PAPER

EFFECT OF PULSED LIGHT ON SELECTED PROPERTIES OF CUT APPLE

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ABSTRACT

The effect of pulsed light (0, 8.8 and 17.5 J cm^{-2}) on selected properties of cut apple (thermal power, oxygen consumption, volatile compounds, colour and firmness) was investigated during storage at 30 °C for up to 6 days. Samples exposed to pulsed light showed lower heat production due to a decrease in tissue respiration. Pulsed light treated samples also showed different evolution of volatile compounds (ethanol, acetaldehyde and ethyl acetate), browning and tissue softening. Modification of tissue metabolisms by pulsed light could be exploited in the processing of fresh-cut fruit and vegetables leading to advantages well beyond its germicidal activity.

Keywords: fresh-cut, isothermal microcalorimetry, light, respiration, volatile

1. INTRODUCTION

Pulsed light processing consists in exposing food to consecutive intense flashes of a radiation with a spectrum similar to that of the sun, including not only ultraviolet light but also visible and infrared radiation (170-2600 nm) (MORARU and UESUGI, 2009; FALGUERA *et al*., 2011). Because of its germicidal effect, pulsed light has been investigated as an interesting technological approach to decontaminate the surface of fresh-cut fruits and extend their shelf life (GÒMEZ-LÒPEZ *et al*., 2007; OMS-OLIU *et al*., 2010; RAMOS-VILLARROEL *et al*., 2011; GÒMEZ *et al*., 2012). The antimicrobial efficacy of pulsed light has been attributed to localized photothermal and photophysical effects but especially to the capability of its UV light component to modify the structure of biomolecules (WEKHOF, 2000; TAKESHITA *et al*., 2003; WANG *et al*., 2005; KRISHNAMURTHY *et al*., 2008). Pulsed light has actually the potential of modifying protein conformation and configuration, leading to significant modification in their biologic activity (MANZOCCO, 2015). These changes promote the death of microorganisms but also the inhibition of oxidative enzymes (GÒMEZ *et al*., 2012; MANZOCCO *et al*., 2013a; 2013b; IGNAT *et al*., 2014). It is thus likely that pulsed light could modulate the metabolic activity of vegetable tissues following adaptation mechanisms to the light stressor (LUCKEY, 1980). A circumstantial evidence supporting this hypothesis is the well-known physiological response of plant tissue to UV-C light, which is part of the pulsed light spectra. Common response to UV-C light in plants actually involves: (i) enhancement of biosynthesis of phenols toxic to pathogens; (ii) reduced ethylene production, and (iii) development of antisenescent putrescine exerting opposite physiological effects to ethylene (STEVENS *et al*., 1998; MAHARAJ *et al*., 1999). Physical effects could also be expected since UV-C light actually promotes moisture redistribution in the plant tissue due to local thermal effects deriving from electron excitation of food components (MANZOCCO *et al*., 2011a). It has been hypothesised that modification of water availability could further modify tissue metabolic activity (MANZOCCO *et al*., 2011b). Nevertheless, limited information is available about effects of pulsed light other than the germicidal one.
The aim of the present work was to evaluate the effect of pulsed light on some metabolism-related properties of cut apple. In particular, Golden Delicious apple tissues were submitted to pulsed light in a fluence range typically applied to inactivate microorganisms and inhibit enzymatic browning (up to 17.5 J cm^{-2}). Samples were then analysed for heat production, oxygen consumption, carbon dioxide formation, evolution of volatile compounds, colour and firmness during storage at 30 °C.

2. MATERIALS AND METHODS

2.1. Sample preparation

Golden delicious apples were purchased at the local market. Fruits were washed with water and rinsed. Apples were cored using a perforated cylinder having 4 mm internal diameter and 50 mm length. The extremities of the cored apple tissue were discarded to get a 35 mm long cylinder of apple mesocarp. A cylindrical shape was chosen to guarantee homogeneous exposure of sample surface to pulsed light and allow sample introduction in the microcalorimetry vials.

