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

Lauana Pellanda de Souza[a], Lêda Rita D'Antonino Faroni[a,*], Fernanda Fernandes Heleno[a], Frederico Garcia Pinto[b], Maria Eliana Lopes Ribeiro de Queiroz[c], Lucas Henrique Figueiredo Prates[a]

[a] Department of Agricultural Engineering, Universidade Federal de Viçosa, Viçosa 36570900, Minas Gerais, Brazil
[b] Institute of Exact and Technological Sciences, Universidade Federal de Viçosa, Rio Paranaiba 38810000, Minas Gerais, Brazil
[c] Chemistry Department, Universidade Federal de Viçosa, Viçosa 36570900, Minas Gerais, Brazil

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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

* Corresponding author.
E-mail addresses: lauana.souza@ufes.br (L.P.d. Souza), lfaroni@ufv.br (L.R.D. Faroni), fernanda.heleno@ufv.br (F.F. Heleno), frederico.pinto@ufv.br (F.G. Pinto), meliana@ufv.br (M.E.L.R.d. Queiroz), lucas.prates@ufv.br (L.H.F. Prates).

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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

O_3 as gas were quantified at the inlet and outlet of the treatment chambers using the iodometric method by indirect titration; for the experiment of O_3 dissolved in water, the ozone concentrations were quantified both at the inlet of the chamber with water and dissolved in water using the iodometric method by indirect titration (Eaton & Franson, 2005; Gottschalk, Libra, & Saupe, 2010). After the passage through the entire system, the residual ozone was directed to a catalyst filter (Ozone & Life, São José dos Campos, SP, Brasil) with the function of transforming ozone into oxygen. Eighteen carrots (≅ 2000 g) were used in each treatment of both experiments.

2.4.1. Experiment 1 – O_3 as gas

The carrot treatment with ozone gas was carried out in a 0.075 m^3 acrylic chamber (0.32 × 0.53 × 0.44 m), with perforated shelves that allowed the gas flow of O_3 inside the chamber. The chamber was fitted with an inlet at the top coupled to the ozone generator. The gas outlet, connected to a catalyst filter, was inserted in the lower part of the chamber.

2.4.2. Experiment 2 – O_3 dissolved in water

The treatment of the carrots with ozone dissolved in water was carried out in a circular chamber of PVC, 50 × 80 cm (diameter x height), containing 10 L of deionized water (0.5 mS m^{-1}). The ozone gas inlet was an aperture in the medial portion of the chamber, and it was coupled to a perforated spiral that ran through the water column until it was concentrated in the bottom of the chamber. A perforated metal plenum was placed over a 10-cm layer of glass beads (2 cm) above the spiral located at the bottom of the chamber to provide support for the carrots and a better distribution of the ozone gas in the water. The outlet of the remaining gas was an aperture in the top of the chamber, and it was connected to a catalyst filter. After each treatment, the carrots were withdrawn from the water and allowed to dry at room temperature (23 °C) for 30 min.

In both experiments, to control the temperature throughout the treatment, the chambers were inserted into a climatic chamber that allowed a variation of ± 1 °C. Before and after ozonation, the samples of three carrots were analyzed for pesticide residues by SLE/LTP-GC/ECD. The analysis of the pesticide residues was carried out in triplicate.

2.5. Experimental design

The ozone treatments were optimized for the removal of the pesticides difenoconazole and linuron in carrots employing a central composite design, with five replicates in the central point (Table 1). Three variables were studied: ozone concentration, treatment time and temperature. The variables were studied at two levels, and the analyses were performed in triplicate. The effects of each variable and the interactions between the variables in the difenoconazole and linuron removal from carrots were calculated using the Statistica 13.0 software (Statsoft Inc., Tulsa, OK, USA). The data were presented in graphs made by SigmaPlot 12.5 software (Systat Software, Inc., San Jose, CA, USA).

2.6. Removal of pesticides in carrots stored after ozonation

Carrots submitted to treatments 9, 10 and 15 (Table 1) were used to study the long-term effects of ozone on pesticide removal. These treatments were selected because they were conducted at the same temperature (14 °C) and treatment time (60 min). Thus, it was possible to evaluate the isolated ozone effect. The carrots submitted to these treatments for both methods of ozone application were stored for up to five days in a climate controlled room (18 ± 2 °C, 80 ± 5% RH). A daily sample of three carrots from each treatment were taken for the verification of the long-term effect of ozone on pesticide residues. The analysis of pesticides residues was carried out in triplicate. The percentages of pesticide removal were subjected to a variance analysis, and the means were compared by the Tukey test at the 5% significance level. Data were analyzed with SAS 9.0 (SAS Institute Inc., Cary, NC, USA).

