Postharvest Biology and Technology 111 (2016) 132–139

Contents lists available at ScienceDirect

Postharvest Biology and Technology

journal homepage: www.elsevier.com/locate/postharvbio

Increased anthocyanin and flavonoids in mango fruit peel are associated with cold and pathogen resistance

Velu Sivankalyani[a], Oleg Feygenberg[a], Sonia Diskin[a,b], Ben Wright[a], Noam Alkan[a,*]

[a] Department of Postharvest Science of Fresh Fruit, The Volcani Center, Agricultural Research Organization, Bet Dagan 50250, Israel
[b] Robert H Smith, Faculty of Agriculture, Food and Environment, Rehovot, Israel

ARTICLE INFO

Article history:
Received 8 December 2014
Received in revised form 30 July 2015
Accepted 2 August 2015
Available online 27 August 2015

Keywords:
Anthocyanin
Mango
Chilling injury
Decay
Storage
Ripening

ABSTRACT

Mango fruit (*Mangifera Indica* L., cv. Shelly) developing at the exterior of canopy and exposed to sunlight acquires a red peel color on the sun-exposed side compared to the green peel fruit that develop within the canopy. Measurements of the red tissue showed a significant increase in total anthocyanin and flavonoids accumulation but not in chlorophyll. The ripening parameters between red and green mango fruit harvested at the same day from the same orchard, including; TSS, firmness and titratable acidity were similar at harvest, during cold storage and further shelf life. However, fruit with a red side or that were mostly green showed a varied response to biotic and abiotic stresses. After three weeks of cold-storage at 5 °C 'green fruit' showed significantly more lipid peroxidation and developed significantly more chilling injury symptoms, such as black spots and pitting, than the 'red fruit'. Furthermore, 'red fruit' were found to be more resistant to a challenge of *Colletotrichum gloeosporioides* fungal inoculation and showed reduction in general decay incidence. Thus, mango fruit with more red color in their peel correlates to anthocyanin and flavonoids accumulation, and showed increased resistance to chilling and pathogens. The results point to new agro-technological approaches to extend shelf life and quality in mango.

1. Introduction

Food waste from the market to the consumer is an issue of growing importance in developed economies as it depletes the environment of natural resources (http://ec.europa.eu/food/safety/food_waste/index_en.htm). Postharvest losses of fruits and vegetables are estimated to be 20-50% (Henningsson et al., 2004; Terry et al., 2011). An Australian case study of mango fruit (*Mangifera Indica* L.) revealed that about 44% of harvested fruit were not eaten (Ridoutt et al., 2010). Postharvest fruit rotting and chilling injuries were major causes of those losses.

As a subtropical and tropical fruit, Mango fruit is highly sensitive to low temperature storage. Chilling injury (CI) occurs when stored at the temperature below 12 °C (Nair and Singh, 2003; Narayana et al., 2012). This limits the application of cold storage to extending mango storage life. CI symptoms of mango fruit are expressed in skin discoloration particularly blackening, sunken lesions on the peel, lenticel discoloration, uneven ripening, poor color, reduced aroma and flavor, and increased susceptibility to decay (Narayana et al., 2012). These are a result of changes in membrane fluidity, lipids composition, proteins and carbohydrates (Leyva et al., 1995).

Many biotic or abiotic stresses induce resistance in plants, termed 'induced resistance', which activates latent defense mechanism in order to protect plant against future attack by pathogens and abiotic stresses (Kuc, 1982; Pieterse et al., 2012; Walters et al., 2013; Pieterse et al., 2014). For example, heat treatment decreases chilling injury in fruit by induction of acquired resistance (Lurie et al., 1997; Lurie, 1998). In mango fruit, heat treatment reduces chilling injury (Mccollum et al., 1993) and disease incidence (Prusky et al., 1996). High levels of UV radiation cause a wide variety of physiological and morphological changes in plants (Stapleton, 1992; Ries et al., 2000). However, low doses of UV may induce resistance to pathogens in many harvested commodities (Wilson et al., 1994). For example, UV-B treatment was shown to enhance chilling tolerance in mango fruit (Ruan et al., 2015). Avocados fruit that was harvested from sun exposed area were more resistant to chilling injuries and *Colletotrichum* infection than avocado fruit from the shaded area of the tree (Woolf et al., 2000).

