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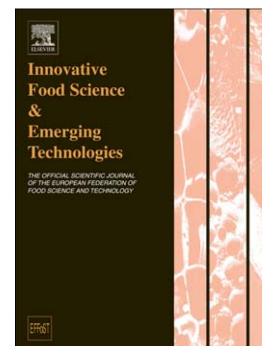

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UV-A light treatment for controlling enzymatic browning of fresh-cut fruits

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Abstract

Enzymatic browning is critical in the preservation of the quality and the shelf-life of fresh-cut fruits. Non-thermal technologies such as UV radiation are emerging for controlling polyphenol oxidase (PPO) activity, the main agent responsible for browning. After determining the best operational conditions of a UV LED illuminator ($2.43 \cdot 10^{-3}$ Wm^{-2} irradiance), the anti-browning effect of UV-A light (390 nm) treatment at 25 °C over increasing time periods up to 60 min was assessed on fresh-cut apples (Golden Delicious, Granny Smith, Fuji) and pears (Abate Fétel, Decana). Colour variation (ΔE) and its percent reduction (%RΔE) were measured using a colorimeter and the greatest effect was observed in apples which showed higher %RΔE values than pears (58% *vs* 25% after 60 min exposure, respectively). Moreover electrophoretic and zymographic techniques demonstrated the inhibitory effect of UV-A irradiation on PPO activity. Anti-browning was found to be related to irradiance, exposure time and the fruit cultivar. Overall this study confirmed UV-A LED technology as an eco-friendly alternative to traditional approaches for reducing browning of minimally processed products.

Industrial relevance: Treatment of fresh-cut products with UV-A LED is not only easy and inexpensive to produce but also presents few hazards for humans. Moreover, the use of LED light sources brings many advantages such as energy savings, device durability, low environmental impact, high luminous efficiency and little thermal effect. UV-A LED technology has great potential to meet the demands of the food industry in the processing of fresh-cut fruits and vegetables. In addition it could also be considered a pre-treatment of fruits and vegetables being processed for the production of snacks.

Keywords: UV-A light, Enzymatic browning, Polyphenol oxidase, Fresh-cut fruits, Colour

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1. Introduction

The main factors affecting the perceived quality of fresh-cut produce are texture and appearance (Rico, Martín-Diana, Barat, & Barry-Ryan, 2007; Toivonen & Brummell, 2008). There is no doubt that the first parameter which consumers take into account is appearance, an attribute which is judged on the basis of multiple factors including size, shape, form, colour, condition and absence of defects all of which can be influenced by several pre-harvest factors (Kays, 1999). In particular colour plays a key role in food choice by influencing taste thresholds, sweetness perception, food preference, pleasantness and acceptability (Clydesdale, 1993) and colour can be used both as a direct and an indirect index of quality (Francis, 1995).

While the market for fresh-cut products is increasing due to their convenience and healthfulness (Ragaert, Verbeke, Devlieghere, & Debevere, 2004), browning represents a problem for fresh-cut fruit and vegetables, especially white-fleshed fruit such as apples and pears. Browning reactions are mainly driven by polyphenol oxidase (PPO; EC 1.10.3.1), a copper-containing oxidoreductase that catalyses two different reactions involving phenolic compounds and oxygen. These reactions produce quinones that react further and lead to the accumulation of melanin and the development of brown- or black-coloured compounds depending on the specific structure of the polyphenolic substrate (Martinez & Whitaker, 1995; Seo, Sharma, & Sharma, 2003; Yoruk & Marshall, 2003; Garcia-Molina et al., 2007; Queiroz, Mendes Lopes, Fialho, & Valente-Mesquita, 2008). PPO is an ubiquitous enzyme found in plants and fungi (Vamos-vigyazo & Haard, 1981; Halaouli, Asther, Sigoillot, Hamdi, & Lomascolo, 2006; Mayer, 2006; Marusek, Trobaugh, Flurkey, & Inlow, 2006),

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bacteria (Claus & Decker, 2006), in the exoskeleton of insects and crustaceans such as shrimp (Zamorano, Martínez-Álvarez, Montero, & Gómez-Guillén, 2009) and also in the human epidermis (Kobayashi et al., 1995; Sanchez-Ferrer, Rodriguez-Lopez, Garcia-Canovas, & Garcia-Carmona, 1995; Olivares, Jimenez-Cervantes, Lozano, Solano, & Garcia-Borron, 2001). In a typical plant cell it is localized in cytoplasmic organelles like chloroplasts while its phenolic substrates are mostly in the vacuole but also in the apoplast/cell wall compartment (Toivonen & Brummell, 2008). Enzymatic browning of fruit and vegetables takes place in the presence of oxygen when polyphenolic substrates are exposed to PPO and/or phenol peroxidases as a consequence of mechanical stress caused by post-harvest handling such as brushing, peeling, cutting and crushing which lead to the breakdown of cell structure (Saltveit, 2000; Degl' Innocenti, Guidi, Pardossi, & Tognoni, 2005).

Most strategies that have been employed to control cut-edge browning have focused on physical and chemical methods to inhibit PPO activity by eliminating essential components such as oxygen, copper ion, substrate or even the enzyme itself.

