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FINAL PROJECT REPORT

STATE-OF-THE-ART POSTHARVEST HANDLING OF "MANILA" MANGOS

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FOR

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1. Executive summary

The Manila variety of mango from Mexico was originally brought from the Philippines by Spanish sailors during the 16th century. In 2010, this variety was the second most widely produced nationally (322,490 tonnes 39,103 hectares), and though it has extraordinary sensory quality, it's participation in the export market is minimal due to its susceptibility to the hot water treatment protocol and limited postharvest shelf life. Research conducted on this variety is extensive and includes aspects related to variety generation, crop handling, postharvest technology and molecular biology studies of the ripening process. A review was conducted of the research results from 14 Mexican research centers that generated 64 published works, 13 projects presented in various forums, 17 thesis papers of different academic grades, and 13 websites.

Given the interest that exists in maintaining a steady production flow, particularly during the off-season, the use of flowering inductors and growth regulators is frequent and can have implications on the quality and the future production of the fruit. For this reason, this review includes the forced production aspect of the fruit at the preharvest stage and the postharvest technology applied to this variety.

The yields per hectare demonstrate annual fluctuations that indicate alternating phenomena in the production cycle and point to a biannual behavior in the tree, all of which is relevant to the responses to the applied agricultural practices and their impact on the expected production volumes. The use of growth regulators such as paclobutrazol (PBZ) or Cultar, as well as the spraying of flowering inductors such as KNO_3 or NH_4NO_3, are common practices in the various mango production regions in Mexico. These are used to regulate production and to produce fruit during the off-season. Nevertheless, these practices can generate metabolic stress that could affect plant development, production, and the development of the fruit in addition to its quality. Given the biannual nature of the mango tree, the research carried out so far has not yielded any general answers

regarding what is happening to the physiology of the trees in the plantations that are subject to the annual use of flowering inductors and PBZ. It's possible that this may be one of the causes of the decreases in mango production from various production areas, and it is not known in what way this factor may be related to climate change. It is for this reason that it is important for these fields of research to be addressed.

Manila mangos are polyembryonic and possess a higher metabolic rate than the Tommy Atkins, Haden, Kent, and Keitt varieties, and it also has key histological differences. It's skin and cuticle are thinner, it has a lesser number of epidermic and hypo epidermic cell layers, as well as thinner cell walls. The cells in the flesh are larger and it is for that reason that it's firmness is lower when the osmotic changes occur during the ripening process, which explains its high susceptibility to decay, mechanical damages, and attack from pathogens.

The harvest indexes used are the same that are used for harvesting other varieties (filling of the shoulders, color of lenticels, etc.). A mathematical equation has been developed that allows us to predict the development of the fruit as a function of the accumulated heat units, and it has been implemented in a procedure to separate immature fruit from mature fruit by way of a flotation separation process in a solution of 1% of common salt in water. This process can be used at the beginning of the harvest season, and whenever there are any difficulties in selecting the fruit based on their maturity.

The ripening process for Manila mangos is partially characterized, and the studies to homogenize or accelerate its ripeness only referred to the use of sodium carbide. There are no reports that refer to the use of ethylene inhibitors to delay the process.

Although the fruit is climacteric, the changes in color, the increase in total soluble solids, and the loss of firmness do not correlate to the respiration pattern of the

fruit. Changes in these factors begin several days prior to the climacteric peak of respiration.

In another polyembryonic variety, the ripening process begins 10 days prior to achieving harvest maturity and shows several optimal levels of ethylene production during its development. It's possible that this fact may be of particular importance for Manila mangos, and it should be further researched.

Studies on preserving Manila mangos under refrigerated conditions indicate that it is sensitive to low temperatures due to the fact that its phase transition temperature is 12° C, and for that reason it must be stored at temperatures above that point. Considering that the export process uses a temperature of 10° C, we could point out that the fruit is subjected to cold stress and it's possible that the damage that occurs after applying the hot water treatment may be caused by the lower storage temperatures, and not necessarily by the hot water treatment itself. A study should be undertaken to assess shelf-life in fruit that is subjected to the hot water treatment (in accordance with the USDA-APHIS protocol), waxed in the usual manner, and stored at 13° C.

Studies on the application of techniques to reduce chilling injuries show that they have not proven effective in achieving a shelf-life that extends beyond three weeks. Although there have been indications that the hot water treatment could reduce these damages, there would have to be a change in the storage temperature. It would also be useful to assess the use of methyl jasmonate with this variety for the purpose of reducing the rate for chilling injuries.

No studies have been conducted on the use of controlled and modified atmospheres for storage. There are only reports that refer to their use as an insecticide treatment where it has been determined that this variety is sensitive to atmospheres that are low in oxygen and rich in carbon dioxide.

Research conducted on how to control decay through the use of wax or plastic film has been incomplete or insufficient. No useful material has been developed that allows for the adequate control of the issue or the manipulation of the food's physiology, particularly after the application of the quarantine treatments.

Studies conducted on the control of anthracnose are based on the the pre-and postharvest use of fungicides, but research has been done on the biological control of the disease through the use of crops that are antagonistic to the pathogen (*Rhodotorula minuta* y *Bacillus subtilis*) that have been able to reduce the rate of the disease by up to 8%. The production of these micro-organisms is currently at the pilot project stage and several experiments have been conducted in the field with other varieties. The effectiveness on this variety has not been determined, as of yet.

The application of the hot water treatment has generated quality issues for the fruit, whereas the treatments based on insecticide atmospheres exhibit physiological restrictions and, as of yet, have not been accepted by USDA-APHIS.

Gamma-ray irradiation treatment (150 to 600 Gy) appears to be the most adequate option, one that is also accepted by USDA-APHIS. Nevertheless, it will be necessary to resolve the fruit decay problem in order to achieve an adequate shelf-life.

The capacity exhibited by this variety to increase it's ascorbic acid content and it's antioxidant capacity after the application of the irradiation treatment provides greater support for resolving the fruit decay problem, hence allowing for the provision of fruit with greater functional properties.

The research on minimal processing is insufficient, and more research is required in order to design technology that will allow for the development of this product with an acceptable standard of quality.

Research conducted on the generation of functional compounds has largely centered on carotenoid content. Nevertheless, increments of quercetin, mangiferin, and iso-mangiferin have been measured in the leaves and bark of trees treated with PBZ and flowering inductors, but have not been measured in the flesh of the fruit; a fact that needs to be studied more closely.

It's necessary to assess with greater detail the presence of lectines in the flesh of the fruit and describe it's functional properties more accurately.

2. Proposed objectives

Collect the scientific information developed in the postharvest technology field for Manila mangos.

Define potential specific research to improve the postharvest handling for this variety.

3. Development of the work

The collection of the scientific information generated by various Mexican research institutions on Manila mangos was conducted through a review of various formal publications, graduate thesis papers, personal interviews, and electronic databanks. Additionally, visits were made to various research centers such as the Instituto Nacional de Investigaciones Forestales Agrícolas y Pecuarias, Colegio de Posgraduados de Chapingo, Universidad Autónoma de Chapingo, Universidad Autónoma Metropolitana, Universidad Veracruzana, Instituto Tecnológico de Veracruz, Centro de Investigación en Alimentación y

Desarrollo de Hermosillo Sonora y de Culiacán Sinaloa, Facultad de Estudios Superiores de la UNAM Campus Cuautitlán México, Centro de Estudios Avanzados del IPN Unidad Irapuato, Instituto de Biotecnología de la UNAM, Centro de Desarrollo de Productos Bióticos del Instituto Politécnico Nacional and the Universidad Autónoma de Querétaro, a review was conducted of farm operation development plans for the states of Veracruz, Guerrero, Nayarit and Chiapas generated by their respective state departments of agriculture. The collection effort included 64 published works, 13 works presented at various fora, 17 thesis papers from different academic grades, and 13 websites. The information was grouped into two sections, preharvest research and postharvest research.

3.1 General characteristics of Manila mangos

Mangos (*Mangifera indica* L.) are recognized as one of the finest tropical fruits in the world. It has been grown for over 6000 years, and it is for that reason that it is considered one of the most ancient fruit crops (Mukerge y Litz 2009). The preference it enjoys at the consumption level is due to its sensory attributes such as odor, flavor, and it's nutritional properties.

Mukerge y Litz (2009) indicated that the manila mango produced in Mexico might have a Filipino origin, given that, prior to the conquest of the Americas, the production of several varieties of mango from Indochina already existed in that country, and that it was brought to our continent on the journeys of Portuguese and Spanish seamen traveling to the New World (Morton, 1987; Anonimous, 1996). According to Mukerge y Litz (2009), the polyembryonic nature of this variety and it's great similarity to the Carabao variety appear to support that assertion.

The Agricultural Marketing Commission for the State of Veracruz (Coveca, 2011) categorizes Manila mangos among the group of varieties that originated in

Indochina and the Philippines, given that they are characterized by their polyembryonic seed, sweet flavor, low fiber content, and low resin flavor.

Differences or similarities among mango varieties are defined by the use of descriptors that allow for their characterization. Knight *et al*., (2009) produced a summary of those descriptors that take into account the characteristics of the plant, leaf, flower, fruit, and seed, and with these developed a wide-ranging description of a large number of current mango varieties. Different aspects are taken into account for each one of those characteristics, for example:

Plant: growth habit and tree height;

- Leaf: shape, length, width, and young leaf color
- Inflorescence: position, shape, flower density, length, color, pubescence, presence or absence of foliar bracteas, and % of average flowers per inflorescence. It could also include the flower's diameter in millimeters, type of flower (pentameral, tetrameral, or both), nature of the disc (swollen, wider or thinner than the ovary), and number of stamen.
- Fruit: length, width and thickness, weight, shape and color of skin, thickness of the skin, texture; flesh, skin, and seed ratio; flesh texture, skin adherence to the flesh, amount and length of fiber in the flesh, stem or peduncle insertion.
- Seed: monoembryonic or polyembryonic; length, weight, veins, vein distribution pattern; presence or absence of fiber, and length.

Also taken into account: ripening period, tree productivity, susceptibility to stress, pests and diseases, as well as consumption quality and fruit appearance. The genetic criteria allow for the precise location of the relationships between the different varieties through the use of molecular markers, cytological characters, and specific genes.

The characteristics for Manila mangos as a function of these descriptors are as follows (Knight *et al*., 2009):

Large vigorous tree, with open canopy; bright yellow fruit, occasionally with slightly pink cheeks, or very small reddish spots; it is long and flat, with a rounded base, with a round apex, and occasionally with a small pointy tip (Figure 1a). Between 15 and 14 cm in length, 5.5 and 6 cm wide, and 5 to 5.5 cm thick, with a weight that fluctuates between 180 and 260 g; thin and slightly hard skin that is easily detachable; juicy flesh with medium firmness; with little or abundant fiber, deep yellow color when ripe, with rich and pleasant flavor, with an eating quality that varies between good and very good. It's seed is polyembryonic, with medium thickness and ligneous. It's ripening period is early to medium and it has a stable harvest.

Given the Filipino origins of Manila mangos, we add the description that these authors developed for the Carabao variety produced in the Philippines. This one has a tree similar to those of green to bright yellow colored fruit, long and flat, between 11 and 13 cm long; 6.5 to 7 cm wide, and the 6 to 6.5 cm thick (Figure 1B). It's weight fluctuates between 270 and 440 g, it's skin is also thin and easily detachable, and it is free of any fiber. It's seed is also polyembryonic with early ripening and it is currently one of the most important varieties in the trade between the Philippines and Japan. Additionally, commercial interest for it in the United States has been on the rise.

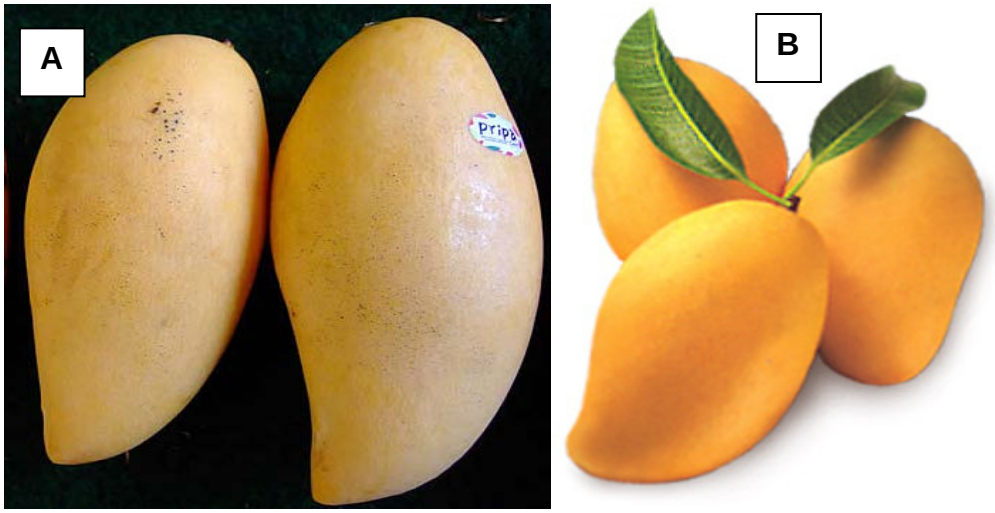


Figure 1. Visual characteristics for Manila (A) and Carabao (B) mangos.

Notwithstanding the descriptors referred to by Knight *et al*., (2009), the weight of the fruit tends to be more wide-ranging and can vary from 120 to 350 g, reason for which they can be packed at the retail level in 4 kg boxes of corrugated carton at sizes 12,14, 16, 18, 20, 22 and 24.

In the literature reviewed, reference is made to two experimental fields located at the Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (INIFAP), an institution that carries out breeding activities for different varieties of mango and has contributed materials from Manila mangos. These experimental fields are located in Aguaruto, Sinaloa y Cotaxtla, Veracruz.

The materials generated in Aguaruto, Sinaloa were characterized by Siller-Cepeda *et al*., (2009) based on their composition and quality. The Manila Rosa variety was identified as having intermediate ripening, like the Tommy Atkins and Kent varieties, however, it recorded the lowest weight for the fruit (209 g) of which 66% corresponded to the flesh, 20% to the skin, and 14% to the seed, all of which are values that contrasted with those of the Tommy Atkins (375 g) and Kent (386 g) varieties, which recorded larger percentages of flesh (75-76%) and lower percentages of skin (14-15%) and seed (10%).

Manila mango varieties have been generated at INIFAP's Experimental Field, located in Cotaxtla, Veracruz, with characteristics described in Table 1 (De los Santos y Mosqueda 1992). Currently, the same research Center has released clones 4, 12, 13, and 15, which exhibit low alternation (less than 25% compared to regional clones) and greater production rates that could be as high as 15.1 tons per hectare (ha^{-1}).

One of the problems related to the production of Manila mangos in Mexico is the excessive growth of its trees, resulting from their great vigor, which provokes the closure of tops and the crisscrossing of foliage in the field, all of which decreases production, and makes the harvesting and phytosanitary control practices more difficult and expensive. Various research projects have demonstrated that grafting into dwarf scions or into intermediate stems leads to increases in their productivity (Sandoval 1987 y García 1992).

