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# Papaya, Mango and Guava Fruit Metabolism during Ripening: Postharvest Changes Affecting Tropical Fruit Nutritional Content and Quality

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## ABSTRACT

The ripening process affects the nutritional content and quality of climacteric fruits. During papaya ripening, papayas become more acceptable due to pulp sweetness, redness and softness, with an increment of carotenoids. Mangoes increase the strong aroma, sweetness and vitamin C, β-carotene and minerals levels during ripening. Ripe guavas have one of the highest levels of vitamin C and minerals compared to other tropical fleshy fruits. Although during these fleshy fruit ripening an increase in nutritional value and physical-chemical quality is observed, these changes could lead to a reduced shelf-life. In order to minimize postharvest losses, some techniques have been used such as cold storage and 1-MCP treatment. The techniques are far from being standardized, but some interesting results have been achieved for papayas, mangoes and guavas. Therefore, this review focuses on the main changes occurring during ripening of these three tropical fruits that lead to an increment of quality attributes and nutritional values but can also cause shelf-life losses. This review also exposes some techniques used to postpone fruit ripening in these tropical fruits.

**Keywords:** ethylene, fruit ripening, 1-MCP, nutritional value, postharvest quality
**Abbreviations: 1-MCP**, 1-methylcyclopropene; **ACC**, 1-aminocyclopropane-1-carboxilic acid; **CI**, chilling injury; **DAH**, dehydroascorbic acid; **DRI**, dietary reference intake; **endoPG**, endopolygalacturonase enzyme

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## PAPAYA

Papaya (*Carica papaya* L.) is a fruit cultivated in most part of tropical countries being one of the most commercialized fruit in the world, and its biology and biotechnology has already been exhaustive discussed in a previous review (Teixeira da Silva *et al*. 2007). Papaya tree carries old features of sexual differentiation that leads fruits to having distinct aspects. Fruits are grown axillary on the main stem, usually singly but sometimes in small clusters, being fruits derived from males or females flowers with an "egg-shaped" aspect. Hermaphrodite fruits, less cylindrical and more oval-shaped, are the biggest crop in the world since their fruits are more appreciated because of their final physical-chemical aspects (Paull *et al*. 2008). Another reason is that the flowers can be self-pollinated (Seymour *et al*. 1993).

Amongst a wide range of papaya cultivars, the ones which produce the smallest fruits are the most important in worldwide commercialization due to its facility for shipment (Paull *et al*. 2008). Nevertheless, these cultivars have some post harvest attributes that favors their consumption acceptability, such as pulp color and sweetness enrichment and pulp melting texture acquired during fruit ripening (Paull 1993). Commercially important cultivars are genetic variations of the 'Solo' variety, defined as the 'Solo' group, which fruits are round and shallowly furrowed in female plants and pear-shaped in bisexual plants. This main variety produces no male plants, and is considered a red-fleshed

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fruit, due to its reddish-orange color pulp. 'Sunrise', 'SunUp' and 'Golden' cultivars are the most appreciated red-fleshed varieties derived from 'Solo' group.

Papaya is considered a climacteric fruit because during ripening it shows a burst of respiration and ethylene production (Fabi *et al*. 2007; Manenoi *et al*. 2007). During and after these burst papaya fruit undergoes to some important physical modifications reflecting the accessibility to important nutrients compounds, such as vitamins, fiber and antioxidants (Fennema 1996). In fact, during the ripening process the nutritional content of papaya alters mostly because of carotenoids synthesis which reaches elevated levels during normal ripening (Cano *et al*. 1996). Yellow-fleshed papaya acquires higher contents of pro-vitamin A in form of β-carotene and β-cryptoxanthin (yellow color), while red-fleshed papaya acquires high levels of both pro-vitamin A and a major antioxidant, the lycopene (red-color) (Chandrika *et al*. 2003). Papaya fruit is also a good source of vitamin C, but this vitamin appears to be constantly synthesized during fruit development, demonstrating no level differences during fruit ripening (Wall 2006; Souza *et al*. 2008). However, papaya fruit is a good source of basic and complex sugars, such as sucrose, inverted sucrose (glucose and fructose) and fibers (Manrique and Lajolo 2004; Shiga *et al*. 2009). Their levels seem to vary during ripening especially sucrose hydrolyses that enhances the commercially desirable sweetness (Gomez *et al*. 2002) and fiber degradation that gives the commercially desirable softness (Shiga *et al*. 2009). Pulp softening can also make possible the rapid accessibility to some nutrients by lessening the main physical barriers, in this case the fruit cell walls. On the other hand, cell wall loosening could lead to postharvest problems such as the reduction of fruit shelf-life and some losses during papaya marketing (Paull *et al*. 1997; Proulx *et al*. 2005).

## RIPENING OF PAPAYAS

### Ethylene regulation of ripening

The plant hormone ethylene is synthesized from amino acid metionine, and the two main enzymes are related to the synthesis and oxidation of the compound ACC (1-aminocyclopropane-1-carboxilic acid), the ACC synthase (EC 4.4.1.14) and ACC oxidase (EC 1.14.17.4), respectively. The amino acid metionine will be recovered in a well known cycle named "Yang cycle" (Miyazaki and Yang 1987), and few molecules of metionine could represent massive production of ethylene. This is what happens with climacteric fruit during postharvest ripening (Bleecker and Kende 2000). ACC synthase and ACC oxidase have already been studied and characterized in a wide range of plants, including papaya fruit. Mason and Botella (1997) identified two papaya fruit ACC synthases, while Dunkley and Golden (1998) and