With regards to chemical inhibition of browning, the data show carboxylic acids such as oxalic and oxalacetic acids, ascorbic acid derivatives such as ascorbic acid 2-phosphate, thiol-containing compounds such as cysteine, glutathione and N-acetylcysteine, phenolic acids such as kojic acid, sodium metabisulfite and 4-hexyl resorcinol have the best effects on apple slices (Son, Moon, & Lee, 2001; Eissa, Fadel, Ibrahim, Hassan, & Elrashid, 2006). The most common commercial anti-browning formulation for fresh-cut products is a mixture of calcium salts with ascorbic acid that act respectively to keep cell structure integrity and to control PPO activity (Rupasinghe, Murr, DeEll, & Odumeru,

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2005). Dipping treatments and edible coatings are the main ways to apply PPO inhibitors to fresh-cut fruit (Rojas-Graü, Soliva-Fortuny, & Martín-Belloso, 2009; Oms-Oliu et al., 2010). As reported by Lante & Zocca (2010), dipping potato slices into β-cyclodextrin improved the brightness of precooked, vacuum-packed potatoes and may be useful for other minimally-processed products.

Some other traditional anti-browning additives, such as ascorbic acid and its derivatives and sulfites, have become considered less useful because of drawbacks including low stability and potential health hazards (McEvily, Iyengar, & Otwell, 1992; Rangan & Barceloux, 2009).

Therefore there is active research into the discovery of PPO inhibitors from natural sources (Kim & Uyama, 2005; Chang, 2009; Loizzo, Tundis, & Menichini, 2012) such as dog rose and pomegranate (Zocca, Lomolino, & Lante, 2011) and other plant extracts (Wessels, Damm, Kunz, & Schulze-Kaysers, 2014) and from by-products of the agro-food industry such as *Brassicacea* processing water (Zocca, Lomolino, & Lante, 2010) and citrus hydrosols (Lante & Tinello, 2015).

Aside from conventional thermal- and chemical-based strategies to preserve cut produce there are alternative non-thermal technologies that are gaining interest. These emerging strategies can be used not only to control microbiological activity to extend the shelf-life of fresh-cut products (Morris, Brody, & Wicker, 2007; Falguera et al, 2011) but also to control enzymatic browning and thus preserve the organoleptic and nutritional qualities of produce better than conventional processes. For example, PPO activity has been shown to be effectively controlled by combined treatment with ultrasound and ascorbic acid

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(Jang & Moon, 2011), pulsed electric fields (Meneses et al., 2013), cold plasma (Surowsky, Fischer, Schlueter, & Knorr, 2013) and pulsed light (Manzocco, Panozzo, & Nicoli, 2013).

Ultraviolet light (UV) radiation is classified into three types (Bintsis, Litopoulou-Tzanetaki, & Robinson, 2000): UV-A (315-400 nm), UV-B (280-315 nm) and UV-C (100-280 nm). The latter is recognized as a simple way to destroy most microorganisms in whole and fresh-cut produce (Allende & Artés, 2003; Allende, Tomas-Barberan, & Gil, 2006; Birmpa, Sfika, & Vantarakis, 2013). Indeed several studies have confirmed its potential as PPO inhibitor in model systems and apple derivatives (Manzocco, Quarta, & Dri, 2009), in fresh apple juices (Falguera, Pagán, & Ibarz, 2011) and in mushroom extracts (Sampedro & Fan, 2014). Unfortunately, the potential for UV-C treatment is limited because of possible adverse effects in food including the alteration of sensory quality attributes such as colour (Refsgaard, Rasmussen, & Skibsted, 1993; Manzocco, Kravina, Calligaris, & Nicoli, 2008), the reduction of antioxidant capacity (Li et al., 2014) and the formation of furan recognized by the WHO as a potential human carcinogen (Fan & Geveke, 2007; Bule et al., 2010; Müller et al., 2013; WHO, 2011) . However, UV-C light is not the only effective range in limiting enzymatic browning. As this regard, irradiation for 120 min with a high-pressure mercury lamp of 400 W emitting UV-visible light between 250 and 740 nm (maximum power of emission from 400-450 nm) effectively inactivated PPO in juices from both apples (Falguera, Pagán, & Ibarz, 2011) and pears (Falguera, Garvín, Garza, Pagán, & Ibarz, 2014). Furthermore the treatments did not induce variations in pH, formol index and the contents of soluble solids, total phenolics and sugars. Kwak, Lee, Murata, & Homma (2004) hypothesized the mechanism of action for the anti-browning effect of UV-visible treatment might stem from the degradation of melanoidins, the polymeric brown

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compounds that result from PPO activity. Ibarz, Pagán, Panadés, & Garza (2005) corroborated the hypothesis studying the effect of UV-visible irradiation in apple, peach and lemon juices. In their study the researchers found increased brightness that was attributed to the photochemical destruction of brown pigments.

The current study was designed to investigate the effectiveness of UV-A irradiation for the control of PPO activity in fresh-cut apples and pears. There are many sources of UV light (Koutchma, 2009; Falguera, Pagán, Garza, Garvín, & Ibarz, 2011) but we chose to use LED technology because it is an inexpensive and eco-friendly source.

2. Material and methods

2.1 Sample preparation

Commercial mushroom tyrosinase (TYR, EC 1.14.18.1, 3,130 U/mg) was obtained from Sigma-Aldrich Chemie GmbH (Steinheim, Germany). Aliquots of 10 ml of TYR aqueous solution (1 mg/ml, 3,130 U/ml) were introduced into Petri plates with 60 mm diameter without cover (layer of 3 mm thickness) and submitted to UV-A LED treatment.

