Food Chemistry 243 (2018) 435–441

Contents lists available at ScienceDirect

Food Chemistry

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

Ozone treatment for pesticide removal from carrots: Optimization by response surface methodology

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ARTICLE INFO

Keywords:
Daucus carota L.
Difenoconazole
Linuron
Storage
Solid-liquid extraction with low temperature partition
Gas chromatography

ABSTRACT

The present study aimed to optimize ozone (O_3) treatments, as gas and dissolved in water, to remove difenoconazole and linuron in carrots. We employed a central composite design to study three variables governing the efficacy of treatments: O_3 concentration, temperature and treatment time. The temperature did not influence the efficacy of treatments. The removal percentage of pesticides increases with increases in ozone concentration and the time of treatment. O_3 application promoted the removal of more than 80% of pesticides when the roots were exposed for approximately 120 min at 5 and 10 mg L^{-1}, respectively, in treatments with O_3 as gas and dissolved in water. After storage, pesticide removal was higher than 98% for difenoconazole and 95% for linuron. The degradation products from the pesticides resulting from treatment were monitored, but none were found. This is the first report demonstrating the removal of difenoconazole and linuron from carrots by ozone.

1. Introduction

Carrot (*Daucus carota* L.) is one of the most important vegetables, with high worldwide consumption, extension of acreage, and great socioeconomic involvement of farmers. The consumption of carrots is critical for human health as a fundamental source of carotenoid precursors of vitamin A (Luengo, Parmagnani, Parente, & Lima, 2011). In the United States, carrot is the fourth most consumed food by the population, with per capita consumption of 4.7 kg per year (PBHF, 2015; USDA, 2016). With a world production of 38.8 million tons, carrots are an economically important crop for the producing countries (FAO, 2014).

The intensive use of pesticides for controlling insects, diseases and invasive plants is necessary for carrot cultivation to minimize losses in productivity and maintain the quality of the final product (Carvalho, Junqueira, Vieira, Reis, & Silva, 2005; Liu, Hu, Xu, & Guan, 2005). Difenoconazole (cis–trans -3-chloro-4- [4-methyl-2- (1H-1,2,4-triazol-1-ylmethyl) -1,3-dioxolan-2-yl] phenyl 4-chlorophenyl ether) is a systemic fungicide of the triazole chemical group, widely used in the cultivation of carrots for the control of alternaria Leaf Blight caused by the fungus *Alternaria dauci* (Carvalho et al., 2005). In the control of several weeds in the crop the linuron herbicide (3- (3,4-dichlorophenyl) -1-methoxy-1-methylurea), a systemic product of the chemical group of urea, is one of the main products used (Andrei, 2017). Carrots are in direct contact with soil, and their roots, covered by a thin permeable film, expose them to contamination by pesticides used in the crop cycle and the residues left in the soil by prior cultures (Souza et al., 2008).

The potential risks of pesticides to health and the growing consumer concern about food quality have evidenced the need to study techniques capable of degrading these residues in food. Technologies currently adopted to reduce or eliminate pesticide residues in foods include the use of chlorine, hydrogen peroxyacetic acid (HPA), cold plasma, ultraviolet radiation, ultrasound, heat treatments and ozone gas (O_3) (Al-Antary, Al-Dabbas, & Shaderma, 2015; Hwang, Cash, & Zabik, 2001; Lin et al., 2012; Misra et al., 2014). However, the use of ozone has been highlighted due to its high oxidative power and easy availability (Santos, Faroni, Cecon, Ferreira, & Pereira, 2016). Ozone is formed from the rearrangement of oxygen atoms and can be generated by electric discharges or the incidence of high-energy electromagnetic radiation in the air. Moreover, O_3 is an unstable molecule that rapidly decays to diatomic oxygen and therefore leaves no residue in food (Gabler, Smilanick, Mansour, & Karaca, 2010). The oxidation of organic

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http://dx.doi.org/10.1016/j.foodchem.2017.09.134
Received 31 May 2017; Received in revised form 22 September 2017; Accepted 26 September 2017
Available online 30 September 2017
0308-8146/

compounds by O_3 can occur through the reaction of the O_3 molecule with organic compounds and the reaction of the free radicals formed by the O_3 decomposition with organic compounds (Chiron, Fernandez-Alba, Rodriguez, & Garcia-Calvo, 2000).