2.2 Pulsed light treatments

Pulsed light treatments were carried out at room temperature by using a pulsed light mobile decontamination unit (Claranor, Avignon, France) equipped with 4 xenon lamps with maximum emission in the range 200-1000 nm (200-400 nm: 41%; 400-700 nm: 51%; 700-1000 nm: 8%). Lamps were positioned at each side of a quartz plaque held in the centre of the cube shaped chamber. Two lamps were symmetrically positioned above and below the apple cylinder at a distance of 1 cm. Two lamps were symmetrically positioned at the lateral side of the apple cylinder at 1 cm distance from the quartz plaque, which corresponded to 3 cm from the sample. Apple cylinders were thus individually exposed to increasing light fluence up to 17.50 J cm^{-2}, by means of increasing number of light pulses. According to the manufacturer's instructions, each pulse delivered to the sample a light fluence of 1.75 J cm^{-2}. Pulse duration was 0.50 μs and repetition rate was 0.50 Hz.
Treated apple cylinders were individually introduced in 1.2 mL capacity vials (Lab Logistic Group GmbH, Meckenheim, Germany), hermetically sealed in the presence of air with butyl septa and metallic caps (Lab Logistic Group GmbH, Meckenheim, Germany), and stored for increasing time up to 6 days at 30 °C (Climacell 222, MMM Group, Gräfelfing, Germany). This temperature was chosen to emphasise metabolic activity of apple tissue.
Analogous sample not exposed to pulsed light were prepared as control.

2.3. Temperature

Temperature was measured by a Testo 805 pyrometer (Testo, Settimo Milanese, Italy). Sample temperature was measured immediately after the pulsed light treatment. Interval time between the end of the pulsed light treatment and sample temperature measurement was less than 10 s.

2.4. Isothermal microcalorimetry

Isothermal calorimetry was performed by a multichannel microcalorimeter (TAM III Air isothermal calorimetry, TA instruments, New Castle, Delaware, USA) equipped with an oil bath thermostat (accuracy 0.0001 °C) operating through a Peltier element. The instrument has a sensitivity of ± 100 nW and allows accurate maintenance of temperature in the 25 ± 5 °C temperature range. Vials containing the apple cylinders were placed in the calorimeter and isothermal traces recorded at 30 °C for 5 days. The thermal profile was expressed as W g^{-1} by normalising the heat flux (W) of each sample based on the sample weight.

2.5 Firmness

Firmness was measured by Warner-Blatzler shear test using an Instron 4301 (Instron LTD. High Wycombe, United Kingdom). The instrumental settings and operations were accomplished using the software Automated Materials Testing System (version 5, Series IX, Instron LTD, High Wycombe, United Kingdom). The blade was lowered into the apple sample perpendicularly to the cylinders at a speed of 5 cm/min. Force was measured over time and sample firmness was taken as the force (kN) required to shear apple cylinders.

2.6 Image analysis

Images of apple cylinders were acquired by using an image acquisition cabinet (Immagini & Computer, Bareggio, Italy) equipped with a digital camera (EOS 550D, Canon, Milan, Italy). In particular, the digital camera was placed on an adjustable stand positioned 60 cm above a black cardboard base where the apple sample was placed. Light was provided by 4 100 W frosted photographic floodlights, in a position allowing minimum shadow and glare. Other camera settings were: shutter time 1/125 s, F-Number F/6.0, focal length 60 mm. Images were saved in *jpeg* format. Image-Pro® Plus (ver. 6.3, Media Cybernetics, Inc., Bethesda, MD, USA) was used to analyzed apple browning. Brown pixels in the apple images presented $103 < R < 194$, $76 < G < 188$, $5 < B < 100$. Browning was defined as the percentage ratio between brown pixels and pixels corresponding to the apple area.