UV light is an important component of sunlight. In peaches, the surface of fruit exposed to sunlight at the exterior of canopy has been shown to develop a red color (Erez and Flore, 1986) due to anthocyanin accumulation in the epidermal cells (Seymour et al.,

* Corresponding author at: Department of Postharvest Science of Fresh Produce, Agricultural Research Organization, Volcani Center, POB 6, Bet Dagan 50250, Israel. Fax: +972 3 9683622.
E-mail address: noamal@volcani.agri.gov.il (N. Alkan).

http://dx.doi.org/10.1016/j.postharvbio.2015.08.001
0925-5214/

1993; Salunkhe and Kadam, 1995). In tomato, the *Anthocyanin fruit* (*AFT*) *gene* is essential for anthocyanin production and accumulation in fruit upon stimulation by high light irradiance (Mes et al., 2008).

Anthocyanins are phenylpropanoids widely distributed in most vascular plants where they serve as inducible sunshields (Grotewold, 2006). Anthocyanins have antioxidant capacity, and as such are important phytonutrients in a healthy diet, having anti-tumor, pro-apoptotic, anti-oxidative, anti-inflammatory and anti-neurodegenerative properties (Buer et al., 2010; de Pascual-Teresa et al., 2010; Spencer, 2010). Cyanidin-3-*O*-galactoside and anthocyanidin-hexoside are the main anthocyanin compounds found in the red color of mongo fruit peel (Berardini et al., 2005).

Both anthocyanin and flavonoids have pleotropic effects and may be involved in plant protection against pathogens. Anthocyanins are involved in pest and disease resistance (Hammerschmidt, 1999; Goodman et al., 2004; Iriti et al., 2004). Anthocyanin and flavonoids are known to increase in response to chilling injuries (Christie et al., 1994; Lo Piero et al., 2005; Sanchez-Ballesta et al., 2007). In red orange fruit, anthocyanin and its biosynthetic genes were induced by low temperature (Lo Piero et al., 2005). In peaches, anthocyanin related SNPs was linked to fruit tolerance to chilling injuries (Martinez-Garcia et al., 2012; Martinez-Garcia et al., 2013). Flavonoids are also known to have antifungal activities (Hammerschmidt, 1999; Treutter, 2006) and UV protection activities (Shirley, 1996). The flavonoids found in mango include catechin, epicatechin, quercetin, isoquercetin (quercetin-3-glucoside), fisetin, and astragalin (kaempferol-3-glucoside) (Masibo and He, 2008).

The purpose of this work was to examine the effect of sunlight induced red color of Shelly mango peel on resistance of mango fruit to low temperature and postharvest pathogens. This study confirmed that the red color of mango peel did not indicate advanced ripening but was correlated with higher anthocyanin and flavonoids. Importantly, those fruit were found to be more resistant to both chilling injuries and pathogens attack, and suggest that agro-technical processes which expose more of the fruit to light may lead to fruit resistant to low temperature and fungal infection.

2. Material and methods

2.1. Plant material

Mango (*Mangifera Indica* L., cv. Shelly) fruit were picked from two different positions of canopy in the orchard: the 'red' colored fruit from 'exterior' position with direct exposure to sunlight, and the 'green' colored fruit from 'interior' position in the shaded part of the canopy. Five hours after harvest, the fruit were transported from 'Mor-Hasharon' storage house, Israel to Agricultural Research Organization, Volcani Center, Israel. Uniform, unblemished fruit weighing approximately 400 g were selected on the basis of peel color, 'red fruit' with more than 60% of fruit peel colored in red and 'green fruit' with less than 25% of fruit peel colored in red. The fruit were washed with tap water and air dried.

