Improvement of the antioxidant status of tropical fruits as a secondary response to some postharvest treatments

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Tropical fruits production, trade and consumption have increased significantly due to their attractive sensory and nutritional properties; nevertheless, their highly perishable nature limits their postharvest life. Postharvest treatments have been used to preserve quality of fresh produce and have been focused mainly on preserving freshness and avoid microbial growth. However, an improvement on the antioxidant system as a secondary response under certain adverse environmental and stress conditions has been observed, including some types of stress used as postharvest treatments. This review focuses on analyzing and proposing some possible mechanisms induced by postharvest treatments affecting the antioxidant status of treated tropical fruits.

Introduction

The production of tropical fruits has increased in the past few years due to their attractive sensorial properties and because they supply an optimal mixture of antioxidants. Tropical fruits contain vitamins, such as C and E, polyphenols and carotenoids, that impart health benefits beyond basic nutrition (Yahia, 2010). Among tropical fruits, banana, mango, pineapple, papaya and guava are the most popular due to their characteristic taste and nutritional value (Gonzalez-Aguilar et al., 2008).

Tropical fruits are very susceptible to qualitative and quantitative deterioration and losses, including sensorial, microbial and nutritional. Major causes of losses are attributed to fungal decay, chilling injury and rapid maturation that enhance senescence process (Chan & Tian, 2006). Several postharvest treatments have been developed to preserve the quality of fresh produce, including ultraviolet light, controlled and modified atmospheres, edible coatings, heat treatments, and natural compounds, among others. Most postharvest treatments involve the alteration of the natural conditions of the fruit in order to prolong its postharvest life. For example, high O2 atmospheres and irradiation cause damage to some vital molecules of food deteriorative microorganisms, in addition of altering some biochemical processes in the fruit (Charles, Tano, Asselin, & Arul, 2009); heat treatments affect a wide range of fruit ripening processes such as ethylene synthesis, respiration, softening and cell-wall metabolism (Zhang, Nakano, & Maezawa, 2009). It has been shown that as a secondary response, some postharvest treatments could induce some mechanisms that affect the metabolic activity of the treated produce, such as the triggering of the antioxidant mechanism of the fruit.

The activation of the antioxidant system as a response to postharvest stress can result in improving the antioxidant status of tropical fruits. The mechanisms by which postharvest treatments induce these types of responses have not been clearly elucidated. There are evidences that permit us to hypothesize the possible mechanisms involved in these processes. The present review analyzes and proposes the possible mechanisms induced by postharvest treatments affecting the antioxidant status of the treated tropical fruits.
Conventional and emerging techniques to extend the postharvest life of fresh-tropical fruits

Fruits and vegetables are living tissues subjected to quality changes after harvesting. There are three important aspects that confer the quality of fresh produce: 1) sensorial quality, that includes aroma, firmness, colour; 2) safety, including pathogens and deteriorative microorganisms; and 3) nutritional value, that includes content and bioavailability of bioactive compounds. Major research has been done focusing on the effect of postharvest treatments on preserving sensorial quality and assuring safety of fresh-tropical fruits. However, reports on the changes of bioactive compounds after postharvest treatments of tropical fruits are scarce.

Ultraviolet light (UV)

UV-C irradiation (240 nm–280 nm) can be applied at lethal and sub-lethal doses. The detrimental effect of UV-C includes tissue structural damage, changes in cytomorphology and water permeability of inner epidermal cells (Lichtscheidl-Schultz, 1985). Nevertheless, low doses of UV-C irradiation stimulate beneficial reactions in biological organs, a phenomenon known as hormesis (Shama, 2007). It has been reported that hormetic doses of UV-C can prolong the postharvest life and maintain the quality of tropical fruits. These effects include delay of senescence process and fruit ripening (Gonzalez-Aguilar, Zavaleta-Gatica, & Tiznado-Hernandez, 2007), induction of natural defence and elicitors against fungi and bacteria (Alothman, Bhat, & Karim, 2009a).