The average yield for parent trees of the Manila Cotaxtla-1 clone over the course of 20 years is 544 kg per tree, and for the Manila Cotaxtla-2 clone it is 503 kg per tree. The agricultural areas for which these varieties are recommended are those with warm to very warm climates, subhumid: Aw_0-Aw_1 of the Koeppen climate classification; these conditions are prevalent in the areas of Actopan, Apazapan, Jalcomulco, Medellín, Jamapa and Cotaxtla, in the state of Veracruz, Mexico.

In order to make the crop more profitable during the first few years of production (6 to 12 years) the recommendation is to use an 8 m x 8 m plantation, which will allow for a density of 156 trees per hectare; after 12 years of age, the suggestion is to change to a system of 16 m x 8 m, and manage 78 trees per hectare; finally, the suggestion is to manage a density of 38 trees, that is 16 m x 16 m.

Table 1. Description of Manila Cotaxtla-1 and Manila Cotaxtla-2 Mango Clones.

Characteristic	'Manila Cotaxtla-1'	'Manila Cotaxtla-2'
Morphological characteristics		
Sprouts or scions	19.3 cm in length. 13.6 leaves per sprout	21.6 cm in length. 10 leaves per sprout.
Leaves	Single, alternate, yellowish green (when new), oblong spear-shaped, pointed tip, 16.2 cm aprox. in length, maximum width 4.5 cm, average width center part 4.4 cm.	Single, alternate, yellowish green (when new), oblong, spear-shaped with rounded edges, pointed tip, length 15 cm, maximum width 4.5 cm, average width center part 4.4 cm.
Inflorescence and flowers	Terminal conical panicle, pale green, average length of principal rachis 26 cm with numerous flowers, hermaphrodites and males, in the same panicle.	Terminal conical panicle, pale green, numerous flowers, hermaphrodites and males, in the same panicle.
Fruit	Medium size, average weight 208 g., ripens with a yellow color, thin skin, yellow flesh, firm, sweet, good flavor, and low fiber.	Medium size, average weight 207 g., ripens with a yellow color, thin skin, yellow flesh, firm, sweet, good flavor, and low fiber.
Seed	Polyembryonic	Polyembryonic
Agricultural characteristics		
Vegetative cycle at first flowering and first harvest	8 years in whole plants; 6 years in grafted plants.	8 years in whole plants; 6 years in grafted plants.
Longevity	Over 40 years.	Over 40 years.
Growth characteristics	Habit: erect, vigorous, dome-shaped tops. Average height: 15.6 m at 29 years of age. Flowering seasons: from January to February, occasionally in march. Harvest season: last two weeks of May until the end of June.	Habit: erect, vigorous, dome-shaped tops. Average height: 14.6 m at 29 years of age. Flowering seasons: from January to February, occasionally in march. Harvest season: last two weeks of May until the end of June.

Source: De los Santos y Mosqueda (1992).

Propagation must be carried out through grafting, given that it is the only way that we can preserve the genetic characteristics of the paternal tree from which it originated (Sandoval 1987 y García 1992). The characteristics of the tree and the fruit for both clones are shown in Table 2.

Table 2. Characteristics of the tree and fruit for both mangos Manila Cotaxtla-1 and Manila Cotaxtla-2.

Characteristic	**'Manila Cotlaxta-1'**	**'Manila Cotlaxta-2'**
Tree		
Age (years)/ Height (m)/ Top (m)	15/11.9/13.6	15/10.6/13.5
	25/14.8/17.9	25/13.8/18.0
	29/15.9/18.9	29/14.6/20.0
Fruit		
Soluble solids %	17.3	18.3
Length (cm)	10.1	11.0
Width (cm)	5.5	6.3
Thickness (cm)	5.0	5.2
Volume (cm)	191	200
Average weight (g)	208	207
Skin		
%	14.0	17.0
Seed		
%	8.9	10.8

Source: De los Santos y Mosqueda (1992).

3.2 Production areas and volumes

From 2000 to 2009, mango production in Mexico increased by 16.3% employing a surface area of 184,000 ha that produced 510,000 tons in the year 2009; in 2010 production increased to 1,630,000 tons (SIAP SAGARPA 2010) making this

activity the second largest based on planted surface area and the fourth largest based on fruit production volume.

The most important commercial varieties in Mexico are Ataulfo, Manila, Haden, Tommy Atkins, Kent and Keitt (Table 3). In 2010, the Manila variety was the second largest in terms of production volume (21.4%), following the Ataulfo variety (27.8%) (SIAP-SAGARPA, 2010).

Table 3. Planted surface area and production volume for different varieties of mango grown in Mexico during the year 2010.

Variety	Planted surface area (Thousands of ha)	Harvested surface area (Thousands of ha)	Production volume (Thousands of Tons) (%)	Production value (Millions of $)
Ataulfo	42.56	42.54	430.26 (27.8)	35.53
Manila	39.10	38.88	322.49 (21.4)	86.49
Haden	25.22	22.67	188.05 (12.2)	22.61
Tommy Atkins	21.51	20.01	215.45 (13.9)	95.63
Kent	17.49	15.56	185.08 (12.0)	76.92
Keitt	8.55	8.38	73.73 (4.8)	75.76
Creoles	12.95	12.56	114.05 (7.4)	44.12
Unclassified	2.59	2.58	18.40 (1.2)	57.16
Totals	**169.97**	**163.18**	**1547.51**	**494.22**

Table 4 shows us the key states in Mexico that have manila mango plantations, and Figure 2 points out that the percentage of distribution of the production volumes for those states. The state of Veracruz represented 56.5% of the total planted surface area, though it only contributed 35% of the total production, whereas the state of Guerrero with 21% of the total planted surface area contributed 41% of the total production. The state of Sinaloa, which represented 7.5% of the total surface area planted, contributed 6% of the total production, and

the state of Nayarit, representing 5.37% of the total surface planted, contributed 7% of the total production (SIAP-SAGARPA, 2010). These five states contributed 89% of the total production for this variety.

Table 4. Planted surface area for Mexican Manila mangos in 2010. Source: SIAP, SAGARPA (2010).

State	**Planted surface area (ha)**	**Planted surface area (%)**
Campeche	204.00	0.52
Colima	546.65	1.40
Chiapas	121.00	0.31
Guerrero	8,201.90	20.97
Jalisco	323.50	0.83
México	9.00	0.02
Michoacán	1,088.29	2.78
Nayarit	2,098.80	5.37
Oaxaca	1,321.00	3.38
Sinaloa	2,932.91	7.50
Tabasco	147.00	0.38
Veracruz	22,109.78	56.54
Totals	39 103.83	100

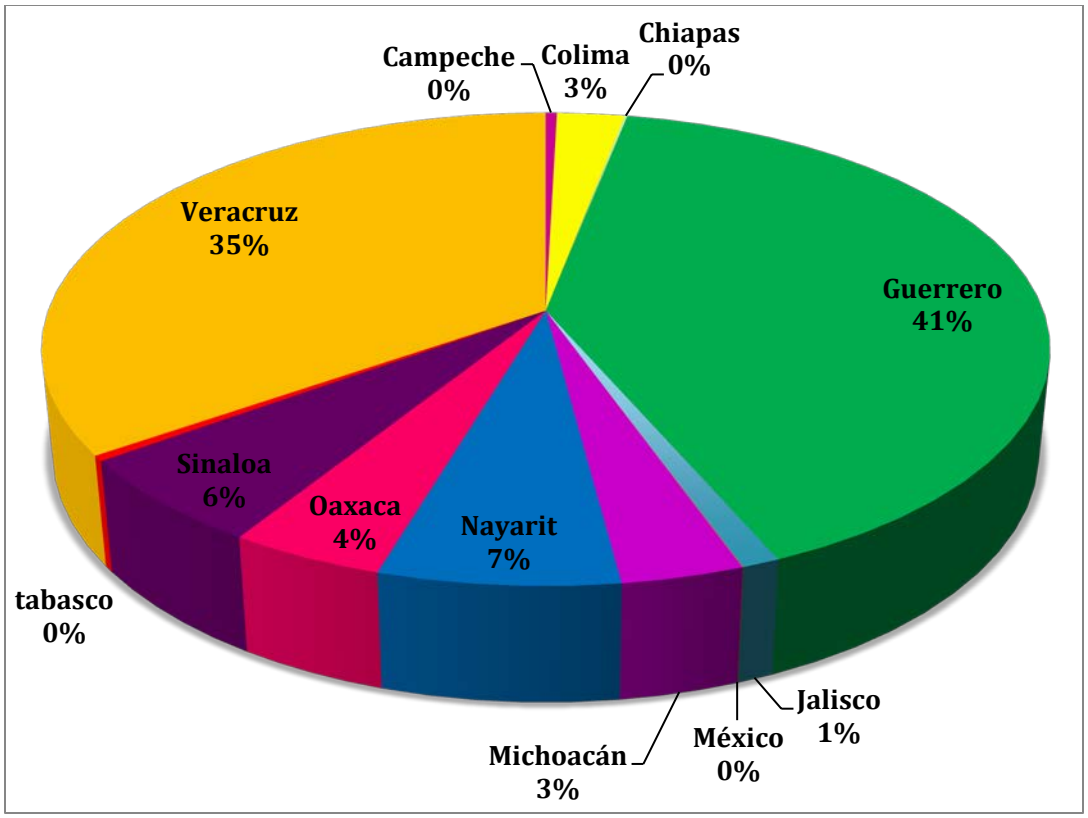


Figure 2. Distribution of manila mango production in Mexico during 2010 as a function of percentage. Source: (SIAP SAGARPA, 2010).

The data from the state of Veracruz stands out because it is the state with the largest planted surface area, but it has low production volumes compared to the state of Guerrero. This suggests that a higher degree of production technology is being employed in the other state. Cabrera-Mireles *et al*., (1996) observed that in the state of Veracruz only 5000 ha benefited from the use of production technology, a fact that is reflected in the per hectare yield for the state (Figure 3). Likewise, by 2011, the state of Veracruz had not included additional orchards in its national fruit fly control program (http://www.senasica.gob.mx/?id=893 review conducted 28 Nov 2011), which confirms that the state does not participate in any export program for this fruit. The governing development plan for the mango product system in the state of Veracruz established that the main variety grown in the state is the Manila variety, and that 97% of the total production is destined for the domestic consumer market due to the aforementioned phytosanitary

issues and this variety's susceptibility to damage resulting from the hot water treatment required for the fruit to be eligible for the export market (COVECA, 2011). In contrast, the states of Guerrero, Michoacan, and Jalisco are registered in the program with orchards that have this variety planted.

The state of Guerrero has better conditions for technology-based production (La Costa Grande region) and, though it has a few commercial packing houses for export, a large part of the production is transported to packing houses in Michoacan. That notwithstanding, the state of Guerrero was the first State that participated with shipments of irradiated Manila mangos to the United States.

Quality certifications. This variety is categorized under the *Calidad Suprema* Mexican quality standard like the Ataulfo, Tommy Atkins, Haden, Kent, Keitt, Irwing, Sensation and Oro varieties (*Mexico Calidad Suprema*, 2005). It is also regulated under the official Mexican standard for fresh products (NOM-129-SCFI-1998).

One aspect that stands out from the historical production data is that the yield per hectare has shown annual fluctuations (Figure 3). This aspect indicates an alternation in the production that could be important to the tree response towards the growing practices and their impact on the expected production volumes.

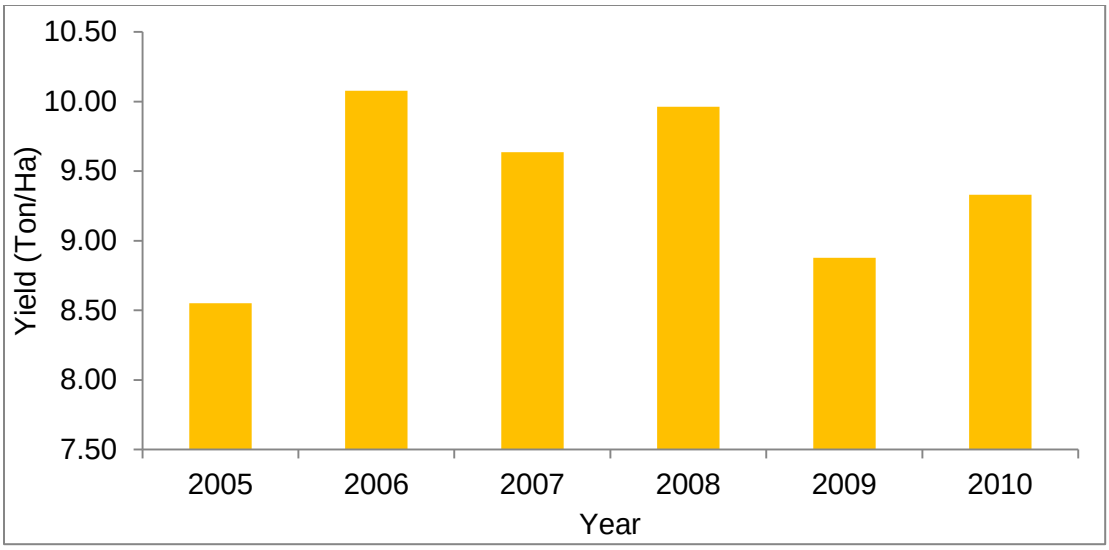
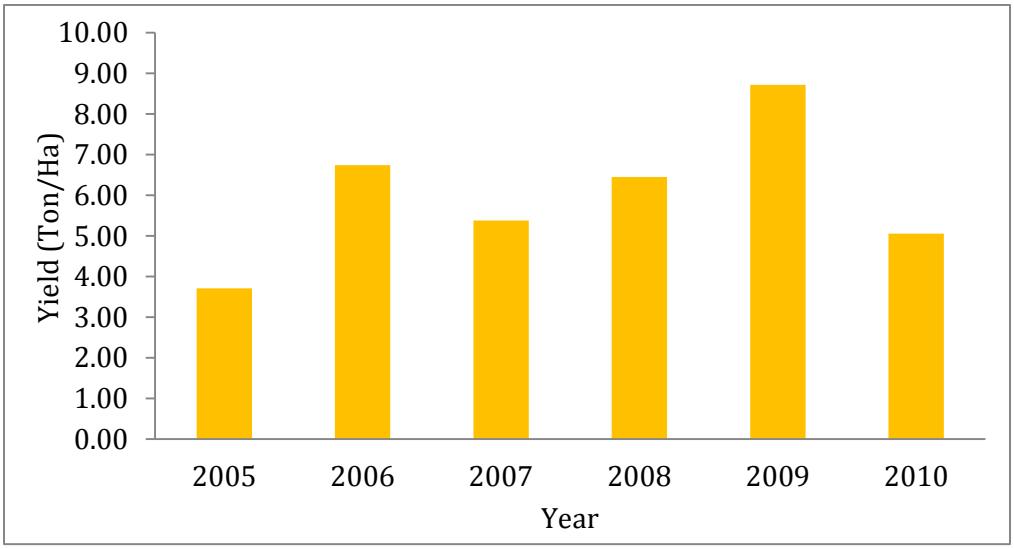


Figure 3. Production yield per hectare for mangos in Mexico. Source: SIAP-SAGARPA, 2010.

Manila mangos, in particular, also show fluctuations in their yield per hectare in the two most important producing states in the nation: Veracruz and Guerrero (Figure 4).



