



# Aqueous ozone solutions for pesticide removal from potatoes

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## Abstract

The presence of pesticide residues in potatoes is of concern because of the potential impact to human health due to the high consumption of this vegetable. In this study, aqueous solutions with and without ozone saturation as postharvest wash treatment at pH 4.0, 7.0, and 9.0 were tested to remove chlorothalonil from potatoes. The method used for pesticide analysis has been validated, presenting recovery values of 94–103%, with variations in the repeatability coefficients of  $\leq 10.6\%$ , and a quantification limit of  $0.05 \text{ mg kg}^{-1}$ . Regardless of pH, treatment with aqueous ozone solutions removed 70–76% of the pesticide present in the potato. In the no-ozone treatments, the percentage average removal of chlorothalonil residues in potatoes was only 36%. Over 24 days of storage, the quality of potatoes washed with aqueous ozone solutions was not significantly different from those washed with pure water.

## Keywords

Potatoes, *Solanum tuberosum*, chlorothalonil, ozonated water, degradation, quality

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## INTRODUCTION

Pesticides are the most widely used form of pest control worldwide. However, when the recommended application doses and harvest intervals are not followed, residual pesticide may remain postharvest (Jardim and Caldas, 2012).

In the cultivation of potato (*Solanum tuberosum*), chlorothalonil fungicide (tetrachloroisophthalonitrile,  $\text{C}_8\text{Cl}_4\text{N}_2$ ) is used to control early blight (*Alternaria solani*) and late blight (*Phytophthora infestans*).

The presence of pesticide residues in potatoes (a highly consumed vegetable) is of concern because of the potential impact to human health. In humans, chlorothalonil can cause dermatitis, severe eye and cuticle irritation, and gastrointestinal problems (Draper et al., 2003).

The development of technologies capable of degrading pesticide residue in food before consumption is important. The use of ozone has been increasing, due to its high oxidative power and the ease of obtaining the gas. Ozone was generally recognized as safe in the USA in 2001 (FDA, 2001), and since that time it is being widely investigated and introduced into some commercial applications in food industry (Karaca et al., 2012).

Some studies have noted the potential of ozone as a pesticide residue degradation agent in apples (Hwang et al., 2001, 2002; Ong et al., 1996), table grapes (Gabler et al., 2010; Heleno et al., 2015; Karaca et al., 2012), strawberries (Al-Dabbas et al., 2014; Heleno et al., 2014; Ikeura et al., 2011; Lozowicka et al., 2016), lettuces (Ikeura et al., 2011, 2013),

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tomatoes (Al-Dabbas et al., 2014; Ikeura et al., 2011, 2013), and lychee fruits (Whangchai et al., 2011), but the literature on ozone treatment of potatoes is limited.

Ozone is an unstable compound that decomposes either spontaneously, producing hydroxyl radicals and other free radical species, or in contact with oxidizable surfaces. Ozone can degrade pesticides via direct or indirect reactions. The type of reaction, direct or indirect, between the ozone and organic contaminants, is pH dependent. In acidic pH, direct reaction mechanisms predominate, namely via molecular ozone. As the pH increases, a larger amount of hydroxyl radicals are formed, and indirect reaction mechanisms predominate. However, at a pH of approximately 7, both types of reactions occur (Özbelge and Erol, 2008).

The aim of this study was to evaluate the removal of chlorothalonil from potatoes by ozonation at different pH values and to evaluate the influence of treatments on the quality of potatoes.

## MATERIALS AND METHODS

### Plant material and chemicals

Commercial potato (*S. tuberosum*) cultivar Ágata, checked in our laboratory to be free of pesticide residue, was purchased from a supermarket in Viçosa city, Brazil.

For the extraction of the pesticides, HPLC-grade ethyl acetate (Sigma-Aldrich, St. Louis, MO, USA) and HPLC-grade acetonitrile (J.T. Baker, Phillipsburg, NJ, USA) were used. Chlorothalonil standard stock solution at a concentration of 1000 mg L<sup>-1</sup> was prepared by solubilization of standard chlorothalonil (Sigma-Aldrich, Steinheim, Germany, 99.3% w/w) in acetonitrile. A working solution containing difenoconazole at 50.0 mg L<sup>-1</sup> was prepared by diluting the stock standard solution with the same solvent. These solutions were stored in a freezer at approximately -20 °C.