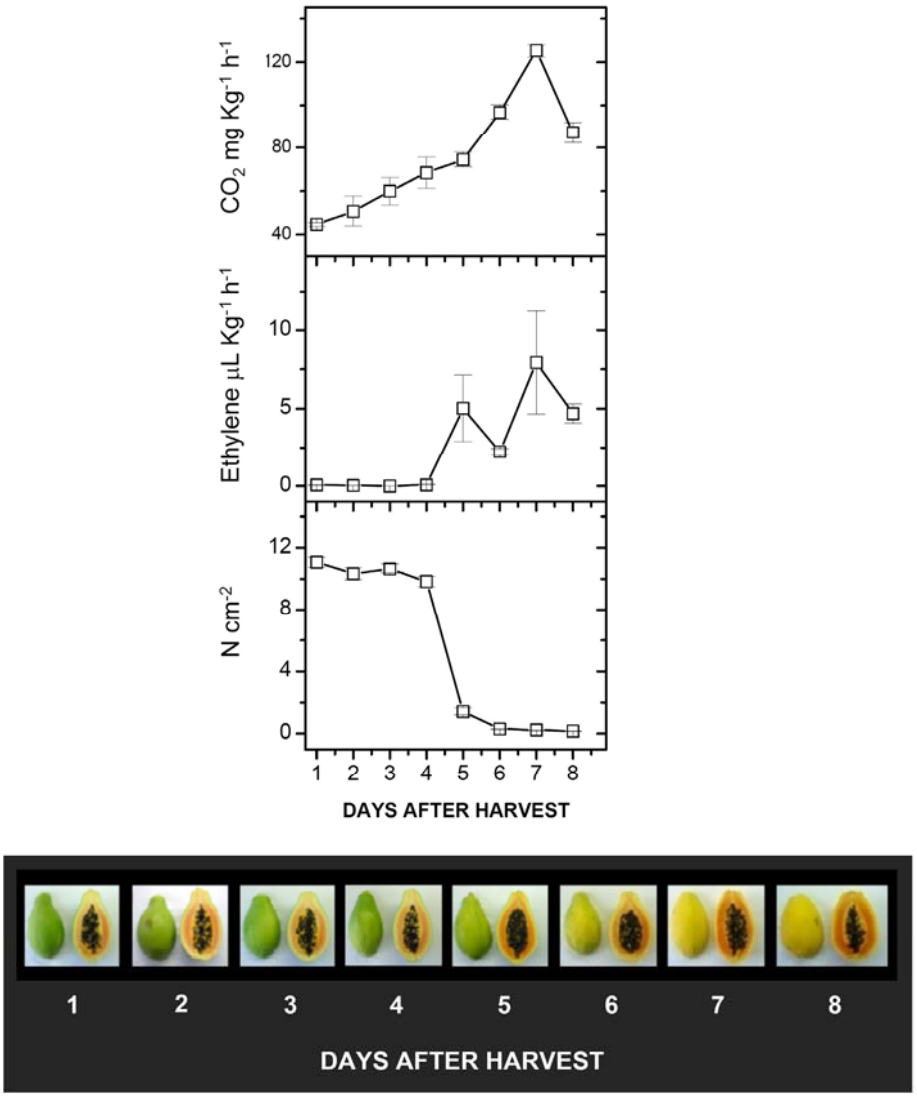


**Fig. 1 Ripening analysis of papaya fruit cv. 'Golden'.** Materials and methods are the same as described in Fabi *et al*. (2007). First row: amount of $CO_2$ produced measured by GC-TCD ($CO_2$ mg $Kg^{-1}$ $h^{-1}$); Second row: amount of ethylene produced by fruits measured by GC-FID (ethylene µL $Kg^{-1}$ $h^{-1}$); Third row: pulp firmness measured with a texturometer (N $cm^{-2}$). Error bars indicate *se* of the mean ($n$ = 6).

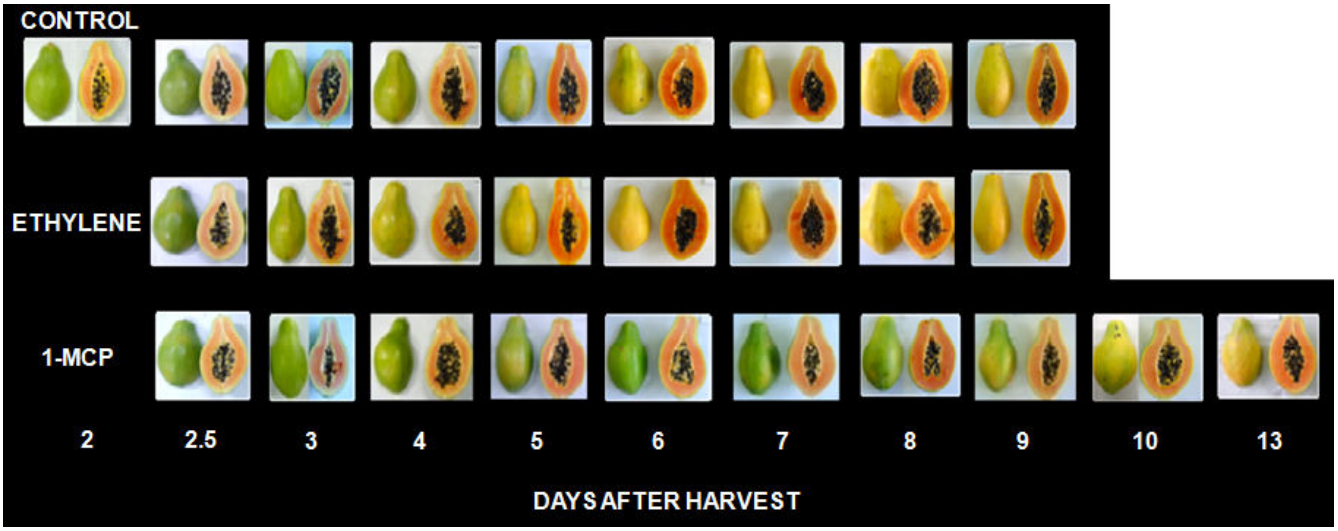


**Fig. 2 Visual ripening analysis of papaya fruit cv. 'Golden'.** Fruits without treatment (CONTROL) and ETHYLENE- and 1-MCP-treated had some physical-chemical parameters of fruit ripening analyzed and published by Fabi *et al*. 2007. Ethylene treatment accelerated chlorophyll degradation while 1-MCP treatment retarded this event.

López-Gómez *et al*. (2004) isolated distinct genes corresponding to ACC oxidase. A new papaya ACC oxidase was discovered by Chen *et al*. (2003) and demonstrated a different pattern of expression when compared to the other isolated genes with the gene expression levels being up-regulated during fruit senescence. A wide range of papaya metionine synthases, ACC synthases and ACC oxydases have already had their putative sequences predicted by *in silico* analyses using the Whole Genome Shotgun sequencing from a Hawaiian cultivar (Ming *et al*. 2008; Paull *et al*. 2008).

Ethylene synthesis, considered an auto-catalytic reaction, has the model of signalization in fruits already proposed (Adams-Philips *et al*. 2004). Self-production of ethylene by climacteric fruits triggers some cascade signalization pathways that lead the fruit to immediate transformations including chlorophyll degradation, respiration and ethylene production and pulp softening. **Fig. 1** shows some physical-chemical parameters of a papaya fruit 'Golden' variety ripening. Observing the figure, it can be speculate that the climacteric of papaya fruit is regulated by ethylene triggering which has already been proposed before (Fabi *et al*. 2007).

Papaya fruit undergoes to some critical transformations for its commercialization. The pulp texture analyzed with a texturometer reveals that in just one day papaya pulp becomes viable for consumption (**Fig. 1**, 4$^{th}$ to 5$^{th}$ day after harvest). However, this event could affect the final quality of the fruit since internal injuries made before and during pulp softening could lead to melting texture spots inside pulp and in the peel (Paull *et al*. 1997; Teixeira da Silva *et al*. 2007). Another interesting proposal is the possible regulation of pulp softening by endogenous ethylene. While the pulp texture decreases in the 5$^{th}$ day after harvesting, there is the ethylene burst. The effects observed by little amounts of endogenous ethylene made some researches rely on the idea fruits had ethylene-sensitive receptors (Payton *et al*. 1996).

In order to minimize the ethylene effects, many compounds were tested as directed competitors of the ethylene receptors, such as 2,5-norbornadieno (NBD), diazocyclopentadiene (DACP) and silver thiosulphate. However, because of their toxicity and negative environmental impact, their use was abolished, and recently the use of 1-methylcyclopropene (1-MCP) is an effective way to control the ethylene effects in some plants (Watkins *et al*. 2000). Using this concept, Sisler *et al*. (2003) proposed to start using this compound to increase climacteric fruit shelf-life. Some studies on papaya fruit treated with 1-MCP have already been done, but the ideal concentration of this compound and the timing of treatment for commercially satisfaction achievement are far from being standardized (Jacomino *et al*. 2002; Ergun and Huber 2004; Manenoi *et al*. 2007; Fabi *et al*. 2007). All authors analyzed either 'Solo' cultivar or cultivars derived from it and despite the results were different for each experiment it was noted 1-MCP treatment leaded to an extended shelf-life after papaya postharvest. All these results support the idea ethylene is the major plant hormone produced in climacteric fleshy-fruits, such as papaya, and inhibition of these effects using chemical compounds could represent a relevant commercially tool to decrease marketing losses.