Resistance to infection by pathogen is correlated with the induction of plant defence mechanism (Gonzalez-Aguilar, Villegas-Ochoa, Martinez-Tellez, Gardea, & Ayala-Zavala, 2007) and DNA damage (Charles et al., 2009). This is manifested through the stimulation of anti-fungal chemical species such as phytoalexins (scoparone and reserveratrol), flavonoids, and degrading fungal cell-wall enzymes (chitinases, glucanases) (El-Ghaouth, Wilson, & Wisniewski, 1998). The induction of plant defence system can also trigger the accumulation of these compounds and other phytochemicals such as carotenoids and vitamin C which exhibit antioxidant potential, improving the nutritional status of the fruit (Alothman et al., 2009a; Alothman, Bhat, & Karim, 2009b; Gonzalez-Aguilar, Villegas-Ochoa, et al., 2007; Gonzalez-Aguilar, Zavaleta-Gatica, et al., 2007).

Heat treatments (HTs)

Extensive commercial application of HTs has emerged over the past two decades and has been used for disinfections and disinfection of various tropical fruits, such as mango, papaya and citrus fruits (Jacobi, MacRae, & Hetherington, 2001). Nevertheless, it has been observed that a wide range of fruit ripening processes are affected by HT, such as control of ripening, fruit softening, pigment metabolism, volatile production, carbohydrate metabolism and disease development (Jacobi et al., 2001; Talcott, Moore, Lounds-Singleton, & Percival, 2006; Zhang et al., 2009). These effects depend on the type of HT applied and duration of fruit exposure.

Heat tolerance of different fruits depends on species, genotype, stage of fruit maturity, type and severity of HT applied, and whether postharvest conditioning treatments have been given before an HT (Jacobi et al., 2001). Several studies have related heat tolerance with the increase of heat shock proteins (HSPs), antioxidant enzymes and phytochemicals such as carotenoids and phenolic compounds. Ghasemnezhad, Marsh, Shilton, Babalar, and Woolf (2008) found an increase in superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) activities after hot water treatments in mandarins. Talcott et al. (2006) found an increase in polyphenols and carotenoids and better antioxidant capacity in hot water treated compared with untreated mangoes. Similarly Djoua, Charles, Lopez-Lauri, Filgueiras, and Coudret (2009) who found an increase in total carotenoids and vitamin C content in mango fruit. These results suggest that HTs prolong postharvest life of some fruits and promote an increase of bioactive compounds. However, the potential effects of HTs on tropical fruits still need to be investigated in more details with regard to increasing the antioxidant status.

Natural compounds

Several natural compounds exhibit positive effects to conserve fruit quality. Amongst these the most studied are volatile compounds (e.g. acetaldehyde, benzaldehyde, hexanal), acetic acid, jasmonates, glucosinolates, chitosan, active principles of some plants, and some plant extracts, among others (Tripathi & Dubey, 2004). The ability of these compounds to control microorganisms was the major reason behind their use as food additives to maintain quality of whole fresh and fresh-cut tropical fruits (Tripathi & Dubey, 2004), and are generally recognized as safe (GRAS) (Smith et al., 2005).

Volatile compounds, such as (E) 2-hexanal, are strongly antifungal in nature and their activity has been reported against Botrytis cinerea inhibiting hyphal growth (Utto, Mawson, & Bronlund, 2008). Compounds released by plant tissue through lipoxygenase (LOX) pathway such as six carbon aldehydes have been found to inhibit hyphal growth of Alternaria alternata and B. cinerea. However, some of these compounds show potential for inducing fruit antioxidant metabolism.

Jasmonates play an important role as signal molecules in plant defence responses against pathogen attack, induce the synthesis of antioxidants such as vitamin C, phenolic compounds and increase the activity of enzymatic antioxidant system (Chanjirakul, Wang, Wang, & Siriphanich, 2006). Gonzalez-Aguilar, Tiznado-Hernandez, Zavaleta-Gatica, and Martinez-Tellez (2004) found that methyl jasmonate (MJ at $10^{-5}$ and $10^{-4}$ M) retarded chilling injury in guava fruit after 5, 10, and 15 days at 5 °C + 2 days at 25 °C,
maintained better appearance, and increased the activity of phenylalanine ammonia-lyase (PAL) and LOX enzymes.

Controlled/modified atmospheres (CA/MA) and edible coatings

The effects of CA and MA technologies are well documented to extend the postharvest life of fruits including those of tropical origin (Yahia, 2009). These effects include reduction of the respiration rate, inhibition of ethylene production and action, retardation of ripening, and maintenance of nutritional and sensory quality (Singh & Pal, 2008; Yahia, 2009). These techniques alter the O2, CO2, and/or C2H4 concentrations in the atmosphere surrounding the commodity to produce an atmosphere composition different from that of normal air.

