Heat treatment effects on ACC oxidase activity of 'Keitt' mangoes

Renar João Bender[1]*, Eduardo Seibert[1], Jeffrey K. Brecht[2]

*[1]Departamento de Horticultura e Silvicultura, Faculdade de Agronomia, CP 15.100 Universidade Federal do Rio Grande do Sul, 91501-970 Porto Alegre, RS, Brasil; [2]Horticultural Sciences Department/IFAS, University of Florida, 32611-0690 Gainesville, FL, USA. * Corresponding author: rjbe@vortex.ufrgs.br*
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With the use of ethylene dibromide for mango disinfestation being ruled out, vapor heat or hot water treatments are the only alternatives for quarantine treatments of mangoes. Physical treatments such as heat treatments have been implicated in higher incidence of physiological disorders and enhancement of ripening processes. Therefore, the objective of the present work was to determine the effects of hot water treatments on ethylene production and on the *in vitro* activity of ACC oxidase. Cv. Keitt mangoes were immersed for 3 min in hot water at 53 °C or 90 min in water at 46 °C. Immediately after the treatments, some of the mangoes were analyzed for ACC oxidase activity and others were stored to be analyzed after 4 days at 12 °C. There was a significant increase in the ACC oxidase activity just after the hot water treatments. After 4 days, only the mangoes treated for 90 min maintained high ethylene production and ACC oxidase activity. Tissue from the outer layers of the mesocarp had higher enzyme activity compared to tissues from the innermost layers of the mesocarp of heat-treated mangoes.

Key words: Ethylene, ethylene dibromide, heat treatment, *Mangifera indica* L.

Efeito de tratamento térmico na atividade da ACC oxidase em mangas 'Keitt': Com a proibição do uso de dibrometo de etileno para tratamento quarentenário, os tratamentos térmicos, água ou vapor quente, constituem as únicas alternativas disponíveis para desinfestação de mosca-das-frutas em mangas. Considerando que os tratamentos físicos têm sido responsabilizados pela ocorrência de distúrbios e pela aceleração do amadurecimento, o objetivo do presente trabalho foi determinar o efeito do tratamento com água quente na produção de etileno e na atividade *in vitro* da ACC oxidase. Frutos da cv. Keitt foram imersos, por 3 min, em água a 53 °C ou, por 90 min, em água a 46 °C. Logo após o tratamento, parte dos frutos foi analisada quanto à atividade da ACC oxidase e, parte, armazenada por 4 dias a 12 °C. Houve um significativo aumento na atividade da ACC oxidase em seguida aos tratamentos. Passados 4 dias, apenas os frutos imersos por 90 min mantiveram tal atividade e a produção de etileno elevadas. Comparando a atividade em três profundidades do mesocarpo, as camadas mais exteriores apresentaram o maior efeito dos tratamentos.

Palavras chave: Etileno, dibrometo de etileno, *Mangifera indica* L., termoterapia.

INTRODUCTION

Physical treatments to eliminate organisms of quarantine concern, especially fruit flies (*Anastrepha fraterculus* and *Ceratitis capitata*) were developed as alternatives to chemical treatments, which face severe restrictions, if not prohibition because of their negative environmental effects (Couey, 1989). For mangoes, hot water dips have been approved. These treatments allow mango shipments out of areas where fruit flies are endemic (Mitcham and McDonald, 1993b). The use of hot water as a disinfestation treatment has spread because of its efficacy and the low incidence of damage to the treated fruit (Jacobi et al., 1995). Nevertheless, some peel disorders as well as quality losses have been observed (Jacobi and Wong, 1991). Among those is accumulation of starch grains in sub-epidermal tissues, probably resulting from heat deactivation of starch hydrolases (Jacobi and Wong, 1992). Negative effects on fruit color were also reported (Joyce et al., 1993). All these effects were more pronounced on mangoes harvested at the mature green ripeness stage than at later stages (Jacobi and Wong, 1992).