## Changes in peel color

One of the transformations papaya undergoes during ripening is the peel color. Peel color changes take place very quickly and it is caused by an up-regulation of genes that encode for chlorophyllases (Jacob-Wilk *et al*. 1999). There are no studies relating chlorophyllases gene expression and papaya fruit ripening; however, some authors studied the loss of peel green color by normal colorimetric assays, such as measuring peel color with a colorimeter (Jacomino *et al*. 2002) or by chlorophyll fluorescence (Bron *et al*. 2004). In both studies, the hue angle (°h), which defines the basic color (0° = red; 90° = yellow; 180° = green; Mcguire 1992), decreased during ripening, and in the second work, the chlorophyll fluorescence also decreased. Fabi *et al*. (2007) verified ethylene treatment accelerate the chlorophyll degradation, while 1-MCP treatment retarded it. This second event was also verified by Jacomino *et al*. (2002) reinforcing the supposition this event could be regulated by ethylene. As can be seen in **Fig. 2**, the visual ripening analyses of papaya fruit ripening gives an idea of chlorophyll degradation occurred in papayas peel during normal ripening and ethylene or 1-MCP treated fruit.

**Fig. 2** shows that ethylene-treated fruit had their peel color changed faster than fruit let to ripen without treatment. However, 1-MCP-treated fruit had their peel color changed slower than fruit let to ripen without treatment. When the figure is compared with the data showed in Fabi *et al*. (2007), it can be speculated chlorophyll degradation is possible regulated by ethylene action. This fact could be supported by the fruits treated with 1-MCP, which recovered their yellowing color by supposedly synthesis of new ethylene receptors as it has already been demonstrated with peaches (Rasori *et al*. 2002).

## Levels of pro-vitamin A, lycopene and vitamin C

Yellow-fleshed and red-fleshed papaya fruit are a good source of vitamin A derived from some pro-vitamin A compounds. Chandrika *et al*. (2003) observed that a red-fleshed papaya had a higher calculated retinol equivalent than a yellow-fleshed cultivar, but the article does not specify the cultivars used in the experiments, with similar results achieved by Wall (2006). Fabi *et al*. (2007) observed lycopene, a powerful antioxidant used to prevent prostate cancer, which is the major carotenoid of 'Golden' papaya variety and gives the red coloration to the fruit pulp. These authors observed that lycopene, β-cryptoxanthine and β-carotene had their quantities increased during ripening, demonstrating an improvement of the nutritional content. These carotenoids seemed to be already produced by fruits before harvesting; however there are no studies concerning papaya carotenoids accumulation during fruit development. The analysis of this article shows that despite pulp color, which changed from moderate to fully red during ripening (increasing of Δa* value) and seemed to become less yellowing (negative values of Δb*), the authors could not correlate these values with carotenoids quantities. In order to exemplify the redness of 'Golden' papaya pulp, an example of a color-spectrum from blue to red evaluated from the pulp of a ripe fruit can be seen on **Fig. 3**.

Although **Fig. 2** and **Fig. 3** did not show any correlation regarding fruit ripening and pulp color, they can give an idea on how the pulp of a red-fleshed papaya cultivar could be analyzed using different spectra of colors. Ripe papaya fruit exhibits a fully red pulp, (**Figs. 1, 2**), and the physical approach used in **Fig. 3** corroborates this visual fact. Indeed, red-fleshed papayas have more lycopene (red color) than β-carotene or β-cryptoxhantine (yellow color), as it can be seen when the comparison of data exposed by Fabi *et al*. (2007) and the higher intensity 630-680 nm than 530-580 nm of **Fig. 3** is done.

As it happens with the pro-vitamin A content of papaya fruit, one average slice (150 g) provides more than twice of dietary reference intake (DRI) for vitamin C (Franke *et al*. 2004; Wall 2006). Vitamin C content seems not to change during fruit ripening (Souza *et al*. 2008), demonstrating this fundamental vitamin has been synthesized during fruit development. However, carefully with papaya fruit postharvest quality is indispensable to maintain vitamin C contents, since its oxidation caused by air-contact of the pulp would form new compounds without vitamin C properties (Souza *et al*. 2008).

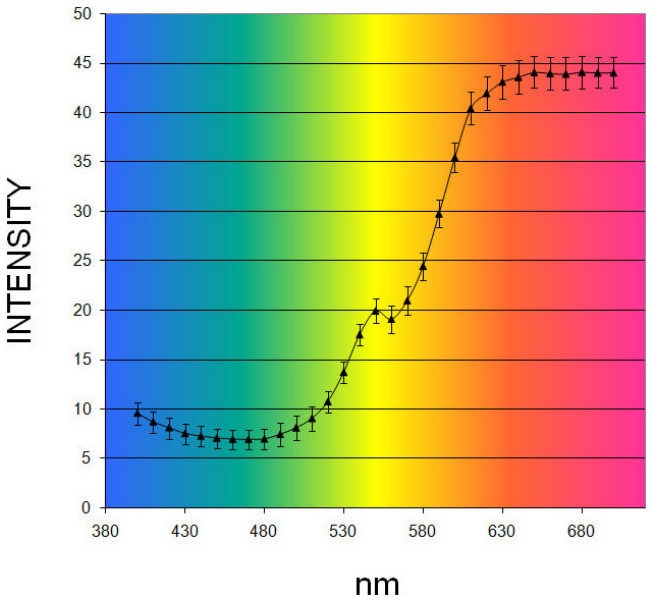


**Fig. 3 Color spectrum analysis of the pulp of papaya fruit cv. 'Golden'.** A slice of a ripe papaya pulp was cut off the fruit and analyzed directly in a HunterLab ColorQuest XE instrument (Hunter Associates Laboratories©) using the same parameters as previously described in Fabi *et al*. (2007).

## Flavor and soluble sugars content

Flavor in papaya could be due to some volatiles compounds produced during ripening .Volatile compounds isolated by a simultaneous distillation/solvent extraction method showed that butanol, 3-methylbutanol, benzyl alcohol and α-terpineol had the highest concentrations in the stage correspondent to full ripeness (Almora *et al*. 2004).

Pulp sweetness is one of the most appreciated characteristics of ripe papayas. The main soluble sugars present in the papaya pulp are sucrose, glucose and fructose (Gomez *et al*. 2002). These sugars give the peculiar sweetness to papaya fruit, and their contents vary during fruit ripening. There is a time-regulated hydrolyzes of the non-reducing sugar sucrose to produce reducing sugars glucose and fructose during ripening (Gomez *et al*. 1999). Both reducing sugars could be used directly to generate energy for all the rapid reactions papaya fruit undertake during ripening. This can be explained by the reducing sugars accumulation in the same day ethylene and $CO_2$ production peak (Gomez *et al*. 1999, 2002; Fabi *et al*. 2007). This accumulation is followed by all ripening-induced changes, showing there is a close relation between glucose and fructose production and energy consumption. Gomez *et al*. (1999) showed sucrose synthesis is somehow still stimulated during papaya ripening. This could happens because as papaya starch content is almost nil, there might be a pathway to avoid harmful accumulation of reducing sugars such as glucose and fructose. There is also a possible incorporation of galactose in this pathway during ripening, a sugar that is probably coming from substantial cell wall degradation, in a synthesis event that has not yet being elucidated (Gomez *et al*. 2002). However, sucrose synthesis rates are lower than sucrose degradation due to increased action of invertases (Gomez *et al*. 1999), maintaining glucose and fructose levels higher during papaya ripening leading to a sweetener pulp.

