UV-B AND UV-C COMBINATION TO ENHANCE PHENOLIC COMPOUNDS BIOSYNTHESIS IN FRESH-CUT CARROTS

Anna Carolina Formica-Oliveira[1], Ginés Benito Martínez-Hernández[1,2], Vicente Díaz-López[1], Francisco Artés[1,2] and Francisco Artés-Hernández[1,2*]

[1] Postharvest and Refrigeration Group. Department of Food Engineering. Universidad Politécnica de Cartagena. Paseo Alfonso XIII, 48, 30203 Cartagena, Murcia, Spain.

[2] Institute of Plant Biotechnology. Universidad Politécnica de Cartagena. Campus Muralla del Mar, 30202 Cartagena, Murcia, Spain.

* To whom correspondence should be addressed: Tel: +34-968-325509; Fax: +34-968-325433. E-mail: fr.artes-hdez@upct.es Web site: www.upct.es/gpostref

Abstract

The single and combined effects of UV-B (1.5 kJ m^{-2}) and UV-C (4.0 kJ m^{-2}) radiation treatments were studied on the phenylalanine ammonia-lyase (PAL) activity, phenolics content and total antioxidant capacity (TAC) of fresh-cut carrot shreds during a 72 h storage period at 15 ºC. Non-irradiated samples were used as control (CTRL). PAL activity of UV-B samples was increased by approximately 500 % after 72 h while it was reduced by <12 % after the remaining treatments. Chlorogenic acid represented 70 % of the sum of phenolic compounds of initial samples. Although single UV-B treatment achieved the highest phenolic accumulation after 72 h with 498 %, combined treatments, regardless of the order (UV-C+UV-B or UV-B+UV-C), still achieved a phenolic accumulation of 440 % after 72 h. Such phenolic data were highly correlated (R^2=0.82) to total phenolic contents throughout storage. Conclusively, combined UV-C and UV-B treatment may be considered a postharvest sanitizing treatment which may greatly enhance phenolic compounds content, and related antioxidant capacity, in fresh-cut carrot shreds during storage.

Keywords: UV radiation; wounding; antioxidants; chlorogenic acid.

1. INTRODUCTION

Nowadays, foods are not only intended to feed, but also to prevent chronic and nutritional-related diseases as well as to improve overall human well-being, mainly linked to the crescent consumer’s knowledge on functional foods. The high contents of phytochemicals from fruit and vegetables have been proven to prevent a grand array of diseases such as degenerative disorders, cancer, cardiovascular among others related to the consumption of these plant products (Slavin and Lloyd, 2012). Enhancement of the health-promoting properties of fruit and vegetables will add value and create new opportunities, even with recent economical drawbacks. Therefore, there is a need to provide technologies to handle fresh products with enhanced health-promoting properties (Jongen, 2002).

Carrot (*Daucus carota* L.) is a popular vegetable among broad strata of the population. The popularity of this vegetable is mainly due to its sensory characteristics and nutritional compounds. Furthermore, carrots do not contribute with high calories intake, however they play a significant source of nutrients, such as carotenoids, vitamins (A, E) and antioxidants on human diet (Sharma et al., 2011). Phenolic compounds are great antioxidants related to several health-promoting properties such as anti-inflammatory, antitumoral, as well as preventing neurodegenerative and chronic disorders. Moreover, those compounds contribute to sensory features to food products. Currently, health recommendations rely on a diet rich in multiple antioxidant compounds than one used based on a single antioxidant (Shahidi and Ambigaipalan, 2015). Plant products have been proposed as biofactories of phenolic compounds through different mechanisms induced by abiotic stresses. Particularly, carrot has been widely used as a model system to understand the effect of different postharvest abiotic stresses on the phenylpropanoid metabolism due to the great enhancement of phenolic compounds observed, with high antioxidant capacity, compared to other vegetables (Cisneros-Zevallos, 2003). Concisely, phenylalanine ammonia-lyase (PAL) is the key enzyme of primary (shikimate) and secondary (phenylpropanoid) pathways and is, therefore, involved in the biosynthesis of polyphenolic compounds (Dixon and Paiva, 1995). It is well reported that this enzyme is induced by an array of biotic and abiotic stress-induced mechanisms, such as wounding, radiation exposure, hyperoxia storage, water stress, chilling injury, low minerals, hormones and pathogen attack, among others (Alegria et al., 2016; Avena-Bustillos et al., 2012; Becerra-Moreno et al., 2012; Jacobo-Velázquez et al., 2011). Consequently, such postharvest abiotic stresses enhance the levels of phenolic compounds like caffeoylquinic (CQA) acid, ferulic acid and their derivates as a defense mechanism of the plant (Jacobo-Velázquez et al., 2011).