Three varieties of apples (*Malus domestica* L cv. Golden delicious, Granny Smith, Fuji) and two varieties of pears (*Pyrus communis* L cv. Abate Fétel, Decana) were purchased at commercial maturity from a local market between January and May of 2014 and stored at 4 °C. Fruits were washed under running water to remove any surface contamination, wiped with blotting paper and manually cut into two symmetrical slices of 5 mm thickness of which one was subjected to UV-A LED treatment while the other was left untreated as a control. Each fresh-cut slice was placed into a 60 mm diameter Petri plate.

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2.2 UV-A LED illuminator prototype

A prototypic UV-A LED illuminator was designed in order to study the effect of UV-A light (390 nm) on enzymatic browning of fresh-cut fruits. The illuminator was constructed from a 50 mm diameter polyethylene tube at one end of which "pin-in-hole" LED (Light Emitting Diode; Bivar, Inc. Thomas, Irvine, California, USA) diodes with emission peaks at 390 nm and emission angles of 30° were installed (Fig. 1). The tube was placed on a support in order to adjust its distance from the fruit slices. The illuminator was shielded from visible light during treatment to avoid any interference with the external environment.

2.3 Setting of UV-A LED illuminator

Three physical parameters of prototype illuminator, the number of LED diodes, voltage and distance from sample, were assessed by evaluating the anti-browning effect of UV-A light on the surface of Golden Delicious apple slices.

In one set of experiments two illuminator levels (with 9 (L9) or 30 (L30) LED diodes) were tested at three different voltages (10 (V10), 15 (V15), and 20 (V20) volts) at a constant distance of 0.5 cm from the sample. In a second set of experiments the L30 illuminator was placed at four different distances from the surface of the apple slices (5, 3, 1, and 0.5 cm) at a constant voltage of 20 V.

The UV-A light treatments were carried out in triplicate at 25 °C over increasing time periods up to 60 min. The irradiance of both illuminators was measured using the radiometer ILT IL-1700 (International Light Technology, MA, USA) equipped with a UV-

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A light probe at the research center Plast-optica (Amaro, Udine, Italy) and was expressed as Wm^{-2}.

2.4 Irradiation of fresh-cut fruits

After determining the best operational conditions for UV-A LED treatment (the L30 illuminator at 20 V set 0.5 cm from the slices with an irradiance of $2.43 \cdot 10^{-3}$ Wm^{-2}) the fresh-cut apple and pear slices were exposed to UV-A light irradiation in triplicate at 25 °C for increasing time periods up to 60 min. Moreover, the anti-browning effect of UV-A LED treatment for 30 min at 25°C on Golden Delicious apples slices was compared in the same experimental conditions with other chemical treatments. In details, an aqueous solution of 1% (w/v) ascorbic acid and 0.5% (w/v) calcium chloride (AAC) as suggested by Soliva-Fortuny, Oms-Oliu, & Martín-Belloso (2002) and 6% (w/v) NatureSeal® (AS1, AgriCoat NatureSeal, Berkshire, England) were applied in accordance to Zocca, Lomolino & Lante (2011).

2.5 Temperature

The surface temperature of the fruit slices was measured before and after irradiation by a thermocouple probe BABUC/M (LSI LASTEM, Settala, Premenugo, Milan, Italy).

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2.6 Colour measurement

Colour analyses on the sliced surfaces of fresh-cut fruits subjected to UV-A LED treatment and their corresponding controls were carried out using a tristimulus colorimeter (Chroma Meter CR-410, Konica-Minolta, Milan, Italy) in the CIE 1976 (L*,a*,b*) colour space. The instrument was standardized against a white tile before measurements. Colour differences (ΔE), calculated as the Euclidean distance between two points in the three-dimensional space defined by L*, a* and b*, were used to estimate the anti-browning effect of UV-A LED treatment and were expressed according to the following equation:

$$\Delta E = \sqrt{(\Delta L^2 + \Delta\alpha^2 + \Delta b^2)^2} = \sqrt{(L_t - L_{t0})^2 + (\alpha_t - \alpha_{t0})^2 + (b_t - b_{t0})^2} \quad (1)$$

where L = lightness (100 for white to 0 for black), a = red when positive and green when negative, b = yellow when positive and blue when negative, t = exposure time of UV-A light treatment, and t_0 = initial time of UV-A light treatment.

The percent reduction (%RΔE) in colour differences was also used to evaluate the anti-browning potential and was calculated as follows:

$$\%R\Delta E = [\ (\Delta E_{control} - \Delta E_{treatment}) / \Delta E_{control}\ ] \times 100 \quad (2)$$

where $\Delta E_{control}$ = colour differences of samples not subjected to UV-A LED treatment, $\Delta E_{treatment}$ = colour differences of samples subjected to UV-A LED treatment.

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2.5 PPO zymography

The inhibitory effect of UV-A LED treatment at the best operational conditions (the L30 illuminator at 20 V set 0.5 cm from the slices with an irradiance of $2.43 \cdot 10^{-3}$ Wm^{-2}) on PPO activity was evaluated through electrophoretic and zymographic techniques (Zocca, Lomolino & Lante, 2011) after irradiating, at 25 °C for 30 min, TYR solutions and Golden Delicious apple slices. Apple PPO was extracted by blending fruit slices with an aqueous solution (1:1) containing 1% (w/v) PVPP and 0.5% (w/v) Triton X-100 (Weemaes, Ludikhuyze, Van den Broeck, Hendrickx, & Tobback, 1998); then the mixture was centrifuged at 48,400 rpm for 15 min at 4°C and filtered through Whatman paper N°1 in a Bűchner funnel under vacuum. The protein content of apple extracts was determined by Bradford (1976) assay.