## Pulp softening

Concerning all ripening-induced characteristics acquired by papaya fruit, pulp softening is one of the most important. This is caused by cell wall disassembling that is achieved mainly by action of glycosidases. This event will enable all the nutrients of the fruit to be more easily released in the intestine of the eating animals, demonstrating a natural developed plant pathway to attract animals for seed dispersal and the consequence of life perpetuation (Giovannoni 2001).

Plant cell walls are mainly composed by cellulose and pectin, being the later one of the most important component of fleshy fruit cell walls. The depolimerization and the consequent solubilization of these compounds reduce cell wall linkage and makes the whole cellular-adhesion system to collapse resulting in pulp softening (Tucker 1993). Pectin is basically composed by homogalacturonans and rhamnogalacturonans type I and II (Carpita and Gibeaut 1993), and during ripening the action of some enzymes would facilitate cell wall disassembling. Significant changes in papaya cell wall pectins have already been associated with pulp softening (Lazan *et al*. 1995; Manrinque and Lajolo 2004; Shiga *et al*. 2009). In papaya, it is believed pulp softening is directly affected by the presence of ethylene, demonstrating that exist a correlation between some enzymes and cell wall solubilization (Brummell and Harpster 2001). Indeed, it has already been demonstrated papaya softening is directly affected by ethylene action (Fabi *et al*. 2007, 2009a, 2009b).

A Malaysian group has already studied the activity of certain glycosidases that acts in the pectin solubilization, such as polygalacturonases, α-galactosidases and pectinesterases (Ali *et al*. 1998; Lazan *et al*. 1995; Ali *et al*. 2004; Lazan *et al*. 2004). Another Hawaiian group studied a gly-

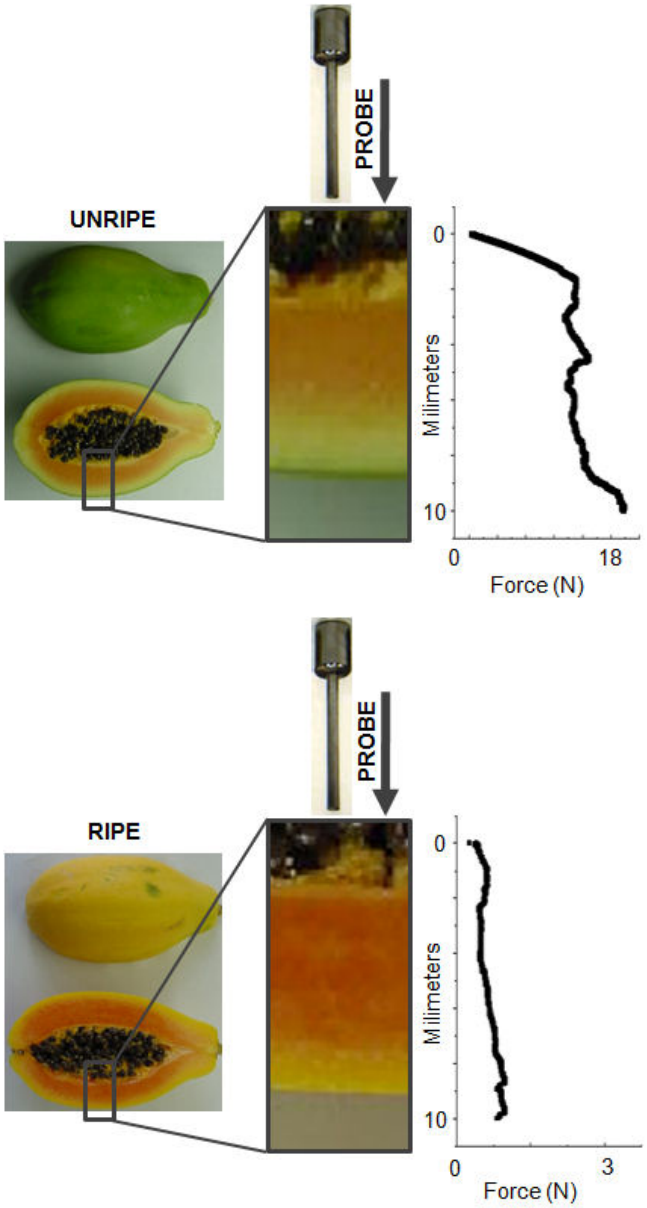


**Fig. 4 Texture analysis of the pulp of papaya fruit cv. 'Golden'.** A slice of a ripe papaya pulp was cut off the fruit in a determined size and analyzed in a texturometer. The parameters of texture analysis were the same as previously described in Fabi *et al*. (2007), despite the probe used changed from 'blade with knife' to a 'penetrating' probe.

cosidase that acts in the cellulose solubilization, an endoxylanase (Chen and Paull 2003). Both groups were able to associate the activity of those enzymes with papaya cell wall depolimerization, but they were not able to point what was the main enzyme that is crucial for papaya pulp softening. In the other hand, Manrinque and Lajolo (2004) and Shiga *et al*. (2009), based on different fractions of papaya cell walls, concluded that only some glycosidases (endopolygalacturonases; endoPG) capable of hydrolyzing the major pectin component (poligalacturonic acid) would give the rapid softening of papaya pulp. In a complementary work, Fabi *et al*. (2009a) isolated an endoPG gene, 13 times up-regulated after ethylene treatment of papaya fruit. This gene was further studied, demonstrating a possible regulation of the papaya endoPG by methyl jasmonic and salicylic acids, gibberellins and heat stress (Fabi *et al.* 2009b).

It is already known papaya fruit softens from inside to outside the pulp as it can be seen in **Fig. 4**. Polygalactoronase activity is higher in the placenta region than the region close to the peel (Chan *et al*. 1981). The beginning of softening is in the placenta, where the seeds are stocked, and the final step of softening occurs near the peel, when the fruit is ready for animal consumption. The main reason for the inside to outside papaya cavity softening is mainly to prevent fungus or insect infestation before ripening maturity.

Therefore, papaya is a very susceptible fruit that undergoes massive transformation during post-harvest ripening, and despite the considerable number of articles published concerning papaya fruit ripening, a well known way to maintain the post-harvest quality is still to be accomplished.

## MANGO

Mango fruit (*Mangifera indica* Linn.) is one of the most important tropical fruit produced around the world, after banana, with more than 150 cultivars. This fruit is known for its acquirement of strong aroma, sweetness taste, and intense peel coloration with a high nutritive value such as high content of vitamin C, β-carotene and minerals (Tharanathan *et al*. 2006; Peroni *et al*. 2008). The amount of organic acids produced during development decreases during ripening while the amount of soluble sugars increases resulting in a sweet pulp (Bernardes-Silva *at al.* 2003; Tharanathan *et al*. 2006; Simão *et al.* 2008).

Mangoes are classified as climacteric fruits, with the ripening period characterized by a series of biochemical changes initiated by the autocatalytic production of ethylene and the consequent increase in respiration. However, peaks for endogenous production and respiration have not been observed during ripening for all cultivars. For a climacteric example, Keitt cultivar presents a short post-harvest life when detached from plant tree. Respiration and ethylene production of this cultivar increase at the onset of ripening followed by a gradual decline (Tharanathan *et al*. 2006; Bernardes-Silva *at al.* 2008).

