Innovative Food Science and Emerging Technologies 33 (2016) 216–224

Contents lists available at ScienceDirect

Innovative Food Science and Emerging Technologies

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

Low fluence pulsed light enhanced phytochemical content and antioxidant potential of 'Tommy Atkins' mango peel and pulp

Mônica M.A. Lopes [a], Ebenezer Oliveira Silva [b], Kirley Marques Canuto [b], Lorena M.A. Silva [b], Maria Izabel Gallão [a], Laurent Urban [c], J. Fernando Ayala-Zavala [d], M. Raquel A. Miranda [a,*]

[a] Federal University of Ceará, Department of Biochemistry and Molecular Biology, Av. Mister Hull 2297, CEP 60451-970 Fortaleza, Ceara, Brazil
[b] Tropical Agroindustry Embrapa, Rua Dra Sara Mesquita, CEP 60511-110 Fortaleza, Ceara, Brazil
[c] Université d'Avignon, UMR Qualisud, Equipe Physiologie de la Qualité des Fruits et Légumes (EA 4279), Rue Baruch de Spinoza 301, BP 21239 Avignon, France
[d] Centro de Investigación en Alimentación y Desarrollo, A.C., Carretera a la Victoria km. 0.6. Apartado Postal 1735, Hermosillo 83000, Sonora, México

ARTICLE INFO

Article history:
Received 28 September 2015
Received in revised form 17 December 2015
Accepted 18 December 2015
Available online 31 December 2015

Keywords:
Radiation
Mangiferin
Antioxidants
Histology
Hormesis

Chemical compounds studied in this article:
Hydrogen peroxide (PubChem CID: 784)
Mangiferin (PubChem CID: 5281647)
Transcinnamic acid (PubChem CID: 444539)
Polygalacturonic acid (PubChem CID: 445929)
Gallic acid (PubChem CID: 370)
Ascorbic acid (PubChem CID: 54670067)
2,2-azinobis(3-ethylbenzthiazoline-6-sulphonic acid) (PubChem CID: 5815211)
thiobarbituric acid (PubChem CID: 2723628)
2-Mercaptoethanol (PubChem CID: 1567)
L-Phenylalanine (PubChem CID: 6140)

ABSTRACT

Physiologically mature Tommy Atkins mangoes were submitted to PL fluence of 0.6 J cm^{-2} (2 pulses) and then, stored for 7 days at 20 °C and 80% RH. Fruit tissues mesocarp (pulp) and epicarp (peel) were separated and evaluated for firmness and associated variables, oxidative stress and constituents of antioxidant metabolism, colour and associated variables and constituents of phenolic metabolism. Pulp firmness and lipid peroxidation degree of cell membranes were not affected; however, PL improved colour due to concomitant increase in total carotenoid content. Hydrogen peroxide content was 20% higher in PL-treated pulp samples and total antioxidant activity increased over 130%. PL treatment also enhanced PAL activity, which consequently, increased phenolic content. Thus, PL triggered the accumulation of enzymatic (SOD and CAT) and non-enzymatic (carotenoid, vitamin C, flavonoid, anthocyanin and total phenolics) antioxidants in 'Tommy Atkins' mangoes pulp; while in peel, activated the antioxidant defense (carotenoid, mangiferin, total phenolics and SOD) system without ROS mediation.
Industrial relevance: Pulsed light technology is considered an alternative to continuous ultraviolet treatments to decontaminate surfaces, packaging or foods. However, applications of pulsed light to increase the health beneficial compounds on fruits and vegetables are scarce. Our results show that application of this technology also can be successfully extended to increase the concentrations of phytochemicals without negative effects on quality criteria of mangoes during storage.

1. Introduction

Pulsed light (PL) is an emerging postharvest process developed initially as non-thermal sterilization technology to maintain safety of vegetable products free of any health risks (Aguiló-Aguayo, Charles, Renard, Page, & Carlin, 2013; Charles, Vidal, Olive, Filgueiras, & Sallanon, 2013). This technology, which may be an alternative to continuous UV light treatment, involves a wide broad-spectrum light in the wavelengths from 100 to 1100 nm with energy emitted in the ultraviolet ranging from 15 to 50% (Ignat, Manzocco, Maifreni, Bartolomeoli, & Nicoli, 2014; Krishnamurthy, Tewari, Irudayaraj, & Demirci, 2010; Maftei, Ramos-Villarroel, Nicolau, Belloso, & Soliva-Fortuny, 2014). During a PL treatment, electrical energy accumulated in a high power capacitor is released over an inert gas (e.g. xenon) generating intermittent and intense pulses of light, which last for microseconds (Krishnamurthy et al., 2010). The potential for using PL as a commercial postharvest technology depends on determining the best conditions: fluence, penetrability of light, which may impart desirable effects on fruit quality without impairing the fresh-like attributes of treated produce.

Regarding PL influence on fruit quality, published studies demonstrated that fresh-cut 'Kent' mangoes responded to postharvest PL application by maintaining their phytochemical levels; however, there was an increment in browning-associated to polyphenoloxidase activity and darkening of samples (Charles et al., 2013). PL also induced darkening of fresh-cut 'Golden Delicious' apples (Ignat et al., 2014). Aguiló-

* Corresponding author.

http://dx.doi.org/10.1016/j.ifset.2015.12.019
1466-8564/

Aguayo et al. (2013) observed that PL reduced microbial contaminants of 'Climberley' tomatoes without compromising its nutritional value; however; it did induce a loss of visual quality. PL treatment influenced fresh-cut 'Abrusen' watermelon physiology through an increment in fruit respiratory rate and decreased ethylene production (Ramos-Villarroel, Maftei, Belloso, & Soliva-Fortuny, 2012).

When plant organs are exposed to abiotic stressful conditions as radiation, deleterious processes are triggered; e.g. generation of reactive oxygen species (ROS), which are normal byproducts of respiration and may accelerate senescence (Sharma, Jha, Dubey, & Pessarakli, 2012). However, plants developed an antioxidant defense system constituted of enzymes catalase (CAT), peroxidase (APX), superoxide dismutase (SOD), glutathione peroxidase (GP) and glutathione reductase (GR), as well as non-enzymatic compounds tocopherols, carotenes, ascorbate and phenolics. Considering that several of these antioxidant compounds present human health-promoting properties, stressful conditions can induce their accumulation or preserve higher levels during postharvest storage of fresh produce. This effect was observed using UV-C (peak emission at 254 nm) to improve the phytochemicals content in fresh fruits and vegetables (Bravo et al., 2012). There may be a specific elicitor as hydrogen peroxide (H_2O_2), a ROS that acts as a signaling molecule associated with stimulation of phenylalanine-ammonia lyase (PAL) activity and therefore, activation of the phenylpropanoid synthetic pathway (Potters, Horemans, & Jansen, 2010). The ROS sources activated upon the application of stress include nicotinamide-adenine dinucleotide phosphate (NADPH) oxidase, localized in plasma membrane and the electron transport chain of mitochondrial respiration (Jacobo-Velázquez, Martínez-Hernández, Rodríguez, Cao, & Cisneros-Zevallos, 2011).

