The influence of pulsed light exposure mode on quality and bioactive compounds of fresh-cut mangoes

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Abstract This study investigated the effect of pulsed light (PL) on the respiratory rate, quality (firmness, color and soluble solid content), bioactive compounds (ascorbate and carotenoid) and total antioxidant activity of fresh-cut “Tommy Atkins” mangoes. Fresh-cut mangoes were subjected to PL treatments: control (0P), 1 pulse (1P; 0.7 J cm^{-2}), 4 successive pulses (4P; 2.80 J cm^{-2}) and 1 pulse per day for 4 days (1P4D; 2.80 J cm^{-2}) before storage for 7 days at 6°C . The 1P and the 4P treatments reduced fresh mass loss during storage, while 4P-treated samples also showed a slower decline of yellow color, as shown by parameter *b* and overall better visual appearance. After 7 days of storage, total ascorbate content was 40% higher in the 1P4D treatment than in control, whereas total carotenoid content ($0.894 \text{ mg g}^{-1} \text{ FM}$) and total antioxidant activity ($144 \mu\text{mol trolox } 100 \text{ g}^{-1} \text{ FM}$) were the highest in the 4P-treated samples. Results suggest that PL mode of application is more important than the fluence or final dose received by fresh-cut mangoes; moreover, 4P is an effective method to preserve, or even improve quality of fresh-cut mangoes.

Keywords *Mangifera indica* · Carotenoids · Ascorbate · Browning · Hormesis

Introduction

Consumption of fruit and vegetables is recommended as part of a healthy diet once these are rich sources of phytochemicals as carotenoids, polyphenols, vitamins and minerals, besides presenting complex carbohydrates and fibers (Heber 2004). Moreover, epidemiological studies have established a relationship between fruit and vegetable consumption and prevention of human diseases (Kaisoon et al. 2012). However, changes in modern life-style led consumers to decrease the intake of fresh produce (Zhou et al. 2015).

Therefore, minimally processed or fresh-cut products rise as alternatives alleviating the inconvenience of consuming whole fruit and vegetables, although conferring the health-associated benefits of a diet rich in phytochemicals. Mangoes are tropical fruit with high antioxidant capacity due to bioactive compounds as phenolics, carotenoids, and vitamin C, besides the pleasant texture and flavor, which have stimulated their postharvest use as minimally processed products (Vijaya Kumar Reddy et al. 2010).

Effective postharvest methods that maintain or even increase the antioxidant potential of fresh produce could contribute to greater consumption of fruits and vegetables, and thereafter, their human health-promoting effects. In the last decade, pulse light (PL) emerged as a non-thermal sterilization technology that evolved from continuous ultraviolet (UV) radiation treatments and involves broad spectrum light with wavelengths ranging from 100 to 1100 nm with, although, approximately 15–50% of the emitted energy in UV range (Gómez et al. 2012). 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. Besides the initial sterilization

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effect, published studies have reported that PL (0.2 to 10 J cm^{-2}) provides a highly effective way for increasing vitamin D₂ content in mushrooms (Koyyalamudi et al. 2011), total anthocyanins and phenolic compounds in figs (Rodov et al. 2012) and increase of lycopene in tomatoes (Aguiló-Aguayo et al. 2013). Koh et al. (2016) reported that PL treatment inhibited microbial growth and extended the shelf life of fresh-cut cantaloupe melons for 8 days, when compared to control.

However, publications that report on the evaluation of PL on fresh-cut mangoes are scarce. One previous study from our group reported that application of 4 successive pulses of 2 J cm^{-2} (8 J cm^{-2}) on fresh-cut mangoes contributed to maintenance of nutritional aspects (Charles et al. 2013). Moreover, there is no published information on the effect of application mode of PL on the postharvest physiology nor quality of fruits. Thus, this work aimed to evaluate the effect of different PL treatment modes, as a 0.7 J cm^{-2} dose (~ 3 fold lower than reported by Charles et al. 2013) was applied solely, as four successive short pulses or as one pulse per day over a period of 4 days, on quality and antioxidant metabolism of fresh-cut mangoes.