Aqueous solutions of the chlorothalonil were prepared (Echo WG, Sipcam UPL Brasil S.A., Uberaba, Brazil) at 2.55 g L<sup>-1</sup>.

The following reagents were used in the experiments: sulfuric acid (96.0% w/v, Vetec, Rio de Janeiro, Brazil), potassium iodide (99.0% w/w, Vetec, Rio de Janeiro, Brazil), sodium thiosulfate (99.0% w/w, Carlo Erba, Milan, Italy), soluble starch (99.6% w/w, Scientific Exodus Hortolandia, Brazil), sodium hydroxide (99.0% w/w, Vetec, Rio de Janeiro, Brazil), phenolphthalein (>99% w/w, Merck, Darmstadt, Germany), and nitric acid (65.0% w/v, Vetec, Rio de Janeiro, Brazil).

### Contamination of potatoes with chlorothalonil

Potato samples, free from pesticide residue, 3.0 kg (around 45 potatoes) per treatment, were immersed for 1 min in 2.55 g L<sup>-1</sup> chlorothalonil solution and were allowed to dry in a ventilated atmosphere for 12 h.

### Preparation of aqueous ozone solutions at different pHs

Ozone gas was obtained from an ozone generator developed by the company Ozone & Life (São José dos Campos, Brazil). Oxygen was used for the generation of ozone (Linde Gases, Canoas, Brazil, 99.5% purity).

Buffered aqueous solutions (6.0 L) at pH 4, 7, and 9 were placed into glass containers (bubbling tanks). These solutions were continuously bubbled with ozone gas at a concentration of 3.0 mg L<sup>-1</sup> at a flow rate of 2.0 L min<sup>-1</sup>.

The solutions were maintained at 15 °C and circulated between the bubbling and washing tanks (Figure 1). The ozone saturation time of each aqueous solution was determined by measuring the concentration of ozone at regular intervals until it

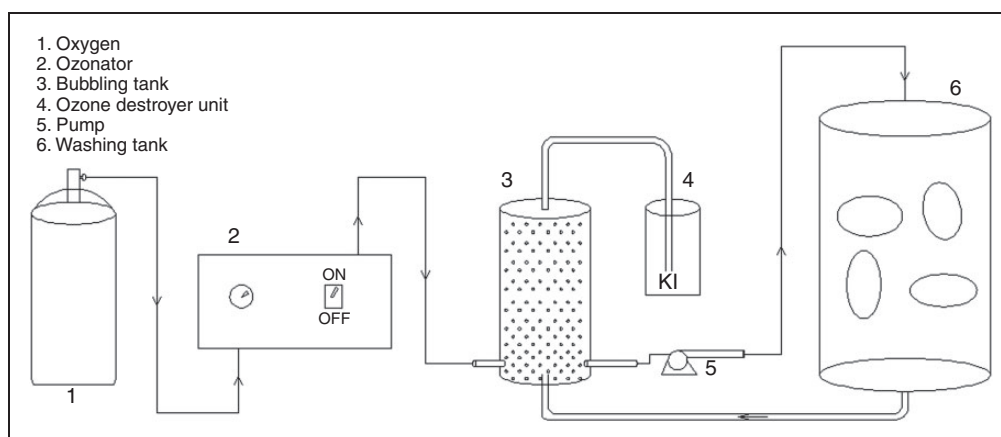


Figure 1. Schematic diagram for the treatment of the potatoes.

remained constant. The concentration of the ozone gas produced was determined by iodometric method (APHA et al., 2005), and the ozone concentration in aqueous solution was monitored by DDPD method using the Ozone Vacu-vials kit (CHEMetrics, Midland, VA, USA).