The positive effects of CA and MA depend on several factors such as type of fruit and cultivar, concentrations of O2, CO2 and others gases in the atmosphere, temperature and duration of storage (Pesis, 2005; Singh & Pal, 2008). Very low levels of O2 and high CO2 favour fermentative processes which lead to ethanol and acetaldehyde production and consequent off-flavour development (Yahia, 2009). In tropical fruits, it has been reported that short-term exposure to O2 levels below 1% and/or exposure to CO2 levels above 12% can reduce incidence and severity of physiological disorders (such as chilling injury), pathogens and insects (Yahia & Singh, 2009). However, concentrations lower than 2% of O2 may result in the development of off-flavours and off-odours (Yahia, 2009). However, there has been little research on the effect of CA/MA on the antioxidant status of fruits. Montero-Calderon, Rojas-Grau, Aguilo-Aguayo, Soliva-Fortuny, and Martin-Belloso (2010) studied the effect of low O2 (12% O2 in combination with 1% CO2) and high O2 (38% O2) on fresh-cut pineapple fruit and found an increment of antioxidant capacity, total phenolics, volatile compounds and vitamin C content in low O2 compared with high O2. These results suggest that optimal conditions of CA/MA can improve the nutritional value of tropical fruits.

Edible coatings can provide additional protection and can be used alone or in combination with CA/MA. Appropriate edible coating formulations may reduce gas exchange rates and water loss, as well as representing an excellent way of incorporating additives to control reactions that are detrimental to quality. Olivas and Barbosa-Canovas (2008) described the different edible coatings used to preserve fruits and vegetables.

Some edible coatings, such as chitosan, have antibrowning characteristics and can maintain tissue firmness and reduce microbial decay of harvested fruits for extended periods. Gonzalez-Aguilar et al. (2009) found that chitosan coating is effective in the preservation of fresh-cut papaya. Jitareerat, Paumchai, Kanlayanarat, and Sangchote (2007) used chitosan to prolong the postharvest life of mangoes and found an increase in total carotenoids and vitamin C content. Chitosan has the potential for inducing defence-related enzymes such as polyphenol oxidase, POD, and PAL, but the possible mechanism of action is little understood.

The use of CA/MA and edible coatings for extending the shelf-life of fresh fruits, including those of tropical origin, is widely recognized, however, more research is still needed on the potential of these technologies to improve the nutrimental quality of fruits, especially their antioxidant status.

Collateral effects of postharvest treatments increasing bioactive compounds of fresh-tropical fruits

Plants have evolved elaborate defence mechanisms for protection against attack of pathogens such as fungi, bacteria and viruses, and also against adverse environmental conditions. One of the suggested mechanisms involves the production of reactive oxygen species (ROS) such as singlet oxygen (1O2), superoxide (O2−), hydrogen peroxide (H2O2) and hydroxyl radical (·OH) (Campos, Carrascal, Coca, Abian, & San Segundo, 2004). Nevertheless, at high concentrations, ROS can have detrimental effects disrupting the normal metabolism by oxidizing nucleic acids, proteins, lipids, or carbohydrates, affecting the integrity of cell membranes and inactivating key cellular functions (Campos et al., 2004).

To prevent injuries, plant cell has developed mechanisms involving some secondary metabolic compounds (flavonoids, lignans, carotenoids, ascorbate, glutathione, among others) and some enzymes (SOD, CAT, POD, ascorbate peroxidase, glutathione reductase, monodehydroascorbate reductase and dehydroascorbate reductase) that convert ROS into less-toxic products. In this regard, ROS are considered as cellular requirement, because they are involved in signalling pathways for the production of antioxidant molecules. Therefore, the importance of cellular equilibrium between the antioxidant system and the levels of ROS must be highlighted.

Postharvest stress-type treatments have been developed to preserve fruits (Fig. 1). The stress can activate some enzymatic and/or non-enzymatic antioxidant systems of the fresh produce, therefore contributing to an adaptation process to stressful conditions and subsequently the maintenance of fruit quality and better antioxidant potential (Lim, Lim, & Tee, 2007).

Effects of UV doses on antioxidants

In recent years, the effect of UV-C on the antioxidant status of fruits has received special attention. The effect of UV-C technology on the synthesis of antioxidant compounds and enzymes can vary depending on the hormetic doses, time of exposure and treated fruit. Alothman et al. (2009a, 2009b) found an increase in phenols and flavonoids in guava and banana after 30 min exposure to UV-C light, contrary to the decrement found in pineapple. Erkan, Wang, and Wang (2008) applied a dose of 5 kJ m−2 for 10 min and showed an increase in the activity of
antioxidants' enzymes. Therefore, the accumulation of antioxidant compounds and nutraceutical quality of fruits treated by UV-C irradiation depends on the doses applied.