The electrophoretic analysis was carried out in triplicate in a Mini Protean II (Bio-Rad, Milano, Italy) at room temperature. Non-reducing SDS-PAGE was performed according to Zocca, Lomolino & Lante (2011) using 12% polyacrylamide gel at 100 V. The running buffer was composed of 25 mM Tris, 192 mM glycine and 0.1% (w/v) SDS.

Before electrophoresis, TYR and apple PPO solutions were solubilized with 700 µl of distilled water and 300 µl of Laemmli buffer (1.33 M Tris pH 7.4, 60% v/v glycerol, 8% w/v SDS; Laemmli, 1970) and centrifuged at 14,000 rpm for 2 min. Each gel lane was loaded with irradiated and untreated enzyme solutions as follows: 5 µl of tyrosinase and 15 µl of apple PPO.

After electrophoresis, the gels were exhaustively washed for 15 min in 50 ml of 0.2 M sodium acetate buffer (0.2 M sodium acetate/0.2M acetic acid) at pH 5.0 and was incubated at 25°C for 30 min with 50 ml sodium acetate buffer at pH 5.0 containing 5 mM

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L-DOPA and 3 mM MBTH (Núñez-Delicado, Serrano-Megías, Pérez-López, & López-Nicolás, 2005). The presence of deep pink bands in treated gel lanes was associated to PPO activity. The gel images were acquired using scanner.

2.7 Statistical analysis

Statistical analysis was performed by subjecting the data to one-way analysis of variance (ANOVA) using the SAS software package (Statistical Analysis System Institute Inc., 2014) after verifying normal distribution and homogeneity of variance. The average data were analyzed by the procedure PROC GLM. Significant difference was determined by Bonferroni's multiple range test ($P \leq 0.05$).

3. Results and Discussion

3.1 Optimizing the operational conditions of UV-A LED treatment

The effects of three physical parameters of the UV-A LED illuminator prototype, number of LED diodes, voltage and distance from sample, on ΔE of the surface of Golden Delicious apple slices were assessed at room temperature at different exposure times (10, 20, 30, 40, 50 and 60 min). As reported in Table 1, the effect of experimental treatment on ΔE data was significant ($P \leq 0.05$) after 30 min of irradiation. Specifically the L30V15 and L30V20 treatments yielded significant differences and the L30V20 irradiation for 60 min achieved the best performance with a %RΔE of treated apple slices (2.86 ± 0.16) of approximately 60% compared to untreated ones (6.83 ± 1.80) (Table 1).

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The L30V20 illuminator was selected for further testing and was placed at different distances between 0.5-5 cm from Golden Delicious apple slices. Table 2 shows distance had an insignificant effect on ΔE data regardless of exposure time. As reported in Table 3, the increase in the number of LED diodes and the voltage that led to a greater anti-browning effect of UV-A light treatment was associated with an increase in the irradiance of illuminator. At a distance of 0.5 cm the irradiance of the L30V20 irradiator was $2.40 \cdot 10^{-3}$ Wm^{-2}, 26 times higher than that of the L9V10 irradiator, $9.23 \cdot 10^{-5}$ Wm^{-2}. Changing the distance from 0.5 to 5 cm did not significantly affect the irradiance, hence the lack of anti-browning effect.

Thus the best experimental conditions of UV-A LED treatment were: illuminator L30, V20 voltage, 0.5 cm distance from sample and $2.43 \cdot 10^{-3}$ Wm^{-2} irradiance. Fig. 2 shows the ΔE of treated (L30V20) and untreated (CL30V20) Golden Delicious apple slices as function of exposure time. It is apparent that the standard deviation was higher in CL30V20 and in the first time points of L30V20 as a consequence of the variability in plant samples despite the maximum standardization of the trial. The increase over time of ΔE values of L30V20 treated samples (61% at 60 min) was less than that of CL30V20 (75% at 60 min). Overall, the %RΔE of treated samples compared to corresponding controls was 28% at 10 min, 49% at 30 min and 53% at 60 min. It is clear that the anti-browning performance of treatment was related to exposure time nearly all the reduction in colour change was seen already after 30 min.

In our study the effectiveness of UV-A LED treatment was a function mainly of increasing irradiance and exposure time, and these results corroborate those by Manzocco, Quarta, & Dri (2009) studying the effect of UV-C (15 W lamps with maximum emission at

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253.7 nm) and visible light (fluorescent tubes with maximum emission from 430-560 nm) treatments on PPO in model systems and Golden Delicious apples at 28 °C. They found the UV-C was more effective than visible light in the inactivation of PPO in an aqueous solution showing inhibitions of 40% and 100% after 60 min exposure to 3.9 Wm^{-2} and 13.8 Wm^{-2} irradiance respectively. Contrastingly the visible light treatment, where exposure time was in the order of hours, was effective only at high doses (12.7 Wm^{-2}) and in fact lower irradiances (11.7 and 9.4 Wm^{-2}) caused an initial PPO activation and only inactivation with increasing exposure time. The PPO inactivation via visible and UV light exposure was associated with direct photo-oxidation arising from the absorption of light by amino acid residues (Trp, Tyr, His, Phe, Met, Cys), the resulting protein denaturation and the formation of high molecular weight aggregates (Davies & Truscott, 2001; Davies, 2003; Lante, Tinello, & Lomolino, 2013) analyzed by HPLC gel permeation by Manzocco, Quarta, & Dri (2009).

