Chilling Injury in Mamey Sapote Fruit (*Pouteria sapota*): Biochemical and Physiological Responses

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Abstract: Problem statement: Cold storage is needed in order to delay senescence and achieve a longer commercial life of tropical fruits like mamey sapote (*Pouteria sapota*), therefore, the physiological and biochemical responses of this fruit to cold storage were evaluated. **Approach:** Samples from different storage temperature (20, 10 and 2°C) were taken at five-day intervals and Chilling Injury Index (CII), decay (%), pulp firmness, weight loss (%), sucrose, fructose and glucose contents, electrolyte leakage (%), ethylene and carbon dioxide production rates and the activities of Peroxidase (POD), Polyphenoloxidase (PPO) and Phenylalanine Ammonia-Lyasa (PAL), were evaluated. **Results:** CII of fruit stored at 2 and 10°C was similar and symptoms included abnormal ripening into trimming zones close to the skin and pulp. Development of CI symptoms was more evident after 10 days of storage at 2 and 10°C. This trend may be associated with decrease of sucrose content but not with fructose that increased on fruits stored at 2°C (p<0.05). For mamey fruits stored at 2°C, the PAL activity was significant lower than in the fruits stored at 10°C, but it did not increase in response to the chilling temperature. No appreciable changes on POD activity were observed in fruit stored at 2°C. PPO activity continuously decreased on fruit stored at 2°C and similar behavior was observed on fruit stored either at 10 and 20°C, during the first 15 days of storage. **Conclusion:** Our results demonstrated the sensitivity to mamey sapote fruits to low storage temperatures (2 and 10°C). Symptoms developed by fruit include abnormal ripening into trimming zones close to skin, as well as darkened zones into middle pulp. Sucrose content could be a better chilling injury indicator in mamey sapote than electrolyte leakage, ethylene production and POD, PPO and PAL activities.

Key words: Peroxidase, polyphenoloxidase, decay, exotic fruits, ethylene

INTRODUCTION

The demand of tropical and subtropical fruits has been increased in the last two decades due to its good sensorial characteristics and high nutrimental value. Particularly, mamey sapote (*Pouteria sapota*) has been considered to have great economic potential because of its typical taste and aroma[1]. However, the postharvest storage life of this kind of tropical fruits is limited by temperature exposure; therefore temperatures below 12°C are commonly used. Exposure of mamey fruit to temperatures below 5°C for more than 10 days causes Chilling Injury (CI) as indicated by dark-brown spots on the peel, failure to ripen, off-flavor development and increased decay incidence after transferring the fruit to higher temperatures[2]. There is a lack of reports regarding the causes of CI symptoms developed in mamey fruit[3].

Under stress conditions such as cold storage, different biochemical changes are associated with fruit physiological disorders. The oxidases, peroxidases (POD; E.C.1.11.1.7), polyphenoloxidase (PPO; E.C.1.14.18.1) and the induction of phenylalanine ammonia-lyase (PAL; E.C.4.3.1.5) have been considered to be a defensive mechanism of plants against stress[4]. These enzymes have been involved in the development of physiological disorders such as CI in many vegetables[5].

Therefore, the aim of this study was to investigate the major physiological and biochemical changes of mamey fruit during storage at chilling (2 and 10°C) and non-chilling (20°C) temperatures.

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MATERIALS AND METHODS

Plant material and storage conditions: Mamey fruits were harvested on April 12, at the beginning of the harvest season (April-May), in an orchard cultivated in Tabasco, Mexico. Fruits were transported and maintained under controlled temperature until arrival to the laboratory, where were selected according to good appearance and freedom of defects. Fruits were separated in three groups of 80 fruits each and stored at 20, 10 and 2°C under 85-90% RH, during 25 days in temperature-controlled chambers. At five days intervals, 10 fruits were sampled from each storage temperature for further evaluation of the physical, physiological and enzymatic parameters during a thirty-day period. Three fruits from each temperature were removed from each group and CO_2 and ethylene production were measured and then, firmness changes were determined. Afterward, remained fruit were sampled for ion leakage and sugars determination and PPO, POD and PAL activities. Three lots of 20 fruit were used to determine weight loss, evolution of CI and decay, in each temperature during the storage period. Initially, fruit were weighted and inspected for decay symptoms.