Besides their use as quarantine treatments, hot water dips are also effective in the control of anthracnose, a critical postharvest pathogen that can cause severe losses in mango (Coates et al., 1993). To control postharvest pathogens, the use of higher temperatures has been recommended, though for shorter exposure times in order to avoid damage to treated fruit.

Mitcham and McDonald (1993a) measured an increase in ethylene production as a response to heat stress. Increases in ethylene production might stimulate ripening processes. However, this stimulus could be limited because of the effects of elevated temperatures on the enzymes of the ethylene biosynthetic pathway (Paull and Chen, 2000). Therefore the objective of the present work was to determine the effects of heat treatments on ethylene biosynthesis, and more specifically on the *in vitro* activity of ACC oxidase.

MATERIAL AND METHODS

Cultivar Keitt mangoes were harvested at the mature green ripeness stage from a grove located in Pine Island/Fort Myers, Florida. Sixteen uniform fruit, average weight of 595.9 ± 45.1 grams, preclimacteric, producing 0.0482 ± 0.0018 μL $C_2H_4 \cdot kg^{-1} \cdot h^{-1}$, were immersed in hot water for either 3 min at 53 °C or 90 min at 46 °C.

Shortly before the heat treatments, ethylene production of each fruit was determined. One-mL headspace samples were retrieved from 1.7 L glass jars and injected in a photoionization gas chromatograph (GC) equipped with a 60/80 mesh 760 x 3.16 mm activated alumina column. The GC detector and injector were operated at room temperature. The column temperature was slightly above ambient because of heating by the UV source.

After the heat treatments the mangoes were left for 30 min at room temperature and then ethylene production of each individual fruit was again determined. The mangoes from each heat treatment were divided into two groups. One group of eight fruit was transferred to a cold store to be analyzed after 4 days at 12 °C. The other eight fruit were immediately analyzed for the *in vitro* activity of ACC oxidase. Tissues from three depths of the mesocarp, 5 mm underneath the epidermis, the median portion of the mesocarp, and from tissues close to the seed, were used to determine enzyme activity.

ACC oxidase activity was determined according to Fernandez-Maculet and Yang (1992) with modifications. Instead of TRIS (Tris[hydroximethyl]aminomethane), 0.4 $mol.L^{-1}$ 3-(N-morpholino)propane sulfonic acid (MOPS) was used in the extraction buffer. To the assay buffer in a test tube were added 1.64 mL of 50 $mmol.L^{-1}$ MOPS (pH 7.2), 10 % glycerol (v/v), 20 $mmol.L^{-1}$ sodium bicarbonate and 0.2 mL of the crude extract of ACC oxidase. Prior to 1 h of incubation in the test tube at 30 °C, 40 μL of a mixture containing 250 $mmol.L^{-1}$ sodium ascorbate, 1 $mmol.L^{-1}$ $FeSO_4.7H_2O$, 50 $mmol.L^{-1}$ dithiothreitol (DTT), and 50 $mmol.L^{-1}$ ACC was added to the assay.

After the 4 days of storage at 12 °C, ethylene production and ACC oxidase activity were determined as described above from tissue samples of three depths of the mesocarp.

RESULTS AND DISCUSSION

The *in vitro* activity of ACC oxidase just after the heat treatment was significantly higher in mangoes treated for 3 min in hot water at 53 °C compared to the 90 min treatment at 46 °C (table 1). After 4 more days of storage at 12 °C, no differences were determined in enzyme activity with regards to water temperatures. However, comparing analysis periods of enzyme activity, just after the treatment and after the storage period, for either 3 or 90 min hot water dips, there was a significantly higher ACC oxidase activity just after the heat treatment in mangoes treated for 3 min at 53 °C. A longer exposure time at a lower temperature did not result in increased enzyme activity.

Table 1. *In vitro* ACC oxidase activity in mesocarp tissues of 'Keitt' mangoes after heat treatments and after 4 days of storage at 12 °C.