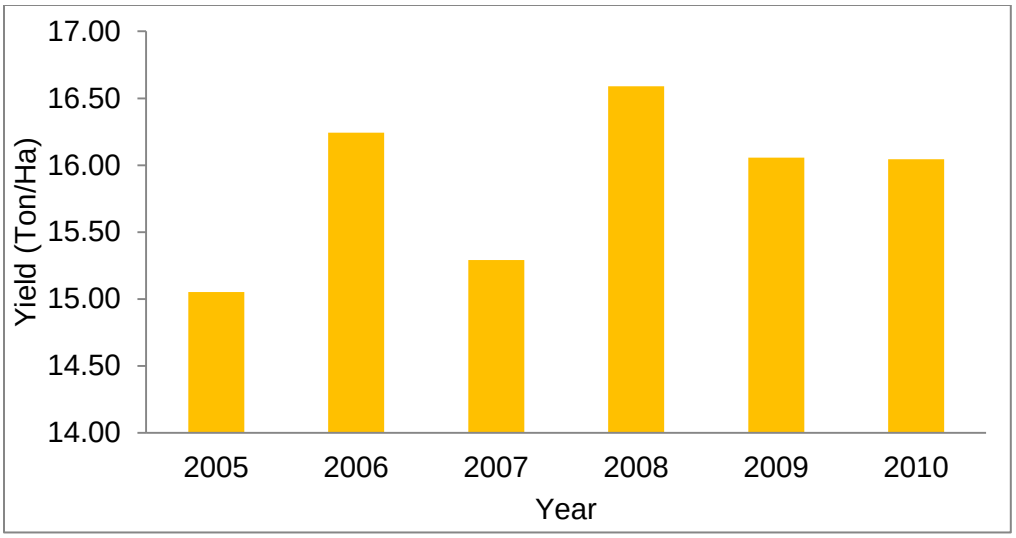


Figure 4. Fluctuations in yield per hectare for the production of Manila mangos in the states of Veracruz (A) and Guerrero (B). Source: SIAP-SAGARPA, 2010.

3.3 State of the Research on Manila Mangos

Due to the production surface area and volume for Manila mangos in Mexico, various institutions have conducted research to contribute additional knowledge in the areas of production and post harvest handling. The objectives of these studies have focused on understanding the behavior of this variety at the preharvest level, where there has been a drive to obtain varieties with low alternation and higher productivity, as well as establishing the best production conditions and evaluate the effects of the applied techniques on the development and quality of the fruit.

In the post harvest arena, the objective has been to extend the fruit's shelf-life through the application of various technologies, and search for quarantine treatments that will allow the fruit to be sent to the export market.

3.3.1 Preharvest Research

Although a review of preharvest research was not the objective of the present work, the practices employed during the production cycle have a great deal of influence in the quality of the fruit, as well as in its behavior during its postharvest life. In that regard, there has been extensive interest in reducing the alternation and forcing production during the off season to achieve better economic benefits. It is for that reason that a great deal of emphasis has been placed on researching the control of the flowering process and the harvest season through the use of flowering inductors and growth regulators. Additionally, research has been carried out to control anthracnose in the field given that it is one of the key diseases that affects this crop.

3.3.1.1 Use of flowering inductors and growth regulators

The use of growth regulators such as paclobutrazol (PBZ) or Cultar, and the spraying of flowering inductors such as KNO_3 or NH_4NO_3 are common practices in the various mango production regions in Mexico. These are used to move up the harvests and produce fruit during the off-season.

A) Studies on the application of flowering inductors

In 1985, Nuñez-Elizea demonstrated that the applications of KNO_3 (80 g L^{-1}) on manila mango trees doubled or tripled the number of panicles in trees belonging to the control group. Likewise, it indicated that fruit set had increased even though their size and quality were not affected by the treatment.

Later on, López - Montoya y Mosqueda - Vázquez (1987), in order to address the production alternation for Manila mangos in the state of Veracruz and the short harvesting period that leads to the concentration of production volume and huge drops in prices, set out to use growth regulators and nitrates to create phase changes for the harvest and achieve production during the off-season. Through foliar applications of KNO_3 (0, 2 y 4%) along with applications of $AgNO_3$ (0 y 250 ppm), they demonstrated that KNO_3 accelerates flowering and $AgNO_3$ inhibits it,

which demonstrates that the flowering process is affected by the synthesis of ethylene. That notwithstanding, contrary to the work carried out by Nuñez -Elizea (1987), these authors were unable to achieve flowering that was greater than that of the trees belonging to the control group.

Rosado - Martínez (1987) studied the effects stemming from applications of Ethrel (400 to 1000 ppm) and KNO_3 (0, 1, 2, 4 and 8%) in order to identify conditions that would induce an acceleration in flowering and a greater number of flowers on Manila mango trees from Actopan, Veracruz, Mexico. The application of Ethrel at 600 ppm on three occasions produced an increase of 112% in the number of panicles compared to the control group, while the application of KNO_3 at 4% on two occasions increased the number of panicles by 120% compared to the treatments with Ethrel at 600 ppm. A double application of KNO_3 and a triple application of Ethrel accelerated flowering for up to one month compared to the control group.

Osuna - Enciso (1998) and Osuna - Enciso et al., (2001) contributed basic information at a histological and biochemical level regarding the changes that occur during the natural flowering process compared to the one induced by the applications of KNO_3 (0, and 40 g L^{-1}), NH_4NO_3 (0 and 20 g L^{-1}), Ethrel (0 and 1 mL L^{-1}) the collaring of stems, and their relationship with the starch and amino acid content of mango buds during their floral induction. Their studies were carried out on Manila trees that were 10 years of age and grown in the state of Veracruz. The applications of KNO_3 and NH_4NO_3 induced a faster transformation from vegetative buds to reproductive buds, while the starch content in the buds did not appear to have any relationship with their reproductive development. Nevertheless, the total amino acid content was greater in buds with floral initiation, and the lower contents were observed in the vegetative buds suggesting that the high levels of amino acids are related to the mango's floral initiation. Polyamines were present during the stage prior to the floral differentiation, and the spraying of Ethrel stimulated the synthesis of ethylene but

did not promote flowering. Additionally, they also observed that a higher content of gibberellins in the buds reduces the percentage of floral buds.

These latest studies provided information regarding how nitrogenated compounds provoke an acceleration in the flowering and harvesting dates, how inhibitors that affect the synthesis of gibberellins contribute to the differentiation between vegetative buds and reproductive buds, and the manner in which the pathways for polyamines in the synthesis of ethylene could be associated with the process of floral differentiation.

Toward the Pacific Coast, Salazar-García *et al*., (2000) sprayed NH_4NO_3 (2 and 4%) on Manila mango trees and other varieties grown on commercial farms in the state of Nayarit, Mexico in order to accelerate the flowering and harvest of those products. The bi-weekly applications of this compound caused the flowering to occur 32 and 34 days earlier than the control group for both concentrations in the study, and moved up the harvest period by 44 days.

After applying KNO_3 (3%) and NH_4NO_3 (1%) on two different dates to accelerate flowering in Manila mangos in Pijijiapan, Chiapas, Mexico, Mendoza - Palacios (2001) determined that the application during the month of October accelerated flowering and the harvest period by 57 days compared to the control group, though the fruit set did not increase, but the sugar content in the fruit was greater compared to the fruit in the control group.

Although the aforementioned research indicated the age of the trees used in the different studies, there was no report regarding the status of the production alternation when they were subjected to analysis. It's possible that this factor can explain the relative inconsistency of the data generated in the reports.

B) Paclobutrazol applications

The action of PBZ is based on the fact that this compound is an inhibitor of the biosynthesis of gibberellins, which inhibits the plant growth of the tree and favors its reproductive activity, and from that point it's used to accelerate flowering, harvest, and the production of fruit. Another commercial product with the same characteristics is Cultar®, which also inhibits the synthesis of gibberellins avoiding the oxidation of kaurene into kaurenic acid, causing a reduction in gibberellic acid in the tissues.

Colón - Candela (2000), after applying dosages of 2.5 and 5.0 g of active ingredient (i.e.) of PBZ in the soil per tree, observed that 100% of flowering occurred 75 days after the application, compared with 34% of the trees in the control group. The application did not affect the length of the vegetative or reproductive bud, or the production periods, number of fruit per inflorescence, or the weight or quality of the fruit, but it did increase the yield per hectare by more than 20% and the production of fruit was moved forward during an alternation year. This effect could better explain the reduced alternation observed in recent years.

Despite these positive factors related to production, it's important to note that the foliar concentration of K and Mn in treated trees was lower during harvest, whereas the N in the skin, flesh, and juice of the fruit increased with the application of 5 g of PBZ. Likewise, there were a higher concentrations of Mg and Mn in the flesh at the same dosage, while the Zn was lower. The extraction of nitrogen from the soil that was directed to the development of the skin and flesh increased with applications of 5 g of PBZ. The average order of magnitude of nutritional extraction was K>N>Ca>Mg>P>Mn>Zn>Cu.

The author points out that the applications of PBZ caused a reduction in the length of the internodes as well as in the crosscut area of the trunk. These data suggest that when fruit production increases, it does so at the expense of the nutritional condition of the plant.

If we consider that mangos plants, by nature, have a biannual habit, the data from the previous study poses an important question that must be researched– what is happening to the physiology of trees on plantations subjected to the annual use of flowering inductors and PBZ?, Is it possible that this may be one of the causes of the decreases in mango production in various production areas?, To what degree is this factor related to climate change? It would seem important to address these fields of study.

Moreno - Aguilar (2006) conducted research regarding the effects on the production and quality of Manila Cotaxtla-1 and Manila Cotaxtla-2 fruit from the application of PBZ (0, 0.5, 1.0, 1.5 and 2 g i.a. per m^2 de top) and KNO_3 (2 and 4%), combined or not, at 1.5 g of i.a. of PBZ. The joint application of PBZ and KNO_3 at high dosages accelerated flowering by 33 days, increased the number of fruit per tree, and improved the yield without increasing the number of panicles. The application of PBZ did not affect the total soluble solids content, titratable acidity, SST/AT ratio, or the firmness of the fruit, though there was a slight increase at the higher dosages. With regard to color, the treated fruit exhibited a more intense yellow coloring and the application did not affect the nutritional condition of the trees. These results contrasted with those observed by Colón-Candela (2000), and although both studies were carried out in the same production area and both recorded the ages of the trees and the manner in which the experiments were developed, the latter study did not indicate the history regarding the alternation status of the trees prior to the study.

Contrary to the references made previously, Rebolledo-Martínez *et al*., (2008a) also researched the effects from the joint application of PBZ (0 to 2 g of i.a. per árbol) and KNO_3 (2 and 4%), in either separate or combined form, on two Mango clones of the Manila variety from the state of Veracruz, Mexico. The observed results once again showed the acceleration of flowering by 53 days and an increase in the number of panicles, while the fruit of trees with the more intense

application of PBZ demonstrated a higher total soluble solids content, less acidity, less firmness, and greater weight loss. These last two parameters indicate potential difficulties for the fruit's shelf-life.

Pérez-Barraza *et al*., (2011), based on reports that indicate that different concentrations of paclobutrazol (PBZ) speed up flowering, generate greater production and better fruit quality, applied PBZ (0, 10 and 20 mL per tree) by itself or combined with 4 % KNO_3 during two production cycles (2008-2009 and 2009-2010) of Manila mangos from Nayarit, Mexico. The joint application of PBZ + KNO_3 moved up flowering by 37 days during the first season and by 23 days during the second season, the flowering intensity was 73% and 94% during both seasons, while the control groups had 50.6% and 64%, respectively. These authors attribute the difference in results to the higher dosages of PBZ used during the first cycle.

Nevertheless, though not questioned by the authors, the data from the control groups also showed a different number of days for flowering (66 days for the first season and 43 days for the second) and a different flowering intensity as well (50.6% for the first and 64% for the second). Unfortunately, no data were reported regarding the number of hours-temperature of flowering induction for both seasons that could help explain the differences between those seasons. Despite this, it's possible that there may be an effect derived from the biannual habit and the natural alternation of the crop as it appears in Figure 5 for the state of Nayarit.

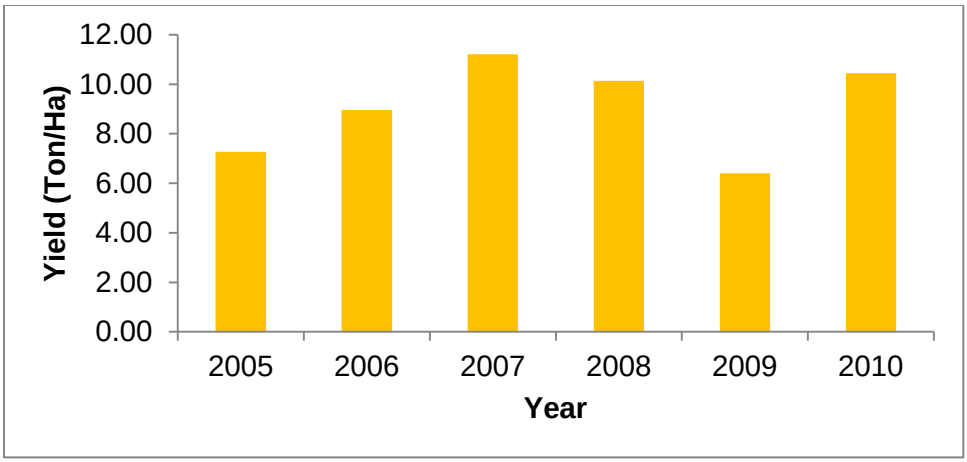


Figure 5. Yield per hectare of Manila mangos for the State of Nayarit. Source: SIAP-SAGARPA, 2010.

The production yield per tree increased compared to the control group, noting that the PDZ only achieved larger yield results during the second season (171 Kg per tree) compared to the 83 kg recorded in trees belonging to the control group, all of which show that these treatments caused a very positive result from the production standpoint. Regardless, the results show that the yields from all of the treatments applied during the first season were lower (from 20 to 30 kg), which, once again, points to the natural alternation of the crop as it is observed in Figure 5.

As far as the weight and the size of the fruit is concerned, the authors observed that during the second season there was a higher weight and larger diameter for the fruit from trees treated with PDZ (275 g and 7.2 mm) compared to those in the control group (223 g and 6 mm). Nevertheless, although these authors don't point it out specifically, their data show that the first season registered lower weights and smaller diameters compared to the second season and, in fact, the control group showed a higher weight (223.7 g) during the second season than the fruit from trees treated with PBZ and KNO_3 (181.9 g). Data show with much greater clarity the effect of the alternation or biannual habit of the crop.

According to the previous statements, it appears that more detailed assessments need to be carried out regarding what may be happening on plantations where these compounds are being applied more frequently during the production cycles.

