

ACTIVITY OF THE ENZYME POLYPHENOL OXIDASE AND SUSCEPTIBILITY TO DAMAGE FROM LATEX IN 'HADEN' AND 'TOMMY ATKINS' MANGOES

F. Díaz de León-Sánchez¹; F. Rivera-Cabrera¹; E. Bosquez-Molina²;
J. Domínguez-Soberanes²; Y. Álvarez-Hoppe¹; L. J. Pérez-Flores^{1¶}.

¹Departamento de Ciencias de la Salud,
²Departamento de Biotecnología. Universidad Autónoma Metropolitana-Iztapalapa,
Av. San Rafael Atlixco Núm. 186, Col. Vicentina, Iztapalapa, D. F. México.
C. P. 09340. MÉXICO. Correo-e: ljpgf@xanum.uam.mx, ljpgf@prodigy.net.mx (¶Corresponding author)

ABSTRACT

Damage from latex (DPL) represents a problem in Mexican mango, causing up to 10 % of annual losses. DPL begins when exuded latex touches the fruit's skin, producing a superficial darkness that diminishes mango quality and commercial value. Previous studies in mango suggest that terpenes favor damage from latex through the activation of polyphenoloxidases (PPO's). The objective of this study was to determine the correlation between damage from latex and the activity of total PPO's of the exocarp and latex in 'Haden' and 'Tommy Atkins' mangoes harvested in April, May, and June in Lázaro Cárdenas, Michoacán, México. Enzyme activity was measured with a spectrophotometer and susceptibility to DPL was evaluated as the percentage of damaged area. Activity of exocarp total PPO's was similar for 'Tommy Atkins' and 'Haden' during the first two harvest seasons, and it only increased for 'Tommy Atkins' in the last harvest season. Susceptibility to DPL showed contrasting performance between both cultivars. In 'Haden' it increased throughout the three seasons while it decreased in 'Tommy Atkins'. Therefore, total activity of exocarp PPO's and damage from latex do not explain susceptibility to DPL in Mexican mango. We suggest including the analysis of other components and latex enzymes in future experiments on susceptibility to DPL.

ADDITIONAL KEY WORDS: *Mangifera indica* L., exocarp PPO, latex PPO, post-harvest losses, fruit quality.

ACTIVIDAD DE LA ENZIMA POLIFENOL OXIDASA Y SUSCEPTIBILIDAD AL DAÑO POR LATEX EN MANGOS 'HADEN' Y 'TOMMY ATKINS'

RESUMEN

El daño por látex (DPL) representa un problema en el mango mexicano, ocasionando hasta 10 % de pérdidas anuales. El DPL empieza cuando el látex exudado entra en contacto con la cáscara del fruto produciendo un oscurecimiento superficial que disminuye su calidad y valor comercial. Estudios previos en mangos sugieren que los terpenos favorecen el daño por látex a través de la activación de polifenoloxidasas (PPO's). El objetivo de este estudio fue determinar la correlación entre el daño por látex (DPL) y la actividad de PPO's totales del exocarpo y del látex en mangos 'Haden' y 'Tommy Atkins' cosechados en abril, mayo y junio en Lázaro Cárdenas, Michoacán, México. La actividad enzimática se midió espectrofotométricamente y la susceptibilidad al DPL se evaluó como el porcentaje de área dañada. La actividad de PPO's totales del exocarpo fue similar en 'Tommy Atkins' y 'Haden' durante las dos primeras temporadas de cosecha, incrementándose en la última temporada en 'Tommy Atkins'. La actividad de PPO's totales del látex fue mayor en 'Tommy Atkins' que en 'Haden' en todas las temporadas de cosecha. La susceptibilidad al DPL mostró un comportamiento contrastante en ambos cultivares. En 'Haden' se incrementó a lo largo de las tres temporadas mientras que en 'Tommy Atkins' disminuyó. Por lo tanto, las actividades de PPO's totales en exocarpo y látex no explican la susceptibilidad al DPL en mango mexicano. Se sugiere analizar en experimentos futuros la participación de otros componentes y enzimas del látex en la susceptibilidad al DPL.