3. Results and discussion

3.1. Method validation

The extraction technique assessed the following parameters of merit of the proposed method: selectivity, linearity, limit of detection (LOD) and quantification (LOQ), precision, and accuracy. The selectivity of the analytical method was evaluated by comparing the chromatograms of the extracts from a pesticide-free array with the chromatograms of the extracts of the matrix fortified with pesticides studied in concentrations equivalent to the Maximum Residue Limit (MRL) (0.2 mg kg^{-1} for difenoconazole and 1 mg kg^{-1} for linuron) (MAPA, 2017). The chromatogram of extracts from carrot samples (obtained by SLE/LTP) containing the pesticides difenoconazole and linuron showed retention times of 3.075 and 9.750 min, respectively. The absence of any signal at the retention time of difenoconazole and linuron indicated that no matrix compounds were present that could give a false positive signal. This demonstrated the validity of the method when used in the study of difenoconazole and linuron in carrot.

The linearity of the response of the method was determined by using matrix-matched calibration by injecting extracts of samples fortified at ten pesticide concentrations (0.25 – 2.5 × MRL) subjected to the SLE/LTP technique. After the chromatographic analysis, analytical curves were constructed, linking the areas of the analytes with the concentrations mentioned. Analytical curves relate the ratio of the analyte areas and their concentrations, thus obtaining the linear equations and the correlation coefficient. The calibration curves of difenoconazole (y = 164349.78x + 3095.73) and linuron (y = 7674.15x − 1179.03) showed a good linearity and a strong correlation between the concentrations and peak area in the studied range. The correlation coefficients of the calibration curves were 0.994 for difenoconazole and 0.990 for linuron. Such values indicate the good linearity of the method in response to the two pesticides at concentrations close to the MRL.

LOD is the lowest concentration of the analyte detectable in the sample by any analytical method, while LOQ is the lowest solute concentration that can be determined with an acceptable level of uncertainty (Abad, Winck, Silva, Caramão, & Zini, 2010; Costa, Queiroz, Neves, Sousa, & Zambolim, 2015; EURL, 2015). LOD and LOQ were determined with a calculation based on 3.3 and 10 times the ratio between the standard deviation of the intercept and the slope estimated from the calibration curve of the analytes (INMETRO, 2016). The LOD and LOQ values were 0.020 and 0.050 mg kg^{-1} for difenoconazole and 0.120 and 0.360 mg kg^{-1} for linuron, respectively. These values were acquired using the method based on analytical curve parameters with a working range of 0.05 – 0.5 mg kg^{-1} for difenoconazole and 0.5 – 2.5 mg kg^{-1} for linuron. The detection and quantification limit values were lower than the MRL prescribed by regulatory agencies such as United States Environmental Protection Agency (USEPA) (0.5 mg kg^{-1} for difenoconazole and 1 mg kg^{-1} for linuron) and Agencia Nacional de Vigilância Sanitária e Ambiental (ANVISA) from Brazil (0.2 mg kg^{-1} for difenoconazole and 1 mg kg^{-1} for linuron) for both pesticides in carrot (USEPA, 1996, MAPA, 2017).

The precision of the method in terms of repeatability was determined by carrot samples fortified with difenoconazole and linuron. The repeatability was verified by conducting injections of 0.5, 1.0, and 1.5 x MRL subjected to the SLE/LTP method, with six repetitions for the standard solution, maintaining all operational conditions constant. The precision values obtained in the three studied levels ranged from 93.4 to 116.6%, the lowest recovery being for difenoconazole and the highest for linuron. For the pesticide residue analysis, the analytical procedure should be able to retrieve an average of 70–120% (ANVISA, 2011) residue at each level of fortification.

The accuracy is the systematic error of the measuring system and

Table 1
Central composite design, with five replicates at the central point (C), to investigate the effects of the concentration of O_3 as gas (experiment 1) and dissolved in water (experiment 2), temperature and treatment time on the removal of difenoconazole and linuron residue in carrots.