Physical and physiological evaluation: Weight loss (%) was determined periodically as percentage of the original weight of the samples. The CII was evaluated on the subgroup of 20 fruits, using a subjective scale according to Martínez-Téllez *et al.*[6], based on the browning intensity: 0 = No pitting; 1 = Slight (until 10% of fruit surface); 2 = Medium (until 20% of fruit surface) and 3 = Severe pitting (more than 20% of fruit surface).

Decay was determined using a subjective scale according to Pérez-Tello *et al.*[7], considering as damage when more than 30% of the fruit surface presented an evident fungal or wooding damage. It was evaluated initially and at each interval of sampling period.

Flesh firmness was measured in three points of the equatorial zone of ten fruits per each storage temperature at each sampling period and expressed as Newtons (N) using an awl (10 mm of diameter) with a Chatillon penetrometer DFG-50 (John Chatillon Sons, Inc. USA) according to the method of Bourne[8].

Ethylene and CO_2 production rates were measured in a gas chromatograph (Varian Star 3400, MFG Corp., USA) using a TCD and FID detectors with a Haysep N column according to the method described by[9] and expressed as μL ethylene kg^{-1} h^{-1} and mL CO_2 kg^{-1} h^{-1}, respectively.

Electrolyte leakage (%) was measured from three randomly selected fruits per each group. Two pulp discs with a diameter of 1 cm and a thickness of 2 mm were excised with a cork borer from the equatorial region of the fruit. The discs were incubated with 0.3 M mannitol for 1 h at ambient temperature and constant shaking. Initial electrolyte leakage was determined following incubation of each tube and final, after boiling the solution for 45 min. Electrical conductivity of the bathing solution was measured using a conductivity meter (Mod. 1481-61, Cole-Parmer Instrument, Co, USA). Rates of ion leakage were expressed as a percentage of the total conductivity [(initial/total)×100].

Enzymatic assays: Acetone powder from mamey fruit was obtained from flesh of three sampled fruits stored at 2, 10 and 20°C and stored at -47°C until used for further enzyme extraction. PPO, POD and PAL activities were measured using a BioSpec 1601 spectrophotometer (Shimadzu, Japan).

The PPO (EC 1.14.18.1) activity was determined according to Pérez-Tello *et al.*[10]. PPO was extracted from 0.2 g acetone powder with a phosphate buffered saline solution, pH 7.2, containing 1 M potassium chloride and 5% (w/w) polyvinyl-polypyrrolidine. Enzymatic activity was measured at 30°C and 410 nm, in reaction mixtures with 1.25 mL of 20 mM 4-methylcatechol as substrate and 25 μL of the enzyme extract. PPO activity was expressed on a dry matter basis as units of PPO g^{-1} min^{-1} of acetone powdered flesh tissue.

POD (EC 1.11.1.7) activity was determined using a modified method of Pérez-Tello *et al.*[10]. The enzyme was extracted from 0.2 g of acetone powder from flesh tissue with 6 mL of 100 mM Tris-HCl, pH 8.0, containing 5 mM β-mercaptoethanol and 5% (w/w) polyvinyl-polypyrrolidine. POD activity was measured at 470 nm for 2 min at 30°C in 2.15 mL reaction mixtures with 10 mM sodium acetate, pH 5.3, containing 0.5% of the substrate guaiacol, 0.25 mL 0.1% hydrogen peroxide and 100 μL extract. POD activity was expressed on a dry matter basis as units of activity g^{-1} of acetone powered flesh tissue min^{-1} (U g^{-1} min^{-1}).