Application of UV-B radiation (280–320 nm) has been proposed as a friendly and cheap non-molecular tool to enhance the phenolic compounds in carrots and other horticultural crops during postharvest life (Castagna et al., 2014; Du et al., 2012; Scattino et al., 2014). On the other side, the high germicidal properties of UV-C radiation (100-280 nm) have justified its use as a sustainable alternative to chlorine washing treatment in fresh-cut products (Allende and Artés, 2003). Then, the application of a combined UV-C treatment with UV-B, just after wounding, could greatly enhance the phenolic accumulation while controlling microbial growth in fresh-cut products extending their shelf life. Nonetheless, to the best of our knowledge, such combined treatment has not already been studied in fresh-cut products. Accordingly, this work studied the singular and combined effects of UV-B and UV-C pretreatment on PAL activity, phenolic compounds and related total antioxidant capacity (TAC) during storage of shredded carrots at 15°C.

2. MATERIALS AND METHODS

2.1. Plant material preparation

Fresh carrots (*Daucus carota* L., cvs. group Nantes, cv. Soprano) were bought in a local market (Cartagena, Spain) on third week of April 2016. According to producer specifications, carrots were harvested on the first week of April in Villena area (northwest area of Alicante region, Spain) without any postharvest treatment, but washing, previous expedition to the market. Carrots were transported to the Pilot Plant of the Universidad Politécnica de Cartagena where they were stored in a cold room at 5 ºC until the next day when the experiment was conducted. Plant material was carefully inspected, selecting those with similar visual appearance and size (14-15 cm long and 2-3 cm diameter). Then, carrots (unpeeled) were sanitized in a cold room (8°C) with chlorine (150 ppm NaClO; 5°C; pH 6.5±0.1) for 2 min, rinsed with tap water at 5 ºC for 1 min and drained in a perforated basket for 1 min. A ratio of 300 g plant material: 5 L chlorine was used. Carrots were wounded to shreds (2 mm×3 mm×40-60 mm) with a food processor (FreshExpress+, Moulinex, Lyon, France). Approximately 9 kg of carrot shreds were prepared for the experiment. Immediately after wounding all samples were submitted to radiation treatments.

2.2. Radiation treatments and incubation conditions

The radiation chamber consisted of a reflective stainless steel chamber with two banks (one bank suspended horizontally over the radiation vessel and the other placed below it) being fitted to each bank 6 UV-B and 7 UV-C (alternatively positioned) unfiltered germicidal emitting lamps (TUV 36W/G36 T8, TL 40W/01 RS, Philips, Eindhoven, The Netherlands). UV-B and UV-C radiations were applied separately controlled by two general keys that switched all UV-C or UV-B at the same time. The radiation chamber also had a ventilator continuously switched on during treatments to renovate the air from inside of the chamber with the cold air from the cold room (8°C). Shredded carrots were placed between the two lines of UV-C lamps at 17.5 cm above and below over a 35 mm thick bi-oriented polypropylene (PP) film mounted on a polystyrene (PS) net (130×68 cm) that minimized blockage of the radiation. The applied UV-B and UV-C intensities of 9.27 and 25.21 W m^{-2}, respectively, were calculated as the mean of 18 UV-C readings on each side of the net using LP 471 UVB (Delta OHM, Italy) and VLX

254 radiometers (Vilber Lourmat, Marne la Vallee, France). Thus, both sides received the same radiation intensities. The UV-C light intensity was kept constant and the applied dose was varied by altering the exposure time at the fixed distance. Applied treatments were:

- CTRL: No radiation treatment used as control.
- UV-B: 1.5 kJ UV-B m^{-2} (162 s). Such UV-B dose was selected based on previous experiments and on Avena-Bustillos et al. (2012) in order to obtain maximum phenolic accumulation in carrots while minimizing heating and evaporation processes during UV-B treatment which may affect the quality of the product.
- UV-C: 4.0 kJ UV-C m^{-2} (159 s). Such UV-C dose was selected based on previous studies in order to achieve a proper microbial reduction and quality while ensuring food safety of the product (Formica-Oliveira et al., 2016; Martínez-Hernández et al., 2015a).
- UV-B+UV-C: 1.5 kJ UV-C m^{-2} followed by 4.0 kJ UV-B m^{-2}.
- UV-C+UV-B: 4.0 kJ UV-B m^{-2} followed by 1.5 kJ UV-C m^{-2}.