The inhibitory effect of UV-A LED treatment on PPO activity was confirmed using non reducing SDS-PAGE electrophoresis and zymographic analysis in order respectively to isolate enzymatic isoforms and visualize the appearance of coloured bands in the gel lanes as indicator of enzyme activity (Fig.3). The UV-A LED irradiation at the best experimental conditions fixed previously was carried out at room temperature for 30 min on a model solution of TYR and on Golden Delicious apple slices subjected to subsequent PPO extraction. The zymograms of TYR (Fig.3A) and apple PPO (Fig.3B) showed only one enzymatic isoform whose activity decreased after irradiation reducing significantly the colour intensity of corresponding band (UV) in comparison to the untreated control (C).

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3.2 UV-A LED treatment of fresh-cut apples and pears

The UV-A LED treatment was applied for 60 min to fresh-cut slices obtained from 3 apple cultivars (Golden Delicious, Fuji, Granny Smith) and 2 pear cultivars (Abate, Decana). As reported in Fig. 4 the effect of fruit type on %RΔE data was statistically significant regardless of the exposure time ($P \leq 0.001$). In particular the apple results (63.6 ± 5.2 % for Fuji, 58.4 ± 2.9 % for Golden Delicious, and 52.8 ± 0.8 % for Granny Smith after 60 min irradiation) differed from those of fresh-cut pears (26.3 ± 3.4 % for Abate and 22.8 ± 4.7 % for Decana after 60 min irradiation) and the higher %RΔE values reflect a stronger anti-browning effect. Of the fresh-cut apples, the Fuji and Golden Delicious were most responsive to treatment and reached half %RΔE after only 20 min exposure (52.3 ± 7.0 and 49.3 ± 4.0, respectively). The effectiveness of UV-A LED treatment has already been demonstrated among several fruit cultivars by Falguera, Pagán, & Ibarz, (2011) and Falguera, Garvín, Garza, Pagán, & Ibarz (2014). These studies showed PPO was inactivated at different rates in various fresh apple and pear juices that were subjected to UV-visible treatment for 120 min with a high-pressure mercury lamp of 400 W which was placed at 22.5 cm distance from juice surface and emitted in a range between 250 and 740 nm with a resulting incident energy of $3.88 \cdot 10^{-7}$ E·min^{-1}.

The anti-browning effect of UV-A on Golden Delicious apple slices was also compared with other chemical treatments. As reported in Fig. 5, L30V20 illuminator showed similar performance to AAC by decreasing significantly ($P \leq 0.001$) the colour change of apple slice surface up to 60% after 30 min irradiation (Fig. 5). Moreover the inhibitory potential of UV-A LED treatment was stronger than 6% (w/v) AS1 commercial formulation whose efficacy in preventing the browning of fresh-cut fruits was demonstrated

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by several authors (Abbott, Saftner, Gross, Vinyard, & Janick, 2004; Toivonen, 2008; Rößle, Gormley, & Butler, 2009).

The surface temperature of the fresh-cut fruit slices was measured in order to exclude any anti-browning effects caused by the thermal denaturation of PPO. The surface temperature of treated fresh-cut fruit slices averaged 22.0± 0.6 °C before irradiation and 26.0 ± 0.6 °C after 60 min exposure time as a consequence of slight heating by the LED diodes whose temperature rose from 20.1 °C to 29.5 °C during treatment. Optimum temperature for PPO activity is a function of the plant source and variety (Yoruk & Marshall, 2003). The optimum temperature for PPO activity in the current study, which we extracted from Golden Delicious apples, was 50 °C in the presence of a catechol substrate at pH 5.0 (data not shown), a value higher than that measured in Monroe apple peels (30°C) under the same assay conditions (Zhou, Smith, & Lee, 1993). The same authors also showed that apple PPO was stable up to 40 °C, rapidly inactivated above 50 °C and completely inactivated in the range of 70-80 °C as a function of time. This finding was further confirmed by Yemenicioglu, Ozkan, & Cemeroglu, (1997) and Soysal, (2009). Ankara pear PPO, which has an optimum temperature in the range of 20-45 °C, showed a similar trend of thermal inactivation (Ziyan & Pekyardimci, 2004). On the basis of these results the non-significant temperature rise in the sliced surface of fresh-cut apples and pears subjected to UV-A LED treatment confirmed that the anti-browning effect was completely due to UV irradiation.

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4.Conclusions

The colorimetric parameter ΔE, which gives an overall evaluation of total colour change including L*,a*,b*, can be considered as a valid, indirect anti-browning index for estimating the inhibition of PPO activity in fresh-cut products. In addition, the zymographic technique is an useful tool to investigate the effect of UV-A LED treatment directly on PPO activity of fresh-cut slices. However further studies on the packaging may be carried out to discuss the effect of environmental conditions such as relative humidity, temperature and inert gas on enzymatic browning.

