Postharvest Biology and Technology 138 (2018) 107–113

Contents lists available at ScienceDirect

Postharvest Biology and Technology

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

UV-C and ozone treatment influences on the antioxidant capacity and antioxidant system of minimally processed rocket (*Eruca sativa Mill.*)

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ARTICLE INFO

Keywords:
Minimal processing
UV-C
Ozone
Rocket
Antioxidant enzymes

ABSTRACT

In this work, treatments with UV-C light (20 kJ m^{-2}) and gaseous ozone (2 mg L^{-1} O_3) were applied to minimally processed rocket (*Eruca vesicaria subsp. Sativa*) leaves to investigate their effects on the antioxidant capacity and antioxidant system throughout 8 ds at 5 °C. As control, water washing was used. On 8 d, the ascorbic acid content was reduced about 50% both control and treated samples. Treatments with 20 kJ UV-C m^{-2} or 2 mg L^{-1} O_3, did not affect the phenolic content neither the total antioxidant capacity, remaining almost constants during storage. The phenylalanine ammonia lyase (PAL) activity had no significant variations during storage, in correlation with the content of phenolics. As to the enzymes involved in the antioxidant system, an increase in its superoxide dismutase (SOD) activity with respect to uncut rocket leaves was detected after the process. In turn, enzymes that remove H_2O_2 like ascorbate peroxidase (APX) and catalase (CAT) showed no significant changes. During storage at 5 °C, the SOD activity remained unchanged while APX and CAT showed a gradual increase in both treated and untreated samples. In conclusion, the UV-C and ozone treatments applied inhibited the growth of spoilage by bacteria as well as by yeasts, extended shelf-life, without exerting significant additional stress with respect to the cutting stage of the leaves, reason by which they did not trigger a greater activation of the antioxidant system.

1. Introduction

The consumption of fruits and vegetables has increased in recent times due to its content in phytochemicals and antioxidant compounds that are beneficial for human health (Lemoine et al., 2010). Koukounaras et al. (2009) reported that in the Mediterranean countries, the rocket (*Eruca sativa* Mill.) is a vegetable very popular with a high interest by consumers. The rocket is a vegetable that is distinguished by its pleasant bitter taste and also by its content of phytonutrients that stimulate health such as provitamin A, vitamin C, flavonoids, sulfur, potassium and fiber (Nunes et al., 2013; Gutiérrez et al., 2016).

Lemoine et al. (2008) reported that both harvesting and processing of vegetables cause severe stress leading to the symptoms of senescence in them. The postharvest chemical treatments used to preserve vegetables and fruits are being less accepted by consumers because of their possible contaminating effects (Shen et al., 2013). Thus in the last years, new physical technologies are of interest in to extend the postharvest life of fruits and vegetables. Among new physical technologies, treatments with low dose UV-C can be effective due to that delaying ripening, producing no pollution and extending the shelf life of various fruits and vegetables (Lemoine et al., 2007). Artés-Hernández et al. (2010) reported that radiation UV-C has germicidal effect and is due to the fact that it damages the nucleic acids of the microorganisms affecting their multiplication. There have been studies in different vegetables on how UV-C radiation influences changes in sensory quality, bioactive compounds and microbial development in vegetables in fresh-cut melon (Manzocco et al., 2011), broccoli (Martínez-Hernández et al., 2011), pineapple (Pan and Zu, 2012) and rocket (Gutiérrez et al., 2015). Different studies have shown that the application of UV-C light improved total phenolics contents and antioxidant capacity of several fruits and vegetables along storage (Erkan et al., 2008; Perkins-Veazie et al., 2008). This agrees with Allende et al. (2007) who reported that UV-C radiation reduced the deterioration of tomatoes and strawberries and produced an increase in antioxidants (Erkan et al., 2008). Besides, Wang et al. (2009) reported an increase of phenolic compounds in UV-C treated blueberries.

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https://doi.org/10.1016/j.postharvbio.2017.12.014
Received 11 September 2017; Received in revised form 27 December 2017; Accepted 30 December 2017

Another sanitizing agent approved by the US-FDA (2001) that is being tested in food industry today is ozone. Ozone breaks down quickly into oxygen and leaves no toxic residue which makes it appealing and useful to food industry (Karaca and Velioglu, 2014). Treatments that use ozone have proven to be effective in extending the shelf life of different fruits such as grapes, pears, oranges and apples (Ali et al., 2014). Furthermore, Jay et al. (2005) reported that 0.15–5.0 mg L^{-1} ozone treatments seemed to inhibit both spoilage bacteria and yeasts development. On the other hand, ozone can cause oxidative stress in plant tissues and in this way induce diverse physiological responses such as synthesis phenolic compounds, antioxidants and other secondary metabolites (Forney, 2003).

Martínez-Hernández et al. (2013) reported that these types of stress can induce the accumulation of reactive oxygen species (ROS) in plant cells and to prevent these damages, both enzymatic and non-enzymatic antioxidant systems are activated. Boonkorn et al. (2012) found that the most important enzymes are catalases (CAT; EC 1.11.1.6), superoxide dismutases (SOD; EC 1.15.1.1) and ascorbate peroxidases (APX; EC 1.11.1.11). The function of these enzymes is that they are ROS scavengers, which cause oxidative damage that are induced by various biotic or abiotic stress such as low or high temperatures, UV radiation, exposure to ozone, pathogenic attack and mechanical damage (Lemoine et al., 2010; Boonkorn et al., 2012). The enzymes act as follows: the superoxide radical (O_2-) is dismutated to H_2O_2 by the enzyme SOD, while that the enzymes CAT and APX metabolize H_2O_2 to H_2O (Martínez-Hernández et al., 2013).