PALABRAS CLAVE ADICIONALES: *Mangifera indica* L., PPO de exocarpo, PPO de látex, pérdidas postcosecha, calidad de la fruta.

INTRODUCTION

Mexico is one of the main exporters of fresh mango in the world. The largest exportation volumes are sent to USA, Canada, Europe and Japan markets. This crop constitutes during the harvest season an important source of employment for peasants in the production regions, as well as a relevant devise source which increases each year (Rojas *et al.*, 1998; Galán, 1999; Galán, 2002).

Mangoes such as 'Tommy Atkins', 'Haden', 'Kent' and 'Keitt' are grown in México due to their demand in foreign markets. These cultivars are characterized by their low fiber and attractive colours (Campbell and Campbell, 1993).

Sapburn is one of the main problems affecting quality in mango for exportation, causing annual production losses of up to 10 % (Rojas *et al.*, 1998; Díaz de León *et al.*, 2000). This damage starts when exudated sap becomes in contact with fruit peel during harvest, producing browning and necrosis around lenticels, causing deterioration in fruit appearance, diminishing its commercial value and shelf life storage (Holmes *et al.*, 1993; Robinson *et al.*, 1993).

Fruit darkening has been associated with polyphenol oxidases and peroxidases enzyme activities which are present in mango exocarp and sap (Mayer and Harel, 1979; Park *et al.*, 1981; Loveys *et al.*, 1992; Thygesen *et al.*, 1995; Saby John *et al.*, 2002).

It has also been suggested that sap terpinolene and 3-carene content are important in sapburn induction, through a direct activation of PPO or indirectly providing the contact between enzyme and its substrate (Robinson *et al.*, 1993).

It has been reported that sap amount and composition depends on cultivar, production region and harvest season (Rojas *et al.*, 1998).

Based upon what it has been reported, sapburn shown by mango fruits depends upon PPO activity as well as on sap chemical composition (in particular terpene levels) (Robinson *et al.*, 1993; Saby John *et al.*, 2002). However, in a previous work we analyzed quantitatively terpinolene and 3-carene levels in the sap content of 'Haden' and 'Tommy Atkins' fruits. Sap major component is 3-carene followed by terpinolene, regardless the cultivar and the harvesting season. It was not possible to establish a correlation between terpene content analyzed (terpinolene and 3-carene) and susceptibility of mangoes to sapburn (Díaz de León *et al.*, 2000).

In this study exocarp and sap PPO's activities were quantified and related with sapburn occurrence analyzed in two mango cultivars grown in Lázaro Cárdenas, Michoacán, México, in three harvest seasons, in order to explore the relationship between sapburn and PPO activity.

MATERIALS AND METHODS

Biological material

Cultivars haden and Tommy Atkins grown in Lázaro Cárdenas, Michoacán, México, harvested in April, May and June, 1998 were used in this study. Sap and mango peel were analyzed for enzyme activity.

Polyphenol oxidase enzyme extraction

1. Peel (Du and Bramlage, 1995): 2 g of peel fresh tissue from five mangoes were weighted, ground with liquid nitrogen and homogenized with 10 ml extraction buffer (50 mM KH_2PO_4 pH 7.0; 0.1 mM EDTA; 0.2 % PVP insoluble; 3 mM MgCl_2). Homogenate was centrifuged at 15,000 g for 15 min at 4 °C (Beckman CS-15R). Pellet was discarded and supernatant was kept in ice as enzymatic source until its use.

2. Sap (Robinson *et al.*, 1993): Three sap samples of 10 ml exuded from five mangoes were collected in vials, and stored at -70 °C until their analysis. Each sample was extracted with three acetone volumes at 25 °C and the precipitate collected by centrifugation at 8,500 g for 10 min, at 4 °C. The extraction procedure was repeated once. Combined pellets were dried, under nitrogen for 30 min, resuspended in 15 ml of extraction buffer. Aqueous extract was centrifuged at 12,250 g for 30 min at 4 °C. Supernatant was kept in ice as the enzymatic source until its use.