Then, approximately 150 g of treated samples were placed in a rectangular polypropylene basket (170 mm×120 mm×60 mm) and covered with a plastic polyethylene bag to reduce water loss. Three baskets (replicates) per treatment were prepared. Samples were stored at 15 ºC (90–95% RH) up to 3 days (sampling days: 0, 1, 2 and 3). Samples were stored at -80 ºC until further analysis of phenylalanine ammonia-lyase (PAL) activity, phenolic compounds and total antioxidant capacity (TAC).

2.3. Phenylalanine ammonia-lyase

PAL activity was analyzed according to Ke and Saltveit (1986) with modifications (Formica-Oliveira et al., 2016). Concisely, 2 g carrot tissue samples were mixed with polyvinylpolypyrrolidone (Sigma, St Louis, MO, USA) (0.2 g) and homogenized (Ultra Turrax® model 18T, IKA-Werke GmbH & Co. KG, Germany) in cold 50 mM borate buffer (pH 8.5) containing 400 µL L^{-1} *β*-mercaptoethanol (Sigma, St Louis, MO, USA). Homogenates were filtered through four layers of cheesecloth and then centrifuged at 10,000×G for 20 min at 4°C. Supernatants were used as enzyme extract. Two sets of UV-Star well plates (Greiner Bio-One, Frickenhausen, Germany) containing 69 µL of PAL extract plus 200 µL ultrapure water were prepared for every sample and pre-incubated at 40 ºC for 5 min. Afterwards, 30 µL of either water (blank) or 100 mM L-phenylalanine substrate solution (freshly prepared before assay) were added to each of the well for every sample set. The absorbances of sample sets were measured at 290 nm, using a Multiscan plate reader (Tecan Infininte M200, Männedorf, Switzerland), at time 0 and after 1 h of incubation at 40 ºC. The PAL activity was calculated as µmol of *t*-cinnamic acid synthesized kg^{-1} fresh weight (fw) h^{-1} using a *t*-cinnamic acid (Sigma, St Louis, MO, USA) standard curve (0-6.75 mM).

2.4. Phenolic compounds

Extraction to determine phenolic compounds and TAC extract was conducted by homogenization (Ultra Turrax®) of 2 g of sample in 8 mL methanol (Sigma, St Louis, MO, USA) for 20 s under ice-water bath. Subsequently, extracts were centrifuged at 13500×G for 20 min at 4 ºC and supernatants were collected and analyzed. Extracts for individual phenolic compounds were further filtered through a 0.22 µm polyethersulphone filter and stored at −80 ºC in amber vials until Ultra High-Performance liquid chromatography (UHPLC) analysis.

Total phenolic content (TPC) was analyzed by Folin–Ciocalteu reagent method (Singleton and Rossi, 1965) with modifications (Martínez-Hernández et al., 2011). Briefly, a 19 µL aliquot of TPC extract was placed on a 96 PS flat bottom well plate (Greiner Bio-One, Frickenhausen, Germany) and 29 µL of Folin–Ciocalteu reagent 1 N (Sigma, St Louis, MO, USA) were added. Samples were incubated for 3 min in darkness at room temperature. After incubation, 192 µL of a solution containing Na_2CO_3 (4 g L^{-1}) and NaOH (20 g L^{-1}) were added and the reaction was carried out for 1 h at room temperature in darkness, measuring the absorbance at 750 nm using the Multiscan plate reader. TPC was expressed as chlorogenic acid equivalents (ChAE) in mg kg^{-1} fw. Each of the three replicates was analyze by triplicate.

