possible interaction between ethephon concentration and immersion time on fruit ripening [8–10,15]. In a previous report, 2-minute exposure to ethephon was more effective in promoting 'Keitt' mango ripening regarding to 1-minute exposure [9]. Further, ethephon may be related to anthracnose development in mango fruits, due to its effects of increasing virulence, spore germination and appressorium formation of *Colletotrichum asianum* from concentrations of 1 µM [16]. The reduction in incidence and expansion lesions of anthracnose was reported to mango fruits 1-methylcyclopropene-treated [17]. However, there are few reports in the literature on the effect of shorter [18] or longer [19] exposure ethephon times on mango ripening and anthracnose development, and further studies on the subject are needed.

Therefore, this report aims to investigate the effect of different concentrations of ethephon and immersion times throughout post-harvest quality of 'Carabao' mangoes. Assessment of soluble solid, titrable acidity, anthracnose occurrence, content of carotenoids and chlorophyl as well as fruit color measurements of mangos subjected to ethephon immersion times at concentrations of 0.5, 1.0 and 2.0 g L^{-1} for 30 seconds and 3 minutes will be evaluated over storage by a factorial design.

MATERIAL AND METHODS

The fruits used were harvested from an experimental orchard in the Agronomy Department of the Federal University of Viçosa (UFV), in the municipality of Visconde do Rio Branco, Minas Gerais, Brazil (21° 00' 13.3" S, 42° 46' 36.2" W, 350 m). Samples of 'Carabao' mango were collected at the beginning of December 2019, when the fruits were at the minimum necessary stage to post-harvest evolution (soluble solids ≥ 7.0%). Immediately after harvesting, the fruits were transported to the packing house of the Agronomy Department of the Federal University of Viçosa (UFV), in Viçosa, Minas Gerais, Brazil. The fruits were selected, cleaned with water and detergent, followed by sodium hypochlorite immersion for 3 minutes (200 mg L^{-1} Cl active). Fruit selection was based on size, epicarp uniform coloration and absence of injuries or field damage for samples homogenization (20). Subsequently, an initial fruit samples (n = 4; 6 fruit $sample^{-1}$) characterization was performed according to the following variables: fruit mass (240.50 ± 33.7 g^{-1}), fruit length (127.2 ± 4.2 mm), larger fruit diameter (64.1 ± 1.3 mm), lower fruit diameter (58.1 ± 1.2 mm), soluble solids (7.9 ± 0.3%), titratable acidity (2.05 ± 0.13%, citric acid equivalent), firmness (18.42 ± 1.8 N), ascorbic acid (38.57 ± 2.0 mg 100 g^{-1}), respiratory rate (66.98 ± 7.7 mg CO_2 kg^{-1} h^{-1}), peel hue angle (106.09 ± 0.76°), pulp hue angle (95.77 ± 2.30°), peel chlorophyll (9.72 ± 1.58 mg 100 g^{-1}), peel carotenoids (0.01 ± 0.01 mg 100 g^{-1}), pulp carotenoids (0.16 ± 0.05 mg 100 g^{-1}).

A completely randomized design was conducted, using six fruits per experimental unit. The treatments were arranged in a 2 × 4^2 factorial, with three replications, totaling 96 experimental units. The fruits were subjected to two immersion times (0.5 and 3.0 min) of four concentrations of ethephon: 0; 0.5; 1.0; and 2.0 g L^{-1} (Ethrel®, 240 g L^{-1} ethephon, Bayer S.A.) over four storage periods (3, 5, 7 and 10 days). The fruits were immersed without agitation, and the ethephon solutions were at room temperature. After immersion, the fruits were dried in the shade. The fruits were stored in a cold room with an air temperature of 21.3 ± 0.2 °C and a relative humidity greater than 95%.

Soluble solids (TSS), titratable acidity (TA), total soluble solids, pulp firmness, ascorbic acid content, mass loss, respiratory rate of fruits was determined to mango fruits subjected to ethephon treatments. TSS was accomplished by digital refractometer (Pocket PAL-1, ATAGO, Tokyo, Japan), after homogenizing aliquots of the fruit pulp. TA was performed by titrating 5 g of mango pulp mass with 0.1 mol L^{-1} NaOH solution, using 1% phenolphthalein as pH indicator. Pulp firmness measured the needed force in which the mango fruits no longer offer resistance, after removing a peel portion from the middle region of the fruits. For this analysis, a penetrometer, with an 8 mm flat stainless-steel tip was employed (model DD200). Ascorbic acid (AA) content was accomplished by a previous method [21]. Respiratory rate by mango fruits was determined by gas chromatography Gow Mac series 550 thermal conductivity detector, according to a previous method [22]. Peel and pulp carotenoids as well as chlorophyll content were determined by maceration of samples in 80% (v/v) acetone using a Fento 600 spectrophotometer, using a slightly modified method (23). Mango peel was measured in the central region, on opposite sides of the fruit while pulp color was measured in the central region on both sides of the fruit. For these analyses, the portable colorimeter (Konica Minolta, CR-10) was employed, providing the values of L*, a* and b* coordinates. In this study, the hue angle of the pulp (h_s) and peel (h_p) was calculated by h = arctan (b*/a*) [24].

Furthermore, the level of incidence and severity of anthracnose (*Colletotrichum gloeosporioides*) in mango peel was determined using a descriptive scale ranging from 0 to 5, where scale 0 indicates absence of anthracnose and scale 5 indicates that more than 50% of the fruit area is affected by the disease symptoms [25]. The frequency of fruit with symptoms (from 1 to 5 on the descriptive scale) determined the incidence of fruit with anthracnose (IA). The McKinney disease index (ID), as previously described [26] was also calculated to estimate the severity of fruit anthracnose.