The application of UV-A light (390 nm) treatment using a LED illuminator, whose irradiance was set to $2.50 \cdot 10^{-3}$ Wm^{-2}, was most effective in decreasing the colour change of fresh-cut apples of approximately 60% after 60 min exposure. While UV-A light irradiation is less powerful than UV-C, browning can be effectively controlled by UV-A light without compromising the organoleptic properties or nutritional quality of fresh-cut fruits. Moreover the use of LED diodes meets the needs for energy saving and reduced environmental impact. Therefore, UV LED technology may be an eco-friendly alternative approach to traditional additives, such as sulfites, and thermal treatments that cause possible adverse effects and involve high energetic costs.

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Fig. 1

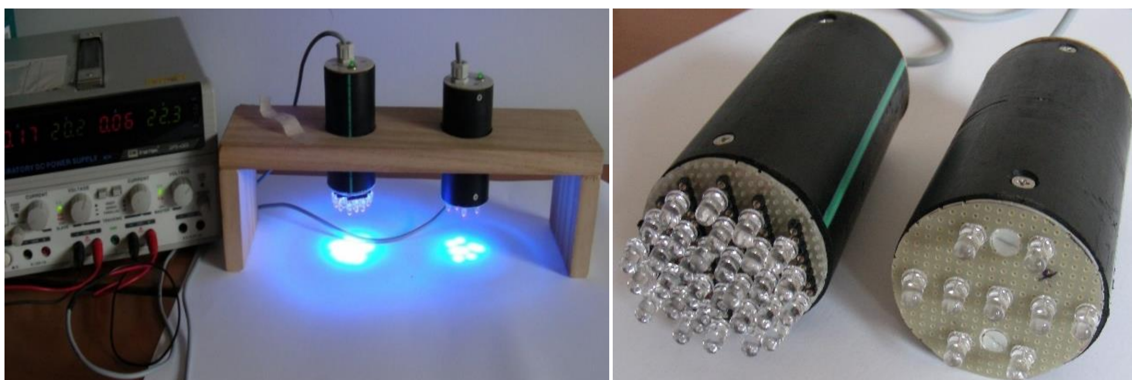

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Fig. 2

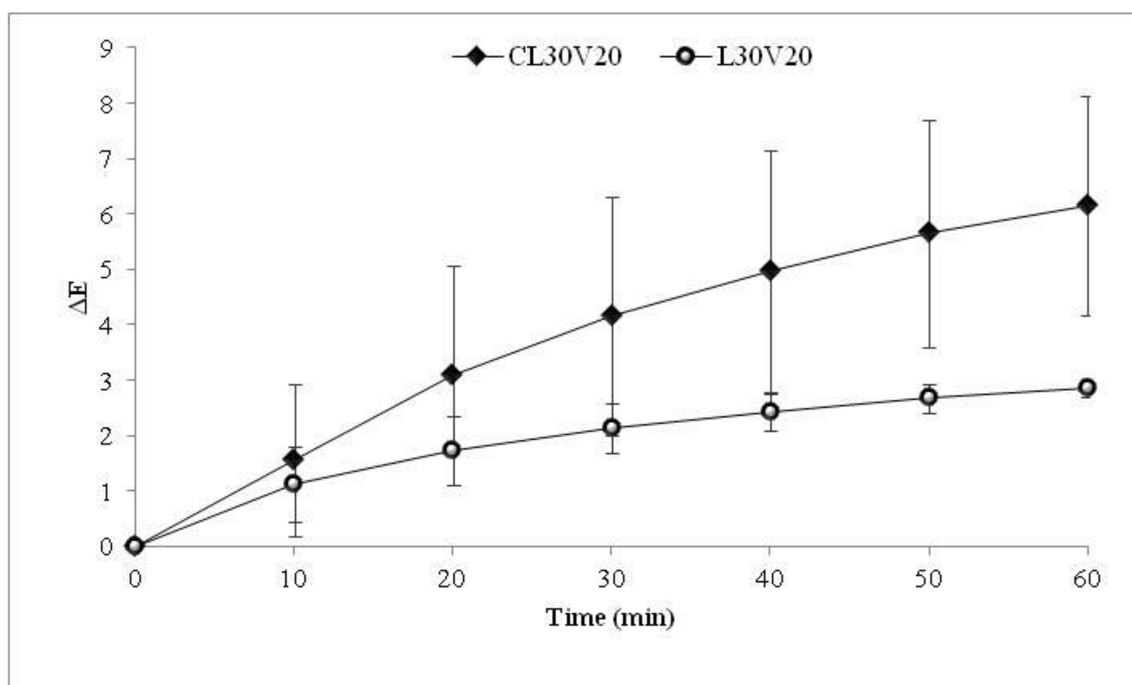

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Fig. 3

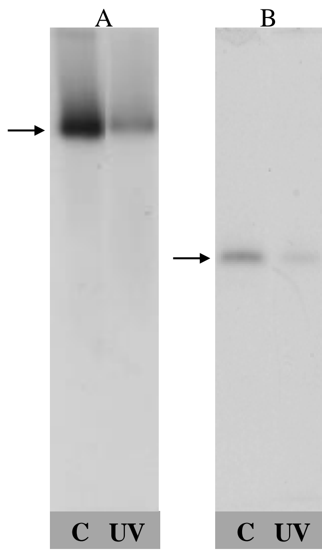

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Fig. 4

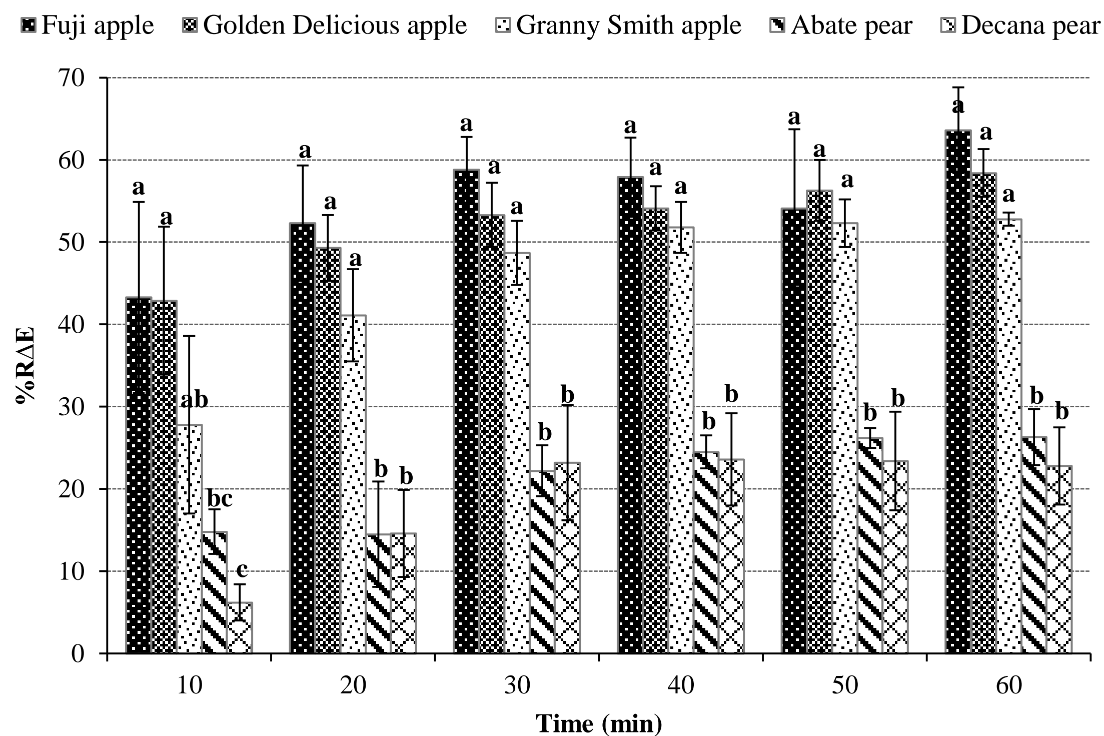