The body of evidence regarding the effects from PBZ, nitrates, and Ethrel on flowering induction, led to a proposed mechanisms that could explain the actions of these compounds during flowering induction. Protacio *et al*., (2009) contributed information to support a potential mechanism proposed by them in which they suggest that the gibberellins act as inhibitors of the transformation process from vegetative buds to floral buds. When said phytohormone is reduced to a predetermined concentration threshold, flowering is initiated. To support this hypothesis, each month they studied the morpho-anatomical changes of the buds and their content of gibberellins in Carabao mango trees treated with PBZ (0 and 1 g of i.a.) and KNO_3 (2%). Their results showed a faster decrease of gibberellins in the buds treated with PBZ compared to the control group, and when the content decreased to less than 100 mg of GA_3 per gram of tissue, bud differentiation began along with flowering, whereas in the untreated buds this did not happen until 30 days later. At the same time, there was also a greater accumulation of starch in the leaves, and the ACC (precursor to the synthesis of ethylene) content reached a maximum even though the production of ethylene was decreasing. The treatment with KNO_3 increased the number of flowers. These results supported the proposed mechanism and pointed out that KNO_3 is not responsible for the transformation from vegetative buds to reproductive buds, that it only induces the rupture of a quiescent state of the buds that have been previously differentiated, and that ethylene is not an inductor of the process but ACC probably is.

This work demonstrates that these regulators, particularly PBZ, alter the normal physiology of the tree in such a way that it gives rise to greater production of fruit, but it's possible that it may also affect the physiological state of the tree even

though there's no data to support this assertion. The use of these regulators in mango production could generate metabolic stress that could be reflected in the development, shelf-life, and general quality of the fruit.

Some partial data regarding what happens with some of the tree's organs is addressesd by Guzmán-Meraz (2006), who quantified the content of functional polyphenols in the bark and leaves of Manila mango trees treated and untreated with Cultar (1.5 and 2 g + KNO3 at 2%), observing that the levels of quercetin, mangiferin, and iso-mangiferin were present in higher concentrations in treated trees compared to the trees of the control group. This subject should be addressed in greater detail with regards to the fruit, given the functional characteristics of these compounds.

With regard to the changes observed in the fruit as a result of the applications of PBZ, Martínez-Castellanos (2004), evaluated the chemical, physical, and physiological changes in Manila mango fruit produced from trees treated with this regulator in the state of Veracruz, pointing out that respiration velocity was lower in fruit from trees treated with PBZ. Contrary to other studies, lower soluble solids and greater firmness was recorded in the flesh. These results contrast with those observed by Rebolledo-Martínez (2008a) who indicated that there was a greater content of soluble solids, less firmness, and greater weight loss, and though the author does not dispute this, this aspect indicates that the treatment could be used as a procedure to extend the shelf-life. This inconsistency in the results could also be related to the alternation of the production carried out in the state of Veracruz (Figure 4).

3.3.2 Studies on Fruit Physiology and Post Harvest Technology

3.3.2.1 Harvest Indexes

There is no report that includes a study on the best harvest index for this variety, nevertheless, the assumption is that the indicators used with other varieties are

applicable to this one and for that reason we take into account the filling of the shoulders (the shoulders are located above the peduncular joint of the fruit) and the minimum content of total soluble solids (7°Bx) as adequate harvest indicators. With this composition the food is capable of achieving up to 20°Bx when it is ripe and ready to eat (Baéz-Sañudo y Contreras-Martínez, 1993).

López-Blancas (2009) collected data on accumulated heat units (UCA) using 10°C as a base temperature to correlate them to the maturity stage of the fruit during harvest and the quality attained during the postharvest. Fruit were harvested from a commercial farm in Veracruz, Mexico with 1100, 1300 and 1500 UCA, and stored at 25°C. The growth of the fruit was completed with 940 UCA, but the fruit harvested prior to reaching 1046 UCA did not attain the minimum weight to be classified within the size codes of the Official Mexican Standard NMX-FF-058-SCFI-2006. For that reason, the suggestion was to harvest at around 1300 UCA, with the following condition, that the fruit be ripened during 6 to 9 days of storage with a shelf-life of 11 days with a sweet flavor. Lizada (1991) suggested 1000 UCA as a harvest indicator for the Carabao variety, nevertheless, her reference temperature was 17.9°C which does not allow for comparisons with the results of this author. Regardless, it's possible that the values of may be very close to one another given that the occurence of 10°C temperatures are quite exceptional in our Manila mango production areas. Lizada (1991) also proposed the separation of fruit based on maturity through a flotation process in solutions with NaCl at 1%. The fruit with greater specific densities would be deemed physiologically ripe fruit, whereas the fruit that floats would be deemed physiologically unripe. This could be a useful application that could be used in packing houses at the beginning of the season, and in that way we could reduce the number of physiological disorders caused by low temperatures and the immature or unripe state of the fruit.

On the other hand, Guzmán-Estrada *et al*., (1997b) developed a mathematical equation that allows us to determine the volume of Manila mango fruit during its

development using the general dimensions of the fruit (total length, width, and basal, average, and apical diameter, displaced water volume) as a reference, and in that way develop a method to predict the physiological maturity of mangos. The key to this equation is that it adequately predicts the development of the fruit based on heat units (base temperature: 10°C), and it indicates that the fruit that accumulated 1278 heat units reached the maximum volume of the fruit and its harvest maturity, which would be consistent with that suggested by Lizada (1991) in mangos of the Carabao variety.

In their own right, when correlating the development of external color of the fruit with its carotenoid content in the flesh, Ornelas *et al*., (2008), proposed that, for Ataulfo and Manila mangos, external color is a good harvest indicator for the fruit given that it is clearly associated with internal changes in the flesh. Nevertheless, more studies are required to be able to implement this system in a selection line.

3.3.2.2 Ripeness Physiology

Mangos are a climacteric fruit whose change in appearance and composition during storage is associated with the ripening process that provides it with the pleasant sensory characteristics experienced by the consumer (pleasant fruit and flesh color, high sugar content, low acidity, adequate firmness, and pleasant aroma). The respiration rate indicates the metabolic activity during storage as well as the fruit's storage potential. Paull (1993) pointed out that mangos have a moderate respiration rate at 20°C (70–150 mg CO_2 kg^{-1} h^{-1}) which he estimates provides the fruit with a shelf-life of 18 days under optimal storage conditions (10-12°C).

Saucedo–Veloz y Lakshminarayana (1977) recorded respiration rates of 242, 255 and 262 mg CO_2 kg^{-1} h^{-1} during the climacteric peak for Manila mangos from Veracruz, Mexico stored at 18, 20 y 25 °C, respectively. More recently, León *et al*., (1997) measured respiration rates at 25 °C of 242 mg CO_2 kg^{-1} h^{-1}, which

makes this variety stand out as one of high respiratory activity and, therefore, one of shorter shelf-life than that suggested by Paull (1993). In support of the aforementioned remarks, Lira *et al.,* (2008) showed data comparing the respiration rates of this variety with those of Ataulfo, Tommy Atkins, Keitt and Haden, indicating that Ataulfo and Manila (polyembryonic mangos) showed the highest respiration peaks at 20°C (265 and 196 mg CO_2 kg^{-1} h^{-1}, respectively) compared to the other varieties (150, 110 and 105 mg CO_2 kg^{-1} h^{-1} for Tommy Atkins, Keitt and Haden, respectively), and adding that Manila mangos showed the shortest shelf-life at 20°C (6 days) compared to the other varieties (11) confirming the higher metabolic activity for this variety and, as a consequence, it's shorter shelf life.

The higher metabolic activity was also associated with greater weight loss that reached 10, 5 and 3.5% at 25, 20 and 18°C, respectively (Saucedo-Veloz y Lakshminarayana, 1977), that caused the visual manifestation of decay that affects it's marketing. Siller-Cepeda *et al*., (2009) pointed out that mangos of the Manila Rosa and Ah-ping (also polyembryonic) varieties exhibited decay when the weight loss was between 5 and 6% at 20°C, which affected it sensory quality, while the Haden, Tommy Atkins, Kent and Keitt varieties exhibited values between 2.5 and 4%, and others like the Diplomático and Edward varieties that lost between 7 and 6% of weight, but did not exhibit any decay.

Susceptibility to decay could be due to the histological characteristics of this fruit. Barbosa-Martínez (2003) and Barbosa-Martínez *et al*., (2009a and 2009b) conducted a histological comparison of the Haden and Manila varieties observing important differences among them that could explain the higher susceptibility that Manila mangos have to weight loss and mechanical damage. The skin of the Manila mango is thinner and proportionally represents less weight compared to the Haden mango, likewise, it showed a lower number of epidermic cell layers and long fibers, and it also recorded a lower number of hypodermic cell layers with thinner cell walls which makes it more susceptible to damages during

mechanical handling. Additionally, it's cuticle is thinner than that of the Haden mango, making it more susceptible to weight loss due to transpiration, and to pathogen attacks. They also indicated that the cells in the flesh are larger compared to the Haden mango, and for that reason it exhibits less firmness when osmotic changes occur during the ripening process. It's also possible that the metabolic activity of the skin may be higher as suggested by Cua y Lizada (1990). The Barbosa-Martínez *et al*., (2009b) study also pointed out that Manila mangos reach their full development more rapidly than the Haden mango, which confirms that it's metabolic activity is higher, and it is for that reason that it has a shorter postharvest shelf life.

In addition to the decay, Manila mangos have less firmness. Mercado-Silva (2010), using a compression test for whole fruit, indicated that this variety has low firmness (18 to 31 N to reach 3% deformation in the fruit) compared to the Tommy Atkins, Haden, Kent, and Keitt varieties (85-185 N) and, likewise, pointed out that even when it's weight-loss was between six and 8% it visually exhibited a higher rate of dehydration that limited its post harvest life to between 13 and 19 days at 20°C and 10°C, respectively. The low firmness is probably associated with a rapid loss of pectines in the tissues during the ripening process as suggested by Lira *et al*., (2008), who observed a decrease in the content of calcium pectates from 0.48 to 0.1 g in 100 g of flesh during the ripening of the fruit, while the Haden variety showed very slight changes (0.65 to 0.45g in 100 g of flesh).

With regard to general changes occurring during the ripening of the fruit at 20°C, Mena-Nevares (1993), studying mangos from the state of Veracruz, Mexico, observed an increase in total soluble solids of 10.48% to 19.3% during nine days of storage, a decrease in titratable acidity of 2.1% to 0.014%, a decrease in chlorophyll content in the skin from 5.0 mg per 100 g of skin, while the content of ascorbic acid remained approximately constant (35 mg in 100 g of flesh). Similarly León *et al*., (1997) observed, in fruit of the same origin, an increase in

total soluble solids and total sugars (up to 20°Bx and 50 mg mL^{-1}, respectively), a rapid loss of acidity in the fruit between 2.4 and 0.6%, as well as a considerable change in external color (96 to 60 °Hue) during five days of storage. These data show that the Manila mango's natural ripening process at 25°C occurs very rapidly (6 to 8 days). For their part, Mercado-Silva (2010) observed particular differences in Manila mangos from the state of Nayarit compared to those reported by other authors (soluble solids only increased up to 16% during 13 days at 20°C), although the ascorbic acid content was greater (50 to 63 mg in 100 g of flesh without showing any significant differences during storage) than the one reported by Mena-Nevares (1993).

In the reviewed literature, no reports were found on the characterization of the ripening process in the interior of the fruit, nor of the moment at which the process initiates, although López-Blancas (2009) indicated the presence of a maximum peak of respiration 89 days after fruit set, that is, prior to the fruit reaching its harvest maturity on the tree. This coincides with the work of Cua y Lizada (1990), who pointed out that the ripening process for Carabao mangos initiated 10 days prior to achieving harvest maturity, and that the production of ethylene in this variety exhibited several maxima during its development, and that the last one among them corresponded to the production of ethylene generated in the skin. It's possible that this knowledge may have particular importance for mangos of the Manila variety, and it's a subject that merits further research.

Among the characteristics that contribute to mango quality, appearance and firmness tend to be the most important attributes. The progressive loss of firmness in mangos is a consequence of the ripening process that occurs due to the changes in the polysaccharides of the cell wall promoted by enzymatic actions on the pectines, hemicellulose, cellulose and starch. Other changes also occur on the permeability of the membrane and in the intercellular spaces. The main phenomena associated with softening is the progressive solubility and depolymerization of the pectic substances of the cell walls. Lira *et al*., (2008)

studied the changes in the enzyme activity related to the cell wall degradation (pectinesterase y cellulase) in the flesh and skin of Manila, Ataulfo, Tommy Atkins, Keitt and Haden mangos, and correlated it with the firmness and pectine content. In particular, Manila mangos suffered a drastic loss of pectine content as the ripening progressed, whereas the pectinerase activity in the skin was high, which showed a high correlation with the loss of firmness in the fruit pointing to the participation of this enzyme in the softening process of the fruit. It's remarkable that the activity of this enzyme in the flesh had a lesser relationship with the loss of firmness. These data suggest that the metabolic activity of the skin in Manila mangos could be important for the visual quality of the fruit. On the other hand, the cellulase activity was notably less associated with the softening process.

Studies conducted at a molecular level showed an overexpression of two counterpart genes (METR1) to the ethylene receptor ETR1 (Gutiérrez-Martínez *et al*., 2001) in the fruit of Manila mangos whose expression increased in accordance with the ripening process, and was related to the production of ethylene (López-Gómez y Gómez-Lim, 1992). Likewise, Cruz-Hernández y Gómez-Lim (1995) described the differential expression of an alternate oxidase gene during the ripening process, which was notably expressed during the respiratory climacteric of the fruit. This indicates that during the respiration climacteric there is a greater release of heat, in addition to an increase in respiratory velocity. The thermogenic nature of this metabolic route is associated with the increases in temperature (around 10°C) recorded in other mango varieties during the respiratory climacteric. In other similar work, Bojorquez y Gómez-Lim (1995) identified the overexpression of a peroxisomal thiolase gene, an enzyme involved in the catabolism of lipids during the ripening process for Manila mangos. At a molecular level, these data confirm the climacteric nature of these fruit, pointing out the importance of alternative respiration and metabolic changes that occur during the ripening process. It's possible that these aspects could explain the metabolic changes that shorten the shelf-life in Manila mangos.

The same research team carried out the first studies to generate somatic mango embryos transformed with ACC oxidase and ACC synthase genes, as well as with alternative oxidase to generate transformed plants with different behavior during ripening (Cruz Hernández y Litz, 1997).

Nevertheless, there are no reports on the generation of plants in the field. However, taking into account what was indicated by Lizada (1991), a potential decrease in flowering capacity is to be expected with these transformed plants.

3.3.2.3 Controlling Ripening

During the course of the conventional handling of the fruit it's common to observe heterogeneity of ripening during the marketing process, which is why this process is manipulated through the application of ethylene to accelerate or delay the ripening process through the use of ethylene action inhibitors. In order to accelerate ripening, the application of calcium carbide (CaC_2) has been used commercially in Mexico which generates ethylene with the mere presence of moisture in the environment. This treatment allows for the harvesting of the fruit at earlier stages of maturity to avoid the collection of fruit with higher rates of fruit fly infestation or anthracnose onset. The fruit harvested in that manner will receive the application of calcium carbide to induce it's ripening.