2.7 Gas chromatographic analyses

A Fisons 8000 Series gas chromatogram, equipped with a thermal conductivity detector Fisons HWD (both from Fisons Instruments, Milan, Italy), was used for analysis of oxygen and carbon dioxide in the headspace of vials containing apple cylinders. Compounds were separated on two glass columns (2 m x 2 mm i.d.), packed with Porapaqs (80/100 mesh), in isothermal conditions (column temperature 70 °C). Carrier gas was nitrogen, at a flow rate of 27 ml min^{-1}; injector and detector temperatures were 180 and 120 °C respectively. The temperature of the filament was 170 °C. Samples were equilibrated at 25 °C before injection; then, 200 μL of headspace was sampled by a 500 μL gastight manual syringe (Dynatech, Batonrouge, Lousiana, USA) and immediately injected in the GC system.
Capillary gas chromatography (GC) and solid-phase microextraction (SPME) were used for the analysis of low-boiling volatile compounds in the headspace of the vials containing apple cylinders. The fiber used was a 1 cm 85 μm Carboxen/PDMS (Supelco, Bellefonte, PA, USA). SPME was run for 2 min at 25 °C; vials were preliminary equilibrated at the operating temperature for 15 min before microextraction. GC injection was carried out in split mode (split ratio 1:10) and the fiber remained in the injector for 1 min. The GC system was a HRGC 8560 Mega Series 2 gas chromatograph (Carlo Erba, Milan, Italy), equipped with a flame ionisation detector (FID); carrier gas was helium, at a linear flow rate of 35 cm s^{-1}. Compounds were separated on a J&W DB-Wax capillary column (30 m x 0.25 mm i.d., 0.25 μm film thickness), purchased from Agilent Technologies Inc. (Santa Clara, CA, USA). The column temperature was programmed as follows: 35 °C for 2 min, then at 4 °C min^{-1} up to 44 °C; temperature was then further increased at 20 °C min^{-1} up to 240 °C, held for 15 min. Injector and detector temperature were set at 250 and 240 °C respectively. The concentration of the volatile compounds was expressed in absolute area units. The identity of the peaks was confirmed by comparing their retention time with those of standard compounds; all the standards were from Sigma-Aldrich (St. Louis, MO, USA).

2.8 Statistical analysis

Analyses were performed on at least duplicated samples. Colour and firmness analyses were performed on at least 8 duplicated samples. Results are reported as mean value ± SD. Pearson correlation analysis was performed by using Statistica for Windows (ver. 5.1, Statsoft Inc., Tulsa, USA, 1997). A p-value >0.05 was set as a statistical threshold for significance.

3. RESULTS AND DISCUSSION

Apple cylinders were submitted to pulse light treatments with increasing fluence at environmental temperature. After the treatment, the temperature of the apple cylinder surface never exceeded 30 °C. The effect of pulsed light was initially monitored by isothermal microcalorimetry. This technique was chosen since the evaluation of heat production provides a direct indication of the metabolic responses of raw materials, such as respiration and reaction to wounding stress (CRIDDLE *et al*., 1991; GÒMEZ GALINDO *et al*., 2005; WADSÖ and GÒMEZ GALINDO, 2009; ROCCULI *et al*., 2012). Apple tissue was thus exposed to increasing fluence of pulsed light and evaluated for heat production under isothermal conditions at 30 °C for up to 6 days (Fig. 1).

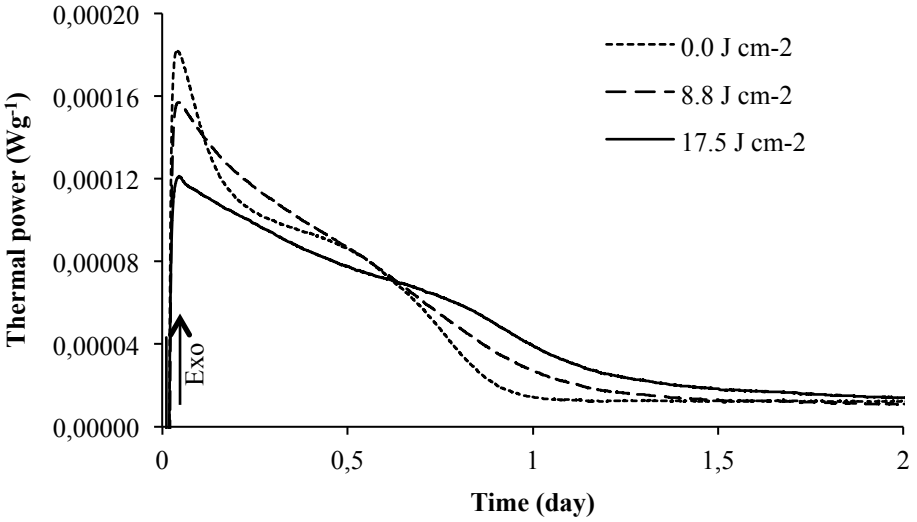

Figure 1. Calorimetric traces at 30 °C of apple tissue exposed to pulsed light with increasing fluence.