The data was submitted to a factorial analysis of variance, and the assumptions were assessed by graphically checking the studentized residuals. The mass loss [$\hat{y}=(y+1)^{0.5}$] and pulp firmness [*rank transformation*] variables were transformed to fit the normal distribution. After checking the effects of each factor ("Immersion time", "Ethephon concentration", and "Storage period") and their interactions, the analysis was complemented by polynomial and non-linear regression analyses. SAS 9.4® software (SAS Institute, Cary NC) was employed for the statistical analyses, while the graphs were made in SigmaPlot 14.0® (Systat Software Inc.)

RESULTS

The highest respiration rate of 'Carabao' mango occurred on the third day of storage, with a rate of 199.2 mg of CO_2 kg^{-1} h^{-1} (Figure 1a). Ethephon concentration (Figure 1b) also affected the respiratory rate, where a maximum of 168.2 mg of CO_2 kg^{-1} h^{-1} at a concentration of 1.73 g L^{-1} of ethephon was obtained. TSS and TA values were affected by storage (Figure 2). The lowest TA value occurred on the 8th day, obtaining an acidity of 0.47. The immersion time affected the average TA values. Immersion time of 0.5 minute of immersion produced fruits with an average TA of 0.70%, while immersions of 3 minutes provided average values of 0.76% (date shown).

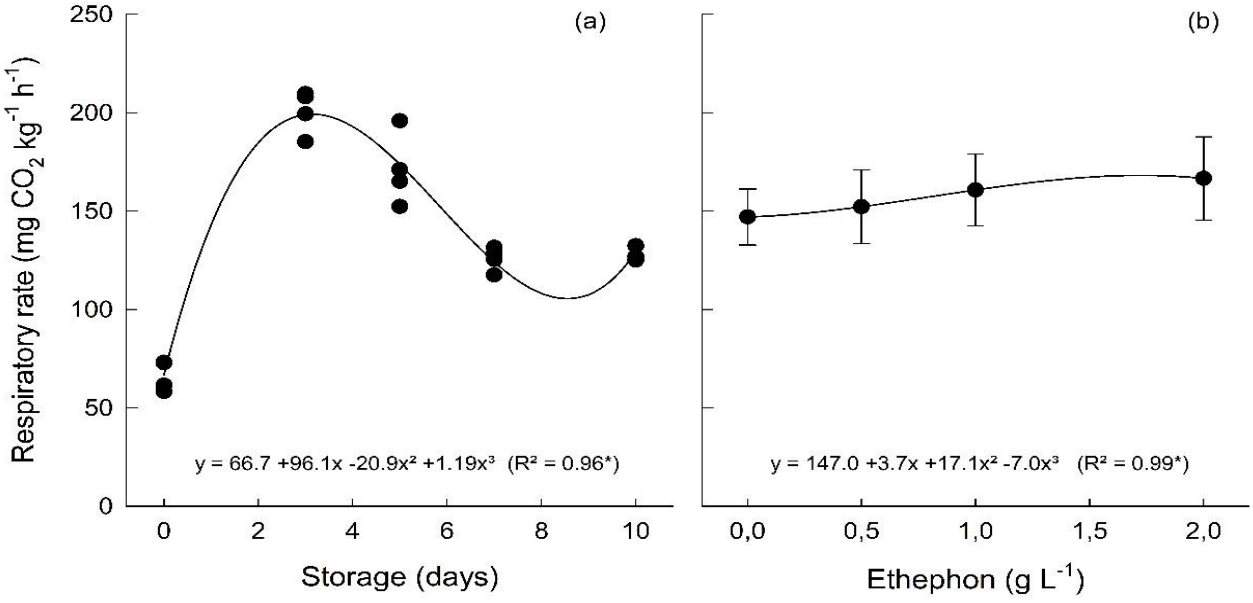

Figure 1. Respiratory rate over storage (a) and ethephon content (b) on respiratory rate of 'Carabao' mango treated with ethephon at different immersion times. Viçosa, Brazil, 2019. * p <0.05.

On the other hand, immersion time and ethephon concentration also affected soluble solids content (Figure 2b). On immersion time of 0.5 minute and 1.2 g L^{-1} of ethephon there was a slight increase in soluble solids, accomplishing a total of 14.4%. Diversely, on immersion time of 3 minutes and 1.3 g L^{-1} ethephon concentration, the total soluble solids reduced, achieving 14%. Slightly higher soluble solid content was observed in immersions for 3 minutes regarding 0.5 minute and in the absence of ethephon (Figure 2b). Although statistically significant, the concentration/immersion time effect caused only subtle differences in SS content, around 0.6%, which may not be relevant in mango ripening management practices.