Low levels of radiation may be at the origin of beneficial responses, a phenomenon known as *hormesis,* i.e. the physiological stimulation of beneficial responses of plants by low levels of stressors which otherwise cause harmful responses. Low levels of radiation can be used as tools in the food industry to create healthier products by enhancing phytochemical levels of either whole or fresh-cut produce (Bravo et al., 2012; González-Aguilar, Villa-Rodriguez, Ayala-Zavala, & Yahia, 2010). It has also been observed that hormetic doses of UV-C radiations altered plant's susceptibility towards diseases, and may elicit plant resistance mechanisms including the production of anti-fungal compounds such as phytoalexins. Hormetic doses of UV-C radiations may also delay the fruit softening caused by changes in activities of enzymes involved in cell wall disassembly, such as polygalacturonase (PG) and pectinmethylesterase (PME), therefore extending shelf life (Pombo, Dotto, Marínez, & Civello, 2009; Ribeiro, Canada, & Alvarenga, 2012).

However, little is known about the physiological basis of the accumulation of phytochemicals as a response to a postharvest stress. Thus, the hormetic use of PL technology arises as a promising tool for maintaining or even, enhancing fruit postharvest quality. Yet, no previous study focused on the effects of this technology on physiology and quality of tropical fruit has been published. Therefore, mangoes were treated with hormetic doses of PL, thus evaluated for metabolic changes associated with quality conservation. Moreover, fruit tissues, peel and pulp were separately analyzed as means to understand the physiological effects of PL treatment restricted penetrability.

2. Material and methods

2.1 Plant material

Mangoes (*Mangifera indica*, cv. Tommy Atkins) were harvested from commercial growing field of Finobrasa Agroindustrial S.A., located at Ipanguaçú-RN, Brazil (05″34′37″S and 36″54′32″O). The climate of this region is hot with average annual temperature of 25 °C, dry with irregular rainfall in average of 670 mm and 70% average relative humidity (RH). Fruit were harvest at physiologically mature stage with yellow colored pulp and average mass of 485 g; moreover, fruits were selected for uniformity in size and absence of injuries.

2.2 Chemicals

Transcinnamic acid, 2,6-dichloro-indophenol (DFI), trichloroacetic acid (TCA), thiobarbituric acid (TBA), ethylenediaminetetracetic acid (EDTA), nitroblue tetrazolium chloride (NBT), guaiacol, hydrogen peroxide (H_2O_2), trolox, ascorbic acid, phenylalanine and polygalacturonic acid were purchased from Sigma-Aldrich (St. Louis, MO, USA). Historesin embedding kit was purchased from Reichert-Jung (Germany). All other chemicals were of analytical grade.

2.3 Pulsed light treatment and storage

Preliminary tests were done to establish the PL treatments with doses ranging from 0 (0 pulse) to 4.8 J cm^{-2} (16 pulses). Treatment with 0.6 J cm^{-2} (2 pulses) showed the best results considering total phenolic content, firmness and overall fruit quality; therefore, this fluence was chosen for this work.

At harvest day, whole mangoes were separated into two equal lots, one was untreated and served as control and the other submitted to PL radiation treatment. Radiation was administered using a XeMaticA-2LXL system (SteriBeam® GmbH, Germany) from a distance of 10 cm applying two pulses with a fluence of 0.6 J cm^{-2}. The chamber was equipped with two xenon flash lamps and Teflon® transparent support that allowed samples to be exposed 360° by both lamps, moreover to ensure uniformity, fruit positions were changed between dose applications. Lamps were positioned vertically, one on the left and another on the right side of fruit, and produced short-time pulses of 0.3 ms, delivering broad spectrum white light (200–1100 nm) with approximately 25% of all emitted energy in the UVC (220–280 nm) and a part of UVB (280–305 nm) range which is effective for sterilization.

Control samples were evaluated at day zero (D0) and after PL treatment, treated samples as well as untreated controls were stored for 7 days at 20 °C and 80% RH and then, evaluated. At each evaluation period, fruit were peeled-off and tissues mesocarp and epicarp were separated, peel (epicarp) was freeze-dried (Liotop® L101, Brazil) and ground, while pulp (mesocarp) was homogenized for 2 min at 15,000 rpm with an omnimixer (Ultra-turrax IKA®, Germany), thereafter all samples were stored at −18 °C. Results were expressed on dry matter basis for peel and on fresh matter basis for pulp.

2.4 Firmness and associated variables

Firmness of pulp was evaluated, after peel removed, twice on opposite sides of each fruit with a texturometer (Brookfield® 25K CT3, USA) to measure the maximum force required to penetrate sample tissue to a depth of 10 mm using a 2-mm diameter cylindrical flat-tipped steel plunger at a shearing speed of 1 mm s^{-1}. Results were expressed in Newton (N).

Biological membrane integrity of both pulp and peel was estimated by lipid peroxidation degree, which was determined by malondialdehyde (MDA) content based on the method described by Zhu, Sun, Liu, and Zhou (2008). Pulp (2 g) and freeze-dried peel (0.5 g) were homogenized in 5 mL of 0.1% trichloroacetic acid (TCA) and centrifuged at 3300 ×*g* for 20 min, at 4 °C. The supernatant (750 μL) was collected and added to 3 mL 0.5% thiobarbituric acid (TBA) in 20% TCA and incubated at 95 °C for 30 min. Following the incubation, tubes were immediately cooled in ice bath and centrifuged at 3000 ×*g* for 10 min. The optical density was measured at 532 nm, corrected for unspecific turbidity by subtracting from absorbance at 600 nm and thiobarbituric acid reactive substances (TBARS) as MDA content was calculated using an extinction coefficient of 155 mmol cm^{-1} (Heath & Packer, 1968) and expressed as nmol MDA kg^{-1}.

Cell wall hydrolases were assayed as following, all procedures were carried out at 4 °C and results were calculated as specific enzyme activity considering the total protein content of each sample, as determined by Bradford (1976), using bovine serum albumin (BSA) as a standard.

The extraction procedure for pectinmethylesterase (PME, EC 3.1.1.11) assay used pulp (5 g) and freeze-dried peel (0.5 g) homogenized with 20 mL of cold NaCl (0.2 M) which were filtered through Whatman n.1 filter paper, filtrate was used as enzyme extract (Jen & Robinson, 1984). Enzyme activity was measured as 5 mL of extract plus 30 mL of citrus pectin substrate (1%) in NaCl (0.2 M, pH 7.0) were titrated with NaOH (0.01 N). One unit activity (UEA) was defined as the amount enzyme capable to remove a methyl group from pectin and results were expressed as UEA min^{-1} mg^{-1} protein (P).