2.2. Cold-storage of red and green mango fruit

Separate four cartons containing red or green fruit, each with 10 fruit, were used for the cold storage experiment. Four cartons for each peel color and different temperature storage were used. Fruit were stored at 5 °C or 12 °C for 3 weeks in the cold-storage rooms. The temperature in cold-storage room was monitored by a DAQ tool - Double Strand wire logger/Data Acquisition control system (T.M.I Barak ltd., Israel). The fruit core temperature was monitored by a MicroLite data Logger LITE5032P-EXT-A (Fourier technologies, Israel), by inserting the probe 5 cm deep in near calyx part of fruit, 2 fruit for each temperature.

2.3. Evaluation of peel color of red and green mango fruit

The peel color of mango fruit was measured at harvest, after 3 weeks of cold storage (5 °C or 12 °C) and further 7 d in shelf life at 20 °C using Chromometer CR-400/410 (Konicka Minolta, Osaka, Japan) at two points on the equatorial line of each fruit (20 measurements/treatment). Hue angle measures color, where 0° = red, 60° = yellow, 120° = green.

2.4. Evaluation of ripening parameters of red and green mango fruit

Physiological parameters of ripening such as firmness (Newton), color change (yellowing), total soluble solids (TSS), and total acidity (citric acid equivalence) of red and green mango fruit were measured at harvest after three weeks in cold storage (5 °C or 12 °C) and after an additional 7 d of shelf life. Fruit firmness (Newton) was determined by an electronic penetrometer force gauge LT-Lutron FG-20KG (Indonesia) with an 11 mm probe at two points on the equatorial line of each fruit (20 measurements/treatment). The color change in red and green fruit upon ripening after 3 weeks in cold storage at 5 °C or 12 °C and further 7 d of shelf life was evaluated qualitatively by visual peel color appearance and represented by color index on a relative scale 1–10 (1 represents no color change, green-fruit; 10 represents fruit with full color change, orange-fruit, 40 evaluations/treatment). Total Soluble Salts (TSS) and Total Acidity: 1 mL of pulp juice of red and green mango was dissolved in 40 mL of double distilled water; the TSS (%) was measured using Palette Digital-refractometer PR-1 (Model DBX-55, Atago, Japan) in total 10 measurements/treatment. The total acidity was determined as citric acid equivalent mass using automatic titrimeter (Model 719 s Titrino Metrohm Ion analysis Ltd., Switzerland) in total 10 measurements/treatment.

2.5. Evaluation of lipid peroxidation in red and green mango by In Vivo Imaging System

Oxidation of linolenic acid produce mainly auto-luminescence at >600 nm, with a major contribution in the wavelength range 640–695 nm (Birtic et al., 2011). In this study, red and green mango fruit cv. Shelly after 3 weeks of cold storage at 12 °C or 5 °C were selected and used to detect lipid peroxidation using Pre-clinical *In Vivo* Imaging Systems (IVIS, PerkinElmer, Massachusetts, USA). Fruit were pre-adapted in complete darkness in a dark cabinet condition for 2 h prior to the evaluation. Lipid peroxidation was detected and visualized by auto-luminescence of peroxide lipids as described (Birtic et al., 2011), using the program sequence set up consist of auto-luminescence for 20 min with emission at 640–770 nm and excitation-block, binning factor 8, and f-factor 1. The auto-luminescence was recorded by highly sensitive charge-coupled-device (CCD) camera. The optical luminescent image data was displayed in pseudocolor that represents intensity in terms of Radiance (photons/sec/cm^2/steradian). The measurements were repeated three times with different fruit, the signal intensity of each optical image was calculated within the region of interests (ROI). The Radiance was summed up and presented as average of total flux (Watts/m^{2}s/steradian) with standard error.

2.6. Analysis of chilling resistance of red and green fruit

Chilling resistance of red and green fruit was assessed on the basis of severity of black spots and pitting in red and green mango fruit after three weeks in cold storage (5 °C or 12 °C) and further 7 d of shelf life (20 °C). Black spot severity index was evaluated and represented on a relative scale 1–10 (1 represent mild black spots and 10 represent severe black spots, 40 evaluations/treatment). Pitting severity index was represented on a relative scale 1–10