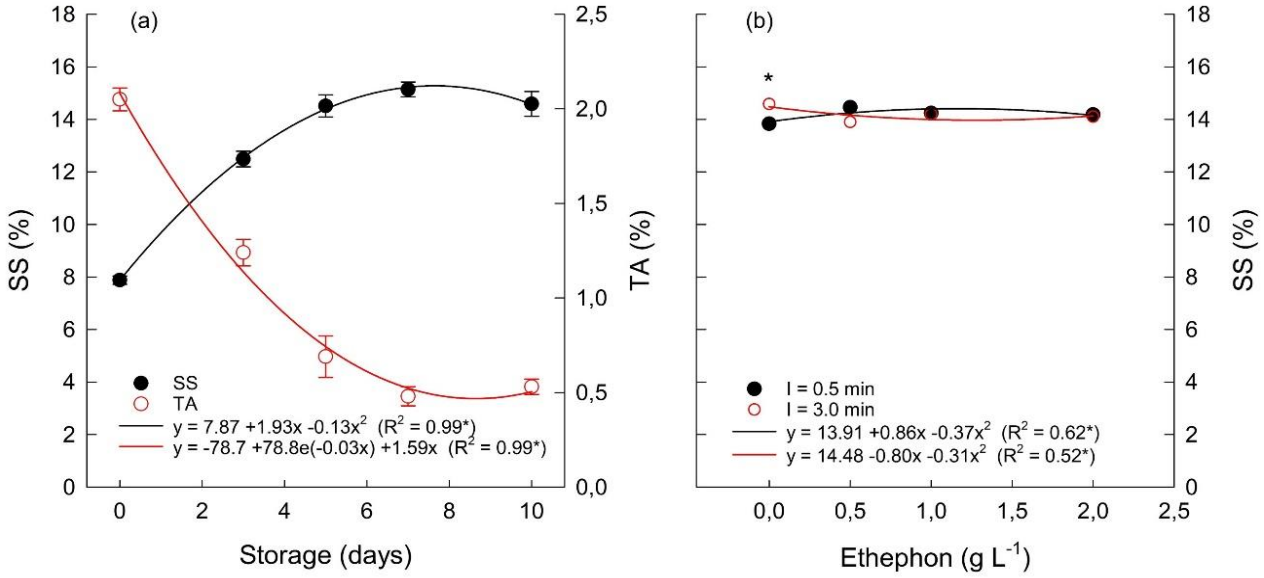

Figure 2. Soluble solid content (SS) and titratable acidity (TA) of 'Carabao' mangos. Viçosa, Brazil, 2019. * p <0.05.

Figure 3 shows the firmness of 'Carabao' mango which was affected by interaction of storage and ethephon content (Figure 3a) and by interaction of ethephon concentration and immersion time (Figure 3b). There was a firmness reduction when mango was subjected to higher ethephon concentrations on immersion time of 0.5 minute (Figure 3b). However, the absence of ethephon or at a concentration of 0.5 g L^{-1} and on 0.5 minute immersion time provided fruits with the highest firmness average values. The greatest reduction in firmness was at a concentration of 2 g L^{-1} of ethephon, of 0.63 N.

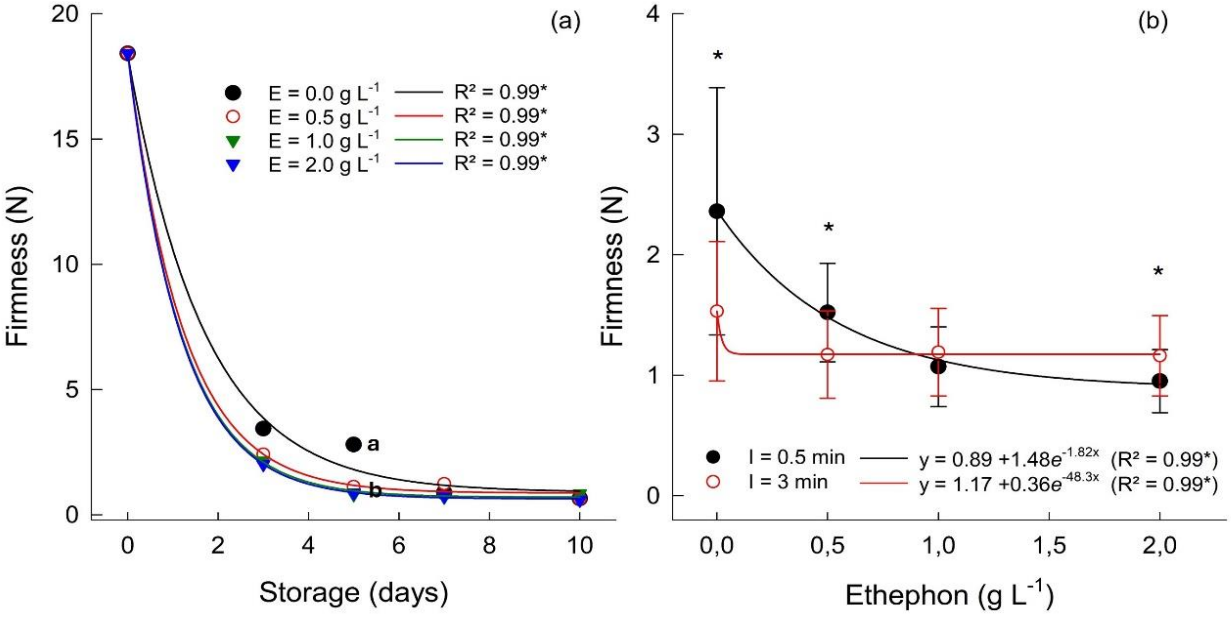

Figure 3. Interaction between storage time and ethephon concentration (a), interaction between immersion time and ethephon concentration (b) on 'Carabao' mango pulp firmness. Viçosa, Brazil, 2019. * p <0.05; Averages followed by equal letters within each storage period do not differ by the Tukey´s test (p <0.05).