Analyses of individual phenolic compounds were conducted as previously described (Formica-Oliveira et al., 2016). Briefly, samples of 20 µL were analyzed using an UHPLC instrument (Shimadzu, Kyoto, Japan) equipped with a DGU-20A degasser, LC-30AD quaternary pump, SIL-30AC autosampler, CTO-10AS column heater and SPDM-20A photodiode array detector. The UHPLC system was controlled by the software LabSolutions (Shimadzu, v. 5.42 SP5). Chromatographic analyses were carried out onto a Kinetex C18 column (100 mm×4.6 mm, 2.6 µm particle size; Phenomenex, Macclesfield, UK) with a KrudKatcher Ultra HPLC guard column (Phenomenex, Macclesfield, UK). The column temperature was maintained at 25 ºC. The mobile phase was acidified water (A; formic acid to final pH 2.3) and acidified methanol (B; formic acid to final pH 2.3). The flow rate was 1.5 mL min^{-1}. Gradient program used was 0/88, 1.2/88, 2.4/85, 8.3/70, 9.4/50, 11.8/50, 20.8/55, 22.0/60 (min/% phase A). Then, column equilibration was conducted at 0 % A for 2.2 min. Chromatograms were recorded at 320 nm. Phenolic acids were quantified as standards of chlorogenic acid (3-CQA), ferulic acid (Sigma, St Louis, MO, USA), isochlorogenic acid A (3,5-CQA) and C (4,5-CQA) (ChromaDex, Irvine, CA, USA). The calibration curves were made with at least six data points. The results were expressed as mg kg^{-1} fw. Each of the three replicates was analyzed by duplicate.

2.5. Total antioxidant capacity

The extracts were analyzed for TAC based on Brand-Williams et al. (1995) with modifications (Martínez-Hernández et al., 2013). Briefly, a solution of 0.7 mM 2,2-diphenyl-1-picrylhydrazil (DPPH) (Sigma, St Louis, MO, USA) in methanol was prepared 2 h before the assay and adjusted to 1.1 (nm) immediately before use. A 21 µL aliquot of the previously described extract was placed on a 96 PS flat-bottom well plate and 194 µL of DPPH was added. The reaction was carried out for 30 min at room temperature in darkness and the absorbance at 515 nm was measured using the Multiscan plate reader. Results were expressed as Trolox (Sigma, St Louis, MO, USA) equivalent antioxidant capacity kg^{-1} fw. Each of the three replicates was analyze by triplicate.

2.6. Statistical Analyses

A complete randomized design in triplicate, with two-way ANOVA (treatment × storage), by Post Hoc Tuckey HSD tests, were used with SPSS software (v. 21, IBM, USA). Possible synergistic effects of the stresses combinations were discarded according to Limpel's formula (equation 1) (Richer, 1987), where the effectiveness of a combination of treatments exceeds the prediction of the effectiveness of their additive action.

$$E_e = X + Y - \left(\frac{XY}{100}\right) \quad (1)$$

3. RESULTS AND DISCUSSION

3.1. Phenylalanine ammonia-lyase activity

PAL is the key enzyme of primary (shikimate) and secondary (phenylpropanoid) pathways and is, therefore, involved in the biosynthesis of polyphenolic compounds (Dixon and Paiva, 1995). Carrots showed an initial PAL activity of 19.7±4.9 µmol cinnamic acid formed kg^{-1} h^{-1} fw (Figure 1). Similar PAL activity has been previously reported for the same carrot cultivar (Formica-Oliveira et al., 2016). No immediate significant ($p<0.05$) changes of PAL activity were observed after radiation treatments on processing day.