Independently on the fluence of the pulsed light treatment, calorimetric traces of all samples showed a main exothermal peak within 1-2 days of observation. Prolonging observation time, no further changes in calorimetric traces were detected (data not shown).

According to the literature, the exothermal peak (Fig. 1) should be related to the thermal effects of microbial growth and/or metabolic activity of the apple tissue (RIVA *et al*., 2001; GÓMEZ GALINDO *et al*., 2005). However, calorimetric peaks due to microbial growth are generally preceded by a plateau signal corresponding to the lag phase of micro-organisms. As clearly shown in Figure 1, no initial plateau signal was detected, suggesting the peak to be mainly attributable to heat generated by the respiration process of wounded apple tissue. It can be inferred that the hypoxic conditions that are quickly generated inside the vial upon apple tissue respiration may inhibit microbial growth making its contribution negligible as compared to that of tissue metabolism.

The calorimetric trace of the control untreated apple showed the occurrence of a sharp and narrow peak (Fig. 1). The peak shoulder could be explained considering the occurrence of physiologic activities with different metabolic rate depending on the storage period. After 1 day of storage at 30 °C, the calorimetric signal reached a plateau value. The latter was above the starting base line and indicated that beyond this storage time, there was still a slight but constant residual metabolic activity. When apple samples were exposed to

pulsed light with increasing fluence, the exothermal peak showed a progressively lower intensity and appeared broader. In addition, the plateau was reached in longer times and its level was lower than in the control sample. These results indicate that pulsed light treatment modifies tissue metabolism. To this regard, the UV-C light component of pulsed light was reported to reduce ethylene production in fresh tomato, favouring the accumulation of putrescine, an anti-senescence agent with an opposite physiological effect with respect to ethylene and delayed the appearance of the climacteric peak (MAHARAJ *et al*., 1999). A similar effect of UV-C radiation has been also reported for other climacteric fruits, such as apples and peaches (LU *et al*., 1991).
To verify the effect of pulsed light on apple tissue respiration, samples were stored for increasing time under the same temperature adopted during the calorimetric analysis (30 °C) and evaluated for oxygen consumption and carbon dioxide formation (Fig. 2).