PAL (EC 4.3.1.5) activity (n mol g^{-1} h^{-1}) was determined according to Martínez-Téllez and Lafuente[11]. PAL was extracted from 0.4 g of acetone powder with 15 mL of 100 mM sodium borate, pH 8.8, containing 20 mM β-mercaptoethanol. Proteins were salted out with ammonium sulphate to a final saturation of 46% and thereafter dissolved in 4.5 mL of 100 mM ammonium acetate, pH 7.7, containing 20 mM β-mercaptoethanol. The reaction mixtures contained 2 mL

of the purified enzyme extract and 0.6 mL of 100 mM L-phenylalanine in a final volume of 6 mL. PAL activity was determined by measuring the absorbance of cinnamic acid at 290 nm for 2 h at 40°C and expressed on a dry matter basis as nmol of cinnamic acid g^{-1} of acetone powder fresh tissue h^{-1} (n mol g^{-1} h^{-1}).

Sugars determination: Sucrose, glucose and fructose contents were quantified according to a modification of the HPLC procedure described in Hunt *et al.*[12] and López-Hernández *et al.*[13]. Sucrose, glucose and fructose, were initially extracted from 10 g of flesh fruit stored at 2, 10 and 20°C, with 50 mL of HPLC grade water and boiled for 15 min. The solution was diluted in HPLC grade water (1:20, v/v), filtered in Wahtman paper No. 40 and separated by ultracentrifugation (Beckman, Co.) at 4500 rpm. Supernatants were filtered through nylon GV membranes (Millipore, Co.) into a glass syringe and the extracts were injected in an HPLC equipment (Varian 9012, USA). Mobile phase was acetonitrile-water (80:20) at 192 nm in the UV region using an UV-VIS detector (Varian 9050, USA) and μ-NH₂ Bondapak column (3.9×300 mm; 10 mm of particle size) and a flow rate of 1.5 mL min^{-1}[12]. Quantitative determinations were made by comparing the sample analyses results of three fruit extracts with the standard mixture of sugars prepared by dilution of 4% w/v solutions of reagent grade in HPLC grade water and expressed as mg g^{-1} Fresh Weight (FW).

Statistical analysis: Statistical analysis of the experimental data were done according to a completely randomized design with three replications by performing ANOVA tests and comparisons of means with the main factors, using the Tukey-Kramer test ($\alpha = 5\%$). The treatments were the time and storage temperatures. The data obtained were analyzed using NCSS (6.0.22 version) and Sigma Plot (8.0 version) computer packages.

RESULTS

Physical and physiological evaluation: Figure 1 shows the Chilling Injury (CI), Weight Loss (WL), firmness and decay of mamey fruits during storage at 2, 10 and 20°C. The highest WL was observed on fruits stored at 20°C, which increased significantly within the storage period. No significant differences in weight loss were observed in fruits stored at 2 and 10°C. The highest firmness loss was observed after 5, 10 and 15 days in fruits stored at 20, 10 and 2°C, respectively. Fruit

firmness decreased constantly with the storage period but at different extent. After five days of storage, fruits stored at 20°C reduced their firmness from 150-30 N. Afterward, it decreased to values of 10 N and was maintained relatively constant with values slightly lower than those fruits stored at 10°C (Fig 1a and b).

CII significantly increased either on fruits stored at 10 and 2°C after 10 days of storage with a similar damage pattern in both treatments, reaching the maximum CI value (1.7) after 20 days of storage. As expected, no symptoms of CI were observed on fruits stored at 20°C (Fig 1c). Even though the CII was similar on fruits stored at 10 and 2°C, apparently fruits stored at 2°C developed other physiological disorders that significantly affected deterioration of fruit measured as percentage of decay (Fig 1d). After 10 days of storage decay of fruit was close to 20%, being more evident on fruit stored at 2 and 20°C that presented similar percentage of decay. Deterioration of fruit stored at 10°C was lower than that observed at 2 and 20°C and started to increase after 15 days of storage (Fig 1d).