An interaction between storage time and immersion time (Figure 4a) and an interaction between ethephon concentration and immersion time (Figure 4b) on ascorbic acid content of 'Carabao' mango is shown. The immersion time of 0.5 minute led to higher degradation rates during the beginning of storage compared to the 3-minutes immersion (Figure 4a). Increasing the ethephon concentration reduced the AA content exponentially only at the 0.5 min immersion time. However, this effect was not observed at the 3 min immersion time (Figure 4b), in which an average content of around 19.48 mg 100 g^{-1} was achieved. In the absence of ethephon, the 0.5-minute immersion time produced higher average AA values compared to 3 minutes, while an opposite behavior was observed with ethephon 2 g L^{-1} (Figure 4b).

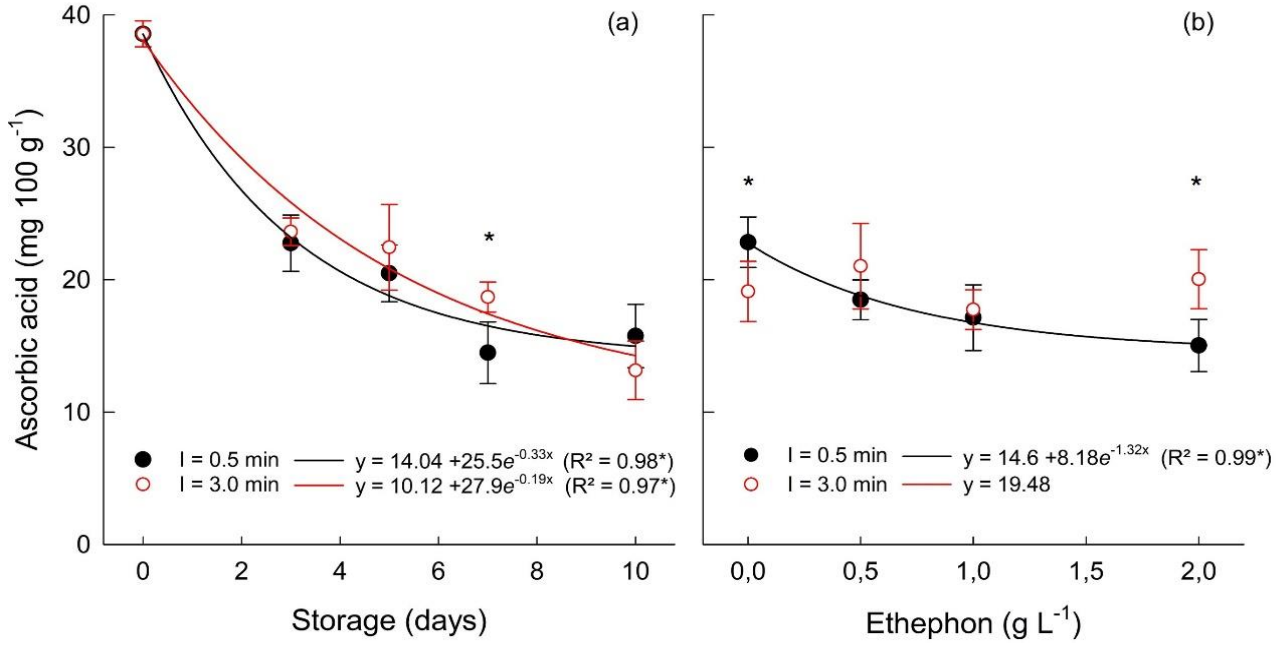

Figure 4. Interaction between storage and immersion time (a), and interaction between ethephon content and immersion time (b) on ascorbic acid content of 'Carabao' mango pulp. Viçosa, Brazil, 2019. * p <0.05.

Regarding weight loss of mango fruit, it was not affected by ethephon concentration. However, mango weight was affected by storage and immersion time. The highest mass loss was 5.6% on the 10th day of storage. A quadratic pattern of mass loss was seen over storage. In the immersion time of 0.5 minute and 3 minutes there was a mass loss of 4.94% and 3.52%, respectively. Also, the weight loss at lower immersion time was statistically different from the one obtained at a longer immersion time.

The carotenoid content was influenced by interaction between storage time and ethephon concentration (Figure 5a) and interaction between ethephon concentration and immersion time (Figure 5b). Throughout the storage period, there was an increase in carotenoids peel which was initially insignificant (0.01 mg 100 g^{-1}). This increase occurred linearly for 'Carabao' mango subjected to ethephon concentrations of 0, 1 and 2 g L^{-1}.

The highest carotenoid content was 5.85 mg 100 g^{-1} obtained to ethephon concentration of 2 g L^{-1}. In turn, ethephon concentration of 0.5 g L^{-1} resulted in a quadratic growth reaching a maximum of 3.37 mg 100g^{-1} on the ninth day of storage (Figure 5a). Ethephon concentration and immersion time affected peel carotenoids (Figure 5). In 0.5 minute of immersion time, the maximum increase in carotenoid was 4.20 mg 100 g^{-1} at ethephon content of 1.48 g L^{-1}. For 3 minutes as immersion time, the highest carotenoid content was 3.72 mg 100 g^{-1} on ethephon concentration of 2.0 g L^{-1} (Figure 5b). The immersion time of 0.5 minute provided higher average peel carotenoids content to ethephon concentrations of 0.5 and 1.0 g L^{-1}(Figure 5b).