### Removal of chlorothalonil residue from potatoes

Potato samples spiked with chlorothalonil were stored in a PVC container (washing tank) and were subjected to eight treatments: distilled water without pH adjustment and buffered aqueous solutions at pH 4, 7, and 9, and the same treatments were saturated with ozone (water, water/pH 4, water/pH 7, water/pH 9, water/O<sub>3</sub>, water/O<sub>3</sub>/pH 4, water/O<sub>3</sub>/pH 7 and water/O<sub>3</sub>/pH 9). The saturated and unsaturated aqueous ozone solutions (6.0 L) were circulated for 60 min between the bubbling and washing tanks (Figure 1). The immersion time used was chosen based on other work (Whangchai et al., 2011). The experiments were conducted in a climate-controlled room maintained at 15 °C and performed in triplicate.

The potatoes subjected to different treatments and the control were separated into two groups. The first group of samples was triturated, and the pesticide residue was extracted and analyzed by gas chromatography using an electron capture detector (GC/ECD). The second group of samples was stored at room temperature in the absence of light.

### Chlorothalonil analysis of potato samples

Chlorothalonil residues were extracted from the potato samples using solid-liquid extraction with partition at low temperature (SLE/LTP), adapted from Rigueira et al. (2013). The method consisted of adding 6.5 mL of acetonitrile, 1.0 mL water, and 1.5 mL of ethyl acetate to 3.00 g of the potato sample. The mixture was stirred on a shaking table (Tecnal TE 420, São Paulo, Brazil) at 25 °C and 200 r/min for 15 min and then was placed in a freezer (Consul, model 280, São Paulo, Brazil) at approximately -20 °C for 12 h for phase separation. After this period, the supernatant was analyzed on a gas chromatograph (model GC-2014, Shimadzu, Kyoto, Japan) equipped with an ECD. An HP-5 capillary column (30 m × 0.25 mm, 0.1 µm thick film; Agilent Technologies, Palo Alto, USA) using nitrogen (Air Products, São Paulo, Brazil, 99.999% purity) as a carrier gas at a flow rate of 1.2 mL min<sup>-1</sup> was used. The temperatures of the split/splitless injector and detector were 280 and 300 °C, respectively. The initial temperature of the column oven was 200 °C, with a heating

ramp of 20 °C min<sup>-1</sup> to 290 °C, and this temperature was maintained for 4.5 min. The injections were split at 1:5, the injected volume was 1.0 µL, and the total analysis time was 9 min.

The chlorothalonil content was determined by direct comparison to a matrix-matched standard. The maximum residue limit (MRL) of chlorothalonil on potatoes established by Brazilian legislation (0.1 mg kg<sup>-1</sup>) was considered in the validation of the method to ensure that the method is appropriate for the determination of residues in this concentration range.

The linearity in the response was studied using matrix-matched calibration solutions prepared by spiking potatoes at six concentration levels, ranging from the quantification limit (LOQ) of the analyte to 0.2 mg kg<sup>-1</sup> (2 × MRL). The limits of detection (LOD) and quantification were calculated as the ratio between the standard deviation of the coefficient of the linear equation and the slope of an analytical curve with low chlorothalonil concentrations. The resulting value was multiplied by 3.3 to obtain the LOD and by 10 to obtain the LOQ, according to the ICH guidelines (ICH, 2005). The precision of the chromatographic method, determined as the coefficient of variation (CV), was obtained from six replicates of the spiked samples at three concentrations (0.05, 0.10, and 0.15 mg kg<sup>-1</sup>) on the same day (repeatability) and on different days (reproducibility). The recovery studies were performed (six replicates) by spiking samples at three concentrations (0.05, 0.10, and 0.15 mg kg<sup>-1</sup>). The results were expressed as the recovery percentage.

### Quality analysis of potatoes

Treated samples and control samples (35 potatoes per treatment) were stored at 25 °C in the absence of light. The quality parameters, titratable acidity, pH, and potato color were evaluated periodically for 24 days.

The acidity was determined by titrating a sample of the juice from the pulp of three potatoes with a standardized solution of 0.1 mol L<sup>-1</sup> NaOH, and the pH measurement was performed by immersion of an electrode in the potato sample prepared to determine the acidity, according to the Instituto Adolfo Lutz (IAL, 2005) methods.