3. Exocarp and sap polyphenol oxidase activity determination (Kar and Mishra, 1976): 500 μl of enzyme and 1500 μl assay buffer (125 mM KH_2PO_4 pH 6.8; 50 mM pyrogallol) were incubated for 5 min at 30 °C. 500 μl of 5 % H_2SO_4 were added to stop the reaction. Control tube contained assay buffer and H_2SO_4 from the start of incubation. Absorbance (420 nm) was determined with a Beckman DU-650 spectrophotometer.

1 U PPO activity was considered as the amount of enzyme which causes an increase of 0.1 in absorbance per minute.

Fruit susceptibility to sapburn injury

Susceptibility to sapburn injury of cultivars Tommy Atkins and Haden peel was tested in five mangoes, applying sap on a area of 5 cm^2 of the fruits. Skin damage was assessed 72 hours after sap application, and reported as average percentage of damaged area.

Statistical Analysis

SAS (6.1 program, SAS Institute Inc., Cary, NC) was used to perform analysis of variance according to a completely randomised design and mean comparisons of the main effects by Fisher and Duncan tests ($P \leq 0.05$).

RESULTS AND DISCUSSION

Enzyme activity showed no significant differences ($P \leq 0.05$) between 'Tommy Atkins' and 'Haden' (Figure 1) in the two first harvest periods (April and May) but in June enzyme activity in 'Tommy Atkins' was significantly higher than in 'Haden' ($P \leq 0.05$).

Sap PPO activity in 'Haden' was similar in the three harvest periods (Figure 2). In 'Tommy Atkins' enzyme activity increased slightly between April and May, and then decreased in June reaching similar levels to those detected in April. Furthermore, 'Tommy Atkins' sap PPO activity was twice higher than 'Haden' PPO's activity in the three harvest periods ($P \leq 0.05$).

Results show no differences in susceptibility to sapburn between cultivars Haden and Tommy Atkins harvested in May (Figure 3) whereas in April susceptibility was slightly higher in 'Tommy Atkins' and in June sapburn was slightly higher in 'Haden'.

It has been reported that 'Kensington' and 'Irvin' mango browning appearing from sap contact is related to the presence of polyphenol group enzymes (PPO's) such as catecolase (EC1.10.3.1) present in fruit exocarp, which catalyzes o-diphenol oxidation to quinones, and lacase (EC 1.10.3.2) present in sap, which in turn catalyses monophenol hydroxylation to o-diphenols (Mayer y Harel, 1979; Loveys *et al.*, 1992; Thygesen *et al.*, 1995).

Previous studies have suggested for mango cultivars Kensington and Irvin that when sap, containing high levels of terpenes as terpinolene and 3-carene becomes in contact with fruit exocarp, breaking of superficial tissues is produced, allowing the interaction of these enzymes and their

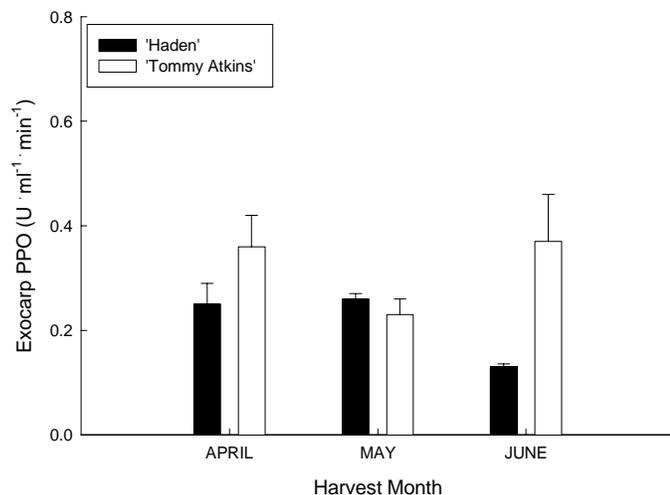


FIGURE 1. Exocarp polyphenol oxidase (PPO) activity in cultivars Haden and Tommy Atkins mango fruits in three harvest periods. Means of three replicates \pm SD.