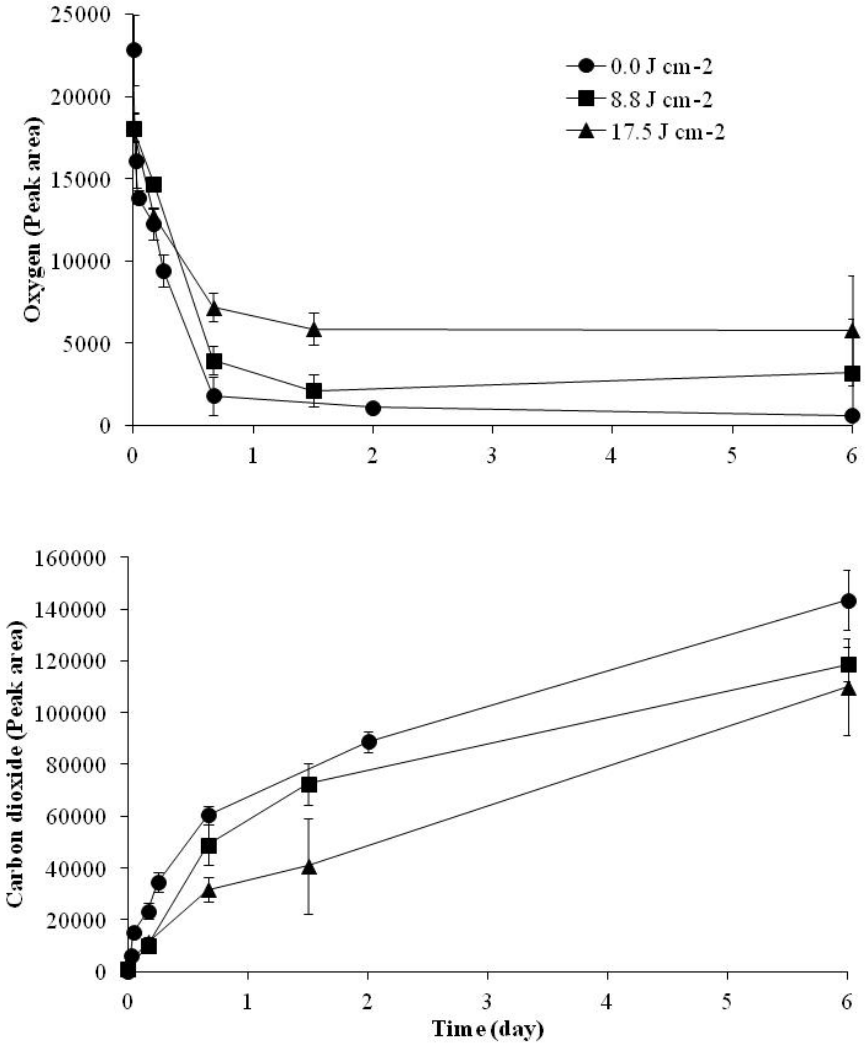

Figure 2. Headspace oxygen and carbon dioxide during storage at 30 °C of apple tissue exposed to increasing fluence of pulsed light.

Headspace oxygen was rapidly consumed during the first days of cut apple storage while carbon dioxide concomitantly accumulated following the typical gas evolution of plant

tissues performing a respiration metabolism. The respiration-driven oxygen consumption and formation of carbon dioxide resulted progressively less intense as the fluence of the pulsed light treatment was increased. This result apparently contradicts with the negligible effect of pulsed light on respiration of endive salad and mung bean sprouts (KRAMER *et al*., 2015). Contradictory results were also reported by RAMOS-VILLARROEL *et al*. (2011) with reference to fresh-cut avocado. These authors showed that pulsed light treatment reduced oxygen consumption but increased respiration products such as carbon dioxide and ethanol. Additional information can be obtained considering the plant respiration effects of UV-C light, which is an important component of the pulsed light radiation. UV-C light was reported to reduce respiration rates of fresh cut cantaloupe melon and intact Fuji apples (LAMIKANRA *et al*., 2005; YUJUAN *et al*., 2015). It can be hypothesised that the different effects of pulsed light on respiration rate may be strongly dependent not only on the nature of the plant matrix and its peculiar respiration metabolism but also on the spectral composition of the light radiation.

The progressive decrease of oxygen partial pressure in the sample headspace (Fig. 2) is well known to be associated to hypoxic conditions, leading to accumulation of ethanol and acetaldehyde involved in post harvest maturation and flavour development (DIXON and HEWETT, 2000; PESIS, 2005). Samples were thus analysed for ethanol, acetaldehyde and ethyl acetate (Fig. 3).

The latter was taken as an example of volatile compound that is expected to play a role in the flavour of fresh-cut apple derivatives (SONG and BANGERTH, 1996; DIXON and HEWETT, 2000). Gas chromatographic analyses were only performed on samples stored up to 2 days of storage due to the minimum metabolic activity on further storage (Figure 1 and 2). Pulsed light treated apple tissues showed significantly lower amounts of ethanol and higher values of acetaldehyde than the control sample (Fig. 3). In addition, when pulsed light was applied at the highest fluence (17.5 J cm^{-2}), it also modified the evolution of ethyl acetate (Fig. 3). As reported in the literature, piruvate is converted to acetaldehyde and CO_2 by the enzyme pyruvate decarboxilase, and acetaldehyde is reduced to ethanol by the enzyme alcohol dehydrogenase (MATHEWS and VAN HOLDE, 1996). Esterification of alcohols, although not fully understood in its biosynthetic pathway, is then responsible for the formation of esters contributing to apple flavour during maturation (DIXON and HEWETT, 2000). Data shown in Figure 3 suggest that exposure to pulsed light radiation could modulate the activity of the enzymes of the anaerobic biosynthetic pathway for the formation of acetaldehyde, ethanol and esters. To this regard, light radiation is known to be absorbed by enzyme proteins due to the presence of endogenous chromophores within their structure (DAVIES and TRUSCOTT, 2001). As a consequence, light would modify the structure of enzymes leading both to their activation or inactivation (MANZOCCO *et al*., 2009). Results acquired in this experimentation indicate that pulsed light is able to modify the metabolic response of apple tissue to the wounding stress, modulating the kinetics of respiration and volatile formation (Figs. 2 and 3). These phenomena would be probably the result of a combination of different biological activities that are concomitantly monitored when measuring the thermal power of the sample (Fig. 1). In order to verify the capability of isothermal calorimetry to study the metabolic consequences of pulsed light treatment, correlation analysis was performed. Table 1 shows the correlation coefficients between thermal power and analytical parameters used to monitor respiration and volatile formation, during storage at 30 °C of cut apple exposed to increasing fluence of pulsed light.