Heat treatments	Analysis period[a]	
	Just after treatment	After 4 days of cold storage
	ACC oxidase activity ($nLC_2H_4 \cdot g^{-1} \cdot h^{-1}$)	
3 min at 53 °C	0.708 a A	0.433 a B
90 min at 46 °C	0.445 b A	0.335 a A

[a]Values followed by the same capital letter in the rows and small letter in the columns do not differ significantly at $p < 0.05$ (Tukey's test).

These increases in enzyme activity are probably a result of the heat stress caused by the hot water treatments. Chan et al. (1998) observed the same stimulus to ACC oxidase activity in papaya treated in hot water.

Antunes and Sfakiotakis (2000) working with kiwis concluded that there is a pronounced inhibitory effect of heat on ethylene biosynthesis. Storage treatments of 38 °C or 40 °C completely suppressed ethylene production. The authors also

concluded that ACC oxidase is more susceptible to elevated temperatures compared to ACC synthase.

These differences in ACC oxidase activity of cv. Keitt mangoes did not result in significant increases in ethylene production following the hot water dips (figure 1). However, mangoes immersed in 53 °C water for only 3 min produced an average of 0.06 µL $C_2H_4 \cdot kg^{-1} \cdot h^{-1}$ more ethylene than mangoes treated for 90 min at 46 °C, which might be attributable to slight increments in enzyme activity, though not statistically different.

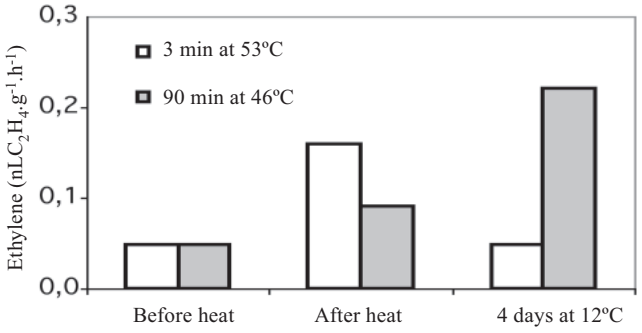

Figure 1. Ethylene production of 'Keitt' mangoes before and shortly after heat treatments and after 4 days of storage at 12 °C.

McCollum et al. (1995) observed the same differences in enzyme activity and ethylene production in heat-treated cucumbers. The authors suggested the possibility of a more complex control mechanism that goes beyond the regulation of the enzymes of the ethylene pathway, specifically ACC oxidase. However, the authors did not indicate which mechanism or mechanisms could be involved in such regulation.
On the other hand, Ievinsh et al. (2001) observed that distinct abiotic stimuli for increases in ACC oxidase activity were followed with synchronous increases in ethylene production of *Pinus sylvestris* needles. This conclusion diverges from our observations with "Keitt" mangoes. Most probably the climacteric ripening pattern of the mangoes and cucumbers in contrast to pine needles is responsible for these differences. After storage for 4 days at 12 °C, ethylene production was higher in mangoes from the 90 min hot water treatment compared to mangoes dipped in hot water for only 3 min. Mangoes from the latter treatment did not exhibit significantly altered ripening processes. After 4 days at 12 °C, they still were preclimacteric. Ethylene production was below 0.2 µL $C_2H_4 \cdot kg^{-1} \cdot h^{-1}$, a concentration indicated by Bender and Brecht (2000) as the beginning of the climacteric rise in ethylene production of cv. Tommy Atkins and cv. Kent mangoes.

On the contrary, mangoes immersed for 90 min in hot water at 46 °C had after 4 days at 12 °C a significant peak of ethylene production, above 0.2 µL $kg^{-1} \cdot h^{-1}$, which might be considered part of the climacteric peak. It might be furthermore considered that the climacteric peak was initiated by the heat treatment. Bender (1996) concluded that the climacteric peak of mangoes lasts at least 5 days with maximum ethylene production rates over 1 µL $C_2H_4 \cdot kg^{-1} \cdot h^{-1}$.