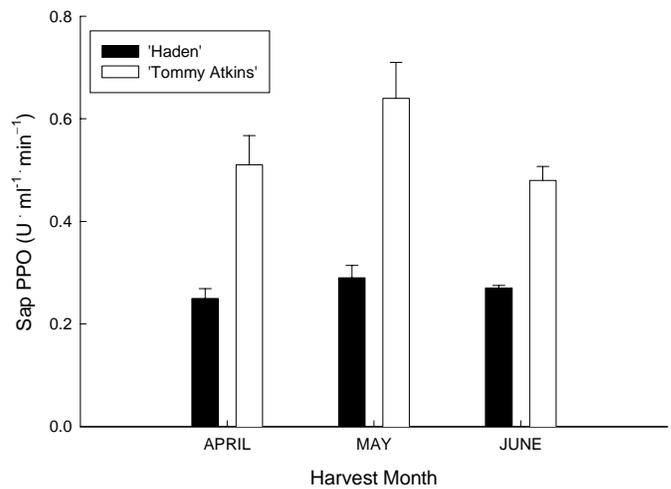


FIGURE 2. Sap polyphenol oxidase (PPO) activity in cultivars Haden and Tommy Atkins mango fruits in three harvest periods. Means of three replicates \pm SD.

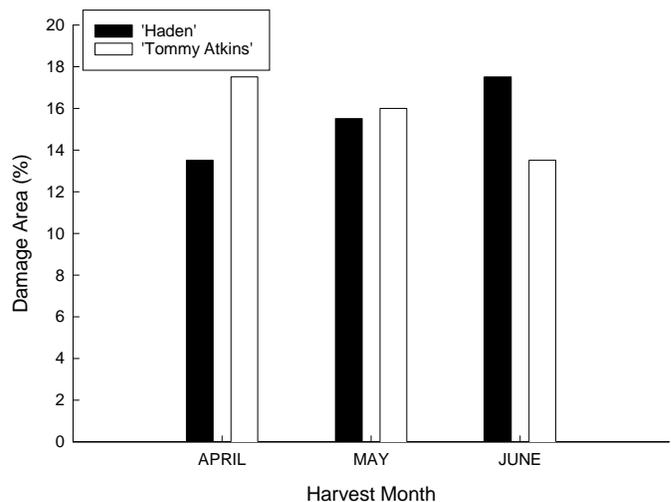


FIGURE 3. Sapburn injury susceptibility in 'Haden' and 'Tommy Atkins' mango fruits, in three harvest periods.

substrates or direct activation of these enzymes by sap terpenes. Thus, a correlation between sapburn polyphenoloxidases activity and terpinolene and 3-carene levels present in sap was observed (Robinson *et al.*, 1993). In Indian cultivars ('Badami', 'Totapuri', 'Malika', 'Malgoa') major terpenoids identified in sap were limonene, ocimene, and β -myrcene. In this study a clear relation was found between the peel PPO and peroxidase activities, the polyphenol content in the peel and the extent of injury (Saby John *et al.*, 2002).

In agreement with reported results PPO enzyme activity was detected in sap and exocarp in 'Haden' and 'Tommy Atkins' mangoes. Activity depended on cultivar and harvest period (Figure 1 and 2). However, these changes did not related with sapburn susceptibility.

It was found that analyzed cultivars, showed low PPO activity levels when sapburn susceptibility was higher, as in 'Haden' which presented the highest sapburn susceptibility in June (Figure 3) whereas exocarp PPO was low (Figure 1). Besides, sap enzyme activity remained constant despite cultivar sapburn susceptibility.

In previous studies we analyzed terpinolene and 3-carene in the sap content of 'Haden' and 'Tommy Atkins' obtained from Michoacán. In contrast to other reported results, our results show that the levels of 3-carene were higher in all cases than those of terpinolene (Rojas *et al.*, 1998, Díaz de León, *et al.*, 2000). However, the sapburn susceptibility of the cultivars harvested in Mexico was not adequately explained only on the basis of terpenes and PPO's activities.

The results suggest that sapburn is a very complex phenomenon in which other sap components, enzymes or seasonal variations in sap amount, composition or sapburn susceptibility may take place.

Therefore in future experiments, it should be analyzed the participation of other sap components as well as other sap and exocarp enzymes in sapburn in Mexican grown mangoes.