The extract for polygalacturonase (PG, EC 3.2.1.15) assay was prepared according to Pressey and Avants (1973), as pulp (12.5 g) and freeze-dried peel (2.6 g) were homogenized with 25 mL of distilled water and then, centrifuged at 3000 ×*g* for 10 min, at 4 °C. PG activity (Buescher & Furmanski, 1978) was expressed based on the difference of reducing sugar determined by DNS. One unit of enzyme was defined as the amount of enzyme able to catalyze the formation of 1 nmol of reducing sugar mg^{-1} min^{-1} and results are expressed as UEA min^{-1} mg^{-1} P.

Light microscopic histological analysis was performed to observed the effect of PL on the tissue structure of treated fruit, started with pulp and peel samples fixed with 4% solution of paraformaldehyde in 0.1 M phosphate buffer, pH 7.2 and 1% glutaraldehyde for 24 h, at ambient temperature (Karnovsky, 1965). Sampled material was then, dehydrated in a graded ethanol series and embedded in Historesin embedding kit (Reichert-Jung®, Germany). Tissue blocks were sectioned at 5 mm on a SLEE CUT® 5062 microtome (Germany). Histochemical reaction was carried out with periodic acid-Schiff's reagent to stain insoluble carbohydrates and proteins and photomicrographs of cell structure were taken using an Olympus® BX41 light microscope (Japan) with a connected camera (model UC30) and computer which used CELL® software.

2.5 Oxidative stress and constituents of antioxidant metabolism

Assay of oxidative stress marker hydrogen peroxide (H_2O_2) levels, as oxidative stress marker was determined as described by Sergiev, Mapelli, and Karanov (2001). Pulp tissue (0.5 g) and peel (0.05 g) were homogenized in ice bath with 5 mL 5% TCA (*w*/*v*) and then, centrifuged at 12,000 ×*g* for 15 min at 4 °C. The 0.5 mL supernatant was added to 0.5 mL of 10 mM potassium phosphate buffer (pH 7.0) and 1 mL of 1 M KI. Optical density was monitored at 390 nm and H_2O_2 content was given on a standard curve expressed as μmol kg^{-1}.

Total antioxidant activity (TAA) was determined using 2,2-azinobis-3-ethylbenzthiazoline-6-sulphonic acid radical cation ($ABTS^{*+}$) method as described by Re et al. (1999). Before the colorimetric assay, the samples were subjected to a procedure of extraction in 50% methanol and 70% acetone (Larrauri, Rupérez, & Saura-Calixto, 1997). Once the radical was formed, reaction was started by adding 30 μL of extract in 3 mL of radical solution, optical density was measured (734 nm) after 6 min and decrement in absorption was used to calculate total antioxidant activity (TAA). A calibration curve was prepared and different trolox concentrations (standard trolox solutions ranging from 100 to 2000 μM) were also evaluated against the radical. Antioxidant activity was expressed as trolox equivalent antioxidant capacity per mass of fruit, μmol trolox kg^{-1}.

Total vitamin C, a non-enzymatic antioxidant, was determined by titration with 0.02% 2,6 dichloro-indophenol (DFI) (Strohecker & Henning, 1967) and results were expressed in mg kg^{-1}.

Activity of antioxidant enzymes was determined using an enzymatic extract pulp (1 g) and freeze-dried peel (0.5 g) which were macerated for 5 min with ice-cold in a (pH 8.0) buffer solution containing 0.05 M Tris–HCl and 0.1 mM ethylenediaminetetracetic acid (EDTA) (Yang, Zheng, & Cao, 2009). Then, homogenates were centrifuged at 12,000 ×*g* for 15 min at 4 °C and supernatant was used as crude enzyme extract. All the procedures were performed at 4 °C.

Superoxide dismutase (SOD, EC 1.15.1.1) activity was determined by spectrophotometry, based on inhibition of the photochemical reduction of nitroblue tetrazolium chloride (NBT) (Giannopolitis & Ries, 1977). Optical density was measured at 560 nm and one unit enzyme activity (UAE) was defined as the amount of enzyme required to cause a 50% inhibition in the NBT photoreduction rate (Beauchamp & Fridovich, 1971), results are expressed as UAE mg^{-1} P. Catalase (CAT, EC 1.11.1.6) activity was measured according to Beers and Sizer (1952). Decrement of H_2O_2 content was monitored by measuring optical density at 240 nm and quantified using molar extinction coefficient (36 M cm^{-1}), results were expressed in μmol H_2O_2 min^{-1} mg^{-1} P. Ascorbate peroxidase (APX, EC 1.11.1.1) activity was assayed according to Nakano and Asada (1981). Reaction was started by adding ascorbic acid, and ascorbate oxidation was measured by optical density at 290 nm, using molar extinction coefficient for ascorbate (2.8 mM cm^{-1}), considering that 1 mol of ascorbate is required for a reduction of 1 mol H_2O_2, results expressed as μmol H_2O_2 min^{-1} mg^{-1} P.

2.6 Colour and associated variables

Colour was determined using a chromameter (Minolta® CR-300 equipped with D_{65} illuminant, Japan) with cylindrical coordinate system and color space for lightness, chroma and hue angle. Peel colour was evaluated four times on each fruit, at the longitudinal area, while pulp colour was measured four times at the center.

Pigments as total carotenoids were measured as described by Lichtenthaler (1987) based on chlorophyll content after optical density was read at 470, 646 and 663 nm and calculated as following: chlorophyll a (mg mL^{-1}) = 12.25 × (A663) − 2.81 × (A646); chlorophyll b (mg mL^{-1}) = 20.31 × (A646) − 2.81 × (A663) and carotenoids (mg mL^{-1}) = (1000 × (A470) − 3.27 × [chlorophyll a] − (104 × [chlo-[chlorophyll b]) / 227, and results were expressed as mg kg^{-1}.

Polyphenolic pigments, anthocyanins and yellow flavonoids were evaluated as described by Francis (1982). One gram of pulp was extracted with a 95% ethanol/1.5 N HCl (85:15) solution, vortexed for 2 min and then, brought to 50 mL with the extracting solution. Protected from light, the mixture was refrigerated at 4 °C for 12 h, then filtered on Whatman n.1 paper and the filtrate was gathered. Optical density of the filtrate was measured at 535 nm for the total anthocyanin content using an absorption coefficient of 98.2 mol cm^{-1} and at 374 nm for total yellow flavonoid content using an absorption coefficient of 76.6 mol cm^{-1}. Both results were expressed as mg kg^{-1}.