Many factors, such as fruit maturity at harvest and orchard management practices, are known to affect the development, maturation and the quality of mangoes. They are usually harvested at a physiologically mature stage being the trade limited due to the highly perishable nature of this fruit. Thus, post-harvest technologies are used to protect the fruits against injury during packaging and transport. The technique of storage at low temperature can substantially reduce the rate of many metabolic processes which lead to fruit senescence, deterioration and poor final quality. Lower temperatures temporarily reduce the ripening of fruit maintaining the ethylene concentrations as low as possible (Seymor *et al.* 1993). Other methods, such as controlled or modified atmospheres and 1-MCP treatment, had been applied to guarantee the desirable quality of mangoes.

### Ripening of mangoes

Mango ripening is an event genetically programmed and highly coordinated involving several biochemicals, physiological and organoleptic changes leading to the alterations in color, flavor, texture and taste (Tharanathan *et al*. 2006; Peroni *et al.* 2008). In order to achieve the desirable post-harvest quality, some fruits are left to ripe until the pulp texture gives an edible aspect to the fruit, as discussed above for papaya. In mangoes, the major textural changes that result in pulp softening are due to the action of different enzymes hydrolyzing the abundant starch content and some others polysaccharides, just like cell wall components (Yashoda *et al*. 2007). These coordinated process leads to a consequent increasing of sweetness with a soluble sugar accumulation. During mango development starch reaches 8% of the fresh pulp, although low amount of soluble sugars (glucose, fructose, and sucrose) is detected. However, when ripening takes place the accumulated starch is degraded and accounts for the synthesis and accumulation of soluble sugars, reaching levels as high as 12% of fresh pulp (Bernardes-Silva *et al.* 2003; 2008; Peroni *et al.* 2008). **Table 1** presents the mean values of soluble sugars determined in different mature mango cultivars. The chemical

**Table 1** Soluble sugars content in 5 cultivars of ripened mangoes.

| Cultivars | Total Soluble Sugars (%)* |
|---|---|
| Keitt | 8.00 ± 0.03 |
| Van Dyke | 7.29 ± 2.19 |
| Haden | 12.75 ± 0.27 |
| Tommy Atkins | 11.89 ± 0.09 |
| Palmer | 9.79 ± 1.18 |

*Mean values of at least three replicates per sample. Source: Bernardes-Silva *et al.* (2003) and Simão *et al.* (2008).

composition of mango pulp changes with the location of cultivation, variety, and stage of maturity. Apparently, there is a large dependence of the sugar amount on cultivar and agronomical practices, but there is a lack of studies dealing with some important chemical and biochemical aspects during both fruit development and ripening.

Many enzymes are involved in the process of starch degradation of mangoes. According to Bernardes-Silva *et al.* (2008), the role of α-amylase, β-amylase and isoamylase are essential on starch granule degradation leading to soluble sugar accumulation. They detected that α-amylase activity increased during fruit development parallel to the starch content, achieving high values at the physiological maturity. On the other hand, β-amylase activity, which was weakly detected during fruit development, presented an increase during ripening clearly correlated to starch degradation. The results of isoamylase showed an increase in the enzyme activity detected when physiological maturity was achieved. The second increase in the activity was observed at the onset of starch degradation. Different from other cultivars, Keitt mangoes present a clear pattern of starch degradation during ripening. These results had allowed the authors to conclude that the degradation of starch was correlated to sucrose accumulation in the pulp of ripe fruits. Depending on the starch botanical source, different pathways or many enzymes can be involved in granule degradation. α-amylase and β-amylase are the main enzymes capable of hydrolyzing the starch granule surface, which would be the first step for its degradation.

Data presented by Peroni *et al.* (2008) confirm these results. The specific activity assay for α- and β-amylases coupled to the surface of granules increased with the onset of starch degradation. Activity assays showed that the enzymes were already bound to the granule surface in the following day after harvest. Similar results were found when starch granules were submitted to immunofluorescence microscopy using α- and β-amylases antiserum. Preliminary immunolocalization results confirmed the presence of both enzymes linked in the surface of the granules isolated from mangoes at mature stage (**Fig. 5**).

There are some considerations to make regarding mango nutritional composition and ripe feature. During mango development, the rapid growth is characterized by an increase in alcohol-insoluble solids. Tharanathan *et al.* (2006) showed the lipid content in pulp of several cultivars ranged between 0.26 and 0.67% at harvest, being the major component a triglyceride with mono- and di-glycerides as minor components. The fruit flavor acquired during mango ripening is due to ester- and carbonyl-like molecules. Volatile compounds produced during ripening are mainly originated from acetyl CoA, which is produced by several metabolic pathways. Monoterpene and sesquiterpene hydrocarbons are the major volatile components representing 70-/90% of total volatiles in all mango cultivars. Vitamins C, K, B1, B2 and folic acid were detected in different cultivars of ripe mangoes (Tharanathan *et al.* 2006). The content of total carotenoids increases gradually as the fruits approached maturity and ripening. Sixteen different carotenoids were identified in mangoes, of which β-carotene was found to account for 60%. Some of the phenolic compounds identified in mango were gallic acid, indigallic acid, gallotannin, quercetin, isoquercetin, mangiferin, and ellagic acid (Lalel *et al.* 2003; Tharanathan *et al.* 2006).

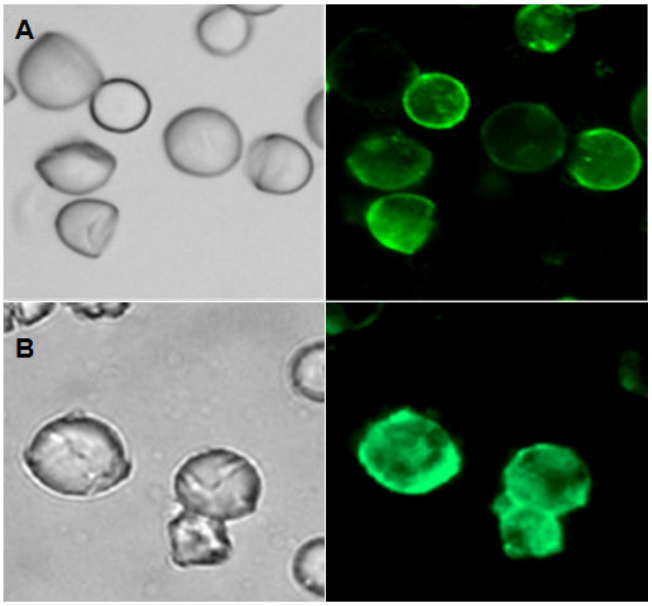


**Fig. 5 Optical analysis of a mango starch granule.** The optical microscopy (gray images) and the respective immunofluorescence microscopy (green images) on mango starch granule surface was done using primary antibodies against (**A**) α-amylase and (**B**) β-amylase. The secondary antibody used was FITC-conjugated antirabbit IgG.