The pathways by which UV-C irradiation enhance the antioxidant potential are not established yet. It is well known that secondary metabolism is activated together with the enzymatic antioxidant system. When a hormetic dose of UV-C irradiation is absorbed by biological material, it can interact with atoms and molecules, mainly water, producing ROS by the univalent reduction of O$_2$ in a rapid and controlled manner (Fig. 2) (Kovacs & Keresztes, 2002). The primary ROS formed in the cell is O$_2^*$ which triggers a cascade of reactions that results in the formation of a variety of ROS and induction of antioxidant enzymes such as SOD, CAT, POD, monodehydroascorbate reductase (MDAR), glutathione (GSH) and oxidized glutathione (GSSG) maintaining redox homeostasis. A key ROS is H$_2$O$_2$ produced by SOD, is involved in cross-tolerance (resistance to a particular stress that also confers resistance to another form of stress), hormonal activity and gene expression (Nyathi & Baker, 2006). This suggests that H$_2$O$_2$ may be responsible for the improvement of antioxidant status of fruits activating gene expression of enzymes (such as PAL, CHS, stilbene synthase) related with the synthesis and accumulation of secondary metabolites with antioxidant capacity (phenolic acids and flavonoids) (Nyathi & Baker, 2006).

Effects of HT

Several authors suggest that the main response to thermal stress is the synthesis of plant hormones such as abscisic acid (ABA), salicylic acid (SA), sulphosalicylic acid (SSA) and MJ (Wahid, Gelani, Ashraf, & Foolad, 2007), which modulate a numerous gene expressions. Cao, Zheng, Wang, Jin, and Rui (2009) reported MJ treatment decreased chilling injury of loquat fruits. MJ delayed the increases in O$_2^*$ production rate and H$_2$O$_2$ content in loquat fruit. Meanwhile, the MJ-treated fruit exhibited significantly higher activities of SOD, CAT and APX, and lower activity of LOX than control fruit during the storage. These results suggest that the reduction in chilling injury by MJ may be due to enhanced antioxidant enzyme activity and higher unsaturated/saturated fatty acid ratio.

Mango fruit treated with SA showed an increase in the levels of H$_2$O$_2$ and O$_2$ as well as PAL and β-1,3-glucanase activities (Zeng, Cao, & Jiang, 2006). It appears that these events may be involved in the enhancement of disease resistance in mango fruit against Colletotrichum gloeosporioides Penz. In addition, plant hormones act as secondary metabolites inducing intermediates of primary carbon metabolism via phenylpropanoid, shikimate, mevalonate or erythritol pathways (Gonzalez-Aguilar et al., 2004).

Among secondary metabolites, carotenoids and some terpenoids are the main compounds enhanced by HTs.
which are involved in photoprotection in the cell due to energy storage capacity. These molecules interact with membrane lipids providing thermostability of membrane and lowering susceptibility to lipid peroxidation under high temperatures (Penuelas & Munne-Bosch, 2005). Carotenoids also potentially play an important role in protecting the fruit tissue by scavenging ROS. In addition, changes in the oxidative metabolism of fruits have been established by several authors (Fig. 2) (Ghasemnezhad et al., 2008). It has been found that HTs induce an increase in the activity of SOD, CAT and the ascorbate–glutathione cycle. It has been proposed recently that HSPs act synergistically with the above-mentioned enzymes or otherwise they might be related to the induction of these enzymes (Wahid et al., 2007). The production of these enzymes, in addition to the induction of carotenoids, is very important in maintaining and prolonging the postharvest life of fruits preventing oxidative damage in cell tissue acting as antioxidants. These antioxidants can help in controlling oxidative reactions caused by ROS and free radical species in living tissues and the inhibition of lipid peroxidation in foods caused by processing and during storage (Cisneros-Zevallos, 2003).

Natural compounds

Natural compounds have been proved as elicitors to activate the defence system in fruits. Examples of these compounds include some volatiles, chitosan and jasmonates (Fig. 3).