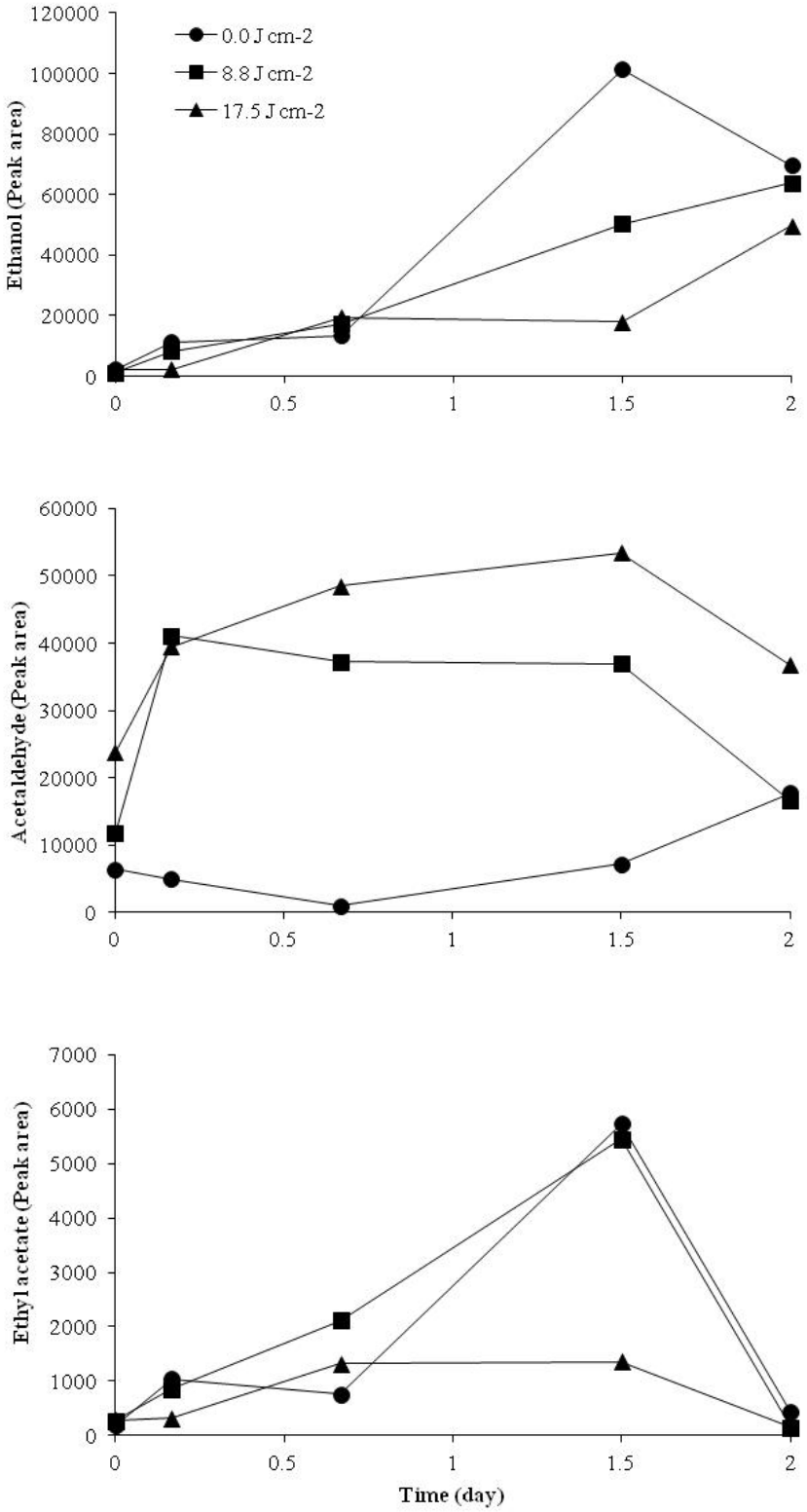

Figure 3. Headspace ethanol, acetaldehyde and ethyl acetate during storage at 30 °C of apple tissue exposed to increasing fluence of pulsed light.

Table 1. Correlation coefficients between thermal power and indices of respiration and volatile formation of cut apple exposed to increasing fluence of pulsed light and stored for increasing time at 30 °C.

	Thermal power
Oxygen	0.77[a]
Carbon dioxide	-0.73 [a]
Ethanol	-0.77 [a]
Acetaldehyde	0.05
Ethyl acetate	-0.32

[a] significant at $p < 0.05$

A very low correlation of thermal power with acetaldehyde and ethyl acetate formation was observed. This can be attributed to the fact that the evolution of these parameters is quite complex, being affected by a number of different concomitant and consequent reaction pathways. By contrast, a high correlation between changes