Materials and methods

Minimal processing and pulsed light treatment of mangoes

Mangoes (*Mangifera indica* L. cv. Tommy Atkins) were purchased at a local market at Avignon, France at commercial maturity stage with light yellow-colored pulp, green skin and average mass of 485 g. Initially, fruit were selected for uniformity in size and absence of injuries, and then, minimally processed as following: peeled, cut into cubes ($3 \times 3 \text{ cm}$), washed in 80 ppm sodium hypochlorite solution for 10 min, and dried with blotting paper.

Pulsed light treatments were carried out using an automatic flash Xenon lamp system (Claranor® S.A, France) composed of three lamps (18 cm each) distant 10 cm from the cube samples. Only one face of each cube was irradiated, in order to reduce handling and to propose a simple method. To determine the total fluence, a VLX-3 W radiometer was used and one pulse was equal to 0.7 J cm^{-2} with duration of 250 μs (input voltage was of the 2500 V corresponding to 400 kW). The wavelength of the emitted light ranged from UV-C to infrared (200–1100 nm) corresponding to 15% of UV-C. Mango cubes were treated with: no pulse as control (0 J cm^{-2}), 1 pulse (1P; 0.7 J cm^{-2}), 4 successive pulses (4P; 2.80 J cm^{-2}) and 1 pulse per day for 4 days (1P4D; 2.80 J cm^{-2}). Then, cubes were placed in 1160 mL glass jars, stored at 6°C and evaluated at day 0, 1, 3 and 7.

Physicochemical analysis

Firmness was evaluated twice on opposite sides of each sample with a Penefel texture analyzer (Setop Giraud-Technologie®, France) to measure the maximum force required to penetrate the geometric center of the cut surface using a 2-mm diameter cylindrical flat-tipped steel plunger at a shearing speed of 1 mm s^{-1} , and results were expressed in Newtons (N). Mass loss was determined at day 0 and after 1, 3 and 7 days with a balance (Precisa® XT 1200C, Switzerland) and expressed as percentage of initial weight. Soluble solids content was determined using a digital refractometer (ATAGO® N1, USA) with automatic temperature compensation and results were expressed in Brix (concentration of sucrose w/w). Fresh-cut pulp color was measured with a chromameter (Konica Minolta® CR-300 with a D65 light source, Japan) for CIE color space coordinates, L* and b* values, after calibration against a standard white reference tile ($L^* = 97.53$; $a^* = 0.09$; $b^* = 1.78$). Luminosity value, L*, indicates sample lightness (varying from 0-black to 100-white) and b* is the grade of blueness/yellowness (also varying from -60 to $+60$).

Respiratory rate

Respiration of fresh-cut mangoes was evaluated through a closed system method using a gas analyzer (Checkmate® 9900, PBI dansensor, Denmark). At 6°C , ten cubes were put in a jar (1555 L) hermetically closed and gas concentration (O_2 and CO_2) were checked on hourly basis for 10 h, at day 0 and 7. The respiratory rate measured both O_2 and CO_2 emission rates, expressed as $\text{mmol kg}^{-1} \text{ h}^{-1}$ (Varoquaux and Wiley 1994).

Antioxidant compounds and antioxidant capacity

Ascorbic acid (AsA) content

The assay of total and reduced ascorbic acid (AsA) content was carried according to Murshed et al. (2008). Mango pulp was lyophilized and 100 mg of sample was homogenized in 1 mL of 6% (w/v) trichloroacetic acid (TCA), centrifuged at $15,000 \times g$ at 4°C for 10 min and the supernatant was collected. Total AsA (plus 20 mM of dithiothreitol-DTT in phosphate buffer 0.2 mM, pH 7.4) and reduced AsA (without DTT) were measured using a 96-well microplate which was incubated at 42°C for 15 min. Then, 10 μL of N-ethylmaleimide 1% (total AsA assay) or phosphate buffer (0.2 mM, pH 7.4) (reduced AsA assay) were added and mixed, followed by 150 μL of a specific reagent (50 μL 10% TCA, 40 μL 42% (v/v) orthophosphoric acid (H_3PO_4), 40 μL 4% (w/v) 2,2-

bipyridyl dissolved in 70% ethanol and 20 μL 3% (w/v) ferric chloride FeCl_3). After incubation at 42 °C for 40 min, the absorbance was measured at 525 nm using a UV/VIS microplate reader (PowerWave HT, BioTek, France). The dehydroascorbate (DHA), oxidized form of AsA, concentration was estimated as the difference between the total and reduced AsA. Commercial L-Ascorbic acid was used for calibration and results were expressed as mg 100 g^{-1} fresh mass (FM).