## Post-harvest of mangoes

As explained above, mango ripening is also affected by an extended pulp softening that could decrease fruit quality and shelf life. In this regard, right procedures for handling, packaging, transporting and storage are essential for an acceptable final quality. Since mangoes are susceptible to a number of biotic and abiotic stresses that leads to a rapid deterioration (González-Aguilar *et al.* 2007), several methods of post-harvest handling have been used to extend the shelf life and to reduce losses. These methods (both physical and chemical ones) include cold storage, controlled and/or modified atmospheric storage, irradiation, polyethylene film packaging and the use of 1-MCP (Tharanathan *et al.* 2006; González-Aguilar *et al.* 2007).

Ethylene can profoundly affect quality of harvested mangoes. Most of the commercial strategies to maintain an expanded shelf-life are based on avoiding exposure to ethylene or minimizing the ethylene production (Seymor *et al.* 1993; Watkins 2006). In this way, inhibition of ethylene perception using 1-MCP has been used as a tool to control the ripening of fruits, and for mangoes it is not different. Alves *et al.* (2004) verified the effects of different concentration of 1-MCP on ripening of 'Tommy Atkins' mangoes. They observed a delay on the climacteric peak, reduced respiration rate, reduced fresh weight loss, a firmer pulp and higher total titratable acidity as compared to non-treated fruits. The fruits harvested in more advanced maturity stages were less susceptible to 1-MCP application. Silva *et al.* (2004) evaluated the influence of 1-MCP on postharvest conservation of exotic mango 'Rosa', 'Jasmim', and 'Espada' harvested as mature-green and pre-climacteric maturity stages. The results showed that 1-MCP was capable to delay ethylene-induced ripening processes for all mango cultivars tested. Mature-green 'Rosa' and 'Espada' mangoes treated with 1-MCP presented lower weight losses and better external appearance. For 'Jasmim' cultivar, by the end of evaluation period, better external appearance was more evident for pre-climacteric fruits.

In fresh-cut fruits the ethylene has also an undesirable effect on the quality of final product. Alternatives to extend post-cutting life of fresh-cut mangoes are the use of ethylene absorbent, anti-browning agents, low temperature and 1-MCP. Vilas-Boas and Kader (2007) verified the effects of 1-MCP applied before or after processing of fresh-

cut mangoes and evaluated the quality of the slices. They observed a delayed of softening and browning when 1-MCP was applied directly on fresh-cut mango.

Other ways to control ripening changes can be achieved by the control of some physical attributes. Despite the use of low temperatures or modified atmosphere during storage can be classified as the main controlled physical parameters, it is not the best method for controlling mangoes ripening. This method is widely used due to its convenience and cheap cost, whereas the use of modified atmosphere may prevent the mold attack and keep the fruit quality for a longer period (Lee and Kader 2000).

Low temperatures are known to decrease the metabolic rates including ethylene production, and can also minimize the microorganism growth leading to a higher storage period. The use of low temperature, however, might be thoroughly investigated to prevent the negative effects that may occur with too low temperature, as cellular collapse may occur as well the appearance of physiological disturbances (Wills *et al*. 1998). In tropical fruit temperatures too low may lead to tissue injuries that turn fruits undesirable to consume (Awad 1993). The temperature to be used must be closely observed in order to avoid chilling injury (CI), which occurs by the physical modifications of the lipid structure. Under low temperatures, the lipid conformation varies from the liquid-crystalline to a solid structure, leading to cell injuries (Lee and Kader 2000). These variations may be determined by the rate of saturated and unsaturated fat acids, but there are other mechanisms that lead to chilling injuries, since there are fruits with the same lipid composition which show different sensitivity to low temperature. During fruit ripening, there is a natural decrease of membrane functions due to its lipid composition and changes on its viscosity and fluidity. In this way, temperatures below 10$^{o}$C can cause a drastic effect on cellular structures leading to probable loss of mitochondrial fluids and membrane disorganization. According to the resistance to cold storage, fruits may be classified as cold tolerant (eg. plum, strawberry, peach, grape) which tolerate temperatures between 0 and 4°C, moderate cold tolerant (e.g. guava, orange, melon and papaya) which tolerate temperatures between 4 and 8°C, and sensitive fruits (e.g. banana, star fruit and mango) which only tolerate temperatures above 8°C (Chitarra and Chitarra 1990).

Mangoes storage in temperatures below 10-13°C presented signs of CI. The most apparent symptoms are dark, discoloration and pitting or sunken lesions on the peel (Lederman *et al.* 1997). It was reported that in these fruits the soluble sugars content was significantly reduced and the starch breakdown was affected with a decrease in amylases activity. Phakawatmongkol *et al.* (2004) verified the development of CI symptoms in six mango cultivars during and after low temperature storage. The more common result was the presence of grayish scald-like spots on the skin becoming subsequently dark brown.

A new alternative for the increasing of mangoes shelf-life is the ultraviolet irradiation (UV-C) treatment. This treatment based on UV-C irradiation from 190 to 280 nm wavelength is able to reduce the fruit ripening and activate the natural plant defense response. González-Aguilar *et al.* (2007) reported that the exposition to low UV-C doses delayed ripening and senescence of apples, tomatoes, oranges, table grapes, mangoes and peaches. They observed that the treatment maintained better the overall appearance, lower decay percentage and increased shelf life of 'Haden' mangoes, becoming a good alternative to other postharvest techniques.

Mangoes are very vulnerable to postharvest losses due to its facility of injuries, including handling and temperature. The numerous studies on the physiology and biochemistry of mangoes could give a clue on how an appropriate technology would enhance the marketable life of this fruit.

## GUAVA

Guava (*Psidium guajava* L.) is a fruit from the myrtle family (Myrtaceae), which contains about 100 species mostly found in tropical countries. The fruits emanate a strong, sweet, musky odor when ripe and they are generally round, ovoid, or pear-shaped with 4 or 5 protruding floral remnants (sepals) at the top. The fruit is a berry consisting of a fleshy pericarp and seed cavity with fleshy pulp and numerous small seeds. The fruit is commonly consumed fresh (Jiménez-Escrig *et al*. 2001). Formerly, round and pear-shaped guavas were considered separate species (*P. pomiferum* L. and *P. pyriferum* L.) but they are now recognized as simple variations. Despite the shape characterization of guava cultivars around the globe, most of them can be classified as red-fleshed (red pulp) or white-fleshed (white pulp). Guavas are rich in vitamins A and C, and if the seeds are eaten too, omega-3 and omega-6 polyunsaturated fatty acids and especially high levels of dietary fiber can be achieved. Guavas contain both carotenoids and polyphenols, the major classes of antioxidant pigments, giving them relatively high dietary antioxidant value among plant foods (Gutiérrez *et al*. 2008). As these pigments produce the fruits color, red-fleshed guavas have more potential value as antioxidants sources than white-fleshed ones (Hashimoto *et al*. 2005).

Ripening of guavas is an important process that involves changes in peel and pulp color, flavor and texture, making the fruit to achieve an edible final quality. The main physiological, biochemical, and structural changes can be resumed as the degradation of starch or other storage polysaccharides, the production of sugars, the synthesis of pigments and volatile compounds, and the partial solubilization of cell wall (Jain *et al*. 2003). On the final phase of maturation, once the fruit reached its maximum size and it is ready to be harvested, the fruit ripening takes place and the transformations mentioned above occur (Wills *et al*. 1998). As most of these changes take place very quickly, fully ripe guavas bruise easily and are highly perishable. This stage of ripening is considered the transition from growth to senescence stages (Bassetto *et al*. 2005). Therefore, guavas handling and post-harvest techniques are made very difficult to maintain the final quality of the fruit or to increase their shelf-life; then many efforts are being done to monitor these changes, aimed to reduce losses on fruit production.