in thermal power and oxygen, carbon dioxide and ethanol was detected. This result definitely indicates that the evolution of the calorimetric signal (Fig. 1) mainly accounts for the metabolic phenomena associated with the respiration process of apple tissue (GÓMEZ GALINDO *et al*., 2005).
The effects of pulsed light on respiration rate could be associated to modification of the metabolism pathways leading to tissue browning and softening. To verify this hypothesis, apple samples exposed to pulsed light were evaluated for the development of browning and the change in firmness. The effect of increasing pulsed light fluence on the percentage of brown colour on the surface of cut apple during storage is shown in Fig. 4.

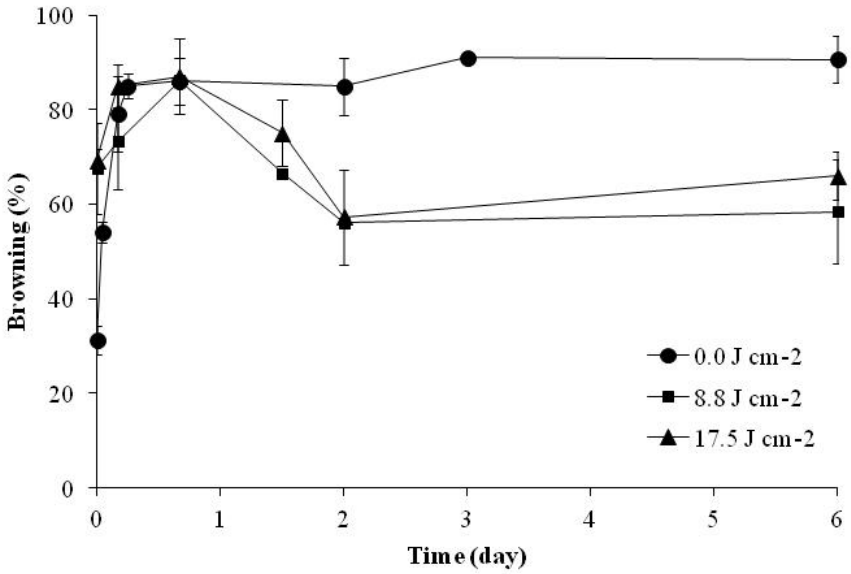

Figure 4. Browning of cut apple exposed to increasing fluence of pulsed light and stored at 30 °C.