In general, PAL activity of shredded carrots increased throughout storage. Latter finding may be explained since PAL is induced by an array of biotic and abiotic stress-induced mechanisms such the applied wounding and radiation exposure (Avena-Bustillos et al., 2012; Formica-Oliveira et al., 2016; Jacobo-Velázquez et al., 2011). Particularly, PAL activity of CTRL and UV-B samples early increased by 214 and 352 % after 24 h reaching the highest PAL increments of 1013 and 804 %, respectively, among the rest of treatments at 48 h. PAL activity of CTRL and UV-B samples decreased after such high enhancements with levels of 155-160 µmol cinnamic acid formed kg^{-1} h^{-1} fw at 72 h without significant ($p<0.05$) differences among them. Similar increments (750 %) of PAL activity after 72 h at 15 ºC have been reported in shredded carrots irradiated with a 1.3 kJ UV-B m^{-2} dose (Du et al., 2012). UV-C showed a similar behavior to CTRL and UV-B with the maximum increase of PAL activity of 267 % at 48 h decreasing its PAL activity to initial levels at 72 h. Such data is in accord to the recently reported detailed photograph (12 h intervals) of PAL and phenolic accumulation in stressed (wounding and UV-C) carrots (Formica-Oliveira et al., 2016). Latter photograph showed that PAL activity and phenolic accumulation in stressed (wounding and UV) carrots during storage at 15 ºC could be divided into three different phases: 1st phase, <24 h: early PAL activity increments; 2nd phase, 24-48 h: moderate phenolic increments concurring with the greatest increase of PAL activity; 3rd phase, 48-72 h: high phenolic increments while a moderate increment of PAL activity is registered. The hereby observed lower PAL activity increase in UV-C samples compared to UV-B may be a result of a feedback modulation or due to the diversion of the synthetic capacity of the cell to the production of other proteins not observed with the UV-B radiation (Alegria, 2015; Boerjan et al., 2003; Saltveit, 2000). Another possible explanation may be a partial PAL denaturation by UV-C (a UV radiation with higher photon energy than UV-B) delaying the stress-enhanced activity of this enzyme (Formica-Oliveira et al., 2016). Combined treatments showed PAL activity increments of 115-144 % after 72 h. The application order for the combined treatments did not affect the PAL activity of samples since no significant ($p<0.05$) differences between UV-B+UV-C and UV-C+UV-B were found throughout all storage period.

3.5. Phenolic compounds

Initial TPC of CTRL carrots was 207.4±43.0 mg ChAE kg fw^{-1} (Table 2). The major individual phenolic compounds identified were 3-CQA, 3,5-CQA, 4,5-CQA and ferulic acid (Table 1). These phenolic compounds accounted 69.6, 11.0, 9.8 and 9.5 % of the sum of individual phenolics, respectively. Similar initial TPC of carrot has been previously reported being hydroxycinnamic acids and their derivatives the major phenolic compounds found (Alegria et al., 2016; Formica-Oliveira et al., 2016; Jacobo-

Velázquez et al., 2011). As expected, radiation treatments did not immediately change ($p<0.05$) the phenolic compounds levels as similarly observed for PAL activity.

Phenolic levels of all samples progressively increased throughout storage. Such increase of these phytochemicals is a response to the applied postharvest abiotic stresses like wounding and UV-C/B radiation (Avena-Bustillos et al., 2012; Cisneros-Zevallos, 2003; Formica-Oliveira et al., 2016). This phenolic biosynthesis has been reported to be a consequence of PAL activation after these abiotic stresses, as previously discussed, being proposed ATP and reactive oxygen species as signaling molecules (Jacobo-Velázquez et al., 2011). UV-B showed the highest TPC increases with 90, 215 and 498 % after 24, 48 and 72 h, respectively (Table 2). The maximum TPC observed at 72 h may be the delayed consequence of maximum PAL activity observed at 48 h as previously reported (Formica-Oliveira et al., 2016). In general, different responses to low or high doses of UV-B have been observed in plants either by stimulating protection mechanisms or by activating repair mechanisms (Frohnmeyer and Staiger, 2003). Biosynthesis of UV absorbing compounds is the most common protective mechanism against potentially damaging radiation (Hahlbrock and Scheel, 1989). These secondary metabolites, mainly phenolic compounds, flavonoids, and hydroxycinnamate esters, accumulate in the vacuoles of epidermal cells in response to UV-B irradiation and attenuate the penetration of the UV-B into deeper cell layers (Avena-Bustillos et al., 2012). Contrary to the reduction observed on PAL activity with UV-C treatment, and its combinations, TPC accumulations in these samples were only slightly reduced (4-12 % after 72 h) compared to single UV-B treatment. Interestingly, samples treated with single UV-C treatment showed 50-170 % higher 3-CQA than CTRL samples after 48-72h. Latter marked difference was not observed in TPC data probably masked by the interference of other antioxidant compounds present in carrots with the Folin-Ciocalteu analysis method. In general, the contribution of 3,5-CQA, 4,5-CQA and ferulic acid to TPC was minimum with no significant (*p*<0.05) changes throughout storage of all samples.