Treatment	Levels of coded variables			Levels of real variables for experiment 1			Levels of real variables for experiment 2		
	X_1	X_2	X_3	O_3 concentration (mg L^{-1})	Time (min)	Temperature (°C)	O_3 concentration (mg L^{-1})	Time (min)	Temperature (°C)
1	−1	−1	−1	1.0	30.0	8.0	2.0	30.0	8.0
2	−1	−1	+1	1.0	30.0	20.0	2.0	30.0	20.0
3	−1	+1	−1	1.0	90.0	8.0	2.0	90.0	8.0
4	−1	+1	+1	1.0	90.0	20.0	2.0	90.0	20.0
5	+1	−1	−1	4.0	30.0	8.0	8.0	30.0	8.0
6	+1	−1	+1	4.0	30.0	20.0	8.0	30.0	20.0
7	+1	+1	−1	4.0	90.0	8.0	8.0	90.0	8.0
8	+1	+1	+1	4.0	90.0	20.0	8.0	90.0	20.0
9	−α	0	0	0.0	60.0	14.0	0.0	60.0	14.0
10	+α	0	0	5.0	60.0	14.0	10.0	60.0	14.0
11	0	−α	0	2.5	9.5	14.0	5.0	9.5	14.0
12	0	+α	0	2.5	110.5	14.0	5.0	110.5	14.0
13	0	0	−α	2.5	60.0	3.9	5.0	60.0	3.9
14	0	0	+α	2.5	60.0	24.1	5.0	60.0	24.1
15 (C)	0	0	0	2.5	60.0	14.0	5.0	60.0	14.0
16 (C)	0	0	0	2.5	60.0	14.0	5.0	60.0	14.0
17 (C)	0	0	0	2.5	60.0	14.0	5.0	60.0	14.0
18 (C)	0	0	0	2.5	60.0	14.0	5.0	60.0	14.0
19 (C)	0	0	0	2.5	60.0	14.0	5.0	60.0	14.0

was calculated by evaluating the values of the coefficients of variation (CVs) of the results obtained (EURL, 2015; INMETRO, 2016). The accuracy related to chromatographic areas ranged from 3.5 to 10.4%. According to Ribani, Botolli, Collins, Jardim, and Melo (2004), CV values up to 20% are acceptable depending on the complexity of the sample. These results demonstrate the good performance of the method.

3.2. Optimization of ozone treatments for pesticide removal

Ozone treatment for pesticide removal is a typical example of gas absorption in a chemical reaction, which affects the mass transfer. Therefore, all process variables affecting these two phenomena will govern the efficacy of the ozone treatment. We studied three important variables (O_3 concentration, temperature and treatment time) governing the efficacy of ozone treatment in gaseous and liquid phase. Temperature variation in the range of 4–24 °C did not have a significant effect ($P > .0972$) on the efficacy of ozone treatments. For the same inlet O_3 concentrations, the O_3 concentrations in the chamber were similar even at different temperatures. This indicates that carrots treated with the same inlet O_3 concentration and same treatment time were exposed to the same O_3 concentration inside the chamber, even with different environmental temperatures (Table 2).

Unlike the gaseous O_3 treatments, the concentration of the dissolved ozone in the water decreased with an increase in temperature (Table 2). However, this variation was not sufficient for the temperature to be a significant factor in the removal of pesticides by O_3 treatments. The solubility of ozone decreases with increasing temperature (Achen & Yousef, 2001). Moreover, ozone decomposes in water to yield hydroxyl radicals (OH). However, the reaction rate of O_3 decomposition is much faster when the water temperature is high; and the relative contribution of these factors in pesticide removal from carrots may compensate each other (Ikeura, Kobayashi, & Tamaki, 2011).

The average concentration of pesticides found in carrots before submission to treatments was 2.500 mg kg^{-1} for difenoconazole and 7.200 mg kg^{-1} for linuron. Both the exposure time ($.018 < P < .048$) and the ozone concentration ($P < .001$) were found to be significant in terms of pesticide removal. Three-dimensional response surface graphs were generated to demonstrate the effects of exposure time and ozone concentration on the removal of difenoconazole and linuron from carrots (Fig. 1). Moreover, the percentage of pesticide removal increases with increases in ozone concentration and treatment time. Both forms of O_3 application caused a significant reduction (over 80%) in pesticide residue, whereas non-O_3 treatments removed less than 20 and 45% of the pesticides in the gaseous and liquid treatments, respectively. The highest percentages of pesticide removal were achieved when the roots were exposed to ozone for approximately 120 min at 5 and 10 mg L^{-1} ozone, respectively, in the gaseous state and dissolved in water.