Carotenoid content

Total carotenoid content was determined according to Lichtenthaler (1987) based on absorbance at 663 nm for chlorophyll a, 645 nm for chlorophyll b, and 480 nm for carotenoids. Mango pulp (0.1 g) was homogenized in 1.5 mL of 80% (v/v) acetone (in darkness at 4 °C) and centrifuged at $15,000 \times g$ at 4 °C for 15 min. Then, absorbance of an aliquot (200 μL) of supernatant was monitored on UV/VIS microplate reader (PowerWave HT, BioTek®, France) and results were expressed as mg g^{-1} FM.

Antioxidant activity

The ABTS^{*+} radical cation assay was used to determine the antioxidant capacity. Mango pulp (5 g) was subjected to the extraction with 4 mL of 50% (v/v) methanol, let to rest for 60 min (in darkness) and then, centrifuged at $25,000 \times g$ at 4 °C for 15 min. The pellet was resuspended and homogenized with 4 mL of 70% (v/v) acetone, allowed to rest for 60 min (in darkness) and then, centrifuged at $25,000 \times g$ at 4 °C for 15 min (Larrauri et al. 1997). The extract was used for determination of the trolox equivalent antioxidant capacity (TEAC) that was carried out using ABTS^{*+} radical cation decolorization assay (Re et al. 1999). Briefly, 7 mM ABTS^{*+} (Sigma®) solution and 140 mM potassium persulfate were mixed and allowed to rest in dark for 16 h to produce ABTS^{*+} radical cation. This solution was diluted with methanol (800 mL L^{-1}) to attain absorbance of 0.700 ± 0.020 , at 734 nm. The ABTS^{*+} diluted solution (3 mL) and 30 mL of blank, standard or sample were mixed and the absorbance was measured at 734 nm after 6 min of the reaction. The standard curve was prepared using Trolox solution (100 to 2000 μM) for calculating antioxidant capacity, which was expressed as μmol Trolox Equivalents (TE) 100 g^{-1} FM.

Statistical Analysis

The experimental design was completely randomized with two factors, PL treatments (control, 1P, 1P4D and 4P) and storage periods (0, 1, 3 and 7 days). Samples were

composed of four jars (repetitions) per treatment with 10 mango cubes in each. Analysis of variance (ANOVA) was performed using ASSISTAT-Statistical Assistance Software v. 7.7. The effect of each factor on the response variable as well as the effect of interactions between the different factors was tested. Significance was considered at $P < 0.05$ by Tukey's test.

Results and Discussion

Quality variables

Firmness of fresh-cut mangoes decreased significantly ($P < 0.05$) in control samples at day 1, however at the end of 7 days of storage, 1P and 1P4D treated samples showed a decline in firmness that was statistically similar to control (Fig. 1a). Control samples showed the earliest and significant softening process reaching 1.52 N, at day 7. Meanwhile, 4P treatment significantly slowed down firmness loss to 2.49 N, after 7 days. The 4P treatment induced a statistically ($P < 0.05$) higher mass loss at day 1 (Fig. 1b), however, the value was constant during storage, thus after 7 days, 4P-treated samples showed the lowest mass loss value (3.05%). The other treatments resulted in statistically greater mass loss at the end of storage, as 1P4D-treated samples lost 22.0% while control samples lost 16.8%.