Chlorogenic acid, the main phenolic compound in carrots, is an ester of caffeic acid with quinic acid with great antioxidant capacity compared to other phenolic compounds (Castelluccio et al., 1995). Carrots occupy the sixth place among the list of most consumed vegetables in the American diet, although the total phenolic content of this vegetable is almost the lowest one (Chun et al., 2005). Hence, the enhancement of those antioxidant compounds during storage could be favored by UV-B treatment as hereby and previously observed (Avena-Bustillos et al., 2012; Du et al., 2012). UV-C radiation is used in fresh-cut (FC) products mainly due to the high germicidal properties of this UV radiation being considered as a sustainable alternative to conventional chlorine washings (Martínez-Hernández et al., 2015b). Accordingly, moderate UV-C doses initially reduced by approximately 1.5 log units mesophiles and yeasts and molds loads in carrot shreds being such microbial loads after 72 h at 15 ºC below the threshold limit (7 log units) which defines fresh-cut products shelf life (Formica-Oliveira et al., 2016). In this sense, the combination of UV-C may reduce microbial loads of FC carrot shreds while still highly (approximately 440 % after 72 h) allowing phenolic compounds accumulation within these wounded tissues.

3.6. Total antioxidant capacity

The initial TAC of CTRL carrots was 121.1±79.8 mg Trolox kg^{-1} fw (Table 2). Radiation treatments did not immediately change (p<0.05) TAC except UV-C+UV-B treatment which showed 3-fold higher TAC than CTRL samples. Such finding may be an experimental artifact resulted from higher extraction of other antioxidant compounds of carrots such as carotenoids due to increased cell wall depolymerization (Alegria et al., 2012; Bhat et al., 2007).

TAC of all samples increased throughout storage similar to TPC. Carrots have a high antioxidant capacity mainly due to their content of phenolic compounds. In this sense, TAC was highly correlated to TPC with R^2 of 0.82 as previously found (Cisneros-Zevallos, 2003). UV-B samples early showed the highest TAC increments with levels 47 % higher than CTRL samples at 24 h. In the same line, UV-B samples showed the highest TAC levels with 3705 mg Trolox kg^{-1} fw at 72 h. The rest of treatments showed final TAC levels of 2537-2890 mg Trolox kg^{-1} fw at 72 h without significant ($p<0.05$) differences among them.

Sufficient antioxidants compounds need to be consumed with foods to prevent or slow the oxidative damage in humans induced by free radicals. UV-B treatment is hereby shown as an excellent sustainable and cheap treatment to be applied by the food industry to even enhance phenolic accumulation, and consequently the antioxidant capacity, in wounded carrots. The combination with UV-C is recommended as a sustainable sanitizing treatment alternative to conventional chlorine washings since the accumulation of such antioxidant compounds was still highly maintained. Although no significant differences were found between UV treatment order, UV-C+UV-B is recommended to rapidly reduce microbial loads by UV-C of samples after wounding of samples.

5. CONCLUSIONS

UV-B radiation has been used as an abiotic stress to enhance accumulation of antioxidant phenolic compounds in many plant products while UV-C is considered as a sustainable sanitizing alternative to NaOCl due to its high germicidal effect. However, the effects of a combined UV-C treatment on the phenolic accumulation achieved by UV-B have not been yet studied. This study showed that phenolic accumulation in fresh-cut carrot shreds after 72 h at 15 ºC could be increase by 30 % applying a UV-B dose of 1.5 kJ m^{-2}. Furthermore, application of a sanitizing UV-C dose of 4 kJ m^{-2} followed by the UV-B treatment did not highly affected phenolic accumulation still allowing an accumulation of 440 % regarding initial levels. Such combined UV-C+UV-B treatment is an excellent opportunity for the food industry to diversify its product offer for an actual consumer increasingly interested in food with high antioxidants contents while meeting the food safety issues.

Acknowledgments

The authors are grateful to the Institute of Plant Biotechnology of the Universidad Politécnica de Cartagena for the use of the UHPLC system.

REFERENCES

Alegria, C., 2015. Heat shock and uv-c abiotic stress treatments as alternative tools to promote fresh-cut carrot quality and shelf-life. PhD Thesis. University of Lisbon.

Alegria, C., Gonçalves, E.M., Moldão-Martins, M., Cisneros-Zevallos, L., Abreu, M., 2016. Peel removal improves quality without antioxidant loss, through wound-induced phenolic biosynthesis in shredded carrot. Postharvest Biol. Technol. 120, 232-239.