In general, fruit metabolism is enhanced by the rise of storage temperature, while maturation of chilling-sensitive fruit could be seriously affected by low temperature storage. In this matter, we observed that fruits stored at 20°C matured normally, in contrast with fruits stored at 2 and 10°C which presented different CI symptoms and abnormal maturation (Fig 1c).
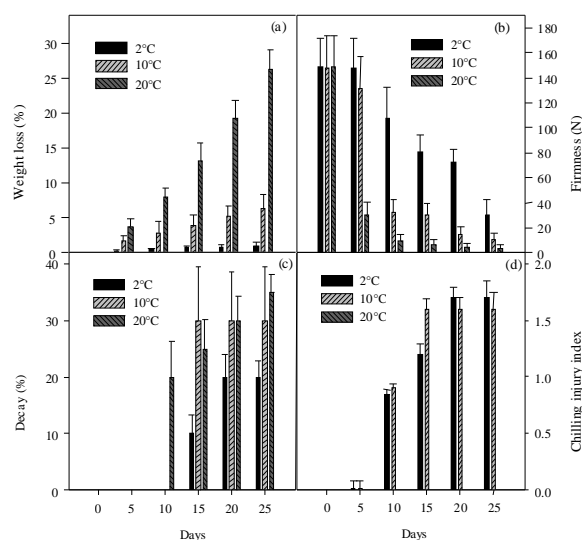

Fig. 1: Weight Loss (a): Firmness; (b): Decay; (c): Chilling Injury Index (CII); (d): In mamey fruits stored at 2, 10 and 20°C, (80-90% RH)

Development of decay symptoms started after 10 days of storage in fruits stored at 20°C (Fig 1c). Afterwards, a sharp increase in the percentage of decay (30%) was observed on fruit stored either at 2 and 20°C. After 25 days of storage, about 50% of the fruits stored at 2 and 10°C showed severe CI symptoms in the pulp (grade 3), caused mainly by abnormal flesh ripening and darkening. Some of these symptoms could be due to some metabolic disruptions that occur at low temperatures.

At the end of storage, most of the fruits stored at 20°C were invaded by green mold but in different extent. The high content of carbohydrates, lipids and water in the pulp, could induce mold growth near to peduncle and affect overall quality of fruits stored at 20 and 10°C.

The CO_2 and ethylene production are shown in Fig. 2. The behavior observed for CO_2 production in fruits stored at 20 and 10°C showed a similar pattern until day 25 of storage (Fig 2a). Also, the CO_2 production rate in control fruits (20°C) decreased during the first 15 days of storage and remained without significant changes. However, after five days, fruits stored at lower temperatures presented a significant decrease in CO_2 production, from 155-25 mL CO_2 kg^{-1} h^{-1}. Afterward, CO_2 production kept gradually decreasing until it reached the lowest values (5-11 mL kg^{-1} h^{-1}) at the end of the storage period. No significant differences on respiration rate were observed in fruits stored at 2 and 10°C.

The highest ethylene production was observed initially (Fig 2b). Afterwards, ethylene production significantly decreased with the storage period. A similar behavior was observed for ethylene production. After five days of storage, a sharp reduction in ethylene production was observed in fruits stored either at 2, 10 and 20°C. Afterward, ethylene was maintained stable with slight changes on fruits stored at 2°C. The lowest ethylene production was observed after 15 days on fruits stored at 20°C. No ethylene was detected after 25 days.