In order to improve this technique, Muñoz-Cano *et al*., (2007) and Ortega-Zaleta *et al.,* (2008) compared the controlled application of calcium carbide during the postharvest and the procedures followed by marketers in the domestic market. They studied different relationships between the compound and the fruit to be treated (0, 0.25, 0.75 and 2.5 g of CaC_2 kg^{-1} of fruit) during 24, 36, and 48 hours at 26°C. under these conditions, they observed that the application of 0.25 g kg^{-1} for 24 hours improved skin and flesh color characteristics, ripening was accelerated by three days, and the loss of firmness was more rapid compared to

the untreated fruit. The accelerated ripening referred to by these authors coincides with that reported by Tirtosoekotjo (1985) and cited by Lizada (1991), who observed an acceleration in the ripening of Carabao mangos when subjected to treatments of calcium carbide (CaC_2). Insofar as the assessment of the commercial process, they pointed out that the proportions of the compound were variable (0.6 to 0.8 g kg^{-1} of fruit), as well as the exposure period (13.5 to 48 hrs), and that the fruit handling conditions in the chambers were deficient, such as inadequate ventilation of the product which caused increases in temperature for the fruit up to 30°C which induced a great deal of variability in the responses and decreased quality in the fruit.

With regards to the use of ethylene to homogenize the ripening process, the available work is only partially complete and was not focused on the study of said effect. Lagunes *et al*., (2007) applied exogenous ethylene in Manila mangos after subjecting them to hot water treatment (65 min at 46.1 °C) and no comparison was made with fruit that was thermally untreated. Kader (2002) suggests the application of 100 ppm of ethylene in the air for a period of 12 to 24 hours at 20°C - 22°C to accelerate the ripening process in mango fruit. Nevertheless, there are no reports on this subject that refer to Manila mangos, and it is an area of opportunity that merits further research.

Currently, the application of ethylene generated by catalyst units stemming from the oxidation of ethanol to promote the ripening of different fruits is a common practice. This technology has been studied with other mango varieties whereby more uniform ripening and greater content of total soluble solids have been achieved (Appaw *et al*., 2009). Other methods that have been employed include the use of Ethephon (2-Chloro ethyl phosphonic acid) or Ethrel in a solution as reported by Sergent *et al*., (1993) which accelerated ripening in Keitt mangos when the fruit were treated with solutions of Ethephon between 1000 and 2000 mL L^{-1}. These two products have been used during the preharvest to accelerate flowering, they have been applied during the post harvest to other varieties to

homogenize ripening but no reports exist for Manila mangos. Lizada (1991) indicated that for Carabao mangos the applications of Ethephon during the preharvest did not significantly affect ripening in terms of its uniformity or period to achieve complete maturity.

Among the techniques to delay the ripening process, studies have been conducted on Kent mangos and the application of 300 mL^{-1} of 1-MCP that allowed for an increase in shelf-life of up to 27 days and a slower loss of firmness than the fruit in the control group (Osuna-García *et al*., 2007 and Osuna-García *et al*., 2009). Likewise, Muy-Rangel *et al*., (2009) applied 400 mL^{-1} of 1-MCP to Ataulfo mango fruit which delayed ripening by three days and reduced the loss of firmness, as well as the activities of the enzymes, polygalacturonase and cellulase.

León *et al*., (2002) applied heating treatments to Manila mangos at 38°C for 36 hours and subsequently stored them at 6°C and 12°C for 20 days, observing that the peak production of ethylene, as well as the synthesis of ACC and the activity of ACC oxidase, was delayed or inhibited by the thermal treatment, though they did not provide data regarding the quality of the fruit to assess the potential use for this procedure.

3.3.2.4 Refrigerated Storage

Refrigerated storage is the conventional method to extend the postharvest life of the fruit. Kader (2002) recommended storing mango fruit at 13°C at physiological ripeness and 10°C for fruit at partial ripeness or consumption ripeness. According to these recommendations, the maturity stage is the main factor to take into account to avoid physiological disorders during storage. Temperatures under those that are recommended generally lead to chilling injuries, however, the chilling injury temperature for Manila mangos has changed over time. Saucedo y Lakshminarayana (1977) compared ripeness at different temperatures (13, 16,

18, 20 and 25°C) registering better development of color, aroma, and flavor at 25° C (92% of the fruit ripened in 12 days), compared to the fruit store at 13°C and 16°C (48% of the fruit ripened in 30 days at 13 °C), indicating that 25°C was the best temperature for ripening the fruit and that the lesser development of those attributes could be attributed to chilling injuries. The criteria observed by these authors were based on the follow-up to the process of constant temperature regimens. The lower metabolic activity of the fruit under continuous refrigeration (less weight loss, lower respiration rate, and lower climacteric peak) alters the normal ripening process after a critical time period for each temperature. This aspect pointed to a need to conduct fruit transfers to determine the actual useful life of the fruit.

Under the same storage system, González (1982) conducted waxing and storage of Manila mangos at physiological ripeness after treating them with hot water (5 min in water at 55ºC and 500 ppm of Benlate®) at 7, 10, 20 and 25 °C with 70–80% Relative Humidity for different time periods. The loss of firmness and increase in soluble solids were not modified by the application of the hot water treatment or the waxes, though they were affected by the temperature. At 20°C and 25°C firmness decreased and total soluble solids increased more rapidly than those stored at 7°C and 10°C. The respiratory pattern of the fruit at 20°C and 25°C showed a maximum level on the fifth and eighth day, respectively. This author compared the respiration patterns with the decreases in firmness, color changes, and the increases in total soluble solids, and the observations in his data show, interestingly, that the changes in those factors initiated several days prior to the climacteric increment in the respiration rate.

Color change was quantified under subjective conditions, observing that in the fruit belonging to the control group the color yellow was achieved more rapidly compared to the treated fruit. For the treated fruit, the change was more rapid in the group subjected to hot-water treatment compared to the group subjected to the waxing process. Additionally, the percentage of marketable fruit was visually

quantified at different storage periods. The treatments that showed the highest percentage of marketable fruit were those whose temperatures were 20°C and 25°C, although only for a period of 10 days. The fruit stored at 7°C that was subsequently transferred to ambient temperature storage lost their commercial value after 10 to 15 days of storage, whereas for those stored at 10°C it didn't occur until after 10 to 22 days. This loss of commercial value was due to the appearance of chilling injury. The previous results allow this author to conclude that the most adequate storage temperature for Manila mangos should be above 10°C.

The symptoms of chilling injury described by this author were: gray superficial spots, pale yellow color, internal darkening, inability to ripen, and greater susceptibility to pathogen attacks. The author concluded that the storage temperature for Manila mangos should be set above the 10°C threshold to avoid chilling injury. Mercado-Silva (2010) also referred to the importance of storing this variety at temperatures above 10°C.

Hidalgo *et al*., (1997) studied the physiological response of Manila mangos at 120 days of development to storage for 16 days at 6°C, 12°C, 16°C, and 25°C, and 85% to 90% relative humidity, and to subsequent transfers to refrigerated storage at 25°C to determine the temperature threshold at which chilling injury symptoms begin to appear. After 12 days of storage at 6°C and 12°C, and their subsequent transfer to storage at 25°C, some chilling injury symptoms were observed visually on the pericarp (superficial depressions of a brown color, and irregular color changes in the skin). However, with regard to the control group, these temperatures did not adversely affect the ripening pattern of the fruit's mesocarp. The fruit exposed to 16°C ripened slowly and did not exhibit any superficial damage when transferred to storage at 25°C. According to these authors, temperatures at 6°C and 12°C are below the optimal temperature level, whereas at 16°C for 15 days they did not observe any evidence of chilling injury,

and they deemed the temperature the most fit for the postharvest handling of Manila mangos.

These authors indicated that, in addition to the symptomatology of chilling injury already referred to, the fruit stored at 6°C showed a high proportion of unsaturated fatty acids in the glycolipids of the membranes compared to the fruit stored at 25°C. Nevertheless, there were no observations regarding the ability to maintain that proportion of unsaturated fatty acids when the fruit were transferred to storage at 25°C.

As evidence that the phase transition of the lipids in the membrane is the primary response to cold stress, León *et al*., (2005) contributed data regarding the composition of fatty acids of the cellular membranes of the skin of Manila mango fruit stored for 20 days at 5°C, compared to fruit ripened at 25°C, observing that the lipids of the membranes subjected to cold stress decreased its contents of fatty acids in different proportions as shown on Table 5.

All of the fatty acids decreased their proportion, though the ones that did so in a more noticeable fashion were the stearic and oleic acids. The ratio between unsaturated/saturated fatty acids was lower at 5°C (0.552) compared to that observed for the group at 20°C (0.898). They also contributed data regarding the changes in fatty acids content after 6 and 9 days of storage at 6°C, 12°C, 16°C and 25°C, and in the fruit transferred from 5°C to 20°C. After six days, the unsaturated fatty acids were recorded in greater proportion compared to the samples stored at 25°C.

Table 5. Changes in the proportion of fatty acids in the lipid content of Manila mango membranes.

Fatty acid	% decrease compared to the group stored at 20°C
Lauric acid	52.8
Myristic acid	88.1
Palmitic acid	71.7
Stearic acid	94.5
Oleic acid	93.9
Linolenic acid	47.7

Modified from León *et al*.,(2005).

As the transfer period was extended, the fatty acid content tended to decrease, whereas it increased in the group stored at 25°C suggesting a lack of capability in the tissue to maintain the production of unsaturated fatty acids. These authors don't suggest a mechanism by which fatty acids are lost, nor do they provide data regarding the content of the various fractions of lipids in the membranes, or the volume of the membranes in the tissues. It's possible that the content of some fractions of lipids may have changed under refrigerated environments or the volume of the membranes may have decreased in the samples under cold stress, given that when the phase transition occurs some membrane sections can separate from the membrane set and change the content of fatty acids of the fractions under analysis.

Aguillón y Lizada (2010) recently described the incidence of chilling injury in Carabao mango at two stages of maturity treated with hot water for the control of anthracnose. Additionally, the chilling injury appeared mainly in the skin and was greater in fruit that was less ripe compared to the fruit that was ripe. Nevertheless, they pointed out that the fruit with less maturity that was treated

with hot water showed a higher incidence of chilling injury (on day 9) without requiring the transfer to higher temperatures to observe the damage.

They also indicated that the content of phospholipids of the membranes decreased drastically after storage at 12°C and 5°C, and that the ratio of saturated/unsaturated fatty acids peaked when the chilling injury symptoms began to appear. The authors indicated that the main fatty acid in the lipids of the membrane was palmitic acid, which explains the greater sensitivity to cold temperature displayed by this variety, whereas the data from León *et al*., shows that the main fatty acid in the skin of Manila mangos is oleic acid (22.1% at 5 °C and 44.5% at 25 °C), followed by stearic acid (11% at 5 °C and 24.4% at 25 °C) and palmitic acid (33% at 5°C and 14.3% at 20 °C), which leads us to assume a greater tolerance to chilling injury given the higher proportion of a oleic acid contained in the membranes of Manila mangos whose phospholipid shows a fusion temperature greater than that shown by the phospholipid of oleic acid. More research is required in this regard.

Gutiérrez *et al*., (1997) described the effect of the hot water treatment to decrease chilling injuries in Manila mangos, pointing out that the fruit treated at 46.1°C for 75 minutes showed brown spots on the skin, internal browning and irregular ripening after 16 days at 6°C plus 9 days at 25°C, and that at 12°C these manifestations of damage occurred after 20 days of storage plus 9 days at 25°C. This last condition could have some commercial interest given that export practices require three weeks of shelf-life for marketing purposes. This fact coincides with the recommendation made by Mercado-Silva (2010) regarding the storage of the fruit at 13°C, which has yet to be demonstrated experimentally, although no shelf-life beyond 15 days was achieved due to decay problems exhibited by the fruit.

Although Gutiérrez *et al*., (1997) did not present data regarding the respiration rate of the fruit transferred from refrigeration to 25°C (only towards the end of the storage period), the effect of storage temperature on the physiology of the fruit appears to be minor during the first 12 days of storage and, oddly, there were no differences between temperatures after that storage period. This behavior does

not correspond with the changes that appeared in the texture, reducing sugars, total soluble solids, and the color changes in the fruit, that did exhibit a logical effect from the temperature (higher temperature greater change). Additionally, these data do not correspond to those reported by Aguilón y Lizada (2010) who pointed to a greater sensitivity to cold for Carabao mango fruit subjected to hot water treatment (55 °C for 10 min). It's possible that the explanation for this difference may be related to the different composition of lipids in the membrane, although there could be another explanation.

León *et al*., (2005) carried out a general review on chilling injuries in mango fruit contributing data regarding the internal changes shown by Manila mango fruit when subjected to chilling injury inducing temperatures, compared to fruit ripened at 25°C, additionally, they describe the procedures to decrease the susceptibility to cold temperatures.

With regard to sensitivity to chilling injury and the maturity stage at harvest, De la Cruz *et al.,* (1999) indicated that fruit with 90 days of postanthesis exhibit the greatest susceptibility to chilling injury compared to the fruit harvested after 105 and 120 days. On the other hand, Beristain *et al.,* (1999) indicated that the temperature for the phase transition of the lipids in the cellular membranes of the pericarp in Manila mangos is set at around 12°C, which could be the critical temperature for this fruit and, hence, the conservation temperature should be set above this one. The data from these two works indicate that the fruit needs to be harvested 105 or 120 days after anthesis and stored at temperatures above 12°C.

Vela *et al*., (2003) studied the changes in the specific activity of the polyphenoloxidase contained in the skin and flesh of ripe Manila mangos (14 °Bx and 1.6 % of acidity) during its storage at 6°C, 12°C, and 25°C, and it's relationship to the manifestation of chilling injury. The enzyme's activity was greater in the skin compared to the flesh, and it was also greater in the fruit stored at 6°C and 12°C. the fruit transferred from refrigeration two 25°C

exhibited greater enzyme activity. The damage to the skin or the darkening rate was not notable during the first 12 days of storage, however, the fruit contained in the group stored at 6°C that were subsequently transferred to 25°C for four days exhibited high rates of darkening (IB=40%). After 16 days at 12°C, the darkening was greater than at 6°C although, once again, the fruit transferred to 25°C exhibited high darkening rates (50% and 80%, respectively) at both temperatures.

The data from these authors show that storage for 12 days at 6°C and 12°C induces chilling injury in the skin, which is visible when the fruit are transferred to 25°C, confirming its high susceptibility to chilling injury as established by Hidalgo *et al*., (1997). The increased activity for this enzyme explained the darkening of the skin on the fruit which is an important indicator of chilling injury in this variety and coincides with that reported by Aguilón y Lizada (2010) for Carabao mangos.

For their part, Trejo-Márquez *et al*., (2010) conducted a comparative study of the PPO and peroxidase (POD) activity in the flesh and skin of mango fruit from the Manila, Kent, and Keitt varieties at different stages of maturity stored at 5°C and 20°C. The skin on Manila mango fruit also showed the highest PPO activity without observing any significant differences between maturity stages. Similarly to what was observed by Vela *et al.,* (2003) the transfers of the product to 20°C registered high rates of activity, particularly during the climacteric and post climacteric of the fruit. One interesting aspect that stood out from the results, is that there were differences among the enzymes in the skin and the flesh, for example, the thermostability of the PPO in the flesh was greater than the PPO in the skin. This could explain why the hot water treatment (50 °C for 5 to 10 min) decreases the manifestation of superficial damage.