### Ripening of guavas

Although some authors described guava as a non climateric fruit, some studies showed an increase on ethylene and carbon dioxide ($CO_2$) production during ripening, indicating that there is a climateric behavior on guava ripening (Jain *et al*. 2003; Bassetto *et al*. 2005). A normal ripening curve can be seen on **Fig. 6** for ethylene and $CO_2$ production of a red-fleshed guava cultivar, with concomitant flesh softening. Analyzing these results, it can be speculated the climacteric of these fruits could be regulated by ethylene triggering.

The synthesis of volatile compounds is a remarkable change occurred during guava ripening. The flavor of ripe white guavas is mainly given by the presence of esters, such as *Z*-3-hexenyl acetate and *E*-3-hexenyl acetate and sesquiterpenes caryophyllene, α-humulene and β-bisabollene, while in unripe fruit, the major compounds are aldehydes just like (*E*)-2-hexenal and (*Z*)-3-hexenal (Soares *et al*. 2007).

In climacteric fruits, changes associated to ripening take place in a relatively short period of time (Seymour et *al.*, 1993). The increase on ethylene concentration seems to trigger ripening changes, and if the gas is externally applied to the fruit, ripening characteristics can be induced in some climacteric fruit, as it was discussed above. The results from our laboratory shows guava fruit treated with ethylene leads to faster loss of firmness during storage, late ethylene production peak and a higher $CO_2$ production, as compared to control fruits (**Fig. 6**). Reyes and Paull (1995) showed

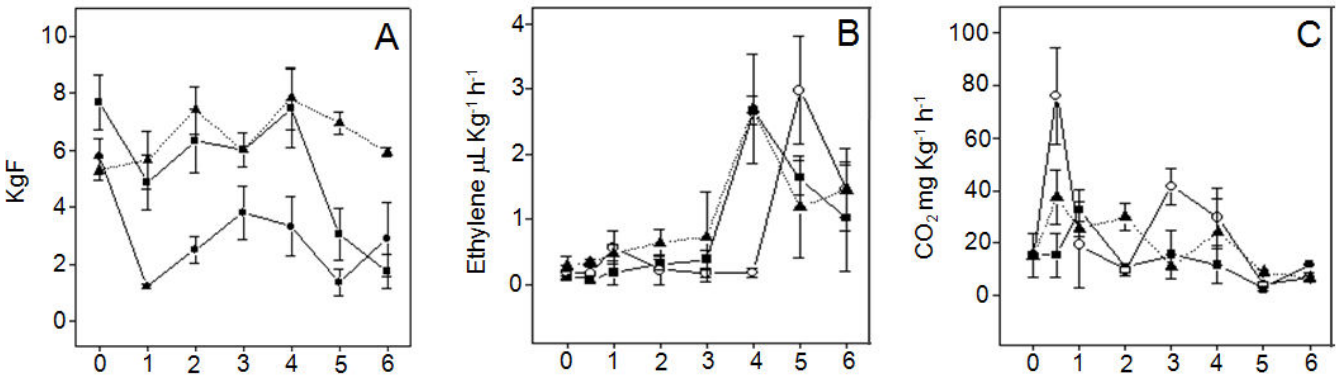


**Fig. 6 Ripening analysis of red-fleshed guava fruit**. Red-fleshed guava fruit were let to ripe normally or after 24 hours of ethylene or 1-MCP treatment just after harvesting (Day 0). The following ripening parameters were compared between control guava fruits (-■-), 100 ppm ethylene-treated fruits (-○-) and 100 ppb 1-MCP-treated fruits (-▲-), as described in Fabi *et al*. (2007). **A**: pulp firmness measured with a texturometer (KgF); **B**: amount of ethylene produced by fruits measured by GC-FID (ethylene µL $Kg^{-1}$ $h^{-1}$); **C**: amount of $CO_2$ produced measured by GC-TCD ($CO_2$ mg $Kg^{-1}$ $h^{-1}$). Error bars indicate *sd* of the mean ($n = 5$).

that the treatment of guavas with ethylene (100 ppm) for 24 h was sufficient to induce fruit skin yellowing in immature-green guava, although immature-green fruit developed 'gummy' pulp when ripened with increased fruit juice viscosity, making the juice probably less acceptable for commercial processing. The same treatment when used on mature-green and quarter-yellow fruit resulted in a minimal reduction in ripening period compared to non-treated fruit. Nevertheless, as it can be seen in **Fig. 6**, 24 hours of 1-MCP treatment did not affect significantly the firmness during early ripening although on final period it delayed considerably the loss of firmness on fruits. Also the respiration rate was lower in 1-MCP treated fruits, as compared to control ones. Bassetto *et al*. (2005) and Singh and Pal (2008b) achieved almost the same results when compare to our laboratory results. The authors used higher concentrations of 1-MCP (300 ppb and 600 ppb) against the 100 ppb concentration used by our group. Most of the physiological and biochemical changes during storage and ripening were affected by 1-MCP treatment in an extensive way, leading to a significantly suppression of ethylene production, respiratory rates and pulp softening. However, a concentration of 900 ppb of 1-MCP did not allow the fruits to ripe normally, demonstrating there are a minimum (100 ppb) and a maximum (900 ppb) limit of 1-MCP to achieve some specific conditions, specially the postponing of guava ripening.

## Post-harvest of guavas

Reyes and Paull (1995) showed that mature-green guava fruit stored at 20°C developed full yellow color within seven days with less disease incidence than fruit that was quarter- and half-yellow at the beginning of storage. Storage temperature of 15°C provided the optimum condition to hold guava prior to processing since it allowed gradual ripening of mature-green fruit while delaying the deterioration of quarter-yellow and half-yellow fruit. Analyzing some results from our laboratory, the 7 days storage of white guavas under 16 and 6°C led to slight modifications on ascorbic acid metabolism when compared to control group (22°C storage), specially in the first day after storage, observing a non significant change on final levels (**Fig. 7**). The dehydroascorbic acid (DHA) contents were also slightly influenced by low temperature just in the second day after storage, being lower in the 16°C and in the 6°C stored samples as compared to the control (22°C). Therefore, the low temperature storage seemed to not stress significantly guava tissues, since there was no increase in the peroxidase detoxification system visualized by ascorbic acid and DHA estimated quantities.