Electrolyte leakage: A noticeable increase in electrolyte leakage was observed in all storage temperatures (2, 10 and 20°C) after five days of fruit exposure, reaching an almost two-fold increment by day 25 (Fig 2c). The increase trend of electrolyte leakage could be associated with the invasion of mold (decay) at 10 and 20°C, chilling symptoms at 2 and 10°C or may also have caused altered ripening and senescence processes at 20°C as sown in Fig 1c and 1d. Loss of cell integrity by senescence was more accentuated at 20 and 10°C than at 2°C, causing an increase of electrolyte leakage. The trend of electrolyte leakage at 10°C showed a similar behavior to the group stored at 20°C along the 25 days of storage.
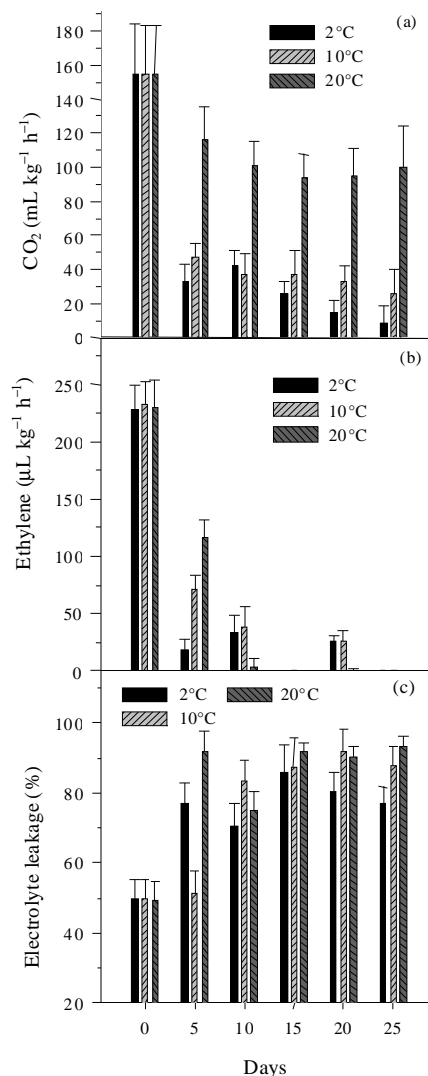

Fig. 2: CO_2 production; (a): Ethylene production; (b): Electrolyte leakage; (c): In mamey fruits stored at 2, 10 and 20°C, (80-90% RH)

Enzyme assays: In this research PAL activity was detected, but showing low values along the 25 days of storage in the three groups of fruits (Fig 3a). PAL activity was higher after five days of storage at 10 and 20°C and decreased along the further 10 days of storage. Moreover, PAL activity increased after 15 and 25 days of storage. Fruits stored at 10°C showed an initial decrease in PAL activity between days 5 and 25 and then a slowly increase was observed after 25 days. For mamey fruits stored at 2°C, the PAL activity was significant minor with respect to the fruits stored at 10°C, but it did not increase in response to the chilling temperature. Such low levels of PAL activity in those fruits could be a result of the age and harvest date.
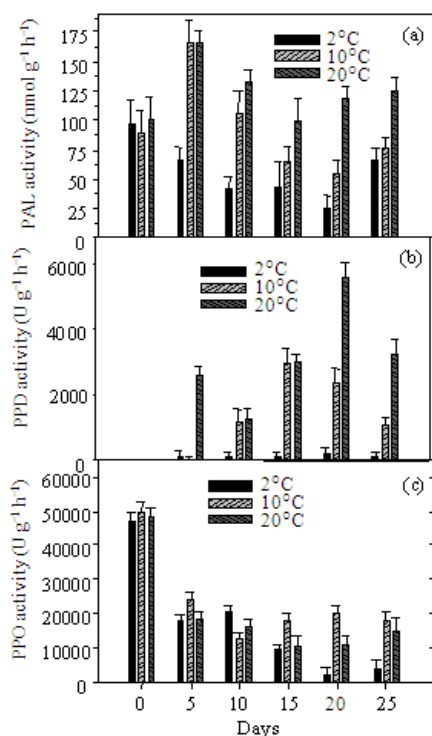
Fig. 3: POD activity; (a): PPO activity; (b): PAL activity; (c): In mamey fruits stored at 2, 10 and 20°C, (80-90% RH)

Mamey fruits used in this work were collected at the beginning of the harvest season (April 12), thereby the magnitude of these responses was not indicative of the chilling susceptibility degree of this specific cultivar.

In this study the initial POD activity was negligent and not detected (Fig 3b). However, POD activity increased with temperature and storage period. The highest POD activity was observed in fruits exposed at 20°C after 20 days of storage, followed by those stored at 10°C. POD activity levels in fruits stored at 2°C was very low during all storage period. After five days at 20°C, a sharp increase on POD activity was observed with respect to other temperatures. However at 10°C the maximum POD activity (3000 U) of fruits was reached after 15 days and then it decreased reaching a value of 1000 U after 25 days of storage. In this research, there were not observed appreciable changes in POD activity in fruit stored at 2°C.