3.3.2.4.1 Treatments to Reduce Chilling Injuries

Applications of Methyl Jasmonate (MJ)

González-Aguilar (2000) and González-Aguilar *et al*., (2001) applied vapors of methyl jasmonate (10^{-4}M) for 24 hours at 25°C on Tommy Atkins and Kent mangos, and observed a decrease both in the incidence of chilling injury and the release of electrolytes, an increase in total soluble solid content, and a delayed color change in the treated fruit; though the respiration rate, weight loss, acidity, and firmness did not show any significant differences.

In all likelihood, based on those experiences, García et al., (2003) compared the application of the vapors of MJ 10^{-4}M to Manila mango fruit both subjected and not subjected to hot water treatment (46.1 °C 65 min) and stored at 6°C, 12°C or 25°C, and observed a higher content of reducing sugars and total soluble solids, less weight loss, and a lower incidence of chilling injury in the treated fruit. Herrera *et al*., (2004) developed a similar experiment to that of García *et.al.,* (2003) indicating that the hot water treatment lessened the sensitivity to chilling injury (14 days at 6 °C and 16 days at 12 °C) pointing out that the fruit treated with MJ exhibited better sensory attributes, and a more rapid and even color development compared to the control group. Unfortunately, these results were not reported or presented in a conference and there is no additional information available.

It is not yet known what mechanism is employed by the methyl jasmonate to reduce the incidence of chilling injury, or how it modifies the metabolism of the carbohydrates or aromas, though it has been observed that the content of polyamines in the fruit treated with MJ increases, but it is not yet known how this process interrelates with the lower incidence for chilling injury. Nevertheless, the findings indicate that the shelf-life attained without any manifestations of chilling injury is shorter than what is required for the export market, where it is important to have three weeks of storage in order to reach the final consumer.

Tasneem (2004) applied both MJ in solution (10^{-4} M 2 min) and diphenylamine (12 mM 2-3 min) to Kent mangos from Ecuador, observing a decrease in chilling

injuries and a greater percentage of marketable fruit. According to the author's data, the use of diphenylamine maintains a higher percentage of marketable fruit (50%) after 21 days of storage at 7°C plus 5 days at 20°C. Notwithstanding this low percentage, the interesting fact is that the fruit used by this author had 17 days of transportation prior to initiating the experiment, which allows us to consider the possibility of using this compound.

The information regarding the application of methyl jasmonate or diphenylamine in Manila mangos is still very scarce, and requires further research to determine the actual possibilities that these compounds offer for the post harvest handling of this product at the commercial level.

Hot water treatments.

Mena-Nevares (1993) studied the effects of commercial hot water treatment (46 °C por 0, 80 y 90 min) on the physiology and quality of Manila mangos from Altamirano, Veracruz, Mexico. The group with treated fruit and the control group were stored at 20°C for 10 days or 10°C and 13°C for 10 and 18 days with the transfer to 20°C for four days. The respiration rate, chlorophyll content, total soluble solids, and color changes did not appear to be altered by the treatments, but the weight loss was greater in the treated fruit compared to the control group (7.2 and 6.9%, respectively), while firmness remained higher in the treated fruit, at the end of the storage period all the fruit had experienced extensive softening. On the other hand, the incidence of decay was lower, particularly with the 90 minute treatment with a difference of 62.96% compared to the control group in fruit stored at 20°C, and 66.71% and 16.66% for those exposed to 10°C and 13°C, respectively. They also found that the treatments decreased the sensitivity to chilling injury by up to 30 to 40% (after 18 days at 10°C).

Gutiérrez *et al*., (1997) described that the hot water treatment used for the control of fruit-flies delayed the manifestation of chilling injury in Manila mangos, pointing

out that the fruit treated at 46.1°C for 75 minutes showed brown spots on the skin, internal Browning, and irregular ripening after 16 days of storage at 6° C plus 9 days at 25°C, and that at 12°C these manifestations of damage occurred after 20 days of storage plus 9 days at 25°C. This last condition could be of commercial interest given that export practices require up to three weeks of shelf-life for marketing purposes.

Although the data contributed by the research group in Veracruz, Mexico found that the hot water treatment decreases the onset of chilling injury, it's worth noting that in commercial practices this treatment has not generated the results observed by the researchers, to the contrary, there have been observations of damages that are attributed to the hot water treatment. It's possible that the preservation temperature used in commercial practices (10°C) may be a probable cause for the observed damage, one that may have to be changed to improve the handling of this fruit, as indicated by Mercado-Silva (2010) who suggested a temperature of 13°C. Nevertheless, these experiments need to be carried out to provide proof of said claims.

Use of controlled atmospheres to reduce chilling injuries

There is information that indicates that the use of controlled atmospheres could contribute to decreases in chilling injuries, nevertheless, the information available that relates to Manila mangos is incomplete, and refers to the use of these as quarantine treatments with potential capability for use as alternative techniques to reduce the onset of chilling injury. León *et al*., (1997) evaluated the impact of the application of insecticide atmospheres (1% O_2 and 30 or 50% CO_2) for periods of three days to observe its effect on the onset of chilling injury in Manila mangos stored at 12°C for 27 days. No beneficial effects were observed from the application of these atmospheres to reduce the symptoms of damage, since the untreated fruit developed damages due to chilling after 24 days, whereas those

treated with 30% and 50% CO_2 exhibited alterations after 21 and 18 days, respectively.

Generally speaking, studies carried out on the preservation of Manila mangos under refrigerated conditions indicate that it is a variety that is sensitive to low temperatures and that it should be stored at temperatures above 12°C. Considering that the preservation temperature for exporting mangos is 10°C, it would be reasonable to assert that the fruit is being subjected to chilling injuries and it's likely that the damages that occurred after the application of the hot water treatment might have been caused by the low preservation temperatures, and not necessarily by the hot water treatment itself. There is a need to conduct a study in which fruit subjected to hot water treatment (in accordance with the APHIS-USDA protocol), should be waxed in the conventional manner, be preserved at 13°C, and be assessed in terms of its potential shelf-life.

3.3.2.5 Controlled atmospheres and surface coatings

Controlled or modified atmosphere technologies, as well as wax coatings or plastic film, are utilized to reduce the metabolism of products or to control the loss of moisture and in that way extend the shelf-life of the fruit (Kader 2007).

(Kader 2002) suggests that the use of controlled atmospheres from 3% to 5% of O_2 and 5% to 8% of CO_2, which delay the ripening process, decrease the respiration rate and the production of ethylene, make it possible to achieve a storage life of 3 to 6 weeks. He also indicates that exposures under 2% of O_2 and above 8% of CO_2 induce alterations in skin color, grayish flesh, and alterations in the flavor. Nevertheless, these conditions have not been studied as of yet for this variety, and the research has focused on the assessment of the use of insecticide atmospheres (for short application periods) and their impact on reducing chilling injuries. In all likelihood, the scarce application of this

technology at the commercial scale and it's low impact on post harvest life may be the reason why so few studies have been undertaken with regard to this crop.

Ortega-Zaleta & Yahia (2000a) and Yahia & Ortega-Zaleta (2000) evaluated the effects of insecticide atmospheres with low oxygen and high carbon dioxide (0 kPa O_2 + 50 kPa CO_2) applied at high temperatures (40, 42, 43, 44, 45, 46, 47 and 49°C and 50% relative humidity) for 160 minutes, on the incidence of damage on Manila mangos from Veracruz, Mexico with 90 days of development and after being stored for 10 and 20 days at 10°C. After 10 days of storage at 10°C, no damage was observed in the treated fruit at temperatures below 44°C, slight damage at 44°C, and serious damage on treated fruit at 45°C or higher. Weight loss was similar between fruit in the control group and fruit in the treated group, and the loss of firmness lessened as the temperature increased up to 46°C. These authors concluded that Manila mangos resisted the treatment with the atmosphere under study at temperatures below 44°C, but are sensitive to higher temperatures, this being a potential limitation to its application as a treatment for the control of insects given that it affects the quality of the fruit.

González-Buenrostro (1982) observed the effects of a waxing Manila mango fruit with "candelilla" wax in addition to the application of the hot water treatment for the control of anthracnose (immersion in water at 55°C for 5 minutes), and compared them with unwaxed fruit during storage at 7, 10, 20 and 25°C for different periods of time. The treatments that resulted in the highest percentage of marketable fruit were those whose storage temperatures were 20 and 25°C, whereas the fruit treated with hot water and waxed maintained a high percentage of marketable fruit (more than 90%) after 20 days of storage, though no evaluation of the sensory characteristics of the fruit was undertaken. This effect was not observed under refrigerated conditions given that the percentage of marketable fruit was null after 12 days at 7°C or after 19 days at 10°C. These data suggest that the use of this type of wax did not surpass the expectations for the mango's shelf-life.

Díaz-Sobac *et al*., (1996) prepared a coating with a solution of 50°Bx of maldodextrine (10 DE) to which they added 3% of carboxymethylcellulose and 10% of esterified fatty acids in addition to a plastifying agent (Sorbac 60 Polisorbac 80). This coating was sprayed on Manila mangos at physiological maturity and stored at 15°C and 25°C. At both temperatures, weight loss was lower in the fruit coated with the film (8 to 9%) compared to the noncoated fruit (13 to 14%). The respiration rate decreased significantly and the changes in acidity, pH, solids insoluble in alcohol (starch content), and total soluble solids were lower (11 and 13 °Bx for 15 and 25 °C, respectively), and the change in color was less significant in the coated fruit compared to the control fruit. These authors pointed out that the fruit reached it's complete maturity after 3 to 4 days at 25°C, though no values were reported for the measured parameters. These authors pointed out that the fruit reached it's complete maturity after 3 to 4 days at 25°C, though no values were reported for the measured parameters. The data provided in this study suggest an alteration of the metabolism of the fruit during its preservation, and no evaluation was conducted as to whether or not any compounds were generated that could compromise the sensory quality of the fruit. Unfortunately, there is no data regarding the production of ethanol or acetaldehyde below these films, or any sensory evaluations that could provide greater certainty as to the commercial viability of the coatings analyzed in this study but, in any case, more information is required with regard to the application of this coating.

Using the aforementioned coatings, Díaz-Sobac *et al*., (1997) evaluated the softening process of Manila mango fruit and the enzymatic activities associated with this process (pectinesterase (PE), polygalacturonase (PG) y cellulase (Cx) during storage at 25 °C for 20 days). The firmness of the fruit treated with the coating remained stable without change during the first eight days of storage, and the softening process began thereafter but at a lower velocity than the one exhibited by the fruit in the control group, while the PE, PG, and Cx activities

reduced, which leads us to conclude that the lower loss of firmness in the fruit coated with the film was due to the lower activity rate for these enzymes.

Despite the effects demonstrated by the film used by these authors, it's necessary to contribute more information regarding whether or not the treatment altered the sensory quality of the fruit. If the coating maintains the quality, it could become a component with potential use in the handling of this fruit.

3.3.2.6 Quarantine Treatments

The presence of pests in mango production areas has been a problem for the marketing and export processes to areas that are free of those pests. Fruit fly infestations of the fruit occur only during specific periods of the development of the fruit, and that particular period when the fruit is susceptible to the infestation is due to changes in the composition of the fruit that make it an attractive element for insect pests. Among the compounds that attract insect pests we include volatile compounds, nevertheless, what can also happen is that other volatile compounds can work during specific stages of development as repellents for said pests.

More knowledge regarding the changes associated with these compounds opens up the possibility of garnering a better understanding of the pest infestation process and designing adequate strategies for controlling them. Jarvio (2006) quantified the changes in the volatile compounds contained in mangos during their development in order to identify those volatiles with potential insecticide activity during the preharvest development of the fruit (*Mangifera indica* var. 'Manila'). Nineteen (19) volatile compounds were identified and quantified in the skin, four of them (3-carene, limonene, pentanal, 2-hexenal) were associated with insecticide activity. These compounds decrease with the development of the fruit and were associated with the increase in sugars, the °Bx/acidity ratio, the development of color, and probably the softening of the skin. According to this

research, the decrease in volatile insecticide compounds and the increase in sugars determines the infestation period for the fruit, which occurs between the 84th and 91st days of development.
These works can serve as a benchmark to implement more adequate prevention measures to avoid fruit fly infestation.

Bagging Technique

Cabrera *et al*., (1996), based on the criteria that asserts that food fly infestation occurs at the beginning of the ripening process, carried out the isolation of the insect fruit through the bagging of the fruit to avoid the use of pesticides. According to these authors, the fruit can be bagged between the 30th and 80th day after anthesis, and the harvest must be carried out on the 90th and 115th days.

Prior to carrying out the bagging process, measures should be taken to control ants, apply fungicides to protect the fruit from anthracnose, fumagina, and scab. The fruit did not exhibit fruit fly infestation and only 10% showed anthracnose during ripening. According to these authors, the bagging process promotes a more uniform color, better firmness and resistance during the post harvest, and lessens drops and unwanted contact with branches and other fruits.

Guzmán-Estrada (2004) compared different types of bags (16 gauge paper, anti-virus mesh, and semi-waxed white bags) on six varieties of mango (Manila, Ataulfo, Haden, Tommy Atkins, Kent and Keitt) from the state of Sinaloa, Mexico. The bagged fruit registered lower weight than the fruit in the control group, but developed a normal color after harvest. The Manila, Ataulfo, Haden, & Tommy Atkins cultivars exhibited a better sanitary quality (without infestations or problems related to anthracnose), whereas the control group required the spraying of agrochemical products. The Kent and Keitt varieties exhibited sanitary problems as a result of said treatment. The Manila mangos registered a

higher quantity of soluble solids (°Bx) in fruit with anti-virus bags, but they were softer. These results coincide with the reports of Lechaudel y Joas (2007), who indicated that the type of bag used could affect the quality of the fruit pointing out that plastic bags promoted greater weight loss and greater loss of firmness in the fruit.

This technique was also used with Carabao mangos in conjunction with hot water treatment (55°C 5 to 10 minutes) resulting in a significant improvement in quality (81% of the fruit was deemed exportable compared with 45% of the fruit that was not bagged), lower incidence of the onset of diseases, and a lower incidence of fruit attacked by fruit flies (Bujante *et al*., 1997). Nevertheless, these researchers also indicated that this technique is not completely effective at avoiding the attack of fruit flies, noting that the bags that were used needed to be opaque.

Given the incidence rate of fruit flies in a large part of the production areas in Mexico, Manila mangos need to undergo treatment through the various quarantine protocols so that it can be exported to the United States. The aforementioned deinfestation protocols for *Anastrepha Ludens*, include the hot water treatment under the APHIS-USDA Plant Protection Quarantine PPQ T-102-a protocol, among the flat oblong varieties there are two treatment alternatives: fruit with weight up to 365 g, 65 minutes, and fruit between 365 and 570 g, 75 minutes. The fruit can also be treated with protocol PPQ T-103-c-1 (Single-stage high temperature forced air). As well as with hot vapor treatment PPQ T 106 – c-3, or with the application of gamma-ray irradiation through protocol PPQ 105-a-1 with a minimum dosage of 150 Gy and a maximum of 1000 Gy.