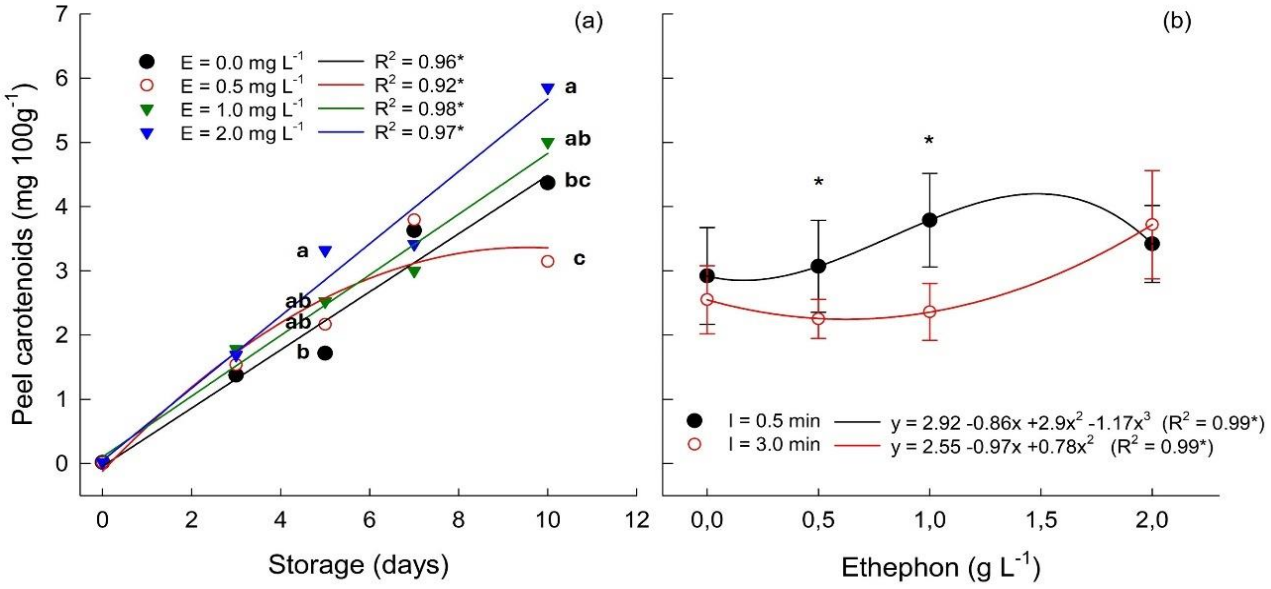

Figure 5. Carotenoids content over storage (a) and interaction between ethephon concentration and immersion time on carotenoids content of peel 'Carabao' Mango (b). Viçosa, Brazil, 2019. * p <0.05; Averages followed by equal letters within each storage period do not differ by the Tukey´s test (p <0.05).

Furthermore, 'Carabao' mango pulp carotenoid content was affected by a three-way interaction between immersion time, ethephon concentration and storage period (Figure 6). At harvest, carotenoids content in the pulp was low, similarly to the peel carotenoids. At an exposure time of 0.5 minute, there was a linear increase for 0.5 and 1.0 g L^{-1} ethephon concentrations, reaching 2.41 e 2.59 mg 100 g^{-1}, respectively, on the 10th day of storage (Figure 6a). Nevertheless, the absence of ethephon and the treatment with ethephon concentration of 2 g L^{-1} resulted in a quadratic increase of pulp carotenoids. A maximum carotenoids content of 2.15 mg 100 g^{-1} on the sixth day of storage and 1.80 mg 100 g^{-1} on the seventh day of storage, were obtained, respectively. At 5 days of storage, the highest pulp carotenoid values were obtained for the treatments without ethephon application. At the end of storage, the highest carotenoid values were achieved for fruits submitted to 1 g L^{-1} of ethephon. (Figure 6b). The treatments effect on pulp carotenoids content was different at immersion times of 3 minutes (Figure 6b). Solely fruits treated with 2 g L^{-1} ethephon concentration had a linear increase in pulp carotenoid, with the highest values of 2.34 mg 100g^{-1}. When concentrations of 0, 0.5 and 1 g L^{-1} of ethephon were employed, the behavior was quadratic, reaching values of 2.55, 2.22 and 1.93 g 100 g^{-1}, respectively. At 7 days of storage, the highest pulp carotenoids content was observed in the absence of ethephon. In turn, at the end of storage the carotenoids values were similar for all treatments (Figure 6b). As previously described for peel carotenoids, pulp carotenoids increase pattern was linked to both immersion time and different concentrations of ethephon. Linear and quadratic increase in carotenoids was favored when an immersion time 0.5 minute and 3 minutes was employed, respectively.