Alegria, C., Pinheiro, J., Duthoit, M., Gonçalves, E.M., Moldão-Martins, M., Abreu, M., 2012. Fresh-cut carrot (cv. Nantes) quality as affected by abiotic stress (heat shock and UV-C irradiation) pre-treatments. Lebensm. Wiss. Technol. 48, 197-203.

Allende, A., Artés, F., 2003. Combined ultraviolet-C and modified atmosphere packaging treatments for reducing microbial growth of fresh processed lettuce. Lebensm. Wiss. Technol. 36, 779-786.

Avena-Bustillos, R.J., Du, W.X., Woods, R., Olson, D., Breksa, A.P., 3rd, McHugh, T.H., 2012. Ultraviolet-B light treatment increases antioxidant capacity of carrot products. J. Sci. Food Agric. 92, 2341-2348.

Becerra-Moreno, A., Benavides, J., Cisneros-Zevallos, L., Jacobo-Velázquez, D.A., 2012. Plants as Biofactories: Glyphosate-Induced Production of Shikimic Acid and Phenolic Antioxidants in Wounded Carrot Tissue. J. Agric. Food Chem. 60, 11378-11386.

Bhat, R., Sridhar, K.R., Tomita-Yokotani, K., 2007. Effect of ionizing radiation on antinutritional features of velvet bean seeds (Mucuna pruriens). Food Chem. 103, 860-866.

Boerjan, W., Ralph, J., Baucher, M., 2003. LIGNIN BIOSYNTHESIS. Annu. Rev. Plant Biol. 54, 519-546.

Brand-Williams, W., Cuvelier, M., Berset, C., 1995. Use of a free radical method to evaluate antioxidant activity. LWT-Food Science and Technology 28, 25-30.

Castagna, A., Dall’Asta, C., Chiavaro, E., Galaverna, G., Ranieri, A., 2014. Effect of Post-harvest UV-B Irradiation on Polyphenol Profile and Antioxidant Activity in Flesh and Peel of Tomato Fruits. Food and Bioprocess Technology 7, 2241-2250.

Castelluccio, C., Paganga, G., Melikian, N., Bolwell, G.P., Pridham, J., Sampson, J., Rice-Evans, C., 1995. Antioxidant potential of intermediates in phenylpropanoid metabolism in higher plants. FEBS Lett. 368, 188-192.

Cisneros-Zevallos, L., 2003. The Use of Controlled Postharvest Abiotic Stresses as a Tool for Enhancing the Nutraceutical Content and Adding-Value of Fresh Fruits and Vegetables. J. Food Sci. 68, 1560-1565.

Chun, O.K., Kim, D.-O., Smith, N., Schroeder, D., Han, J.T., Lee, C.Y., 2005. Daily consumption of phenolics and total antioxidant capacity from fruit and vegetables in the American diet. J. Sci. Food Agric. 85, 1715-1724.

Dixon, R.A., Paiva, N.L., 1995. Stress-Induced Phenylpropanoid Metabolism. The Plant Cell 7, 1085-1097.

Du, W.X., Avena-Bustillos, R.J., Breksa, A.P., 3rd, McHugh, T.H., 2012. Effect of UV-B light and different cutting styles on antioxidant enhancement of commercial fresh-cut carrot products. Food Chem. 134, 1862-1869.

Formica-Oliveira, A.C., Martínez-Hernández, G.B., Aguayo, E., Gómez, P.A., Artés, F., Artés-Hernández, F., 2016. UV-C and hyperoxia abiotic stresses to improve healthiness of carrots: study of combined effects. Journal of Food Science and Technology, 1-12.

Frohnmeyer, H., Staiger, D., 2003. Ultraviolet-B Radiation-Mediated Responses in Plants. Balancing Damage and Protection. Plant Physiol. 133, 1420-1428.

Hahlbrock, K., Scheel, D., 1989. Physiology and Molecular Biology of Phenylpropanoid Metabolism. Annu. Rev. Plant Physiol. Plant Mol. Biol. 40, 347-369.