Hot Water Treatment

Studies on the effects of hot water treatment are scarce, nevertheless, Lagunes *et al*., (2007) studied the content of 1 aminocyclocarboxylic acid, or ACC, ACC

oxidase activity, ethylene production, changes in the content of total soluble solids or SST, acidity and firmness in Manila mango fruit from 215 to 260 g and 105 days post anthesis subjected to hot water treatment (46.1 °C for 65 min and cooled in water for 35 min), subsequently treated with 0, 0.5, 0.75, and 1.0 mL L^{-1} of ethylene for 6, 12 and 18 hours, and thereafter stored at ambient temperature. The fruit not treated with ethylene decreased it's ACC content, increased it's ACC oxidase activity, and the ethylene production reached a maximum on day 5 of storage, whereas the total soluble solids increased from 11 to 16 °Bx, and acidity and firmness decreased from 4% to 2% and from 7.5 to 4.5 kgF, respectively. Although the data presented by these authors only corresponds to five days after the treatment and there was no comparison with fruit not subjected to the hot water treatment, the high content of acidity and the higher firmness towards the end of the period appear to indicate that the ripening was incomplete, though they also don't include data on weight-loss or the visual characteristics of the fruit. The fruit treated with 0, 0.5 and 0.75 mL L^{-1} of ethylene for 6 and 12 hours increased their ethylene production, exhibited greater ACC oxidase activity or ACO, achieved a higher content of total soluble solids (SST) (18°Bx), lower acidity (<1.0%), and lower firmness values (around 3 kgf), all of which suggests an alternative to induce ripening after the hot water treatment. The fruit treated with 1 mL L^{-1} ethylene for 18 hours registered lower values for ACC, ACO activity, ethylene production, and SST (17°Bx), and acidity decreased down to approximately 2% indicating that this treatment inhibited the ripening process.

At the international food technologist conference (IFT), Peralta *et al*., (2004) presented results on the application of ethylene (750 □L of ethylene L^{-1} in the atmosphere for 6 hours at 25°C) to Manila mango fruit after having been subjected to hot water treatment (46.1°C for 65 minutes) and stored for two days at 12, 18, and 25°C. after the treatment with ethylene, the fruit were stored at 6, 12, and 18°C for five days. The best composition and appearance results for the fruit were obtained in the fruit treated with ethylene that was stored at 18°C. Nevertheless, the observation period was very short and there is still a lack of

information regarding what happens with the shelf-life of the fruit in order to estimate the application of the technique in a commercial setting where a minimum shelf-life of three weeks is required.

Dry Hot Air

As an alternative quarantine treatment, Yahia *et al*., (2000) studied the effects of a dry hot air treatment (44°C 50% relative humidity) for 160 and 220 minutes on the physiology and quality of Manila and Oro mangos at 17 and 10 weeks of development from Oaxaca, Mexico, which were stored at 10°C for 20 and 32 days. After 20 days of storage at 10°C, the dry hot air treatment did not cause superficial damage to the Manila mangos. According to the authors, Manila mangos stored at 10°C, did not exhibit damage due to heat or cold, reason for which they proposed that the treatment could help alleviate issues related to chilling injury. In the majority of cases, the treatment applied for 160 minutes delayed the ripening of the fruit but none of the two time periods used controlled the onset of anthracnose. The treated fruit exhibited better texture, and the internal and external color did not register any statistically different results. The researchers concluded that the treatments evaluated in this study did not cause any adverse or negative effects in the quality of the fruit, and therefore could be used to control pests such as *A*. *ludens* y *A*. *obliqua.*

According to USDA–APHIS regulations, the approved protocols that have a certain similarity to this treatment include PPQ T103-c-1, or hot air at high temperatures, which includes application requirements of minimum air temperature of 50°C and minimum flesh temperature of 48°C, which operate above the experimental conditions used by these researchers. This points to a need to continue a process so that the United States authorities can approve said treatment.

Another treatment is the T106-a or hot vapor treatment, which entails gradually elevating the temperature in the flesh of the fruit until its center reaches that temperature in 8 hours and subsequently maintains that temperature for 6 hours. These treatments are very aggressive for the physiology of the fruit, nevertheless, they are approved procedures and, if these are to be changed, a process needs to be started with the phytosanitary authorities in that country in order to modify them. Therefore, this treatment would be subject to the evaluation of the phytosanitary authorities for its possible application.

Uses of insecticide controlled atmospheres and hot dry air.

Yahia & Ortega Zaleta (2000) studied the effects of 21 treatments of controlled air or atmospheres at high temperatures and different application periods on the *in vitro* mortality of third stage larvae of *Anastrepha ludens* y *A*. *oblicua*, and observed 100% mortality when applying controlled air currents or atmospheres of different composition (0 or 13 kPa of O_2 and 0, 20 or 50 kPa of CO_2) at 48°C during 220 minutes. Nevertheless, in the case of both species' eggs, more severe conditions were required to achieve 100% mortality (air at 51°C for 240 minutes or 52°C for controlled atmospheres for 240 minutes, these authors suggest that temperatures above 44°C and exposure periods longer than 160 minutes could cause damage to Manila mangos).

Ortega-Zaleta y Yahia (2000b), based on research on the effects of controlled atmospheres on the quality of Manila mangos reported by Ortega-Zaleta & Yahia (2000a) and Yahia & Ortega-Zaleta (2000), studied the effects of insecticide atmospheres (0 kPa of 0_2 and 50 kPa of CO_2) applied at different temperatures (35, 37, 39, 40, 42, 43, 44, 45, 46, 47, 48 and 49°C) for 160 minutes on fruit infested with first and third stage larvae as well as eggs from two species of fruit fly (*Anastrepha oblicua* y *A*. *Ludens*). The combination of the controlled atmosphere with temperatures higher than 40°C produced a 100% mortality in

the eggs and larvae of the fruit infested artificially or naturally, providing an indication of the potential use for this treatment on this fruit.

Although quarantine treatments with insecticide atmospheres are not approved by USDA–APHIS for mangos that are exported to the United States, this is an alternative that could have industrial applications in the future even though there is a need to determine the degree to which these conditions must be changed to accommodate larger sized fruit.

Application of modified atmospheres and hot air.

Yahia *et al*., (1997) reported on the use of hot dry air (44 to 48°C) in an atmosphere that changed composition during the treatment period (22% at 0.8% of O_2
and of 0.035% at 67% of CO_2) for 160 and 220 minutes in Manila and Oro mango fruit from Oaxaca, Mexico. The treatments at 44°C for 220 minutes caused damage in both varieties and some fruit did not ripen after the treatment. Nevertheless, at 44°C for 160 minutes, both in the air as well as in the modified atmosphere, no damage was caused and in most cases ripening was delayed, though anthracnose was not controlled. Not having observed the presence of fruit fly larvae in the treated fruit, and only occasionally in the fruit belonging to the control group, the authors point out that the process could be efficient for the control of insects. Their results on the mortality of the eggs and third stage larvae of *Anastrepha ludens* and *A. oblicua* showed a 100% mortality in fruit treated at 44°C for 160 minutes with atmospheres with low O_2 and high CO_2 is efficient as a treatment to control these insects.

Treatments through superficial coatings

Diáz-Sobac *et al*., (2000) indicated that the coating described by the same workgroup (Díaz-Sobac *et al*., 1996) for the control of softening can also have an

effect on the development of fruit fly larvae. No infestations of fruit flies were carried out on the fruit in that research, and the fruit that was used was from areas that have fruit fly prevalence, parting from the hypothesis that the infestation of the fruit in the sampling orchard was generalized and homogeneous, all of which they could use to estimate the effect of the films on the development of larvae after a comparison of the incidence of infestation in the fruit belonging to the control group and the fruit covered with the film. According to these authors, none of the fruit that was covered with the film and stored at 15°C and 25°C exhibited any incidence of fruit fly larvae (the method employed to determine said incidence was not described), whereas the control fruit exhibited an incidence of 2.06 larvae per fruit. After six days of storage with the film, the incidence of fruit fly larvae in the fruit was the same for both the treated fruit as well as the control fruit, after nine days the incidence of larvae was present in 40% of the fruit and when the film was removed through a washing process, the incidence in the fruit was 25%. Nevertheless, the potential use of this film as an element for fruit fly control must be supported with controlled studies of fruit with a known degree of infestation, and consideration should be given to the fact that the use of coating treatments is not deemed to be a quarantine treatment by the phytosanitary authorities of the importing countries.

Irradiation quarantine treatment

Mercado-Silva (2011) developed a summary of the potential uses for irradiation as a technique for the de-infestation of horticultural products, and outlines the issues related to its application, as well as the limits to which the different varieties of mango need to be irradiated to avoid irradiation damage or spongy tissue.

Mercado-Silva (2010) y Guerrero (2010), studied the changes in the general quality of the fruit, as well as the changes in the antioxidant activity of Manila mango fruit from Nayarit, Mexico, at two stages of maturity ($^{1}/_{4}$ y ¾), subjected to

different dosages of Gamma-Ray irradiation (0, 0.15, 0.6 y 1.0 kGy), and stored at 10°C and 20°C. The fruit at $^{1}/_{4}$ maturity, irradiated with a dose of 1.0 kGy (0.96–1.33 kGy) and stored at 10°C, developed spongy tissue which demonstrated its susceptibility to those irradiation dosages. The results showed that Manila mangos have a shelf-life of 13 and 19 days when stored at 20°C and 10°C, respectively. The factor that drastically affected its visual quality was similar to the one registered for the Ataulfo variety, which did not exhibit that loss in visual quality. This decay was more noticeable in the fruit stored at 20°C.

The irradiation caused a delay in the fruit's change of color from green to yellow, although the high dosages did cause some browning of the skin as well as the development of spongy tissue, reason for which the recommendation was made to use an optimum dosage interval for this variety from 0.15 to 0.6 kGy, at which point the general quality of the fruit is preserved and the antioxidant properties improve.

Something that stood out from the study is that the content of ascorbic acid in this variety tends to increase as the fruit ripens, something that did not happen with the other varieties in the study. Likewise, the content of phenolic compounds increased, recording its maximum values in the fruit irradiated at 0.6 and 1.0 kGy.

The sensitivity to the higher dosages of irradiation observed by these authors is consistent with the findings of Manoto *et al*., (1992) who, after applying irradiation dosages of 0.1, 0.15, 0.25, and 0.350 kGy to Carabao mango fruit, indicated that they did not observe any impacts on the quality of the fruit in dosages up to 0.25 kGy, but that at 0.35 kGy there was a slight incidence of browning in the flesh. This also appears to show evidence of a relationship between both varieties.

3.3.2.7 Controlling Anthracnose

The National Mango Board published a literary review regarding the procedures employed for the control of this disease, which is available on their website: http://www.mango.org/media/55712/resumen_ejecutivo_antracnosis_en_mango.pdf, therefore, we will only refer to research carried out on Manila mangos here.

The development of equipment for measuring anthracnose damage.

Corkidi *et al*., (2006), at the *Centro de Ciencias Aplicadas y Desarrollo Tecnológico del Instituto de Biotecnología de la UNAM* (UNAM Biotechnology Institute's Center for Applied Science and Technological Development), developed a machine to measure the degree of damage caused by anthracnose. The machine records the fruit's color changes with a digital camera that takes 360 photographs (one for each degree of rotation of the fruit), which subsequently are downloaded into a hypothetical cylinder through which the surface damaged by the fungus is measured by way of imaging analysis.

Field experiments:

Rebolledo-Martínez *et al*., (2008) studied the effectiveness of controlling anthracnose in Manila mango production operations through the use of various products approved for that purpose. The products that were evaluated included: Benomil (Benlate®); Mil Stop Plus 2 & 4 (Potassium bicarbonate 85%); Sulfocop 3 & 6 (Copper sulfate and Sulfur); Master cop 1.2; Garlic 2 y 4 (Garlic extracts 99%), which were applied for four separate periods during the growing cycle in Cotaxtla, Veracruz, Mexico, as well as an additional bagging treatment for the fruit. The treatment that provided the best control for the disease (79% of the fruit were healthy at physiological maturity) was the one that involved four applications of Sulfocop 6 (6 L ha^{-1}), followed by the bagging treatment (59%). At the same time, the researchers observed that the bagging process did not outperform the use of the other products for controlling diseases, and therefore should be accompanied by other procedures.

Use of coatings

Using the same formulation as the films reported by Díaz-Sobac *et al*., (1996) and described in the section referring to chilling injuries (50 °Bx of maltodextrine (10 DE); 3% de carboximethylcellulose; 10 % of esterified fatty acids and a plastifying agent), Díaz-Sobac *et al*., (2000) evaluated their potential use as elements to reduce the damages due to anthracnose. The authors parted from the hypothesis that the *Colletotrichum gloesporoides* infection was natural, that it was present in all of the analyzed samples and was evenly distributed, therefore, after conducting a comparison with the untreated group, they obtained an indication of the potential impact of the coating with regard to controlling the disease. Under this assumption, the control group exhibited a severe manifestation of infection (100% of the fruit on day 12 at 25°C), whereas the groups coated with the film only exhibited traces of the disease under the same conditions. On the 18th day the infection was mild (20% of the infected fruit). It's possible that the metabolic alteration caused by the coating also delayed the decrease in phenolic compounds of the fruit, which led to a slower development of the microorganism. In any case, the potential use for this coating as a measure for controlling diseases requires controlled experimentation with fruit inoculated with known concentrations of the pathogen, in addition to evidence regarding whether or not the wax compromises the sensory quality of the fruit.

Biological control

In his literature review, conducted on behalf of the Mango Board on the subject of anthracnose in mangos, which is available at: http://www.mango.org/mango/sites/default/files/download/anthracnose_of_mango.pdf, Ploetz points out that, at the time of his review, *Bacillus licheniformis* bacteria had been used as a control measure, however, at the time no biological control procedure had been as successful as the application of fungicides.

Nevertheless, there are new approaches being used at the present time in the field such as the use of microorganisms that are antagonistic to *Colletoctrichum gloesporoides* that could signify an important strategy for the control of this disease. The use of antagonist microorganisms for biological control is based on the fact that the antagonists use one of the following mechanisms to eliminate or displace the pathogen: they produce lytic enzymes to combat the pathogen, they compete for nutrients and space with the pathogen, or they induce resistance in the fruit towards the pathogen (Hernández-Lauzardo *et al*., 2007).

The Institute of Biotechnology of the Autonomous University of Mexico has conducted studies in this regard with applications in the field. Carrillo-Fasio *et al*., (2005) evaluated the effectiveness of controlling anthracnose in Kent mangos through the use of two antagonist microorganisms (*Rhodotorula minuta* y *Bacillus subtilis*), isolated from the mango skin and grown in the lab in order to be applied during the development of the fruit. The application of the mixture of both microorganisms allowed for a noticeable reduction in the incidence of the disease (80%) compared to the untreated fruit (60.5%), or 25% and 12%, respectively, when the application of the antagonists was done separately.

Based on these results, Patiño-Vera *et al*., (2005) escalated the production of *Rhodotorula minuta* in a 100 L fermenter, and the product was applied in the field on Kiett and Kent mangos in the state of Sinaloa, Mexico. The severity of the infection was lower in the control group and similar to the fruit treated with a commercial fungicide. Nevertheless, these studies have not been conducted on Manila mangos which are sensitive to anthracnose, have a thinner skin, and a more accelerated metabolism which could generate different responses to these control procedures.