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Fig. 5

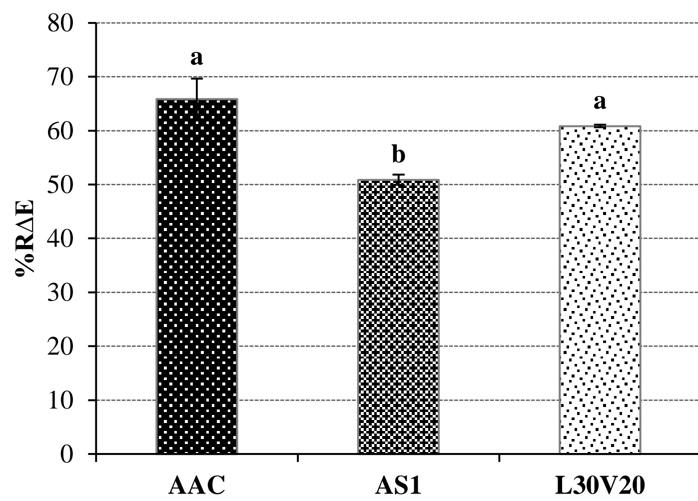

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Table 1

Time (min)	ΔE							
	Treatment [1]							
	Control	**L9V10**	**L9V15**	**L9V20**	**L30V10**	**L30V15**	**L30V20**	***P*-value [2]**
10	2.11^{a}±1.27	2.47^{a}±1.13	1.66^{a}±0.29	2.24^{a}±1.23	1.56^{a}±0.61	1.35^{a}±0.50	1.12^{a}±0.69	NS
20	3.84^{a}±1.69	4.15^{a}±1.38	3.30^{a}±0.40	3.43^{a}±1.33	2.80^{a}±0.95	2.13^{a}±0.52	1.72^{a}±0.62	NS
30	4.98^{a}±1.77	5.07^{a}±1.53	4.09^{a}±0.52	4.02^{a}±1.57	3.54^{a}±1.12	2.47^{a}±0.55	2.13^{a}±0.46	NS
40	5.79^{a}±1.82	5.66^{a}±1.64	4.68^{ab}±0.67	4.31^{ab}±1.03	3.97^{ab}±1.10	2.65^{b}±0.49	2.42^{b}±0.33	*
50	6.41^{a}±1.80	6.07^{a}±1.70	4.93^{ab}±0.78	4.52^{ab}±0.95	4.27^{ab}±1.12	2.87^{b}±0.55	2.67^{b}±0.26	*
60	6.83^{a}±1.80	6.36^{a}±1.70	5.17^{ab}±0.91	4.66^{ab}±0.82	4.48^{b}±1.11	2.90^{b}±0.47	2.86^{b}±0.16	**

[1] Control: no UV-A light treatment. L9V10: combination of 9 LEDs and 10 V. L9V15: combination of 9 LEDs and 15 V. L9V20: combination of 9 LEDs and 20 V. L3V10: combination of 30 LEDs and 10 V. L30V15: combination of 30 LEDs and 15 V. L30V20: combination of 30 LEDs and 20 V.