The objective of this study was to analyze the effects of UV-C and ozone on several chemical components and on enzymes related to the antioxidant systems of minimally processed rocket stored at 5 °C.

2. Materials and methods

2.1. Plant material and chemicals

The rocket (*Eruca Sativa* Mill.) was harvested from the field of a farmer in the province of Santiago del Estero, Argentina. Its stemless leaves were transported to the laboratory for their processing.

2.2. Sample preparation, treatments and storage conditions

The rocket leaves were sorted at 8 °C in a disinfected area in order for the removal of damaged or dehydrated leaves. After selection, the leaves were washed with running water 5 °C for 1 min and drained on a stainless-steel mesh. They were then cut into 20 mm strips with a sharp stainless-steel knife and were washed for 2 min and drained again. The remaining water was removed using a manual centrifuge and the leaves submitted to sanitizing treatments using UV-C and O_3 afterwards.

The treatments applied were as follows: 0 (control); 2 mg L^{-1} for 10 min O_3; 20 kJ m^{-2} (303 s) of UV-C dose (the exposure time was calculated according to the radiation intensity). Both the UV-C dose and the O_3 concentration were selected according to the results of preliminary experiments (Gutiérrez et al., 2016). In each treatment, 60 g of leaf strips were placed on 600 mL polypropylene (PP) trays wrapped with a 35 μm bi-oriented PP film thermally sealed on the topside to generate a passive MAP.

The transmission rate was 2.58×10^{-6} mol O_2 $m^{-2} s^{-1}$ and 9.30×10^{-6} mol CO_2 $m^{-2} s^{-1}$ at 20 °C and 90% RH. The trays were stored in a dark cold room (5 °C). Five trays per treatment and storage time were performed. The various parameters were measured after 1, 4, 5 and 8 d of refrigerated storage.

2.3. UV-C and ozone treatments

The UV-C apparatus was a reflective stainless-steel chamber equipped with 6 unfiltered germicidal lamps (254.7 nm, TUV 36W/G36, Philips, Amsterdam, The Netherlands), on its topside and 6 at its bottom. This equipment is fully described in a work by Gutiérrez et al. (2016). A constant light source of 254 nm was used (intensity of radiation 0,066 kW) and applied upon the samples for different exposure times according to the test. The UV-C radiation doses was measured with a digital radiometer (Cole-Parmer Instrument Company, Vernon Hill, IL).

A 1 g h^{-1} ozone generator (Bio3 Ozone Generator, TDZ-1 model, Uruguay) was used for the tests. The ozone concentration within the chamber was recorded via an ozone analyzer (Gas Alert Extreme O_3 − BW Technology, Honeywell, Canada). The O_3 flow and treatments employed were described by Gutiérrez et al. (2016).

2.4. Total phenolic compounds

The total phenolic compounds concentration was determined following Singleton et al. (1999). The phenolic compounds of the samples were extracted as described by Gutiérrez et al. (2015); For each day of measurement, samples (10 g) of each replicate treatment were frozen at −80 °C (Ultrafrezzer Righi, Argentina) and stored until chemical determinations were performed. The 4 g frozen rocket samples were homogenized using 20 mL of methanol and centrifugated for 15 min at 6000 × g at 4 °C. The supernatant of each sample was used as an extract. A 0.5 mL aliquot of each extract mixed with 8 mL of distilled water altogether were mixed in turn with 0.5 mL of the Folin-Ciocalteu reagent (that is, 1:1 v/v, diluted with distilled water). Three minutes later, 1 mL of a 5% Na_2CO_3 solution was added to the solution while shaking the solution vigorously. The resultant mixture was then incubated in darkness at 25 °C for 1 h and its absorbance measured at 765 nm using a UV–vis spectrophotometer (JASCO V-630). The total phenolic content was expressed as chlorogenic acid equivalents (CAEq) in g kg^{-1} (expressed on a fresh weight basis). All the measures were made in triplicates.

2.5. Antioxidant capacity

The total antioxidant activity of rocket was determined as described by Brand Williams et al. (1995) out of each extract obtained as described above. Thus, a 150 μL aliquot of each extract was added with 2850 μL of a 0.1 mM DPPH solution (prepared with ethanol) and the mixture kept in dark for one hour at room temperature. The absorbance at 515 nm was measured at different times with a spectrophotometer (JASCO V-630, UV-vis). The calibrating curve was depicted using Trolox as standard and the results are expressed as Trolox equivalents (Trolox Eq) in g kg^{-1} (expressed on a fresh weight basis). All the measurements were made in triplicate.

2.6. Ascorbic and dehydroascorbic acid content

The samples of rocket leaves (2 g) were added to 20 mL of 6% (w/v) trichloroacetic acid (TCA). The mixture to be extracted was kept in darkness for an hour. The homogenate was centrifuged at 12,000 × *g* at 4 °C for 20 min. The supernatant was used for measurements. Both the ascorbic acid (AA) and dehydroascorbic (DHA) acids contents were determined using the spectrophotometer described above by following Kampfenkel et al. (1995). Thus, a standard AA solution was employed for identifying and quantifying these contents in reference to a standard curve while DHA content resulted out of the difference between the total Vitamin C content and AA. The results were expressed as AA in g kg^{-1} (expressed on a fresh weight basis). All the measures were taken in triplicate.

2.7. Superoxide dismutase activity

A solution made from 2 g rocket leaves samples in 2 mL of buffer solution (namely 1 mM phenylmethylsulfonyl fluoride (PMSF), 100 mM sodium phosphate pH 7.8, 0.1 mM EDTA, 10 g L^{-1}