One aspect, yet to be explored in the field of biological control, is the resistance induced in mango fruit through the use of microorganisms that induce resistance in the fruit allowing it to overcome its attack. Ugay (2003) employs a non-

pathogenic mutating strain of *Colletoctrichum gloesporoides* to induce resistance to this disease in Carabao mango fruit, thereby reducing the severity of the incidence of anthracnose by up to 49% (http://agris.fao.org/agris-search/search/display.do?f=2004%2FPH%2FPH04003.xml%3BPH2004000586).
The application of the non-pathogenic strain prior to the inoculation of the pathogen induced a greater synthesis of ACC and, hence, of ethylene even when there was no recorded attack from the disease. This fact made the researchers consider that these microorganisms provoke a response in the fruit that could lead to the formation of resistance elements that allow it to resist any attack from the pathogenic microorganism. This work is notable because the Carabao variety may have many histological similarities to the Manila variety, and it's possible that they could exhibit similar responses.

Nevertheless, more information is necessary to really delimit the scope of these techniques, the application of which needs to be broadened in much greater detail from the commercial standpoint to address the issue of controlling anthracnose.

Hot water treatments.

In addition to treatments with fungicides, physical treatments such as immersion of the fruit in hot water (53°C for 5 to 10 minutes), irradiation, refrigeration, and controlled atmospheres have their spectrum of application with regard to pathogen control. In particular, the hot water treatment of mango fruit has a positive effect for the control of anthracnose (Spalding y Reeder 1986; McMillan *et al*., 1987). Said treatment frequently incorporates fungicides to enable its action.

Mena-Nevarez (1993) studied the impact of the hot water treatment (46°C for 80 and 90 minutes) during the development of anthracnose in Manila mangos stored at 20°C for 10 days, indicating that the 90 minute treatment presented a slight

and moderate incidence of the disease of 8.5% and 2.1%, respectively, whereas the untreated group registered 52% and 17% of incidence. Nevertheless, the data from this author show that the hot water treatment that was applied for 80 minutes increased the incidence of damage compared to the control group (60% and 29%). According to the sizes of this variety, the hot water treatment would be applied for 75 minutes and, based on the data recorded by the author, it appears that it's possible to have a higher incidence for the pathogen in this variety when it is subjected to this process. Additionally, It's possible that the treatment for 80 minutes may affect the resistance of the fruit more than the resistance of the pathogen making it more feasible for its subsequent development in the fruit. At 90 minutes it's possible that the resistance of the pathogen may have been altered, a fact that would have decreased it's incidence in the fruit, though the fruit would've also been affected.

The use of ozone

Ozone has an oxidizing potential 1.5 times greater than chlorine and 3000 times greater than hypochlorous acid, likewise, it's contact periods for antimicrobial actions are 4 to 5 times shorter than those of chlorine, which makes it a useful compound for disinfection procedures involving fruits and vegetables.

Barbosa-Martínez (2003), in addition to conducting a comparative study on the development of Haden and Manila mango fruit, also conducted a comparative study on the effects resulting from the application of ozone (0, 0.8 and 2 mg L^{-1}) for controlling anthracnose in both varieties artificially inoculated with a suspension of *Colletotrichum gloesporoides* spores, and evaluated the development of the disease 120 hours and 11 days after the inoculation. There was up to 98% of spore germination inhibition observed in *in vitro* cultures. The *in vivo* inoculation studies indicated that the Manila variety exhibited the first symptoms of the disease after the 96th hour, whereas in the Haden mango they appeared after 120 hours. After 11 days of incubation, the severity of the attack

on the Manila mango was 45% of the total surface, while it was only 5% in the Haden mango, which was a clear indication that the Manila mango had a higher susceptibility to anthracnose compared to the Haden mango. According to this author, the greater susceptibility displayed by this variety is due to the lesser thickness of the epicarp, and to a higher metabolic activity which causes a more accelerated softening, and for that reason the greater ease of attack from the microorganism. The applications of ozone to the fruit inoculated with spores from the microorganism resulted in a the formation of a necrotic area slightly inferior to that exhibited by the control fruit, which was an indication that it is very difficult to control the development of the disease once it has been established in the fruit.

The use of irradiation for controlling anthracnose

The application of Gamma-Ray irradiation for the control of pathogens in mangos has been widely used (Jhonson *et al*., 1990), in some varieties a certain degree of control of the disease has been achieved, though not to commercially accepted standards and is only successful when the treatment is combined with fungicides or hot water treatment, but the irradiation by itself does not protect the fruit from the disease, in addition to the fact that some varieties are susceptible to irradiation damage when they are exposed to dosages above 600 Gy, as demonstrated by Mercado-Silva (2010) for Manila mangos. Mercado-Silva *et al*. (2011, Data not published) evaluated the impact from the hot water treatment (6 min at 53°C) for controlling anthracnose, as well as the subsequent irradiation of the fruit at 500 Gy, observing that the fruit are adequately preserved for 18 days at 10°C.

3.3.2.8 Minimally processed Manila mangos

The available information regarding the development of products that are minimally processed is quite scarce and was only found in a presentation made at a conference (Espinoza *et al*., 2004) in which the researchers reported having

developed a minimally processed product based on slices of 1X4X5 cm that were submerged in citric acid, calcium chloride, sodium benzoate, and hydrogen peroxide, packed in high density polyethylene, and stored at 24, 12, and 6°C for 17 days. The storage at 6°C decreased the respiration rate and maintained firmness, whereas at 12°C there was a greater concentration of ascorbic acid and glucose, and because of the use of additives the shelf-life can be as long as 17 days. Nevertheless, no data were presented regarding the microbial quality or the sensory evaluations of the products.

3.3.2.9 Manila mangos as a functional food

The National Mango Board charged Dr. Lucas from Oklahoma State University with developing a study on the modulation of body fat and glucose and lipids in the plasma of rats fed a high-fat diet plus dehydrated mangos. The results are available on the website: http://www.mango.org/mango/sites/default/files/download/glucose_and_lipids_research%5B1%5D.pdf the results indicate that adding 1% of freeze-dried dust of Tommy Atkins mango flesh to the high-fat diet fed to rats is capable of decreasing the glucose levels in the bloodstream, and of helping to regulate the cholesterol and fat levels in the organism. With regard to regulating the glucose in the plasma, the aforementioned study indicates that the mechanism by which this effect takes place is not through increasing glucose absorption absorption through the muscles, and that a potential mechanism could be at the mango directly or incorrectly makes glucose absorption in the long intestine more difficult.

It has been reported recently that mango protein extract could have therapeutic properties for immune system diseases and diabetes given the presence of lectins in the fruit. Lectins are plant proteins that intervene in generation processes, defense mechanisms, the transportation and mobilization of reserve

metabolic substances, as well as in immune, antitumor, nutraceutical and antiviral functions.

Based on this background, as well as research carried out at the *Instituto de Ciencias Básicas de la Universidad Veracruzana* (Institute of Basic Sciences of the Universidad Veracruzana) in Xalapa Veracruz, Mexico, Alarcon-Aparicio (2005) and Reyes-Pool (2008) isolated, identified, and characterized the functionality of the lectins present in the leaves, skin, and flesh of Manila mangos at 60, 75, and 90 days of development from Jalcomulco, Veracruz, Mexico. These proteins have different functional properties, one of them being their capacity to attach to carbohydrates which allows them to interact with systems that transport sugars in the membranes of the digestive tract making the absorption of glucose more difficult. Nevertheless, this aspect requires further research, particularly the verification of the action of these proteins. The content of these proteins appears to change between varieties and with the development of the fruit. The contents for undertaking the selection of fruit were 3.91 and 2.08 mg /100g compared to the content in Manila mangos (2.59 and 2.04mg/100g SS for leaves and skin, respectively).

The lectins in Manila mangos exhibited antibacterial activity against gram-positive and gram-negative bacteria, though their activity appeared to rely on the origin of each one. Some of them were capable of inhibiting the growth of *Salmonell epidermis*, whereas others that originated from other samples registered activity against *S. epidermis, E. coli,* and *Peudomonas. Aureginosa* and others inhibited the growth of *Candida. albicans*. Further research is required to identify the reason why these proteins are not homogeneous in the various materials.

Additionally, one of the isolated lectins was identified as *Mangifera indica* aglutinine, or MIA. These data appear to open new mango properties to human nutrition as a factor of assistance in the treatment of diseases such as diabetes

or obesity. Nevertheless, there may be other compounds such as polyphenols that could interfere with the transportation of glucose, as shown in other studies (Carrero-Berzal, 2010).

These results show that mango lectins can have applications in other research sectors related to food biosecurity, biomedicine, and agriculture.

In a different study carried out on behalf of the National Mango Board, Dr. Talcott from Texas A&M University carried out research of the attributes of mango phytochemicals that provide benefits to human health which is available on the website: http://www.mango.org/mango/sites/default/files/download/mangos_and_cancer_cells_final_report_eng.pdf where it shows that the Ataulfo variety possessed the highest content of polyphenols, followed by the Haden variety; that the Ataulfo Mango was capable of inhibiting colon cancer growth by up to 72%; that mango polyphenols have no adverse effect on normal colon cells; that mango polyphenols caused the suicide death of cancer cells, and that the fruit contains moderate quantities of carotenoids.

As supplemental information to this research, Ornelas et al., (2007) compared the carotenoid content of seven varieties of Mexican mangos (Ataulfo, Manila, Criollo, Paraiso, Haden, Kent y Tommy Atkins). All of the varieties had a similar pattern of carotenoid compounds having separated 25 different compounds, the most abundant of which were all-trans □□carotene, dibutyrate esters of all-trans-violaxanthin and 9-cis violaxanthin. They also showed that the mango samples studied only contained a tocopherol. The Haden variety registered the highest content for the three main carotenoids, while the Ataulfo Mango showed the lowest contents of xantophylls; the Manila mango exhibited lower contents of carotenoids compared to the Haden mango, but higher than those of the Ataulfo mango. In the opinion of these authors, the content of carotenoids and a

tocopherol in Mexican mangos is high compared to mangos from other geographical locations.

Given that color is a quality parameter in food that affects the acceptance and the perception of sweetness and flavor, and that carotenoids are the responsible elements for the yellowish-orange color of the mesocarp in mangos, Ornelas et al., (2008) identified the most important carotenoids in the mesocarp of Manila and Ataulfo mangos, and quantified the relationship between the concentration of those carotenoids and the changes in the color of the mesocarp or the epidermis during the ripening of the fruit. The main carotenoids identified in the fruit were all-tran β carotene, all-tran violaxanthin and 9-cis-violaxanthin (like dibutirate). For Manila mangos, the concentration of these compounds increased during ripening from $0.25x10^{-3}$ to $35.57x10^{-3}$, $0.40x10^{-5}$ to $31.97x10^{-3}$ and 0 to $16.81x10^{-3}$ gKg^{-1} for the three carotenoids, respectively, and these exhibited a very high coefficient of correlation with the color parameters of the mesocarp or of the skin, which permitted these authors to develop useful equations to calculate the content of these carotenoids parting from the data related to skin color.

3.3.2.10 Processed products

The drying process for fruit degrades physical (texture, color) and nutritional quality. Indicators such as ascorbic acid content are used to determine the impact of the treatments on the nutritional quality of the food. Ortiz-Yescas et al., (2007) conducted a study in which they assessed the degradation of the ascorbic acid during the drying process of Manila mango and Maradol papaya slices at different operating conditions. The degradation kinetics for the ascorbic acid were carried out at different temperatures (40, 50, 60, and 70°C), two air flow velocities (1.5 and 2.5 m/s) and two thicknesses for the slices (1 and 1.5 cm). The results indicated that the degradation kinetics of the ascorbic acid follow a first order model. The model described by these authors presents an Arrhenius type of

dependence with regards to the temperature and an empirical relationship with regard to the moisture. The use of the model along with the differential equations of the drying process will allow for the optimization of the process of nutrient retention.

Combined methods to preserve the color of mango purée

Jiménez et al., (2001) carried out a study for the purpose of searching for stability in the color changes of manila mango purée during storage, utilizing combine methods formulated with different values for water activity or a_w, concentration of sodium sulfite and hot water treatment times. For this purpose, once the mango purée is prepared, it is subjected to a thermal treatment at 75°C and subsequently stored at 35°C for 120 hours. The results allowed for the development of a prediction model for the browning index with which it would be possible to determine combinations of a_w, sodium sulfite, and thermal treatments to obtain a mango purée with minimal changes in color, yet acceptable from a sensory standpoint.

Characterization of the starch in Manila mangos

Bello-Pérez et al., (2005) isolated, characterized, and evaluated the functional properties of the starch contained in Manila mangos, as well as creole mangos, indicating that this starch could be a potential source for use in the food industry. They also pointed out that the starch in Manila mangos has a higher content of protein compared to corn starch, as well as a greater capability for water retention and swelling, reason for which it could be used in the deli meat industry.

4 Proposed research to improve the postharvest handling of Manila mangos

The review conducted of the research carried out in Mexico with this variety demonstrates a need to continue working in the following areas:

1. Research the effects of continued use of flowering inductors and PBZ in the physiology of trees subjected to the annual use of these compounds, and determine whether or not their continued use causes decreases in production.
2. Given the histological and metabolic differences in this variety, it is necessary to design adequate strategies to increase its shelf-life and, in particular, decrease the problems of decay and susceptibility to anthracnose.
3. Although the harvest indexes employed are the same ones used for the harvest of other varieties, there is a need to evaluate the applicability of the mathematical equation to predict the development of the fruit as a function of the accumulated heat units.
4. There is a need to characterize the ripening process of the fruit using different approaches to the ones being used at the present time that will allow us to identify whether or not this variety registers different climacteric peaks in its development so we can subsequently develop better preservation strategies.
5. There is a need to carry out studies on the application of ethylene inhibitors in order to delay the ripening process and predict how much the shelf-life could be extended.
6. Given the susceptibility that Manila mangos have towards chilling injuries when exposed to temperatures under 12°C, there is a need to conduct research in which the fruit subjected to the hot water treatment (in accordance with the APHIS–USDA protocol) would be waxed in the conventional manner, preserved at 13°C, and their shelf-life would be assessed.
7. Evaluate the use of methyl jasmonate with this variety for the purpose of decreasing its susceptibility to chilling injury.

8. There is a need to develop or search for material that will allow us to control the problem of decay and manipulate the physiology of the fruit, particularly after the application of the quarantine treatments.

9. It's important to identify whether or not the biological control for anthracnose is feasible in this variety by capitalizing on the experience obtained with other varieties.

10. Gamma-Ray irradiation treatments, until now, have been the most adequate option and is accepted by APHIS-USDA, nevertheless, there is a need to resolve the problem of decay in the fruit to achieve an adequate shelf-life.

11. The research on minimal processing is in its infancy and requires more studies to design a technology that will allow for the development of this product with an acceptable level of quality.

12. The capability that this variety has to increase its content of ascorbic acid and it's antioxidant capacity after the irradiation treatments provides greater support to resolve the problem of decay in this fruit and, hence, offer fruit with better nutritional properties.

13. The research on the generation of functional compounds has focused on carotenoid content, however, increments of mangiferin have been measured in the leaves and bark of trees treated with PBZ and flowering inductors, but they were not measured in the flesh of the fruit, which is something that needs to be assessed in order to determine whether or not this fruit has functional potential.

14. There is a need to assess in greater detail the presence of lectins in the flesh of the fruit and accurately describe its functional properties.

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