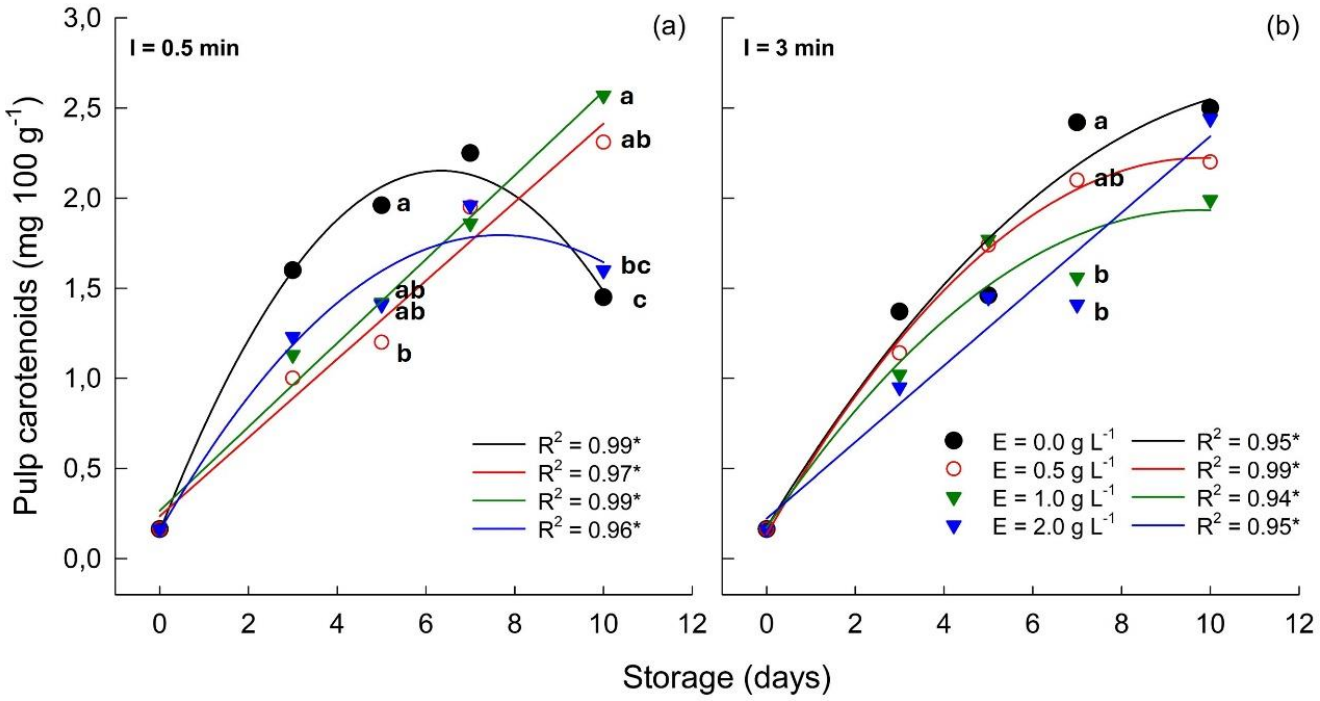

Figure 6. Interaction between ethephon concentration and 0,5 min immersion time (a) and interaction between ethephon concentration and 3 min immersion time on carotenoids content of pulp 'Carabao' Mango (b). Viçosa, Brazil, 2019. * p <0.05; Averages followed by equal letters within each storage period do not differ by the Tukey´s test (p <0.05).

The total chlorophyll content of 'Carabao' mango was affected by storage (Figure 7a) and by ethephon concentration (Figure 7b). The total chlorophyll content had an exponential reduction (Figure 7a). The concentration of ethephon statistically affected the chlorophyll content although it was not possible to fit a curve, obtaining an average content of content was 5.40 mg 100 g^{-1} (Figure 7b).

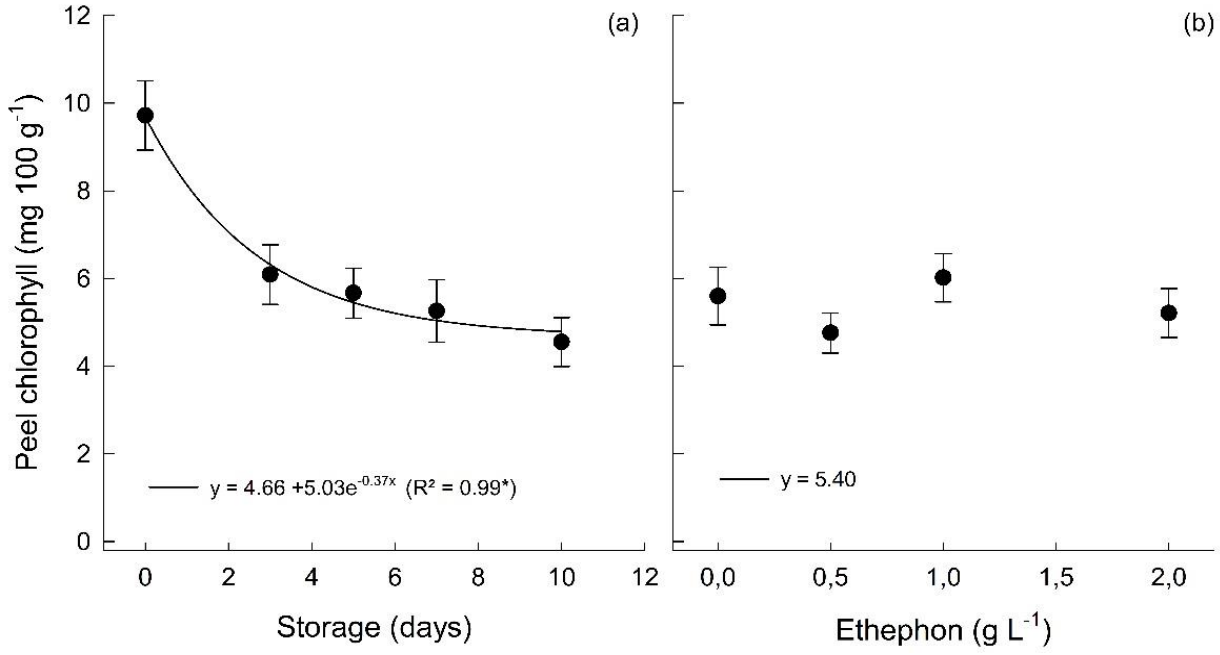

Figure 7. Storage effect (a), ethephon content effect (b) on 'Carabao' mangos total chlorophyll content. Viçosa, Brazil, 2019. * p <0.05.