These results corroborate with other previous studies that showed continuous or pulsed UV radiation improved firmness and quality of fresh-cut produce (Barka et al. 2000; Hemmaty et al. 2007; Liu et al. 2011; Charles et al. 2013). Firmness is a main attribute influencing quality of fresh commodities and indeed, it is determinant for consumers acceptance as it impacts organoleptic quality, flavor perception, shelf-life and transportability (Seymour et al. 2002). Increments on firmness by UV-C have been associated with suppression of cell wall loosening through a direct effect on cell wall hydrolases structure and activity, or through increases in polyamine content which would inhibit the cell wall hydrolases as well as enzymes responsible for breakdown of starch and protein (Barka et al. 2000; Hemmaty et al. 2007). Liu et al. (2011) showed that tomato firmness is negatively influenced by UV-B radiation, as fruit treated with lower doses ($1\text{--}4 \text{ J cm}^{-2}$) were firmer than those treated with higher doses (8 J cm^{-2}), after 37 days of storage. Manzocco et al. (2011) observed that radiation did not affect firmness of fresh-cut apples and inferred that exposure to low fluence UV-C light for short periods of times promoted dehydration of the surface of apple slices without leading to additional cell damage, besides that caused by processing itself.

An increase ($P < 0.05$) in soluble solids (SS) content was observed during storage of fresh-cut mangoes with no

Fig. 1 Firmness (a), mass loss (b), soluble solids content (c), luminosity L^* (d) and color parameter b^* (e) of fresh-cut mangoes cv. Tommy Atkins treated with pulsed light (0.7 J cm^{-2}) and stored at 6°C for 7 days. Treatments: 1 pulse (1P, solid line with square); 4 pulses (4P, dotted line with square), 1 pulse per day for 4 days (1P4D, dashed line with triangle) and no pulse (Control, dashed line with diamond). *Means the effect of PL treatment is significant at $P < 0.05$

significant difference between PL treatments (Fig. 1c), thus at day 7, SS values were 10.38 and 9.49 °Brix for 4P and 1P4D samples, respectively. Increases in soluble solids content most probably result from moisture loss and polysaccharide hydrolysis thus; may act as an indicator of soluble sugars and sweetness levels (Saltveit 2005). When apples were submitted to UV-C radiation associated to hot

water and 4% CaCl_2 treatments (Hemmaty et al. 2007), the SS content decreased while there was an increase in firmness which was attributed to a lower cell activity of wall hydrolases resulting in lower sugar solubility. However, mangoes have higher starch content ($7 \text{ mg g}^{-1} \text{ FM}$) than apples ($3 \text{ mg g}^{-1} \text{ FM}$) (Feng et al. 2014), thereby, the increase in SS here observed may be due to

Fig. 2 Visual appearance of fresh-cut mangoes cv. Tommy Atkins treated with pulsed light (0.7 J cm^{-2}) and stored at 6°C for 7 days. Treatments: 1 pulse (1P); 4 pulses (4P), 1 pulse per day for 4 days (1P4D) and no pulse (Control)

depolymerization of other carbohydrates than those present in the cell wall.

Color variables, luminosity L^* and parameter b^* that represent lightness and color spectrum from blue to yellow, respectively, decreased significantly ($P < 0.05$) through storage of fresh-cut mangoes (Fig. 1d, e, respectively). While, luminosity was not significantly affected by PL treatment, parameter b^* values were statistically different. Thus, at the end of storage, the 4P-treated samples showed an L^* value of 64.22 and the highest parameter b^* value (41.90) with a more yellowish color. This result may be corroborated by their visual appearance as observed in Fig. 2, which shows that control and 1P4D treatments negatively affected fresh-cut mango color.

Lightness and visual appearance are the first sensory attributes to change when fresh-cut mangoes are stored at 5°C (Salinas-Hernández et al. 2015). Darkening of samples may be associated to oxidative reactions catalyzed by enzymes peroxidase and polyphenoloxidase (PPO) that transform quinones to melanin, a dark-colored pigment (Jolivet et al. 1998). Shayanfar et al. (2014) reported the retention of the initial color in fresh-cut fruit due to use of anti-browning agents and Manzocco et al. (2013) reported that PL doses higher than 8.75 J cm^{-2} led to complete PPO inactivation due to protein structural modification as cleavage and unfolding/aggregation phenomena. According to Charles et al. (2013), as a superficial treatment, PL is to be applied directly onto samples as a means to improve color, however, our results show this effect on fresh-cut mango color is significantly influenced by dose and/or time of exposure.