Activity of enzymes involved in tissue browning was assayed. Guaiacol peroxidase (G-POD, EC 1.11.17) activity was determined as described by Amanko, Chen, and Asada (1994). Aliquots of 90 μL of 0.1 mM EDTA 0.1 M in potassium phosphate (pH 7.0) buffer, 50 μL of 0.02 M guaiacol, 50 μL H_2O_2 and 10 μL of extract were homogenized, incubated at 30 °C and optical density was monitored at 470 nm. Activity was calculated using the molar extinction coefficient of tetraguaiacol (26.6 mM cm^{-1}) considering that 4 mol of tetraguaiacol is required for a reduction of 1 mol H_2O_2, results expressed as μmol H_2O_2 min^{-1} mg^{-1} P.

To determined polyphenoloxidase (PPO, EC 1.14.18.1) activity, an extract was prepared as described by Sojo, Nuñez-Delicado, and García-Carmona (1998). Samples of fresh pulp (1 g) and of freeze-dried peel (0.25 g) were macerated in a pre-chilled mortar for 5 min with 0.1 M potassium phosphate buffer (pH 6.1) containing 4% (*v*/*v*) triton X-100 and 0.02 g of polyvinylpolypyrrolidone (PVPP). PPO activity was assayed as described by Robinson (1987), the reaction consisted of 80 μL of potassium phosphate buffer (100 mM, pH 6), 5 μL pyrocatechol (0.1 M) and 100 μL of extract. It was determined by measuring the rate of increment in optical density at 395 nm during an incubation period of 30 min at 30 °C. One unit of enzyme activity (UEA) was defined as a change in absorbance of 0.001 and results expressed as UEA min^{-1} mg^{-1} P.

2.7 Constituents of phenolic metabolism

Total phenol content of mangoes was measured by a colorimetric assay using Folin-Ciocalteau reagent as described by Obanda, Owuor, and Taylor (1997). Samples were subjected to extraction in 50% methanol and 70% acetone as described by Larrauri et al. (1997); 1 mL Folin–Ciocalteau reagent (1 N), 2 mL Na_2CO_3 at 20% and 2 mL of distilled water were added to extracts. Results were expressed as gallic acid equivalents (GAE) mg kg^{-1}.

Mangiferin was also quantified in mango peel, after an initial extraction in Dionex® ASE 350 accelerated solvent extractor (USA). Freeze-dried peel (2 g) and diatomaceous earth (4 g) (ThermoScientific®, USA) were mixed and defatted with 100% (*v*/*v*) of hexane, and afterwards, with 100% (*v*/*v*) methanol. Operating conditions of the solvent extractor were: purge time of 200 s, 3 static cycles, temperature of 80 °C, heating period of 5 min, rinse volume at 60%, static time of 10 min and time of extraction of 60 min for each sample. Methanolic extracts were distilled to dryness using a rotary evaporator (IKA® RV10, Germany) at 40 °C and 60 rpm. Dry extracts (3 mg) were diluted in 3 mL of 20% (*v*/*v*) aqueous methanol, and filtered through a 0.45 μm polytetrafluoroethylene (PTFE) membrane (Biotechla®, Bulgaria). Mangiferin was determined through HPLC-DAD according to Canuto, Neto, Garruti and Lima (2010), using a LC-20A HPLC system coupled with a SPD-M20A diode array detector and a SIL-20AC autosampler (Shimadzu®, Japan). Samples were separated with ODS C18 column (4.6 × 150 mm) in a thermostatic oven at 30 °C. The mobile phase consisted of (A) 1% (*v*/*v*) trifluoroacetic acid and (B) methanol and gradient elution was performed by isocratic method: 0–10 min, 35% of B; 11–16 min, 100% of B; 17–21 min, 35% of B. Flow rate was at 1 mL/min, injection volume of 20 μL, and UV detection at 350 nm. In order to plot the mangiferin standard curve, a serial dilution was made from a mangiferin stock solution (Sigma®, USA), which was then, quantified based on its peak area, results were expressed as mg kg^{-1}.

Phenylalanine ammonia lyase (PAL, EC 4.3.1.24) catalyzes the initial reaction in polyphenol synthetic pathway and its activity was determined in response to PL treatment. PAL activity was assayed as described by Mori, Sakurai, and Sakuta (2001) and El-Shora (2002), with modifications. Fresh pulp (1 g) and freeze-dried peel (0.25 g) were homogenized for 3 min at 4 °C with 100 mM Tris–HCl buffer (pH 8.4), and centrifuged at 10,000 × *g* for 10 min at 4 °C. The recovered supernatant was used for testing enzymatic activity, 100 μL was homogenized with 20 μL of ß-mercaptoethanol, 580 μL of 100 mM Tris–HCl buffer (pH 8.4) and 200 μL of 40 mM L-phenylalanine. The reaction was stopped with 100 μL of 6 M HCl and optical density was monitored at 290 nm. PAL specific activity was expressed as μmol transcinnamic acid h^{-1} mg^{-1} P.

2.8 Statistical analysis

The experimental design was completely randomized (2 × 2, treatment and storage) with four replications with four mangoes each and all analyses were performed by triplicate. Data were subjected to analysis of variance (ANOVA) using ASSISTAT® Statistical Assistance Software v. 7.7 Program and means were compared by Tukey's test at 5% probability.

3. Results and discussion

3.1 PL effects on structural organization of mangoes

Influence of PL on the structural organization of 'Tommy Atkins' mangoes was evaluated through different variables (Table 1). Pulp firmness, which is a main quality indicator was not affected by PL treatment, but decreased significantly ($P > 0.05$) over 40% during storage, to 10.7 N. Fruit integrity or firmness depends on the overall structure and spatial organization of cellular morphology as well as on physical properties of dermal tissues to prevent water loss by transpiration (Aurand et al., 2012). Plant cells are limited by a plasma membrane surrounded by a much more resistant cell wall, although both structures contribute largely to tissue integrity.

Table 1
Firmness and associated variables of "Tommy Atkins" mango submitted to pulse light (PL) radiation and stored for 7 days (D) at 20 °C. Values in the same column followed by different letters indicate significant difference at $P < 0.05$. – not evaluated.