Jacobo-Velázquez, D.A., Martínez-Hernández, G.B., del C. Rodríguez, S., Cao, C.-M., Cisneros-Zevallos, L., 2011. Plants as Biofactories: Physiological Role of Reactive Oxygen Species on the

Accumulation of Phenolic Antioxidants in Carrot Tissue under Wounding and Hyperoxia Stress. J. Agric. Food Chem. 59, 6583-6593.
Jongen, W., 2002. Fruit and Vegetable Processing: Improving Quality. Woodhead PublishingLimited Cambridge (England).
Ke, D., Saltveit, M.E., 1986. Effects of calcium and auxin on russet spotting and phenylalanine ammonia-lyase activity in iceberg lettuce HortScience 21, 1169-1171.
Martínez-Hernández, G.B., Artés-Hernández, F., Gómez, P.A., Formica, A.C., Artés, F., 2013. Combination of electrolysed water, UV-C and superatmospheric O2 packaging for improving fresh-cut broccoli quality. Postharvest Biol. Technol. 76, 125-134.
Martínez-Hernández, G.B., Gómez, P.A., Pradas, I., Artés, F., Artés-Hernández, F., 2011. Moderate UV-C pretreatment as a quality enhancement tool in fresh-cut Bimi® broccoli. Postharvest Biol. Technol. 62, 327-337.
Martínez-Hernández, G.B., Huertas, J.-P., Navarro-Rico, J., Gómez, P.A., Artés, F., Palop, A., Artés-Hernández, F., 2015a. Inactivation kinetics of foodborne pathogens by UV-C radiation and its subsequent growth in fresh-cut kailan-hybrid broccoli. Food Microbiol. 46, 263-271.
Martínez-Hernández, G.B., Navarro-Rico, J., Gómez, P.A., Otón, M., Artés, F., Artés-Hernández, F., 2015b. Combined sustainable sanitising treatments to reduce Escherichia coli and Salmonella Enteritidis growth on fresh-cut kailan-hybrid broccoli. Food Control 47, 312-317.
Richer, D.L., 1987. Synergism—a patent view. Pestic. Sci. 19, 309-315.
Saltveit, M.E., 2000. Wound induced changes in phenolic metabolism and tissue browning are altered by heat shock. Postharvest Biol. Technol. 21, 61-69.
Scattino, C., Castagna, A., Neugart, S., Chan, H.M., Schreiner, M., Crisosto, C.H., Tonutti, P., Ranieri, A., 2014. Post-harvest UV-B irradiation induces changes of phenol contents and corresponding biosynthetic gene expression in peaches and nectarines. Food Chem. 163, 51-60.
Shahidi, F., Ambigaipalan, P., 2015. Phenolics and polyphenolics in foods, beverages and spices: Antioxidant activity and health effects – A review. Journal of Functional Foods 18, Part B, 820-897.
Sharma, K.D., Karki, S., Thakur, N.S., Attri, S., 2011. Chemical composition, functional properties and processing of carrot—a review. Journal of Food Science and Technology 49, 22-32.
Singleton, V.L., Rossi, J.A., 1965. Colorimetry of Total Phenolics with Phosphomolybdic-Phosphotungstic Acid Reagents. American Journal of Enology and Viticulture 16, 144-158.
Slavin, J.L., Lloyd, B., 2012. Health Benefits of Fruits and Vegetables. Advances in Nutrition: An International Review Journal 3, 506-516.

FIGURES AND TABLES CAPTIONS

Figure 1. Phenylalanine ammonia lyase activity of carrot shreds treated with UV-C and UV-B, and their combinations, during storage up to 72 h at 15 ºC (n=3±SD). Different capital letters denote significant differences ($p < 0.05$) among different treatments for the same sampling day. Different lowercase letters denote significant differences ($p < 0.05$) among different sampling days for the same treatment.

Table 1. Total phenolic content and total antioxidant capacity of carrot shreds treated with UV-C and UV-B, and their combinations, during storage up to 72 h at 15 ºC (n=3±SD). Different capital letters denote significant differences ($p < 0.05$) among different treatments for the same sampling day. Different lowercase letters denote significant differences ($p < 0.05$) among different sampling days for the same treatment.

Table 2. Individual phenolic compounds of carrot shreds treated with UV-C and UV-B, and their combinations, during storage up to 72 h at 15 ºC (n=3±SD). Different capital letters denote significant differences ($p < 0.05$) among different treatments for the same sampling day. Different lowercase letters denote significant differences ($p < 0.05$) among different sampling days for the same treatment.