In the food industry, ozone is used for the decontamination of fruits and vegetables to preserve food during storage without modifying its physical–chemical and organoleptic characteristics. Al-Antary et al. (2015) found that the use of O_3 dissolved in water (4 μg L^{-1}) to treat juice-producing tomatoes removed 100% of the carbosulfan residue in the final product. Moreover, Heleno et al. (2014) studied the effect of ozone gas on difenoconazole removal and found that O_3 treatment reduced the pesticide residue in strawberries from 5 to 0.5 mg kg^{-1}. The use of ozone in pesticide removal has been demonstrated in other agricultural products such as lettuce, grape, apple, mustard, lemon, orange, grapefruit, corn, wheat, and lychee (Gabler et al., 2010; Heleno et al., 2015; Lozowicka, Jankowska, Hrynko, & Kaczynski, 2016; Wu, Luan, Lan, Lo, & Chan, 2007). Although the potential of ozone in the removal of pesticide residues is known for several foods, there are no reports on the use of this gas for the removal of pesticide residue in carrots.

The effectiveness of ozone applied as gas or dissolved in water depends on factors such as the time of exposure, temperature and chemical composition of food (Misra, 2015). Therefore, the application parameters of ozone cannot be generalized, and specific studies are necessary for obtaining information about the ozonation process of each food. Thus, this study aimed to optimize the use of ozone in gaseous form and dissolved in water as an immediate and long-term degradation agent of difenoconazole and linuron in carrots. The pesticide degradation products in the carrots were also evaluated.

2. Materials and methods

2.1. Reagents and solutions

The solutions employed in this study were prepared from the analytical standard of fungicide difenoconazole 99.2% w/w and the herbicide linuron 99.3% w/w using acetonitrile 99.9% w/w as solvent, all from the Sigma-Aldrich brand (St. Louis, MO, USA). Acetonitrile was also used as an extraction solvent. Stock solutions of 1000 mg L^{-1} of pesticides in acetonitrile were prepared and subsequently diluted to obtain different concentrations according to the stages of the study. Commercial formulations with 25% difenoconazole fungicide (Score 250EC, Syngenta, Basel, Switzerland) and 45% linuron herbicide (Afalon 450SC, Adama, Airport, Israel) were applied on carrot fields.

2.2. Carrot field cultivation

Carrots (Carandaí variety) were grown from the final days of the winter until the begining of the summer at the Universidade Federal de Viçosa (UFV), (20.7626° S, 42.8640° W), Viçosa - MG, Brazil, in beds (1 m x 10 m) previously prepared and fertilized according to the soil analysis. The cultural practices carried out until the harvest followed the recommendations of the Manual of Safety and Quality for Carrot Culture (EMBRAPA, 2004). Throughout the cultivation, the average temperature was 21 °C, with 75% of average relative humidity, 723.387 kJ m^{-2} of average radiation and 307.4 mm of total volume of rain (INMET, 2017). No pesticides, other than those studied, were used in the cultivation of carrots. Each pesticide was applied by leaf spraying individually in different areas of the planting to ensure that each batch of roots possessed the residue of a single product. Each pesticide was applied 80 days after planting (BBCH 49) in doses equivalent to five times the recommended dose in the product packaging (totalizing 3 L ha^{-1} of Score 250EC and 11 L ha^{-1} of Afalon 450SC). Three days after pesticide application, approximately 30 kg of carrots at expansion complete stages with typical form and size of roots reached (BBCH 49–51)were harvested, placed in plastic boxes (60 cm × 40 cm × 20 cm) without the aerial part of the plant and immediately transported to the Postharvest Laboratory of the Agricultural Engineering Department of the UFV at room temperature. The carrots were washed with tap water and submitted to solid–liquid extraction/low-temperature partition (SLE/LTP) for pesticide analysis. Pesticide extractions were performed in triplicate. The analyses were performed by a gas chromatograph equipped with an electron capture detector system (GC/ECD) and a gas chromatograph coupled to a mass spectrometer (GC/MS).