Variables	Treatment	Pulp	Peel
Firmness N	Control D0	25.1 ± 1.8a	–
	Control D7	10.7 ± 1.0b	–
	PL D7	11.7 ± 2.5b	–
Lipid peroxidation degree nmol MDA kg^{-1}	Control D0	17 ± 2b	15 ± 1b
	Control D7	23 ± 4a	37 ± 2a
	PL D7	27 ± 1a	37 ± 1a
Activity of associated enzymes			
Pectinmethylesterase, PME UEA min^{-1} mg^{-1} P	Control D0	1232 ± 31a	49,629 ± 205a
	Control D7	1050 ± 4b	17,635 ± 125b
	PL D7	858 ± 24c	10,263 ± 96c
Polygalacturonase, PG UEA min^{-1} mg^{-1} P	Control D0	742 ± 23b	44,410 ± 442c
	Control D7	886 ± 52a	69,220 ± 370a
	PL D7	454 ± 35c	64,560 ± 486b

PL treatment did not influence the degree of lipid peroxidation of cell membranes of neither pulp or peel of mangoes. In both tissues, lipid peroxidation degree increased significantly ($P < 0.05$) during storage, over 135% in pulp and 246% in peel, as observed with firmness loss. Cell membrane integrity may be estimated through its lipid peroxidation degree as degradation of polyunsaturated fatty acids due to peroxidation, producing MDA, induces membrane rigidification and cell death. Therefore, changes in MDA level in a tissue can be a good indicator of the structural integrity of membranes and often, is induced by free radicals (Ahmad, 2014). Increment in peroxidation degree and, consequently in electrolyte leakage may be expected at the end of ripening or when the fruit is exposed to stressful conditions (Vicente, Martínez, Chaves, & Civello, 2006).

Enzymes that catalyze cell wall dissolution or disintegration influence tissue firmness and in both mango peel and pulp, PME activity decreased significantly during storage. However, PL treatment induced an even stronger and significant inhibition of this cell hydrolase ($P < 0.05$), which was almost 20% lower in treated pulp than in control. In mango peel, PME activity was much higher than in pulp (up to 40× greater), although, it presented the same behavior decreasing significantly during storage and even more, in treated samples. On the other hand, during storage, PG activity increased significantly ($P < 0.05$), in control (+20%) pulp and decreased in PL-treated samples (−40%), when compared to control at day 0. While in peel, it increased significantly during storage ($P < 0.05$), although this increment was lower in PL treated samples (145%) than in control (155%) when compared to day 0. As observed for PME, PG was also much more active in peel (up to 70× greater) than in pulp.

These hydrolases act coordinately as PME catalyzes demethylation of pectic chains present in both primary cell wall and middle lamellae, meanwhile PG hydrolyzes glycosidic bonds releasing galacturonic acid residues from these chains. Their contrasting behaviors regarding PL influence, may explain why pulp firmness declined without statistical differences between control and treated mangoes. Barka (2001) observed reduction in activity of cell wall-degrading enzymes PG, PME, cellulase, xylanase, and β-D-galactosidase in tomato fruit submitted to lower doses of UV irradiation. While, another author reported that PG, endoglucanase and PME activities were not affected or also inhibited in UV-irradiated strawberries (Pombo et al., 2009).

Histological evaluation of mango pulp and peel showed that peel cells have more rigid structure when compared to pulp cells, which are larger, vacuolated, with thinner cell walls and greater intercellular spaces (Figure 1). In cross-section subjected to the PAS reaction to stain insoluble carbohydrates as pectin and starch, the epidermal layers are constituted of compact and rounded cells with different sizes, which become paler in color after 7 days of storage indicating dissolution of

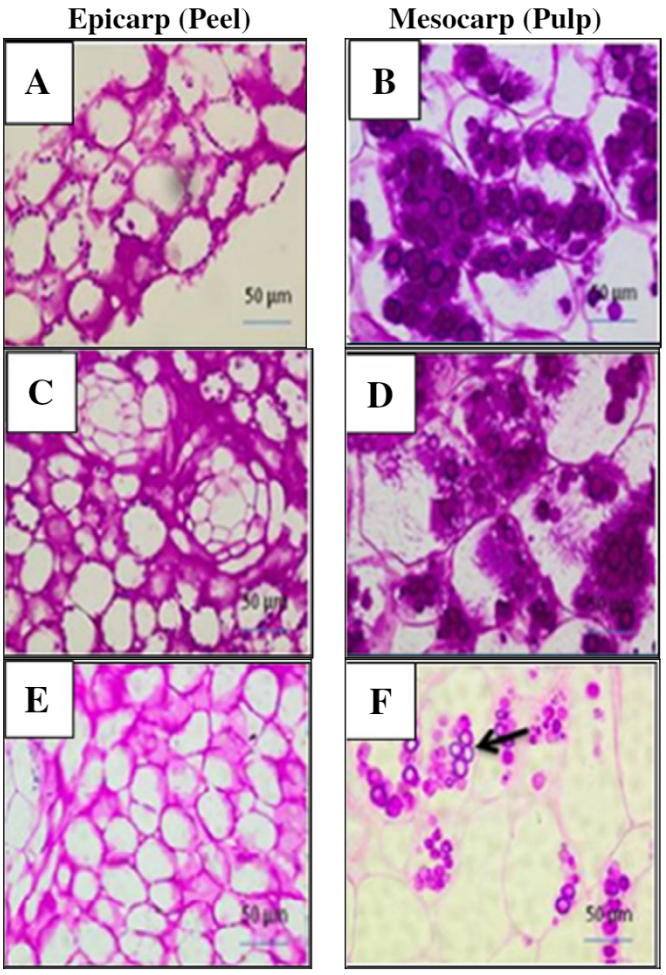

Fig. 1. Periodic Acid-Schiff (PAS) stained tissues of 'Tommy Atkins' mango submitted to pulse light (PL) radiation and stored for 7 days at 20 °C. A/B) control tissues at 0 day; C/D) control tissues at 7 day and E/F) PL-treated tissues at day 7. Arrow points to starch grains. Scale bars 50 µm (A–F) using a 10× objective.

cell wall. Parenchyma cells of pulp present large quantities of starch grains that decline during storage indicating disruption of its structure. This may be explained by the increment in PG activity (Table 1) during storage leading to pectin depolymerization and tissue softening. However, no obvious differences were observed between PL-treated and control fruit corroborating with their firmness results (Table 1) and ratifying that PL does not exert any significant effect on mango structure.

Thus, the fluence here applied (0.6 J cm^{-2}) was low enough to not induce any changes in anatomical characteristics of both dermal (peel) and ground (pulp) mango tissues, which may directly influence the appearance and therefore, quality of fruit. As fresh-cut apples were exposed to PL radiation increasing from 0 to 15.75 J cm^{-2}, pulp cells showed a loss of compartmentalization with activation of an oxidative phenomenon and leading to dehydration (Ignat et al., 2014). Thus, PL fluences higher than 1.7 J cm^{-2} induced structural changes as rupture of cell membranes, a decrease in intracellular volume and loss of turgidity causing both browning and weight loss.

3.2 PL effects in color and associated variables of mangoes

When color was evaluated (Table 2), no significant difference was found for ºHue or luminosity between control and PL-treated mangoes pulp and peel (data not shown). However, PL induced a significant increment ($P < 0.05$) in Chroma values to 140% in the peel and 130% in the pulp, when compared to control at day 7. Chroma is an expression of the purity or saturation of a single color; therefore, different colors may

Table 2
Colour and associated variables of "Tommy Atkins" mango submitted to pulse light (PL) radiation and stored for 7 days (D) at 20 °C. Values in the same column followed by different letters indicate significant difference at $P < 0.05$.