The h_s was influenced by the interaction between ethephon concentration, immersion time, and storage period (Figures 8a and 8b), while h_p was affected by storage and immersion time (Figure 8c). h_s on storage had a sigmoidal fit (Figures 8a and 8b). At the immersion time of 0.5 minute, the lowest hue angle was obtained at 10 days of storage with ethephon at concentrations of 0.5 g L^{-1} and 1 g.L^{-1} achieving values of 72.37° and 72.40°, respectively (Figure 8a). The greatest development of peel color (along with a lower h_s) was noted for fruits treated with 0.5 and 1.0 g L^{-1} ethephon from 3 days to 10 days of storage, in 0.5 minute of immersion (Figure 8a). Besides, lowest h_s values were obtained at the end of storage, varying between 77° and 81.83°, on immersion time of 3 minutes. At 7 days of storage, the use of ethephon reduced h_s values regarding fruits not treated with ethephon (Figure 8b). In turn, the h_p had a reduction throughout storage, from an initial one of 95.75° to 72.87° on the 10th day of storage (Figure 8c). In 0.5 minute of immersion time, there was an average value of 77.87°, smaller than the hp obtained in 3 minutes of 80.65° ($p<0.05$) (data not shown).

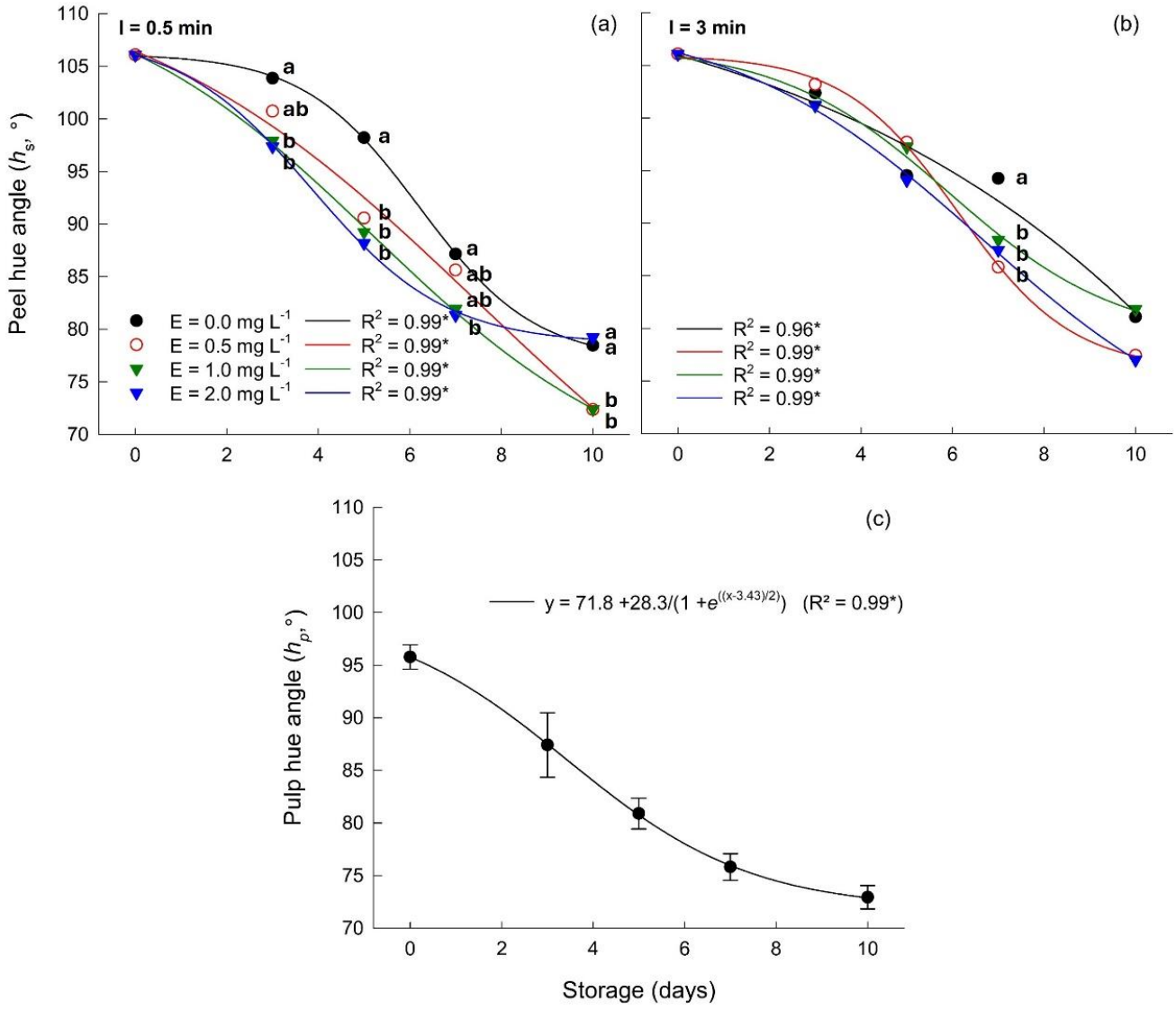

Figure 8. Interaction between ethephon concentration and immersion time at 0.5 minute (a), interaction between ethephon concentration and 3 minutes immersion time of 'Carabao peel Hue angle (b), pulp Hue angle (h^o) of 'Carabao' mango over storage (c). Viçosa, Brazil, 2019. * p <0.05; Averages followed by equal letters within each storage period do not differ by the Tukey´s test (p < 0.05).