Ozone is a well-known gaseous chemical agent capable of oxidizing a variety of organic and inorganic compounds in the gaseous phase, as solid substrates and in aqueous solutions, either by direct attack or through a radical-mediated mechanism involving the hydroxyl radical (Segat et al., 2014). Recently, several studies have reported effective removal of pesticide residues from fruits and vegetables by ozone (Gabler et al., 2010; Heleno et al., 2015; Heleno et al., 2016; Lozowicka et al., 2016). Although the use of ozone in pesticide removal has been proven for many agricultural products, its effect on the removal of difenoconazole and linuron from fruits and vegetable has not been well studied. Heleno et al. (2014) observed that the concentration of difenoconazole in strawberries reduced drastically as the concentration of ozone dissolved in water increased. After 1 h of exposure to ozone at a concentration of 0.800 mg L^{-1}, the fungicide residue showed a 95% reduction compared with the fungicide concentration before ozone treatment.

The oxidation of organic compounds by O_3 can occur through two specific reactions: the reaction of the O_3 molecule with the molecules of the organic compounds and the reaction of the free radicals (-O) formed by O_3 decomposition, with organic compounds. In aqueous solution, the organic compounds are oxidized by the hydroxyl radical action formed from the oxygen atoms resulting from O_3 degradation with free hydrogen atoms in solution (Chiron et al., 2000).

The pesticides difenoconazole and linuron have aromatic rings and double bonds in their molecular structure (Table 1S). The double bond is most susceptible to ozone attack, leading to the formation of a primary ozonide, which, being unstable, dissociates into a stable compound and a corresponding intermediate. The intermediates undergo further decomposition via O-atom elimination, the ester channel, or the hydrogen peroxide channel or might stabilize after collision with another body (Al Rashidi, Chakir, & Roth, 2013).

In some cases, the disappearance of the pesticide residue does not indicate safe treatment because the degraded products may be as toxic as the parent compounds (Wu et al., 2007). The intermediates from the degradation of difenoconazole and linuron by O_3 were also monitored by GC/MC analysis, but none were found in this study. Ten intermediates from the degradation of linuron by O_3 were identified by Rao

Table 2
Average ± SD of O_3 concentration (mg L^{-1}) in the inlet, in the chamber, and dissolved in water at different temperatures (°C) and treatment times (min).

Treat.	Time (min)	Temp. (°C)	O_3 concentration			
			Experiment 1 (mg L^{-1} of air)		Experiment 2 (mg L^{-1} of water)	
			Inlet (mg L^{-1})	In chamber (mg L^{-1})	Inlet (mg L^{-1})	Dissolved in water (mg L^{-1})
1	30.0	8.0	1.0	0.354 ± 0.100	2.0	0.689 ± 0.073
2	30.0	20.0	1.0	0.366 ± 0.072	2.0	0.513 ± 0.054
3	90.0	8.0	1.0	0.488 ± 0.143	2.0	0.379 ± 0.062
4	90.0	20.0	1.0	0.610 ± 0.132	2.0	0.243 ± 0.022
5	30.0	8.0	4.0	1.952 ± 0.133	8.0	0.689 ± 0.073
6	30.0	20.0	4.0	2.440 ± 0.231	8.0	0.513 ± 0.054
7	90.0	8.0	4.0	2.928 ± 0.132	8.0	0.629 ± 0.021
8	90.0	20.0	4.0	2.806 ± 0.120	8.0	0.533 ± 0.056
9	60.0	14.0	0.0	0.000 ± 0.000	0.0	0.000 ± 0.000
10	60.0	14.0	5.0	2.930 ± 0.291	10.0	0.683 ± 0.002
11	9.5	14.0	2.5	1.464 ± 0.112	5.0	0.229 ± 0.033
12	110.5	14.0	2.5	1.579 ± 0.103	5.0	0.397 ± 0.042
13	60.0	3.9	2.5	1.708 ± 0.193	5.0	0.454 ± 0.032
14	60.0	24.1	2.5	1.532 ± 0.086	5.0	0.234 ± 0.022
15–19	60.0	14.0	2.5	1.590 ± 0.193	5.0	0.337 ± 0.032