Respiratory rate

The respiratory rate indicates the metabolic activity level and thus, the potential storage life of fresh produce. After

7 days of storage (Fig. 3), control samples had a high respiratory rate of $0.24 \text{ mmol kg}^{-1} \text{ h}^{-1}$ that was statistically similar to 1P4D-treated samples ($0.28 \text{ mmol kg}^{-1} \text{ h}^{-1}$). Meanwhile, 1P treatment ($0.16 \text{ mmol kg}^{-1} \text{ h}^{-1}$) and 4P-treated samples ($0.15 \text{ mmol kg}^{-1} \text{ h}^{-1}$) reduced the respiratory rate of fresh-cut mangoes with a positive impact on quality during storage with maintenance of yellow color (Fig. 1e) and lower mass loss (Fig. 1b). Allende et al. (2006) treated lettuce with different UV-C doses and concluded that higher doses (i.e., 0.71 J cm^{-2}) resulted in higher respiratory rate and CO_2 levels leading to greater softening and darkening of samples, when compared to lower doses.

Fig. 3 Respiratory rate of fresh-cut mangoes cv. Tommy Atkins treated with pulsed light (0.7 J cm^{-2}) and stored at 6°C for 7 days. Treatments: 1 pulse (1P); 4 pulses (4P), 1 pulse per day for 4 days (1P4D) and no pulse (Control). Different capital and lowercase letters indicate significant differences at $P < 0.05$ between storage periods and PL treatments, respectively

Bioactive compounds and antioxidant activity

The changes on bioactive compounds of fresh-cut mangoes are shown in Fig. 4. After an initial decrease, carotenoid content increased during storage reaching significantly ($P < 0.01$) higher levels in 4P-treated samples, $0.89 \text{ mg g}^{-1} \text{ FM}$ (Fig. 4a). Since, carotenoids are the main pigments responsible for the yellow color of mangoes, the higher levels stimulated by 4P treatment are probably associated to the higher parameter b^* value (Fig. 1e) and better visual appearance (Fig. 2) observed in the samples.

Mangoes are climacteric fruits and therefore, during ripening, depend on ethylene to regulate carotenoid biosynthesis that is concomitant to the dismantlement of the photosynthetic apparatus with the differentiation of chloroplasts into chromoplasts, loss of cellular integrity, hexose accumulation and decrease in organic acids (Karlova et al. 2011). The regulation of carotenoid biosynthesis seems to be tightly coordinated with developmental processes and carotenoid accumulation could be a photoprotective antioxidant defence response to oxidative stress caused by high UV radiation (Solovchenko and Merzlyak

Fig. 4 Carotenoids (a), total ascorbic acid-AsA (b), reduced AsA (c), dehydroascorbate-DHA (d), AsA:DHA ratio (e) and total antioxidant activity-TAA (f) of fresh-cut mangoes cv. Tommy Atkins treated with pulsed light (0.7 J cm^{-2}) and stored at 6°C for 7 days. Treatments: 1 pulse (1P, solid line with square); 4 pulses (4P, dotted line with square), 1 pulse per day for 4 days (1P4D, dashed line with triangle) and no pulse (Control, dashed line with diamond). *Means the effect of PL treatment is significant at $P < 0.05$

2008). In addition to that, Castagna et al. (2013) observed that carotenoid and ascorbic acid levels were incremented as UV-B responses mediated by gene LeDDB1-a, in tomato.

Total ascorbic acid (AsA) content of fresh-cut mangoes increased significantly ($P < 0.05$) during storage and 1P4D treatment resulted in the greatest content ($33.76 \text{ mg} \cdot 100 \text{ g}^{-1} \text{ FM}$) (Fig. 4b), which was $\sim 40\%$ higher than control and other treatments. Total ascorbic acid represents both reduced and oxidized, dehydroascorbic acid (DHA), and although, regarding reduced AsA content (Fig. 4c), treatments did not differ statistically from control ($17.29 \text{ mg} \cdot 100 \text{ g}^{-1} \text{ FM}$); DHA levels (Fig. 4d) were statistically incremented by 1P4D-treatment ($15.65 \text{ mg} \cdot 100 \text{ g}^{-1} \text{ FM}$), at the end of storage.