[2] 'NS' $P > 0.05$; '*' $P \leq 0.05$; '**' $P \leq 0.01$.

[a,b] All values were based on three different samples. All assays were done in triplicate and data are presented as means ± SD. In each sample's row, values followed by the same letter are not significantly different ($P > 0.05$), as measured by Bonferroni's multiple range test.

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Table 2

	ΔE				
	Distance (cm)				
Time (min)	**0.5**	**1**	**3**	**5**	***P*-value[1]**
10	1.12±0.69	1.25±0.28	1.23±0.44	1.11±0.50	NS
20	1.72±0.62	1.83±0.35	1.90±0.39	1.69±0.50	NS
30	2.13±0.46	2.10±0.37	2.25±0.32	1.99±0.46	NS
40	2.42±0.33	2.30±0.40	2.48±0.35	2.19±0.42	NS
50	2.67±0.26	2.41±0.36	2.63±0.33	2.36±0.38	NS
60	2.86±0.16	2.59±0.46	2.79±0.35	2.50±0.37	NS

[1] 'NS' $P > 0.05$.

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Table 3

Distance (cm)	L9[1]			L30		
	V10[2]	V15	V20	V10	V15	V20
0.5	$9.23 \cdot 10^{-5}$	$7.80 \cdot 10^{-4}$	$1.50 \cdot 10^{-3}$	$1.85 \cdot 10^{-4}$	$1.28 \cdot 10^{-3}$	$2.40 \cdot 10^{-3}$
1	$8.09 \cdot 10^{-5}$	$6.80 \cdot 10^{-4}$	$1.30 \cdot 10^{-3}$	$1.60 \cdot 10^{-4}$	$1.11 \cdot 10^{-3}$	$2.07 \cdot 10^{-3}$
3	$4.93 \cdot 10^{-5}$	$4.08 \cdot 10^{-4}$	$7.80 \cdot 10^{-4}$	$1.69 \cdot 10^{-4}$	$1.17 \cdot 10^{-3}$	$2.20 \cdot 10^{-3}$
5	$5.17 \cdot 10^{-5}$	$4.15 \cdot 10^{-4}$	$7.78 \cdot 10^{-4}$	$1.92 \cdot 10^{-4}$	$1.34 \cdot 10^{-3}$	$2.50 \cdot 10^{-3}$

[1] L9, L30 refer to UV-A light illuminators with 9 and 30 LED lamps respectively.
[2] V10, V15, V20 refer to treatment voltages of 10, 15, and 20 V respectively.

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Figure and Table captions

Fig. 1 UV-A illuminator prototypes with 9 LEDs and 30 LEDs.

Fig. 2 Colour change (ΔE) of Golden Delicious apple slices treated over time with a UV-A illuminator with 30 LEDs set to 20 V (L30V20) and corresponding controls (CL30V20).

Fig. 3 SDS-PAGE 12% zymograms of TYR (33 U per lane, A) and PPO extracted from Golden Delicious apple slices (7.38 µg of protein loaded per lane, B) before (C) and after UV-A LED irradiation at 25°C for 30 min with L30V20 illuminator (UV).

Fig. 4 Effect of UV-A LED treatment with L30V20 illuminator on the reduction in colour change (%RΔE) of apple and pear fresh-cut slices for increasing exposure times at 25°C.

Fig.5 Reduction in colour change (%RΔE) of Golden Delicious apple slices treated for 30 min at 25°C with AAC and AS1 as anti-browning references and L30V20 illuminator.

Table 1 Effect of UV-A LED treatment obtained by combining illuminator LEDs number and voltage after setting 0.5 cm distance from sample on the colour change (ΔE) of Golden Delicious apple slices for increasing exposure times at 25°C.

Table 2 Effect of the distance between L30V20 illuminator and sample surface on the colour change (ΔE) of Golden Delicious apple slices for increasing exposure times at 25°C.

Table 3 Irradiance (Wm^{-2}) of L9 and L30 illuminators set at different voltages and distances from the sample surface.

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Industrial relevance: Treatment of fresh-cut products with UV-A LED is not only easy and inexpensive to produce but also presents few hazards for humans. Moreover, the use of LED light sources brings many advantages such as energy savings, device durability, low environmental impact, high luminous efficiency and little thermal effect. UV-A LED technology has great potential to meet the demands of the food industry in the processing of fresh-cut fruits and vegetables. In addition it could also be considered a pre-treatment of fruits and vegetables being processed for the production of snacks.

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HIGHLIGHTS

- The effect of UV-A LED irradiation on fresh-cut apples and pears was studied
- Anti-browning was found to be related to irradiance, exposure time and the fruit cultivar
- The effect of UV-A LED treatment on PPO activity has been demonstrated by colorimetric and zymographic assays
- The use of LED diodes meets the needs for energy saving and environmental impact
- UV-A LED treatment has great potential in the processing of fresh-cut products

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