Ketsa et al. (1999) though determined a 5-day delay in the upsurge of the climacteric peak in heat-treated cv. Nam Dormai mangoes. Verlinden and Woodson (1998) also reported a 24-hour delay in the ethylene peak of carnations treated for 1 day at 44 °C.

These differences in the start of the climacteric peak might be related to the duration of the heat treatment. Ketsa et al. (1999) applied hot air at 38 °C for 3 days, while in the present experiment the longest treatment period was of 90 min. Most probably treatment duration is an important factor to consider in the indication if there are or are not effects on ripening processes of mangoes.

Analyzing the differences in the *in vitro* activity of ACC oxidase extracted from different depths of the mango mesocarp treated with hot water for 3 or 90 min, higher enzyme activity was observed only just after the 3 min treatment and only in the outermost layers of the mesocarp (table 2). In the remainder of the tissues no stimuli to ACC oxidase activity were observed.

Table 2. *In vitro* ACC oxidase activity in tissues from three mesocarp layers of 'Keitt' mangoes determined after either 3 min at 53 °C or 90 min at 46 °C and after 4 days of refrigerated storage at 12 °C.

Treatments	ACC oxidase activity[a] (nL $C_2H_4 \cdot g^{-1} \cdot h^{-1}$)		
	Mesocarp layers	Just after heat treatments	After 4 days of storage at 12 °C
Initial activity		0.667	
3 min at 53 ºC	Outermost	0.805 a	0.348 b
	Intermediate	0.600 a	0.363 a
	Innermost	0.718 a	0.589 a
90 min at 46 ºC	Outermost	0.378 a	0.361 a
	Intermediate	0.365 a	0.269 a
	Innermost	0.609 a	0.359 a

[a]Values followed by the same letter in the rows do not differ significantly at $p<0.05$ (Tukey's test).

Increased enzyme activity observed in the outermost layer of mango tissue after the 3 min hot water dip at 53 °C indicates that there is a tissue response to the treatment, how-

ever, this response does not permeate to the core of the mango mesocarp. Yet there is a tendency for a slight increase in enzyme activity determined in tissues from the median portion of the mesocarp, comparing 0.365 nL $C_2H_4 \cdot g^{-1} \cdot h^{-1}$ to 0.600 nL $C_2H_4 \cdot g^{-1} \cdot h^{-1}$ in table 2.

With the more prolonged heat treatment, 90 min at 46 °C, no increases in enzyme activity were determined. Reduced enzyme activity, nonetheless could be an indication of a more remarkable and lasting effect of the hot water dip on ACC oxidase. Most likely a lasting effect of the prolonged heat treatment was the cause of the reduced ACC oxidase activity in agreement with the observations of Jacobi and Wong (1992) regarding starch hydrolysis of heat-treated mangoes.

In interpreting the effects of the hot water dips on the *in vitro* activity of ACC oxidase determined immediately after treatment application, we need to consider that mangoes initiate ripening processes in the innermost layers of the mesocarp (Mitcham and McDonald, 1993a). This behavior becomes evident in examining enzyme activity before treatments (0.667 nL $C_2H_4 \cdot g^{-1} \cdot h^{-1}$) to the activity of tissues close to the seed after the heat treatment and after 4 days at 12 °C. In both analyses there is an indication of lower enzyme activity, possibly a consequence of the increased temperatures at the core of the mangoes as is required for quarantine treatment (Sharp, 1986).

The significant increase in the *in vitro* activity of ACC oxidase of cv. Keitt mangoes immersed for 3 min in hot water at 53 °C and the return to initial levels of activity after 4 days at 12 °C indicates that the effect of short exposure to heat only has a temporary effect on ethylene biosynthesis.

Higher rates of ethylene production during storage at 12 °C of mangoes treated for 90 min with hot water at 46 °C probably derives from enhanced ACC oxidase activity of epidermal tissues since the innermost tissues there is no increment in enzyme activity.

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