The accumulation pattern of ascorbic acid differed among treatments, as total ascorbic acid increased in 4P-treated samples, at day 3, and remained constant thereafter, while 1P4D treatment resulted in an increment at day 7. The increase observed in 1P4D-treated samples was mainly due to increments in DHA oxidized form, once reduced AsA did not differ among treatments. The relation between reduced AsA:DHA has been proposed as an abiotic stress-marker in plants (De Gara 2003) and fresh-cut mango cubes treated with 1P4D had the lowest AsA:DHA ratio (1.16), indicating this condition induced an oxidative imbalance, at day 7 (Fig. 4e). The increase in oxidized ascorbic acid forms could be an adaptive response to oxidative stress; moreover, the idea of such oxidative imbalance is corroborated by the overall decline in visual appearance of 1P4D-treated samples (Fig. 2).

Ascorbic acid plays an important role as non-enzymatic antioxidant. In the ascorbic acid-glutathione (GSH) cycle, ascorbate peroxidase (APX) catalysis the first step of the hydrogen peroxide (H_2O_2) scavenging pathway by oxidizing AsA and producing monodehydroascorbate (MDHA) which can dismutate spontaneously to either AsA or DHA, or be enzymatically reduced to AsA by GSH-dependent DHA reductase. Oms-Oliu et al. (2010) demonstrated that PL treatment of intermediary fluence (4.8 J cm^{-2}) conserved the total vitamin C content of fresh-cut mushroom. When fresh-cut cantaloupes were subjected to PL treatment, lower fluence (2.7 and 7.8 J cm^{-2}) treatments maintained total ascorbic acid content, while at higher fluence (11.7 and 15.6 J cm^{-2}), it was significantly reduced (Koh et al. 2016). The authors credited this to thermal damage. In contrast, a previous study showed that PL treatment (8 J cm^{-2}) had no significant effect on vitamin C content of fresh-cut “Kent” mangoes (Charles et al. 2013).

The total antioxidant activity (TAA) was significantly ($P < 0.05$) affected by both storage time and PI treatments (Fig. 4f). TAA increased after the first day for all

treatments and continued to increase in 4P-treated mangoes to $144.9 \mu\text{mol TE } 100 \text{ g}^{-1} \text{ FM}$, which was significantly higher than control. In this work (data not show; $P < 0.05$), it was found a low positive correlation between TAA and ascorbic acid ($r^2 = 0.302$). According to other published studies, the total antioxidant activity of mangoes is positively correlated to ascorbic acid, but it is mainly attributed to phenolic compounds (Ma et al. 2011; Palafox-Carlos et al. 2012; Ibarra-Garza et al. 2015). Over the last years, the antioxidant activity has become an important parameter for evaluation of food quality, therefore several researches have focused on UV-C influence on antioxidant metabolism in fruits, suggesting the increase in TAA by UV-C treatment was due to a combined effect of irradiation-induced stress and storage (González-Aguilar et al. 2010; Shen et al. 2013).

In this study, we observed that fragmenting the PL dose as one pulse per day during 4 days (2.80 J cm^{-2}) had a completely different impact on quality of fresh-cut mangoes, when compared to of 4 successive pulses (2.80 J cm^{-2}). Once the best results (total carotenoids, color parameters, firmness, SS and TAA) were induced by 4 successive pulses, these results indicate that PL mode of application is more important than fluence or final dose received by fresh-cut mangoes. Moreover, the response of fresh-cut mangoes to PL was systemic, i.e., the fact of treating only one face of the mango cube does not impede the effects from being transmitted to the whole cube.

Conclusion

Our observations confirm that PL can substantially impact quality criteria of fresh-cut mangoes ‘Tommy Atkins’ after harvest, notably the contents of health-promoting phytochemicals and the antioxidant activity. However, there are important differences according to PL mode of application. Our observations indicate that 4 pulses of 0.7 J cm^{-2} each, delivered in short succession are the most effective for maintenance of firmness, color, and soluble solids and total carotenoids contents, and for increasing the antioxidant activity after 7 days of storage at 6°C . Considering the different effects observed with single pulses repeated over a period of 4 days, it would be interesting to test additional combinations, for instance 4 pulses in 2 days. Moreover, it would certainly be useful in the future to use approaches of transcriptomics and metabolomics to unravel the mysteries of PL effects and give a more solid basis to techniques of PL treatment after harvest.

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