The assessment of color was performed with a CR 400 Minolta colorimeter (Ramsey, USA), and the color difference value of three points on three potatoes was measured. The color difference value was calculated using the CIE system (Commission Internationale de l'Éclairage) with direct reading of the reflectance of the coordinates "L\*" (lightness), "a\*" (from green to red), and "b\*" (from blue to yellow). With the values of these coordinates, it was possible to calculate

parameters related to the total color difference,  $\Delta E$  (equation (1)) (Meir et al., 1992; Macdougall, 2002).

$$\Delta E = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \quad (1)$$

### Statistical analysis

Chromatographic data were analyzed using GC solution (Shimadzu, Kyoto, Japan). The validation data were subjected to analysis using descriptive statistics based on the parameters of the measurement of the central tendency (mean) and the measurement of dispersion (standard deviation, relative standard deviation and CV). The descriptive statistics were calculated using Excel (Microsoft Corp., Redmond, USA).

The data obtained from the tests to assess potato quality and the removal of chlorothalonil residues of potatoes were subjected to analysis of variance (ANOVA) using Statistica 8.0 (StatSoft Corp.,

Tulsa, USA).  $P < 0.05$  was considered statistically significant.

The data of the saturation of buffer solutions with ozone were subjected to regression analysis “Linear Response Plateau” as a function of time, using SAEG software (UFV, Viçosa, Brazil).

## RESULTS AND DISCUSSION

### Extraction method validation parameters

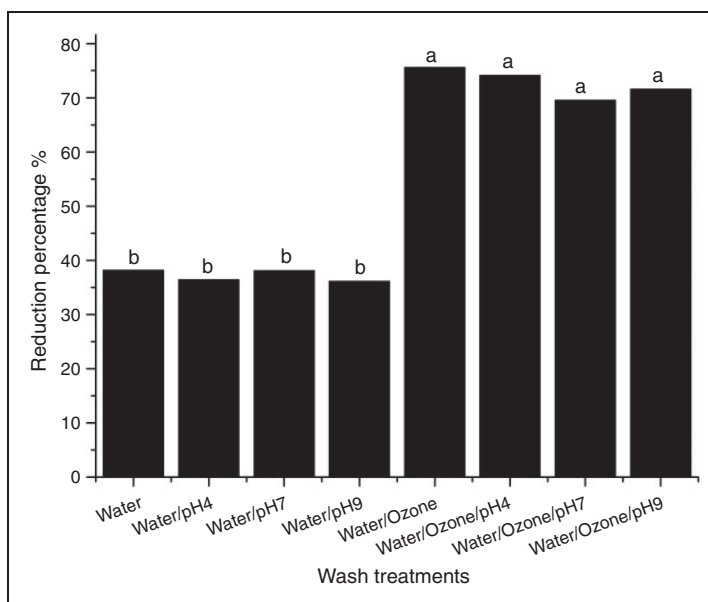
The extraction method of chlorothalonil residues in potato samples was adapted from the chlorpyrifos and thiamethoxam extraction method employed by Rigueira et al. (2013).

To assess the linearity of the method, we obtained the linear regression fit of the analytical curve from 0.05 to 0.2 mg kg<sup>-1</sup> chlorothalonil. Six concentration levels were employed, resulting in a correlation coefficient (r) of 0.99. This value indicates the high linearity of the method for chlorothalonil.

To determine the extraction precision and accuracy of chlorothalonil, potatoes were spiked with chlorothalonil at three concentration levels (0.05, 0.10, and 0.15 mg kg<sup>-1</sup>). The average recoveries of chlorothalonil were 98 ± 12% (0.05 mg kg<sup>-1</sup>), 103 ± 14% (0.10 mg kg<sup>-1</sup>), and 94 ± 11% (0.15 mg kg<sup>-1</sup>). To assess the repeatability, the CVs for the tests at each concentration level for a total of six repetitions were used. The results were 10.1, 10.6, and 8.7% at the concentrations of 0.05, 0.10, and 0.15 mg kg<sup>-1</sup>, respectively. For inter-day precision, the values were 12.7, 11.8, and 11.1%. The method detection limit and

**Table 1.** Concentration and time to saturation of the buffer solutions with ozone as a function of pH at 15 °C

pH	Saturation time/min	Concentration saturation/(mg L <sup>-1</sup> )
4	26.9	0.848
7	9.5	0.910
9	9.3	0.428



**Figure 2.** Removal of chlorothalonil residues from potatoes after 60 min by washing with aqueous solutions at different pH values, with and without ozone saturation. Values with the same letters are not significantly different ( $P > 0.05$ ).