Variables	Treatment	Pulp	Peel
Chroma (C)	Control D0	44 ± 1b	24 ± 2c
	Control D7	45 ± 1b	35 ± 1b
	PL D7	65 ± 0a	58 ± 1a
Pigments			
Total carotenoids mg kg^{-1}	Control D0	34 ± 0b	392 ± 14b
	Control D7	59 ± 0b	442 ± 48b
	PL D7	266 ± 1a	853 ± 18a
Total chlorophyll mg kg^{-1}	Control D0	50 ± 8a	1857 ± 70a
	Control D7	8 ± 0c	147 ± 6b
	PL D7	15 ± 1b	154 ± 3b
Total yellow flavonoids mg kg^{-1}	Control D0	101 ± 0c	564 ± 80b
	Control D7	170 ± 0b	801 ± 60a
	PL D7	242 ± 0a	663 ± 78b
Total anthocyanins mg kg^{-1}	Control D0	31 ± 3c	58 ± 8a
	Control D7	47 ± 3b	53 ± 7a
	PL D7	57 ± 7a	47 ± 7a
Activity of associated enzymes			
Polyphenoloxidase, PPO UEA min^{-1} mg^{-1} P	Control D0	14 ± 2a	923 ± 39b
	Control D7	15 ± 2a	907 ± 20b
	PL D7	15 ± 2a	1113 ± 43a
Guaiacol peroxidase, GPOD µmol H_2O_2 min^{-1} mg^{-1} P	Control D0	0.0079 ± 0.0b	0.58 ± 0.0a
	Control D7	0.0076 ± 0.0b	0.59 ± 0.0a
	PL D7	0.1040 ± 0.0a	0.64 ± 0.0a

have the same chroma values. Moreover, during ripening of 'Tommy Atkins' mango, different colors (green, pink and yellow) are present simultaneously. Thus, in mango peel, chlorophyll is degraded from green to colorless compounds at the same time that carotenoids and phenolics are synthesized, while in the pulp, carotenoids accumulate from colorless precursor (phytoene) to β-carotene (orange) and hydroxylated carotenoids (yellow) (Doreyappa Gowda, 2002).

Fruit color is a critical parameter of quality and is often, directly related to pigment concentration, after 7 days of storage PL treatment induced a significant increase of 450.84% (266 mg kg^{-1}, $P < 0.05$) in total carotenoid content in pulp of ripe 'Tommy Atkins' mangoes, when compared to control, 59 mg kg^{-1} (Table 2). In peel, PL also induced an increase to 853 mg kg^{-1} (190%) in carotenoid content of mature mango when compared to control, 442 mg kg^{-1}. As a climacteric fruit, mango presents an increment in ethylene, which is involved with dismantlement of the photosynthetic apparatus and concomitant differentiation of chloroplasts into chromoplasts (Karlova et al., 2011). In agreement with such statement, chlorophyll content significantly ($P < 0.05$) declined in mango pulp and peel as ripening progressed, during storage for 7 days (Table 2). In peel, chlorophyll content declined over 90% with no differences between treatments, while in pulp, PL treatment significantly ($P < 0.05$) delayed (15 mg kg^{-1}) such loss when compared to untreated samples (8 mg kg^{-1}). These results indicate that effects of pulse light on carotenoid accumulation contribute more strongly to Chroma, than its delay in chlorophyll degradation.

Phenolics also contribute to mango color and among them; anthocyanin and yellow flavonoids are the most important (Table 2). Anthocyanin is responsible for dark red to bluish color nuances found in plants and its content did not change in mango peel with storage nor with treatment, while PL treatment induced a significant ($P < 0.05$) increment in pulp to 180% when compared to untreated samples, 150%, after storage. Similar results were observed for yellow flavonoid content in PL-treated pulp (1.5× greater), although in peel, PL treatment significantly inhibited ($P < 0.05$) its accumulation (117%) when compared to control (140%), after storage. Quercetin is the most abundant flavonoid in mangoes and present the greatest antioxidant activity, IC_{50} 0.004 mg mL^{-1} (Meneses, Caputo, Scognamiglio, Reverchon, and Adami, 2015).

Plants produce such phytochemicals as protectants against stressful radiation, amidst other roles. Under high light condition, ROS

are generated and as several of these pigments present antioxidant potential, they are able to limit their accumulation or neutralize them. By metabolizing ROS, carotenoids may protect lipids and phenol quinones from photooxidation (Merzlyak & Solovchenko, 2002). Moreover, these results suggest that during ripening of mango, carotenoids are the main pigments involved in physiological response to radiation in external tissues (peel) as chlorophyll and anthocyanin levels are not influenced by such condition. Anthocyanin accumulation in mango peel is probably a high irradiance response that is proportional to the exposure time to sunlight and may not be reverted or enhanced by postharvest treatments. While, chlorophyll degradation is more influenced by its developmental stage (ripening) than by PL treatment. These results also show that PL penetrates and induces physiological responses in more internal tissues as all of the evaluated pigments were influenced by it, in mango pulp.

Besides pigments, enzymes PPO and GPOD catalyze oxidative reactions that result in dark-colored products and affect tissue color. In mango pulp, PPO activity was not influenced by the PL nor storage, while GPOD activity was significantly ($P < 0.05$) stimulated by PL over 1000%, when compared untreated samples at day 7. In peel, a contrasting result was found as PPO activity significantly increased 120% in comparison to control, at day 7, while GPOD was not influenced by PL nor storage. These results, as those found for pigments, indicate that PL induces different physiological variables in dermal and ground (pulp) tissues. However, no association may be inferred between color variables (Table 2) and browning-responsible enzymes, PPO and G-POD.

In accordance with the results here shown, an immunohistochemical study developed by Ortega-García, Blanco, Peinado, and Peragón (2008) showed that PPO, in leaves, was mainly localized in epidermis, parenchyma and companion cells, while in fruit, it was mainly present in epidermis. Thus, stressful conditions that lead to browning reactions depend upon the type of plant tissue harmed. Zhao, Cao, Jiang, Gu, and Zhao (2009) also suggested that ripe mangoes showed a stronger resistance to stress due to their higher antioxidant capacity, mainly due to phenolics and that changes in PPO activity would not result directly in darkening of fruit tissue.

3.3 PL effects on oxidative/antioxidant metabolism

Hydrogen peroxide (H_2O_2) is a reactive oxygen species that, under oxidative stress conditions, acts as a signal inducing antioxidant defense mechanism (Jacobo-Velázquez et al., 2011). In mango pulp, H_2O_2 content increased during storage for both treatments (Table 3), although it was 20% higher in PL-treated samples. However, in peel the increment in H_2O_2 content was only influenced by storage period.