The control sample showed a quick development of enzymatic browning during the first hours after cutting (SAPERS and DOUGLAS, 1987). No significant further colour changes were detected when the sample was maintained at 30 °C for up to 6 days. Apple samples exposed to pulsed light appeared browner just after their preparation (0 days). This is in

agreement with the enhancement of phenol biosynthesis as a common response of plant tissue to UV-C light (STEVENS *et al*., 1998; MAHARAJ *et al*., 1999). After 16 hours of storage, pulsed light treated samples showed a browning level analogous to that of the control sample. A significant decrease in browning was observed when pulsed light samples were stored beyond one day. This sample whitening could be attributed to the modification of the phenolic metabolism controlling the formation of brown polyphenols in apple tissue. However, it is not excluded that brown polyphenols could be further degraded to uncoloured compounds. A similar effect was reported as a consequence of pulsed light treatment of protein rich ingredients, such as egg white (MANZOCCO *et al*., 2013a). In that case, sample bleaching was attributed to melanoidin electronic transitions following light radiation absorption. Colour changes could also be accounted for by changes in physical structure of apple tissue. Water distribution could actually play a role in determining the overall sample colour as well as changes in apple tissue firmness. To this regard, Fig. 5 shows the evolution of firmness during storage at 30 °C of apple samples exposed to increasing fluence of pulsed light.

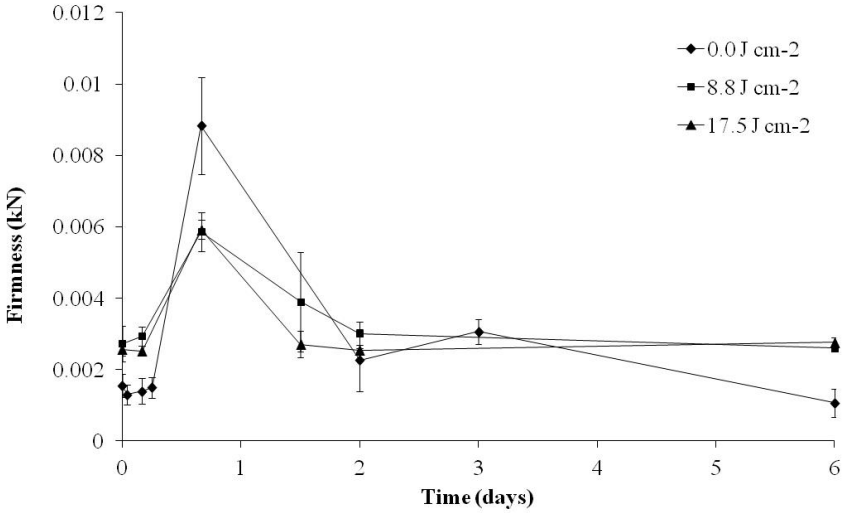

Figure 5. Firmness of cut apple exposed to increasing fluence of pulsed light and stored at 30 °C.

All samples showed an initial increase in firmness, reaching a maximum value after 16 hours of storage. Beyond this storage time, firmness decreased so that a maximum value was identified, followed by a plateau. The increase in firmness could be attributed to sample dehydration while the following decrease could account for progressive pectolityc activity (OMS-OLIU *et al*., 2010). The maximum value of firmness resulted lower in the case of the samples exposed to pulsed light. These result is in agreement with the fact that radiation can promote dehydration of a thin surface layer of the wounded plant tissue, begetting a protective film that hinders moisture migration from the inside to the environment (MANZOCCO and NICOLI, 2015).

4. CONCLUSIONS

Pulsed light has been investigated as an interesting technological approach to decontaminate the surface of fresh-cut fruits and extend their shelf life. Results reported in this work demonstrate that pulsed light exerts additional complex effects of different

metabolism-related properties of fresh-cut vegetables. In the case of apple, exposure to pulsed light modified the kinetics of respiration, volatile formation, browning and tissue softening. The possibility of pulsed light implementation in fresh-cut processing will be strictly dependent on the availability of detailed information about its consequences on tissue metabolisms, especially in relation to different temperature and atmosphere conditions during storage. These data should be merged with those relevant to the antimicrobial activity of pulsed light in order to select optimal fluence to be adopted during fruit treatment. In this context, isothermal calorimetry could represent a very useful methodological tool since potentially allowing to concomitantly quantify both metabolic and microbial activity.

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