2.3. Pesticide residue analysis

2.3.1. Samples of carrot and SLE/LTP extraction

The method SLE/LTP, adapted from Araújo et al. (2016), was used to extract the difenoconazole and linuron residues from the carrot samples. For the preparation of samples, three whole carrots (approx. 330 g) were minced in a mini food processor (Britânia, Curitiba, PR, Brazil). After being processed, 4.00 g of carrot was transferred to 22 mL vials, and 2 mL of deionized water (0.5 mS m^{-1}) and 4 mL of acetonitrile were added. The vials were subjected to agitation on an orbital shaker at 200 rpm for 10 min and were later subjected to centrifugation at 3000 rpm for 3 min. The samples were stored in a freezer at -20 °C for 4 h. After this time, the matrix and aqueous phase freeze, allowing the extraction of the organic phase with pesticides. The organic phase was collected with a micropipette and transferred to 1.5 mL glass vials for later chromatographic analysis.

2.3.2. Analysis by GC/ECD

The optimized conditions for the GC/ECD (Shimadzu GC-2014, Kyoto, Japan) analysis of the pesticides in carrot samples were as follows: the injector temperature was fixed at 280 °C, the detector temperature was operated at 300 °C, the injected sample volume was 1.0 μL, carrier gas flow was applied (nitrogen at 1.3 mL min^{-1}), the initial column oven temperature was 100 °C (0.4 min), with a heating rate at 25 °C min^{-1} up to 290 °C, and this temperature was fixed for 1 min. The total running time was 12 min. The separations were performed on an HP-5 capillary column (Agilent Technologies, Palo Alto, CA, USA), 30 m, 0.25-mm inner diameter, and 0.10 μm film thickness, with the stationary phase consisting of phenyl 5% and dimethylsiloxane 95%.

2.3.3. Analysis by GC/MS

The presence of the degradation products from difenoconazole and linuron residues in carrot was analyzed on a GC/MS system composed of a 7820A gas chromatograph coupled to a 5977B mass spectrometer (Agilent Technologies, Palo Alto, CA, USA). The GC/MS was operated in full scan mode (mass acquisition range m/z 50–450) using an ionization energy of 70 eV. The gas chromatograph was operated in splitless mode with an injector temperature of 280 °C. The initial column oven temperature was 100 °C (0.4 min), with a heating pad at 25 °C min^{-1} up to 290 °C, which was maintained for 9 min. Helium was used as the carrier gas with a column flow rate of 1.2 mL min^{-1}. The initial solvent cut time was 2.9 min. The injected sample volume was 1.0 μL, and the data acquisition time was 17 min. A capillary column HP-5ms (Agilent Technologies, Palo Alto, CA, USA) 30 m x 0.25 mm i.d. x 0.25 μm film thickness with stationary phase 5% diphenyl/95% dimethyl polysiloxane was used for analysis. The MS spectrum was compared with the NIST mass spectra database.

2.4. Optimization of ozone treatment conditions for pesticide removal

Two experiments were carried out separately, one for the optimization of the use of O_3 as gas and the other with O_3 dissolved in water. In both experiments, ozone was obtained through the ozone generator O & L3.ORM (Ozone & Life, São José dos Campos, SP, Brasil). The ozone generator used a constant oxygen flow of 2 L min^{-1} from the Mark 5 Plus Concentrator Oxygen Concentrator (Nidek Medical Products, Birmingham, AL, EUA). The ozone concentrations in the experiment of