Controlled atmospheres storage can extend the shelf-life of some tropical and subtropical fruits by reducing some metabolite-degrading processes during ripening (Yahia 1998). A relevant problem regarding controlling the atmosphere is the accumulation of some fermentative metabolites that will lead to the development of undesirable off-flavors reducing the commercial value of the fruit (Beaudry 1999). Kader (2003) recommended 2–5% $O_2$ and 0–1% $CO_2$ for controlled atmosphere storage of guava at 5–15°C. The short-term exposure of guava fruit to high $CO_2$ levels did not influence the respiration rates, but reduced ethylene production during ripening (Pal and Buescher, 1993). In the

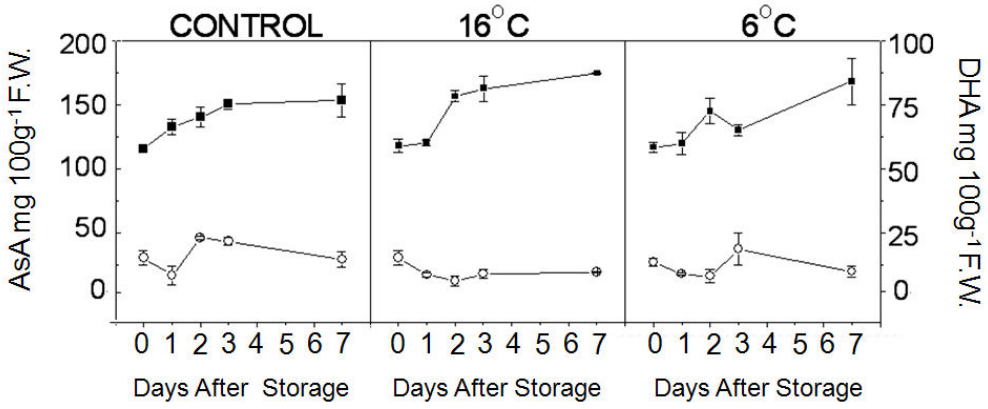


**Fig. 7 Ascorbic acid and dehydroascorbic acid (DHA) variation contents during cold storage of white pulp guavas.** The cold storage, at least for 16°C and 6°C, seemed not to influence both components content when compared to control group (22°C storage). Amount of both acids measured by HPLC as described by Genovese *et al*. (2008) (acid mg $100g^{-1}$ F.W.). Ascorbic acid (AsA, -■-); dehydroascorbic acid (DHA, -○-). Error bars indicate *SD* of the mean ($n = 5$).

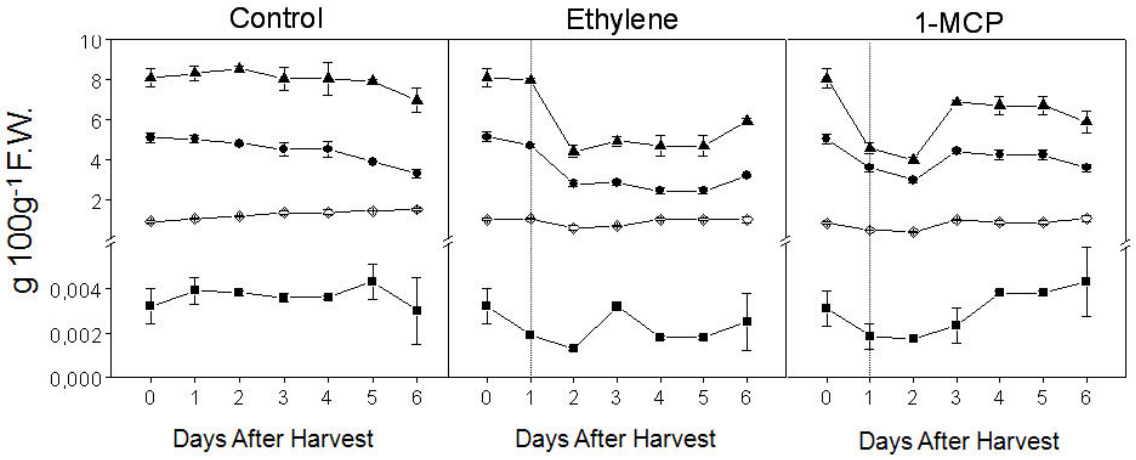


**Fig. 8 Soluble sugars content measured during ripening of untreated and treated white-fleshed guava fruits.** Guava fruits were exposed to 100 µL $L^{-1}$ of ethylene and 100 nL $L^{-1}$ of 1-MCP, and the content of galactose (-■-), glucose (-●-), fructose (-▲-) and sucrose (-◊-) were measured by HPLC as previously described in Fabi *et al*. (2007). Sugars were measured during untreated (Control) and gas-treated (Ethylene and 1-MCP) fruit ripening. Both vertical lines in Ethylene and 1-MCP columns show the end of the gas treatments. Error bars indicate *SD* of the mean ($n$ = 3).

same way, guavas treated with 10% $O_2$ + 5% $CO_2$ for 24 h (just before the storage in air at 4°C during 2 weeks) delayed color development and reduced CI when compared to fruit held in air (Benito-Bautista and Mercado-Silva 1997). All these data are supported by a more recently study, where Singh and Pal (2008a) described that fruits stored in low $O_2$ (≤5 kPa) atmospheres, when compared to those stored in 8 or 10 kPa $O_2$ levels, had their respiratory and ethylene peaks delayed and suppressed during ripening, while high $CO_2$ (>5 kPa) exposure had some negative effects, such as the reduction of ascorbic acid levels. The changes in soluble solids content, titratable acidity, ascorbic acid, and total phenols were retarded by controlled atmosphere, being all these characteristics dependent upon cultivar and atmosphere composition.

Thus, the physical controlling of some climacteric fruit ripening can be achieved by both atmosphere temperature and gas composition. In guavas, some physic-chemical attributes acquired during ripening can be delayed or suppressed using these two techniques separately or combined. However, the optimal conditions are not well established, part because of the number of these kinds of studies and part because of the wide range of guava cultivars.

Guava fruits have a considerable quantity of starch reaching 5% of fresh weight (Jain *et al*. 2001). The pleasant sweetness of guava pulp could be due to starch breakdown during ripening with a possible concomitant release of reducing and non-reducing sugars (Jain *et al*. 2001). However, some results achieved by our laboratory point that pulp sweetness acquired during guava ripening could be due to the reduction of pulp firmness instead, which confers the "melting sensation" of the pulp and increasing the sugar availability related to the sweet post-sensation. Our laboratory conclusion is based on the HPLC sugar content measurement showed in **Fig. 8**. Using the same fruits which had some physical-chemical parameters exposed on **Fig. 6**, it can be seen a decrease in glucose and fructose content during control fruit ripening, what it was not expected. Both ethylene and 1-MCP treatments induced the decreasing of both glucose and fructose, with a recovering after two days of treatment. Meanwhile, sucrose content has a marginal increase and did not reflect a possible starch breakdown. This might mean that the increased reducing and non-reducing sugars content described by Jain *et al*. (2001) is released mainly by cell wall solubilization and other reactions, since there is no evident accumulation of sugars derived from starch breakdown (sucrose, glucose and fructose). It can be speculated that there is a possible *de novo* synthesis of hemicellulose, cellulose and lignin in over-ripe guavas and a degradation of pectin during ripening (Jain *et al*. 2001). Although the results discussed above, it is not reasonable to point which event is the main one regarding the sweeter pulp acquired during guava ripening.

Guava is a fruit that during ripening acquires some edible characteristics very appreciated all round the world. However, there are few articles concerning the control of guava ripening by physical-chemical techniques, such as cold storage or 1-MCP treatment. In this way, it is not possible to presume what would be the best choice to increase guava shelf-life without compromising the final quality of the fruit.

## CONCLUDING REMARKS

Papaya, mango and guava fruits have some characteristics changed during normal ripening and in most of the cases exhibit some climacteric pattern variations either in $CO_2$ or ethylene production. These tropical fruits respond within different intensities to the phenomenon of ripening affecting the nutritional content and quality. Papayas undergo intensive pulp softening, while mangoes breakdown starch making the pulp sweeter and guavas have an increment on vitamin C content. All these changes are directly influenced by enzymes up-regulated during ripening. In this way, the ripening process is fundamental to the development of nutritional and quality attributes and deep understating of this event could lead to postharvest improvement of these tropical fruits.

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