CONCLUSIONS

Results demonstrated that sap and exocarp PPO's activity depended on cultivar and harvest periods. However, this activity did not relate with sapburn susceptibility in Mexican grown mangoes. Thus, when Haden cultivar presented the highest sapburn susceptibility the exocarp PPO's activity was low and sap PPO's activity remained constant. In general, in 'Tommy Atkins' sapburn susceptibility decreased through the three harvest periods, whereas exocarp and sap PPO's activities showed no significant differences through harvest periods ($P \leq 0.05$). Therefore, other factors (sap components, enzymes, etc.) should be involved in sapburn susceptibility in Mexican grown mangoes.

ACKNOWLEDGEMENTS

Universidad Autónoma Metropolitana-Iztapalapa gave financial support for the present research. We greatly ap-

preciate the support of Frutícola San Blas Packinghouse (Lázaro Cárdenas, Michoacán) for providing mango fruits for this research.

LITERATURE CITED

- CAMPBELL, R. J.; CAMPBELL, C. W. 1993. Commercial Florida mango cultivars. *Acta Horticulturae* 341: 55-59.
- DÍAZ DE LEÓN, F.; FIGUEROA, S.; KERBEL, C.; SÁNCHEZ-DÍAZ, D.; PÉREZ, L.; BÓSQUEZ, E. 2000. Relationship between sap chemical composition and incidence of sapburn in Mexican mango. *Acta Horticulturae* 509: 321-328.
- DU, Z.; BRAMLAGE, W. J. 1995. Peroxidative activity of apple peel in relation to development of poststorage disorders. *HortScience* 30: 205-209.
- GALÁN, V. 1999. Importancia económica del mango, pp. 52-67. *In: El Cultivo del Mango*. GALÁN, V. (ed.). Ediciones Mundi-Prensa. España.
- GALÁN, V. 2002. Mango: Emerging tropical export crop. *Chronica Horticulturae* 42: 14-17.
- HOLMES, R. J.; LEDGER, S. N.; MACLEOD, W. N. B. 1993. Handling systems to reduce mango sapburn. *Acta Horticulturae* 341: 528-532.
- KAR, M.; MISHRA, D. 1976. Catalase, peroxidase, and polyphenoloxidase activities during rice leaf senescence. *Plant Physiology* 57: 315-319.
- LOVEYS, B. R.; ROBINSON, S. P.; BROPHY, J. J.; CHACKO, E. K. 1992. Mango sapburn: components of fruit sap and their role in causing skin damage. *Australian Journal of Plant Physiology* 19: 449-457.
- MAYER, A. M.; HAREL, E. 1979. Polyphenol oxidases in plants. *Phytochemistry* 27: 2189-2193.
- PARK, Y. K.; SATO, H. H.; ALMEIDA, T. D.; MORETTI, R. H. 1981. Polyphenoloxidase of mango (*Mangifera indica* var. 'Haden'). *Journal of Food Science* 45: 1619-1621.
- ROBINSON, S. P.; LOVEYS, B. R.; CHACKO, E. K. 1993. Polyphenol oxidase enzymes in the sap and skin of mango fruit. *Australian Journal of Plant Physiology* 20: 99-107.
- ROJAS, T.; FIGUEROA, S.; ACUÑA, M. C.; RIVERA, F.; DÍAZ DE LEÓN, F.; SANDOVAL, M. O.; SÁNCHEZ-DÍAZ, D. M.; BÓSQUEZ, E.; MADRID, R.; KERBEL, C.; PÉREZ, L.; VÁZQUEZ, C. 1998. Composición química del látex de mango de exportación. *Revista Iberoamericana de Tecnología Postcosecha* 1: 26-31.
- SABY JOHN, K.; BHAT, S. G.; PRASADA RAO, U. J. S. 2002. Involvement of peroxidase and polyphenol oxidase in mango sap-injury. *Journal of Food Biochemistry* 26: 403-414.
- THYGESEN, P. W.; DRY, I. B.; ROBINSON, S. P. 1995. Polyphenol oxidase in potato. *Plant Physiology* 109: 525-531.