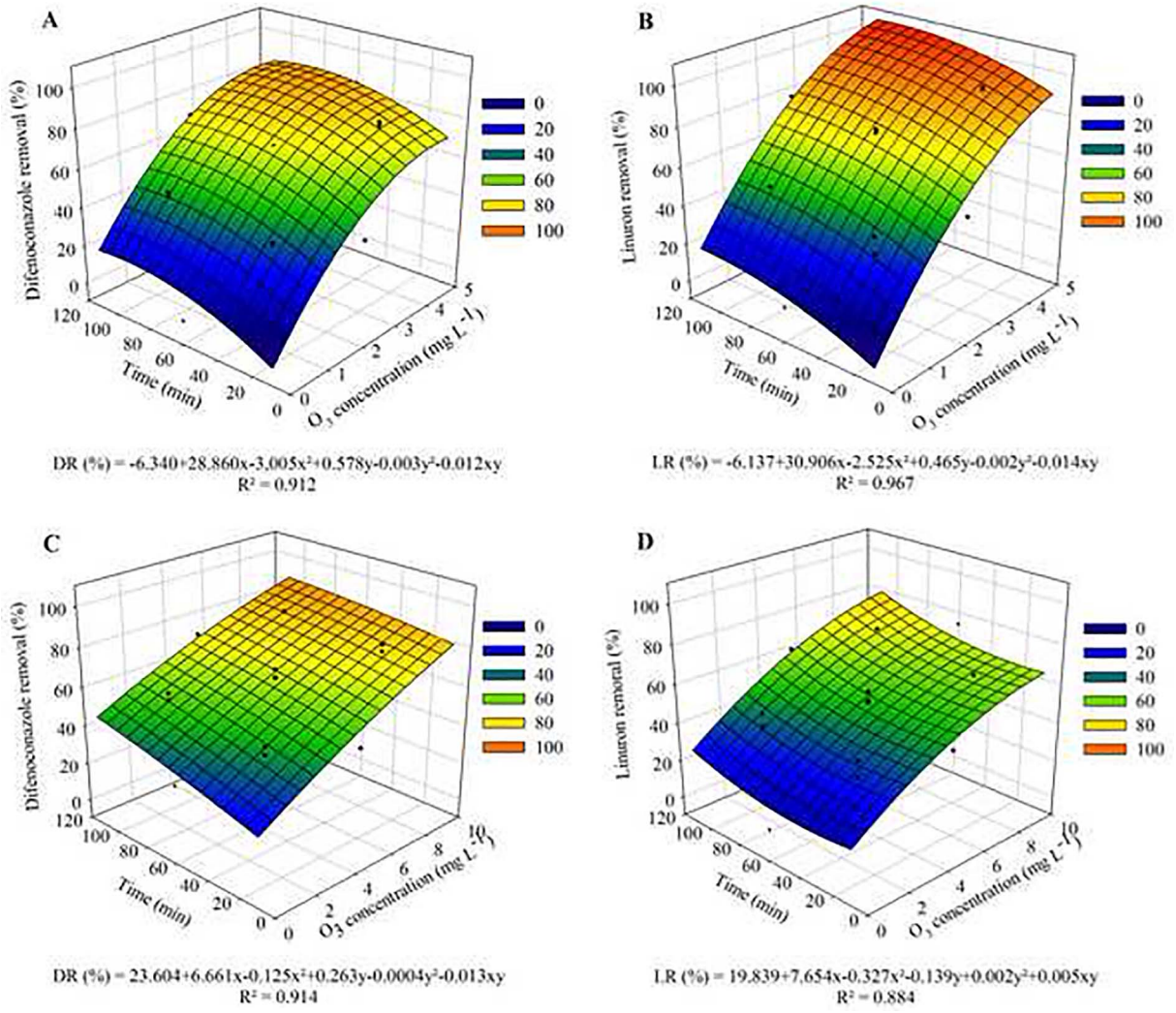

Fig. 1. Response surface showing the effect of x = O_3 concentration as gas (A and B) and dissolved in water (C and D) and y = treatment time on the removal of difenoconazole (DR) and linuron (LR) residue in carrots.

Table 3
Effect of O_3 concentration (at 14 °C for 60 min) on the removal of difenoconazole and linuron residue (%) from carrots in storage (18 ± 2 °C, 80 ± 5% RH).