The arise on POD activity was correlated with the deterioration of fruits stored at 10 and 20°C but not with the CI symptoms observed in those fruits stored at 2 C. PPO activity continuously decreased (48332-15151 U) on fruits stored at 2°C (Fig. 3c). Similar behavior was observed on fruits stored either at 10 and 20°C, during the first 15 days of storage. Afterwards,
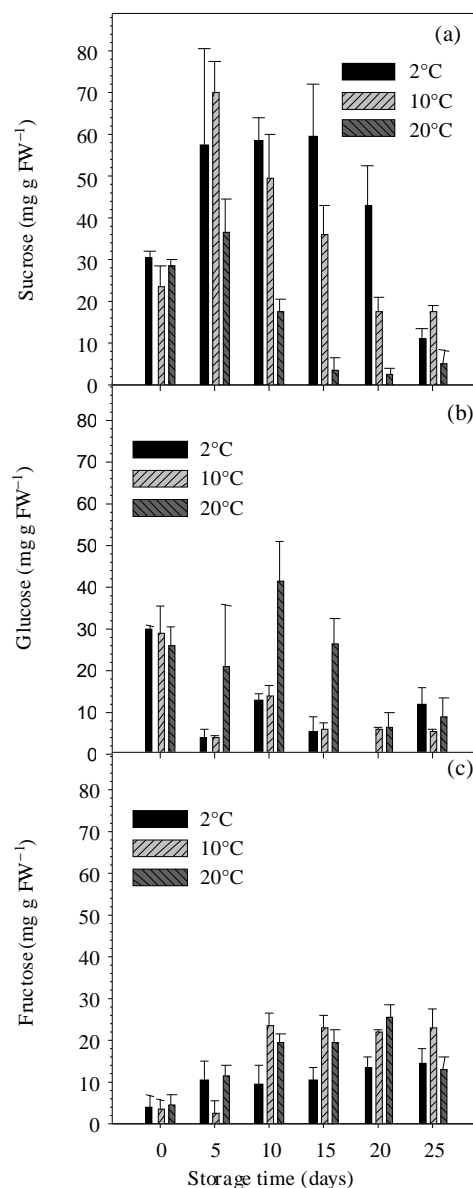

Fig. 4: Sucrose (a): Glucose (b): Fructose content (c): In mamey fruits stored at 2, 10 and 20°C, (80-90% RH)

PPO activity was maintained stable until the end of the storage period. Even when PPO and POD are involved in deteriorative processes of tissue such as browning, activity of both enzymes could vary depending upon the fruit, cultivar and storage conditions.

Carbohydrates content: The pattern of changes in soluble carbohydrates of fresh mamey sapote stored at 2, 10 and 20°C are shown in Fig. 4. The major carbohydrate detected in all samples was sucrose,

followed by glucose and fructose. Sucrose content of mamey sapote decreased during storage at 20°C (Fig. 4a), where the highest sucrose level in this group was observed on the 5th day, with a 1.2-fold increase. However, fruits stored at 10°C reached the highest sucrose level after five days of storage, showing a 3-fold increase of this carbohydrate. Fruits stored at 2°C showed a constant value in sucrose content between days 5-15; a further decline was observed from day 20th onwards. A significant and uniform decrease of sucrose content was observed in fruits stored at 10°C along the last three sampling periods (days 10, 15 and 20). Glucose levels of fruits stored at 20°C increased after 10 days and further decreased between days 15 and 20 of storage as shown in Fig 4b. The increase of glucose was probably due to sucrose degradation by invertase into glucose and fructose, which may be a more useful form for fruit respiration. Glucose content showed an initial 7.2-fold decrease in fruits stored at 10 and 2°C and kept a similar behavior from days 5-25, while the highest value of glucose levels was reached by fruits stored at 2°C after 10 days of storage (Fig. 4b).