As ROS are normal byproducts of respiratory process, the increment in H_2O_2 content in untreated samples may be explained by the climacteric respiratory peak that leads to greater ROS production promoting an oxidative process, which contributes to a general deterioration of cellular metabolism intrinsic to fruit ripening and senescence (Jimenez, Gomez, Navarro, & Sevilla, 2002). Thus, higher H_2O_2 levels explain the greater lipid peroxidation degree observed in cell membranes contributing to mango softening (Table 1), as they ripened during 7 days of storage, regardless of PL treatment. Another evidence of respiratory chain-origin of H_2O_2, is that at harvest of mature fruit, chloroplasts have been transformed in chromoplasts, thus ROS cannot originate from photosynthesis as the pulp has lost its photosynthetic machinery.

The increment in H_2O_2 in pulp may be considered an indicator of oxidative imbalance due to PL radiation. In pulp of stressed fruits, ROS may originate from reactions catalyzed by NADPH oxidase located in plasma membrane and by xanthine oxidase in peroxisomes, besides from perturbations on respiratory mitochondrial electron transport chain (Del Río, Sandalio, Corpas, Palma, & Barroso, 2006). Thus, ROS formed (superoxide, $O_2^{\bullet-}$ and H_2O_2) under stressful condition play an important role as defense mechanisms by acting as a signal that increases the respiratory rate inducing even higher ROS levels (Jacobo-Velázquez et al., 2011Rhoads, Umbach, Chalivendra, & Siedow, 2006).

In response to an oxidative imbalance, cells produce antioxidants of different chemical nature as defense molecules that may eliminate or neutralize free radicals. In accordance to this, the total antioxidant activity was 130% higher ($P > 0.05$) in PL-treated pulp (Table 3) and 145% higher in PL-treated peel, when compared to control, after storage. However, when vitamin C was evaluated (Table 3), a well-known antioxidant, PL only affected the pulp retaining a 40% higher content than control, which decreased through storage to 4412 mg kg^{-1}. In peel, total vitamin C decreased 50% without any significant difference between treatments, after storage. Ascorbate plays a major role in scavenging of H_2O_2 via ascorbate peroxidase (APX) or as reductant itself; thereby its content depends on the rate of its biosynthesis, recycling by APX or oxidation by ascorbate oxidase (Wang, Wang, Meng, & Meng, 2012).

After 7 days of storage, the evaluated antioxidants contributed differently to the greater antioxidant potential found for the PL-treated mango tissues. In PL-treated pulp, significantly higher ($P < 0.05$) values were observed for vitamin C (150%), total carotenoids (450%), chlorophyll (187%), anthocyanins (180%) and yellow flavonoids (142%). In mango peel, however, PL treatment only induced higher levels of total carotenoid (190%).

Together with non-enzymatic antioxidants, antioxidant enzymes are frontline defense against ROS surplus. Superoxide dismutase (SOD) scavenges radical $O_2^{\bullet-}$ by converting it to H_2O_2, which may be, subsequently neutralized by catalase (CAT) or APX (Mittler, 2002). ROS, as H_2O_2, production was induced during storage in both tissues, although it was also further influenced by PL-treatment in pulp. SOD activity was also significantly ($P < 0.05$) stimulated by both PL and storage (Table 3), increasing 158% in both tissues of PL-treated mangoes, when compared to control. Similar increments ($P < 0.05$) were observed for CAT activity in PL-treated mango pulp (204%), but in peel, this was only due to storage (Table 3). Although, APX activity was only influenced by storage, not by PL (Table 3).

In plants submitted to environmental stresses as radiation, increments in APX activity are often associated to higher levels of oxidation of the ascorbate pool, which was not observe here at day 7 (Table 3). Moreover, as APX and CAT have different affinities for H_2O_2, these results suggest that APX might be responsible for the fine modulation of ROS for signaling, whereas CAT might be responsible for removal of excess ROS under stress (Mittler, 2002). Results obtained here, higher

Table 3
Oxidative and antioxidant metabolism of "Tommy Atkins" mango submitted to pulse light (PL) radiation and stored for 7 days (D) at 20 °C. Values in the same column followed by different letters indicate significant difference at $P < 0.05$.

Variables	Treatment	Pulp	Peel
Hydrogen peroxide (H_2O_2) µmol kg^{-1}	Control D0	0.0006 ± 0.0c	0.004 ± 0.0b
	Control D7	0.029 ± 0.0b	0.02 ± 0.0a
	PL D7	0.036 ± 0.0a	0.02 ± 0.0a
Antioxidants			
Total antioxidant activity µmol trolox kg^{-1}	Control D0	57 ± 2c	49 ± 0b
	Control D7	70 ± 5b	49 ± 3b
	PL D7	90 ± 6a	85 ± 4a
Total vitamin C mg kg^{-1}	Control D0	8549 ± 15a	4083 ± 130a
	Control D7	4412 ± 25b	1916 ± 188b
	PL D7	7037 ± 38a	2095 ± 137b
Activity of associated enzymes			
Superoxide dismutase, SOD UEA mg^{-1} P	Control D0	4.6 ± 0.8c	447 ± 20c
	Control D7	15.5 ± 0.5b	788 ± 7b
	PL D7	24.5 ± 0.2a	1250 ± 10a
Catalase, CAT µmol H_2O_2 min^{-1} mg^{-1} P	Control D0	36 ± 1c	96 ± 6c
	Control D7	278 ± 10b	717 ± 42b
	PL D7	568 ± 19a	790 ± 30[a]
Ascorbate peroxidase, APX µmol H_2O_2 min^{-1} mg^{-1} P	Control D0	0.6 ± 0.0b	8.5 ± 0.3b
	Control D7	1.3 ± 0.0a	10.3 ± 0.5a
	PL D7	1.4 ± 0.2a	10.2 ± 0.5a

Table 4
Phenolic metabolism of "Tommy Atkins" mango submitted to pulse light (PL) radiation and stored for 7 days (D) at 20 °C. Values in the same column followed by different letters indicate significant difference at $P < 0.05$. – not evaluated.

Variables	Treatment	Pulp	Peel
Total phenolics mg GAE kg^{-1}	Control D0	6153 ± 17b	37,609 ± 164b
	Control D7	5832 ± 19b	36,303 ± 113b
	PL D7	7566 ± 37a	71,656 ± 252a
Mangiferin mg kg^{-1}	Control D0	–	2746 ± 151b
	Control D7	–	2323 ± 124c
	PL D7	–	4013 ± 149a
Activity of associated enzyme			
Phenylalanine ammonia lyase, PAL µmol transcinnamic acid h^{-1} mg^{-1} P	Control D0	3.5 ± 0.6b	216 ± 18b
	Control D7	2.4 ± 0.2b	245 ± 12b
	PL D7	5.9 ± 0.8a	487 ± 22a

H_2O_2 level and SOD activity, agree with findings that enzymatic antioxidant system is induced in response to oxidative stress counterbalancing the stressful effect of UV radiation (Jaleel et al., 2009).