The induced resistance by jasmonates and chitosan protect the fruit from environmental stresses and pathogen attack. The earliest event in plant cells exposed to an elicitor (MJ and chitosan) is its recognition mediated by plasma membrane-localized receptors (Kaku et al., 2006). Elicitor perception activates receptor-coupled effectors such as GTP-binding proteins or protein kinases and phosphatases which further mobilize or generate diverse signalling molecules directly or indirectly such as free calcium, NO₂ and ROS (Faurie, Cluzet, & Merillon, 2009). These inducible mechanisms have led to a broad spectrum of metabolic modifications, such as cell-wall reinforcement, production of antimicrobial metabolites, and enzymes involved in oxidative stress protection, lignifications, and frequently the hypersensitive response (Cao, Zheng, Yang, Wang, and Rui, 2009; Cao, Zheng, Yang, et al., 2009; Gonzalez-Aguilar, Villegas-Ochoa, et al., 2007; Gonzalez-Aguilar, Zavaleta-Gatica, et al., 2007).

Changes in antioxidant capacity and antioxidant enzymatic system in fruits treated with MJ have been reported. Cao, Zheng, Yang, and Rui (2009) observed that MJ increases the content of organic acids, total phenolics, total flavonoids and the activity of SOD, CAT and APX in loquat fruit and the treatment also maintained significantly higher antioxidant activity. Similarly, Gonzalez-Aguilar et al. (2004) showed the activation of defence mechanism in guava fruit by increasing the activity of PAL and LOX enzymes and the total phenolic content, preventing chilling injury. Similarly, Jin, Zheng, Tang, Rui, and Wang (2009)
Fig. 3. Hypothetical model representing the recognition of natural elicitors by plant cell membrane and the induction of signal molecules, secondary metabolites and enzymatic antioxidant system.

Fig. 4. Proposed model representing the controlled anaerobic stress caused by CA/MA atmospheres and edible coatings triggering the synthesis of some antioxidant enzymes and secondary metabolites.
found the same effect in peach and an increase in SOD and PAL activities compared with the control. On the other hand, chitosan induced mRNA accumulation of PAL, induced the activity of POD and enhanced the synthesis of phenolic compounds (Wang & Li, 2006). This suggests that once MJ and chitosan are recognized by the cell membrane, the production of a cascade of signalling molecules is carried out, mainly ROS (Faurie et al., 2009). These molecules activate the expression of genes related to the synthesis of antioxidant enzymes and secondary metabolites increasing their bioactive compounds.

CA/MA and edible coatings

The benefits of CA/MA and edible coatings for tropical fruit and vegetables have been well described recently by Yahia (2009). The application of CA/MA and edible coating in tropical fruit maintains the contents of phenolic compounds and ascobic acid, and may cause an increase in the antioxidant capacity of the fruit (Fig. 4) (Simões, Tudela, Allende, Puschnmann, & Gil, 2009). The accumulation of phenolic compounds may be promoted by PAL activity which is activated under stress conditions. Previous studies showed that low O2 (2.5%) and high CO2 (7%) atmospheres induce a greater production of phenolic compounds during storage of fresh-cut melons, which was related to oxidative stress (Oms-Oliu, Soliva-Fortuny, & Martín-Belloso, 2008). However, the production of volatile compounds such as ethanol and acetaldehyde appears to play an important role in the induction of the antioxidant activity.

Ethanol has been related with the induction of ROS scavenging enzymes such as SOD, POD, CAT, among others (Chanjirakul et al., 2006), while acetaldehyde induces antifungal compounds such as limonene and phytoalexins (Fisher & Phillips, 2008). Therefore, these volatile compounds may also induce the accumulation of antioxidant compounds, inactivating ROS by scavenging enzymes improving antioxidant status of produce and promoting health benefits to consumer.

Future trends

More research on tropical fruits is still needed to obtain data on the overall effect of postharvest treatments on the nutritional value. Investigation on the processes describing the correlation between the physiology, biochemistry and nutraceutical induction by postharvest treatments is needed. Although physiological mechanisms of stress tolerance are relatively well understood, further studies are essential to determine the physiological basis of assimilate fruit phenotypic flexibility which leads to stress tolerance, and the factors that modulate tropical fruit stress response as well as the possible interaction between different stresses and the response of the tissue. Applications of genomics, proteomics and transcriptomics approaches to a better understanding of the molecular basis of fruit response to postharvest stress-type are imperative. Molecular knowledge of response and tolerance mechanisms will pave the way for engineering tropical fruits with optimal postharvest stress-type and could be the basis for increasing the nutritional value, therefore promoting an improvement in the health of consumers.

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