Fructose content increased along with the storage time in fruits stored at 20°C; the maturity and senescence processes can be related to this event. Fructose content showed a similar pattern at 10 and 2°C, but it was higher at 10°C during the 25 days of storage (Fig. 4c). The trend of fructose content at 2°C showed a similar behavior than the group stored at 10°C; this occurrence could be attributed to a response to chilling stress.

DISCUSSION

It has been reported that firmness of tropical fruits decreased during ripening and senescence processes[2]. Different enzymes are involved in the firmness reduction of flesh tissue and their activity increase along with increases in storage temperature. Therefore, it is expected that fruits stored at higher temperatures had the highest firmness and weight losses as observed in the present study. Firmness could be an important physical indicator for mamey fruit quality. However, the firmness evaluation on mamey sapote pulp, necessarily involves a destructive technique and the major problem of mamey fruit is the variation in fruit maturation. Recently, it has been reported that a non-destructive technique such as vibration, may be useful to detect slight changes in firmness of tomatoes treated with methyl jasmonate and stored at different temperatures[14]. This technique could be used as an alternative to evaluate mamey firmness. However further studies are needed in order to demonstrate the

advantages that offer these techniques in this type of fruit.

Tropical fruit normally develop chilling injury symptoms below 10°C. Results obtained in the present study, confirmed those obtained previously in Mamey sapote where CI symptoms were observed on fruit stored at 10°C[15]. Nevertheless, other authors reported that lower temperatures (5°C) develop this physiological disorder in this tropical fruit[6]. In this study we observed that fruits stored at 2 and 10°C presented similar patterns of CI symptoms development. However, mamey fruits stored at 12°C did not show chilling injury development[16]. These results confirmed that storage duration and temperature are critical factors for CI development in sensitive fruits.

Respiration rate of mamey fruit could be influenced by different factors such as cultivar, storage temperature among others. Kader reported a CO_2 production between 25 and 35 mL kg^{-1} h^{-1} in mamey sapote stored at 20°C[2]. Our data contrast with data reported by this author, but nevertheless the CO_2 production observed in this study was similar to an earlier study on Mexican mamey sapote stored during 30 days at 20°C (90-137 mL CO_2 kg^{-1} h^{-1})[17]. Alia *et al.*[15] reported a CO_2 production of 25-85 mL kg^{-1} h^{-1} during 12 days of storage at 20°C. These authors reported that mamey fruit had the maximum climacteric peak (85.3 mL CO_2 kg^{-1} h^{-1}) 8 days after harvest. However, in the present study fruit stored at 20°C did not present any peak during storage and followed a pattern similar to those fruits stored at 2 and 10°C.

Ethylene affects maturation processes and can be stimulated by different stresses such as low and high temperature storage. The slight increase of ethylene observed in fruits stored at 2 and 10°C after 10 and 20 days, could be associated with the CI development. Alia *et al.*[15] reported that mamey fruit stored at 5 and 10°C and then transferred at 20°C, increased the CO_2 and ethylene production as a consequence of cold stress, which was associated with the incidence of CI symptoms. Citrus fruit stored at 2°C, also presented an increased ethylene production as response to cold storage of chilling sensitive mandarins[18]. Although the respiratory responses of many commodities and tissues have been related to chilling temperatures, the course and rate of respiration are not uniform among these commodities and tissues[19]. In some commodities ethylene production is stimulated during chilling; in others, ethylene production does not occur until chilled tissues are transferred to a warmer non-chilling temperature[19]. The results in this work, suggest that ethylene production is not a good indicator of chilling

injury in mamey sapote stored at 2 or 10°C. However, it appears that fruits used in the present experiment had a higher metabolic rate than those used previously.

Despite electrolyte leakage has been established as a parameter to evaluate chilling injury[20,21], the results in this study varied significantly (Fig 2c). Therefore, a conclusion regarding tissue damage in mamey fruits cannot be drawn from this parameter.