Pesticide	O_3 concentration (mg L^{-1})	Removal of pesticides (%)		
		Storage time (days)		
		1	3	5
Ozone in gas				
Difenoconazole	0	18.1 b	53.1 b	53.2
	2.5	94.9 a	95.7 a	> 98.0*
	5	95.3 a	95.7 a	> 98.0*
	P value	*.0007*	*.0001*	–
Linuron	0	12.1 b	19.4 b	22.9 b
	2.5	73.9 a	74.2 a	78.1 a
	5	> 95.0*	> 95.0*	> 95.0*
	P value	*0.0021*	*0.0101*	*0.0027*
Ozone dissolved in water				
Difenoconazole	0	33.1 c	40.3 b	44.8 b
	5	70.3 b	79.0 a	88.8 a
	10	88.7 a	91.1 a	96.0 a
	P value	.0015	.0014	.0020
Linuron	0	7.4 c	13.0 c	15.8 b
	5	50.9 b	57.7 b	63.2 a
	10	75.7 a	78.2 a	79.8 a
	P value	*.0003*	*< .0001*	*.0034*

* Removal percentages higher than 98% for difenoconazole and 95% for linuron could not be quantified because they correspond to values lower than the LOQ of the analytical method used (0.05 and 0.36 mg kg^{-1}, respectively). Values followed by the same letters in the column are not significantly different by the Tukey test at the 5% significance level ($P < .05$).

and Chu (2009). The authors suggested that *N*-terminus oxidation should be the major mechanism of linuron decay by ozonation, and all the intermediates should be possibly susceptible to the ozonation process. However, the intermediates may not accumulate to an appreciable level due to fast decays. Our study corroborated the results of Rao and Chu (2009), who did not found intermediates in the solution when 99% linuron degradation was achieved.

3.3. Removal of pesticides from carrots stored after ozonation

In addition to the immediate effect on pesticide removal, our research also evaluated the possible long-term effect of ozone treatment on carrots. As seen in Table 3, in all treatments, at 14 °C for 60 min, the removal percentages of pesticides increased up to the fifth day of storage. However, the removal percentages reached higher values in the ozone treatments.

The removal percentages of difenoconazole were the same statistically ($P < .0007$) in all treatments with O_3 as gas and from the third day in treatments with O_3 dissolved in water ($P < .0020$). The same behavior was observed for linuron on the fifth day of storage after treatment with O_3 dissolved in water ($P = .0034$). Toward the end of the carrots' storage period, the maximum removal values of the pesticides by ozone as gas were greater than 98% for difenoconazole and 95% for linuron. In the treatments with O_3 dissolved in water, the removal percentages of difenoconazole and linuron reached values of up to 96 and 79.8%, respectively.

In our experiments, approximately 92% of the difenoconazole residue and 86% of the linuron residue needed to be removed so that the MRLs set for Brazil and USA were respected simultaneously (MAPA, 2017; USEPA, 1996). Values equal to or lower than the MRLs were reached on the first day of storage of the carrots after 60 min of treatment with O_3 as gas at a concentration of 2.5 mg L^{-1} for difenoconazole and 5 mg L^{-1} for linuron. However, in treatments with O_3 dissolved in water, the MRL was only reached for difenoconazole on the fifth day of storage of carrots treated with at least 2.5 mg L^{-1}. Nevertheless, due to its systemic nature, the pesticides could have penetrated deeply the carrots. Even though, the ozone treatments achieved high levels of systemic pesticides residues removal.

The ability to remove difenoconazole and linuron from carrots without the formation of toxic intermediates strengthens the GRAS (Generally Recognized as Safe) status for the treatment, storage and processing of food and water recognized by the United States Food and Drug Administration (FDA, 2001). O_3 is environmentally friendly to produce and does not leave any residues in the food when it dissociates into oxygen; thus, it has a distinct advantage over other oxidants.

4. Conclusion

This is the first study to investigate the effect of ozone in the removal of difenoconazole and linuron in carrots. Ozone treatments in gaseous form and dissolved in water were effective methods for post-harvest removal of the pesticides residues without the formation of toxic intermediates in carrots. Removal percentages increased with increases in ozone concentration and treatment time. In addition to being a strong oxidant, ozone also has the advantage of not leaving a decontaminant residue in treated foods because it quickly decomposes to oxygen.

Conflict of interest

The authors declare that they have no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.foodchem.2017.09.134.

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