Moreover, exposing 'Carabao' mangoes to ethephon concentrations between 0.5 and 2 g L^{-1} for 0.5 minute of immersion increased carotenoid concentrations in the peel (Figure 5b) and pulp (Figure 6a). This combination resulted in more developed colors of 'Carabao' mangoes' peel, as evidenced by lower h_s values (Figure 8a).

Neither ethephon concentrations nor immersion times impacted on anthracnose incidence in ‘Carabao’ mango (data no show). The average incidence of anthracnose after 7 and 10 days after storage were 74.3% ± 12.0 and 63.1% ± 13.5, respectively. The averages disease index after 7 days of storage was 32.5% ± 5.7 while after 10 days after storage, it was 46.9% ± 2.8.

DISCUSSION

As mangoes are climacteric fruits, they have a climacteric peak that is indicative of their increased respiratory activity [6,27]. In other report, the exogenous application of ethephon also caused an increase in respiration compared to untreated fruits [28]. The postharvest application of ethephon in fruits can be an interesting approach to enable/increase the content of compounds with health benefits. Monoterpenes production increased when concentrations of 0.5 g L^{-1} and 2 g L^{-1} ethephon were applied to mango, affecting the production of volatile aromatic compounds. Furthermore, the application of 2 g L^{-1} of ethephon also increased the concentration of linoleic and linolenic acid in mango pulp [28].

The soluble solids content of a fruit is an important measure of postharvest quality as it is related to a human acceptance [29]. ‘Osteen’ mango harvested earlier showed lower sensory acceptance due to their lower SS, lower than 12% [30]. Therefore, soluble solids determination is a fundamental feature related to sensory acceptability and probability of fruits consumption. As the fruit ripens there iso also a decrease in acidity [29], due to the reduction of organic acids which are employed as respiratory substrates [31]. In other report, ‘Carabao’ mango had a 0.3% of TA after ripening [32]. A quadratic increase was obtained for soluble solids over storage, which was previously reported [13]. A maximum of 15.3% of TSS on the 7th days of storage was obtained (Figure 2a). Similarly, the absence of ethephon application on ‘Carabao’ mango, soluble solids of 18 and 19% were obtained on the seventh day after harvest, and the starch content varied from 11% to close zero during this period [32].

Firmness is reduced on fruit ripening due to the pectin methyl esterase, polygalacturonase and beta-galactosidase activity on the fruit cell walls [31]. An increase in pectins concentration in ‘Carabao’ mango with increasing ripening [32] explains the reduction in firmness even for fruits not subjected to exogenous ethephon application (Figure 3a). Likewise, storage time [8,10] as well as higher ethephon content caused a decrease in mango firmness [8–10]. Moreover, ‘Keitt’ mango also showed lower firmness when higher ethephon concentration and exposure times of 1 and 2 minutes were employed in postharvest ripening [9].

Ascorbic acid is an enzymatic cofactor in vegetables, and its content varies between fruit development and ripening [33] where the exponential decay of AA for ‘Carabao’ denotes fruit ripening [32]. Also, post-harvest treatment of mango with ethylene significantly reduced ascorbic acid content after 15 days of treatment [5].The increase in carotenoid content is a consequence of the ripening process due to increased respiration [31]. In this sense, climacteric peak of ‘Carabao’ mango occurred concomitantly mesocarp yellowing [34]. Moreover, the pulp carotenoids increase was similar to the one from peel, denoting the ripening process [35]. During ripening, along with ethylene expression increase there is an increase in carotenoid biosynthesis and in flavonoids content [36]. On the other hand, both storage and different ethephon concentrations reduced total chlorophyll content [37].

The decrease in hue angle is expected as mango ripening [31,38], since higher ones indicate greener and firmer fruits [31]. Hue angle decreasing values are related to the lower firmness of the fruits, which correlates with fruits greater chewiness and juiciness [39]. By comparing the hue values from peel and from pulp, lower immersion time favored hue angle reduction. Even in the absence of ethephon treatment, 0.5 minutes immersion time led to a lower hue values.

Regarding anthracnose, there was no increase in the severity of anthracnose on the fruit, similar to a previous study in which ethephon and 1-MCP were applied to ‘Carabao’ mangoes [18]. The high occurrence of anthracnose in this report may be due to the non-use of fungicides and hydrothermal treatments [9]. Furthermore, the lowest immersion time with ethephon provided the greatest degreening of oranges and limes [20]. Likewise, the shortest ethephon immersion time was enough to allow ‘Carabao’ mango ripening in this report. Hence, the effect of ethephon immersion time on mango postharvest ripening needs to be further investigated since there are no previous reports approaching the impact of ethephon immersion time on anticipating or delaying mango ripening.

CONCLUSION

The immersion time or its interaction with ethephon concentration or storage time influence ripening process and the fruits quality characteristics. Ethephon concentrations between 1 and 2 g L^{-1} are the most effective in increasing respiration and the ripening process in ‘Carabao’ mangos. At higher ethephon concentration, lower immersion times improves postharvest ripening, mainly by reduction of firmness and of

ascorbic acid content. Ethephon increases the 'Carabao' mango external color and peel carotenoid content more effectively when used at lower immersion time. The use of ethephon in postharvest did not increase the occurrence of anthracnose in ‘Carabao’ mango. Therefore, there is a need for a more in-depth investigation over the interaction between immersion time and ethephon concentration on mango ripening, aiming to optimize postharvest mango ripening process. Furthermore, employing lower ethephon immersion time may be useful to fruits with similar features to mango.

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