3.4 PL effects on phenolic metabolism

When compared to control, PL treatment increased significantly ($P < 0.05$) total phenolic content of both peel and pulp of 'Tommy Atkins' mangoes, which was 130% higher in pulp and 197% higher in peel, when compared to control after storage (Table 4). Besides anthocyanin and yellow flavonoids (Table 2), the xanthone mangiferin was evaluated in mango peel (Table 4). PL-treatment induced a significant ($P < 0.05$) increment over 170% in mangiferin content when compared to control, after storage. In mango peel, mangiferin is the predominant phenolic constituent reported to display a variety of pharmacological effects as anticancer and anti-inflammatory agent and potent antioxidant (Meneses et al., 2015; Mohan et al., 2013; Peng, Yao, Yang, Tang & Huang, 2015; Ribeiro et al., 2012). Mangiferin has also been detected in leaves and barks of *M. indica* due to its importance in plant protection against biotic and abiotic stress (Barreto et al., 2008). These results represent a possibility to further exploit the peel of mangoes as they are usually discarded during processing of fruit in the industry.

PAL catalyzes the synthesis of the first compound, trans-cinnamic acid, in the phenolic pathway at expenses of amino acid phenylalanine and its activity was significantly ($P < 0.05$) enhanced by PL treatment in both mango tissue (Table 4). After storage, PAL activity was 2.5× greater in PL-treated pulp samples and 2× greater in PL-treated peel than control justifying higher total phenolic contents found in PL-treated mango tissues. Regardless of light radiation treatment, PAL activity contributed to anthocyanin, yellow flavonoids and mangiferin levels. Several reports pointed out that UV radiation results in the accumulation of flavonoids, which may be implicated in the resistance of fruit and vegetables to microorganisms and senescence, that act as protective filter against radiation (Aguiló-Aguayo et al., 2013; Charles et al., 2013; Li et al., 2010; Shama & Alderson, 2005).

PAL has been used as an indicator of stressful conditions for it links the primary to secondary metabolism of plants (Sreelakshmi & Sharma, 2008) and together with higher H_2O_2 levels (Table 3), the increase in PAL activity indicates that PL induced an oxidative imbalance in cells of mango pulp and peel. Besides the fact that phenolic biosynthetic pathway may, itself, be under ROS/redox control, which is consistent with knowledge of the gene-controlling role of redox-sensitive systems (Potters et al., 2010). Thus, the oxidative imbalance induced by radiation would be even greater than originally produced during ripening and senescence, thus establishing a stress and in response to such high light condition, defense mechanisms are activated. Under excess light condition, fruit defensive responses depend on genetics, radiation intensity and exposure time, as well as its physiological maturity stage (González-Aguilar et al., 2010).

4. Conclusion

PL as postharvest treatment on 'Tommy Atkins' mangoes induced different responses in fruit tissues, pulp and peel (Figure 2). In mango pulp, an oxidative stress was established due to overproduction of ROS, which would have acted as signals triggering enzymatic (SOD and CAT) and non-enzymatic (carotenoid, vitamin C, flavonoid, anthocyanin and total phenolics) antioxidants. While in mango peel, the antioxidant defense system (carotenoid, mangiferin, total phenolics and SOD) was stimulated without ROS mediation. However, PL did not affect structural variables of neither mango tissue. These results suggest that PL penetrated mango peel reaching the pulp and influencing its physiology, although there is also a possibility that stress-signaling molecules generated at the peel would have migrated and influenced pulp physiology. Once the

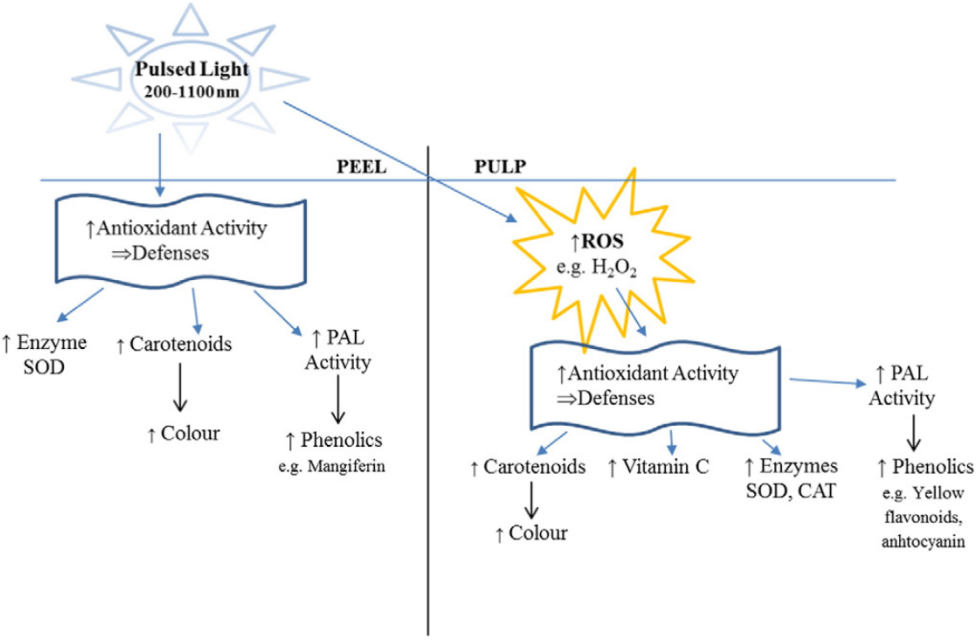

Fig. 2. Proposed mode of action of a pulse light postharvest treatment on 'Tommy Atkins' mango tissues, pulp and peel.

responses to PL are beneficial as they promote higher antioxidant levels, without any permanent serious damage to plant tissue or cell, this condition would be referred to as hormesis.

PL does positively affect the postharvest physiology of 'Tommy Atkins' mangoes enhancing its health-promoting phytochemicals and antioxidant potential. Thereby, understanding how plant tissues and their specific secondary metabolic pathways respond to different abiotic stresses would be the basis for defining strategies to use fruits as biofactories of nutraceuticals, as shown by the very promising results with mangiferin. However, using PL, specifically, as a tool to improve fruit quality requires greater knowledge of the physiological processes involved and understanding how these are influenced by such stressing agent. Thus, further studies are necessary to investigate the penetrability of broad spectrum PL and constituting wavelength ranges in different fruit tissues, as well as perception/response mechanisms involved.

Acknowledgements

We thank Instituto Nacional de Ciência e Tecnologia de Frutos Tropicais-INCT/CNPq (Project No.573781/2008-7) for finacial support and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior-CAPES, Brazil for doctorate scholarship.

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