PAL activity is a key enzyme of secondary metabolism, expressed as a defense mechanism in response to different stresses. In 'Fortune' mandarins fruits, PAL activity could be a protective response of the fruit to repair the damage originated by chilling[22]. It was observed that PAL activity was higher in mamey fruit after five days of storage at 10 and 20°C and decreased along the further 10 days of storage. Moreover, PAL activity increased after 15 and 25 days of storage. This behavior at 20°C probably indicates a physiological response to fruit senescence and to mold attack by regulating the defense mechanisms. Fruits stored at 10°C showed an initial decrease in PAL activity between days 5 and 25 and then a slowly increase was observed after 25 days. Probably PAL enzyme was activated by chilling at 10°C at the middle of storage and inhibited at the beginning[23,24].

Studies in Fortune mandarins have indicated that the effect of cold stress in increasing PAL activity was clearly higher in fruit harvested in February (1400 n mol $g^{-1} h^{-1}$) than in those harvested in January (400 n mol $g^{-1} h^{-1}$)[25].

With these results we concluded that PAL activity was not a good biochemical marker for chilling susceptibility in mamey sapote fruits.

POD and PPO activities play an important roll in the oxidative degradation of phenolic compounds, as they lead the production of brown polymers[26]. Fruit stored at 10°C reached its maximum POD activity (3000 U) after 15 days and then it decreased reaching a value of 1000 U after 25 days of storage. These values are in accordance with previous studies in mamey fruit that reported a POD activity of 1600 U-POD g^{-1}[15]. These authors reported a maximum POD activity of 2400 U g^{-1}, after 25 days at 10°C. In this research, no appreciable changes in POD activity in fruit stored at 2°C were observed.

The arise on POD activity was correlated with the deterioration of fruits stored at 10 and 20°C but not with the CI symptoms observed in those fruits stored at 2 C. In previous studies on mamey sapote fruit, the high POD activity was related with the CI development observed at the end of storage[27]. Some authors mentioned a relationship between POD activity and some physiological disorders in vegetables[4,5].

However, storage of fruit at chilling temperature (2°C) did not show to induce a significant increase in POD activity along the 25 days of storage. The increase in POD activity detected in fruits stored at 10 and 20°C, could be influenced by the observed accelerated metabolism and decay.

Even when PPO and POD are involved in deteriorative processes of tissue such as browning, activity of both enzymes could vary depending upon the fruit, cultivar and storage conditions. Previous results suggest that POD activity was inhibited in pulp of mamey fruits chilled at 2°C, while PPO activity was not affected[27].

It has been shown that increased resistance to CI is strongly associated with the carbohydrate content[28,29]. A significant and uniform decrease of sucrose content was observed in fruits stored at 10°C along the last three periods of sampling (days 10, 15 and 20). The relative increase in sucrose content at 2 and 10°C in the first period of storage (day five) could be attributed to a physiological response of tissues to chilling injury[30,31]. The trend of fructose content at 2°C showed a similar behavior than the group stored at 10°C; this occurrence could be attributed to a response to chilling stress. Nevertheless, the relationship between chilling injury and content of sucrose, fructose and soluble sugars varies respect the fruit and is not clear yet.

CONCLUSION

Our results demonstrated the chilling sensitivity of mamey sapote fruits to storage temperatures of 2 and 10°C. Symptoms developed by fruit included abnormal ripening into trimming zones close to skin, as well as darkened zones into middle pulp. The lower the storage temperature the higher the chilling injury symptoms. Fruit sucrose content increased as the temperature storage decreased. Ethylene production and electrolyte leakage were not correlated with the chilling injury symptoms under our experimental conditions. Enzymatic activities of POD, PPO and PAL in mamey sapote fruits stored at 2 and 10°C changed in different extent and were not correlated with the other evaluated variables. According to previous studies, it is difficult to establish a good biochemical indicator of chilling injury, due to the intrinsic physiological characteristics of mamey fruit. Therefore additional studies are needed in order to elucidate the possible mechanism by which chilling injury occurs in mamey sapote fruit.

ACKNOWLEDGEMENT

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