**Table 2.** Average values of titratable acidity, pH, and total color difference ( $\Delta E$ ) during storage

Parameter	Treatment	Storage days									
		1	6	8	10	13	15	17	20	22	24
Titratable acidity	Water	0.20a	0.23a	0.18a	0.19abc	0.19a	0.12ab	0.11ab	0.09ab	0.11a	0.08bc
	Water/pH 4	0.17a	0.13c	0.15ab	0.19abc	0.08b	0.10ab	0.09b	0.07ab	0.09a	0.11a
	Water/pH 7	0.15a	0.17b	0.17ab	0.21ab	0.08b	0.08b	0.09b	0.07ab	0.08a	0.06c
	Water/pH 9	0.20a	0.16bc	0.14b	0.22a	0.10b	0.07b	0.10ab	0.08ab	0.09a	0.08bc
	Water/ozone	0.18a	0.12c	0.16ab	0.14c	0.19a	0.13ab	0.18a	0.10a	0.07a	0.10ab
	Water/ozone/pH 4	0.18a	0.18b	0.15ab	0.16bc	0.16a	0.12ab	0.15ab	0.10a	0.09a	0.11a
	Water/ozone/pH7	0.14a	0.15bc	0.16ab	0.17bc	0.22a	0.15a	0.13ab	0.09ab	0.08a	0.08bc
	Water/ozone/pH 9	0.19a	0.17b	0.18a	0.16bc	0.18a	0.12ab	0.14ab	0.06b	0.09a	0.12a
pH	Water	6.13cd	6.44a	6.34ab	6.31bcd	6.23a	6.46abc	6.40a	6.43a	6.18c	6.31b
	Water/pH 4	6.32a	6.45a	6.41a	6.45ab	6.50d	6.43bcd	6.29a	6.28a	6.37ab	6.52a
	Water/pH 7	6.29ab	6.43a	6.47a	6.50a	6.33abc	6.53ab	6.35a	6.32a	6.40a	6.55a
	Water/pH 9	6.25abc	6.28b	6.41a	6.33bcd	6.44cd	6.56a	6.25a	6.35a	6.33ab	6.49a
	Water/ozone	6.08d	6.27b	6.33ab	6.32bcd	6.50d	6.44abcd	6.43a	6.41a	6.36ab	6.18c
	Water/ozone/pH 4	6.11d	6.48a	6.17c	6.28cd	6.28ab	6.32d	6.41a	6.34a	6.37ab	6.24bc
	Water/ozone/pH 7	6.17bcd	6.35ab	6.19bc	6.25d	6.30ab	6.44abcd	6.29a	6.36a	6.31ab	6.31b
	Water/ozone/pH 9	6.13d	6.38ab	6.24bc	6.40abc	6.36bc	6.34cd	6.27a	6.27a	6.25bc	6.35b
$\Delta E$	Water	0.00a	4.23a	4.89ab	5.09a	4.48ab	4.65a	5.11a	5.55a	5.89a	6.35ab
	Water/pH 4	0.00a	3.26ab	3.88ab	4.20a	4.63ab	4.14a	4.34ab	5.18a	5.85a	5.37ab
	Water/pH 7	0.00a	2.85b	3.34b	3.38a	3.64b	3.82a	3.47b	4.53a	4.31a	4.66b
	Water/pH 9	0.00a	2.62b	3.83ab	3.93a	4.57ab	3.85a	4.11ab	5.36a	4.81a	5.24ab
	Water/ozone	0.00a	3.61ab	3.95ab	4.40a	4.45ab	4.17a	5.20a	5.76a	6.09a	5.37ab
	Water/ozone/pH 4	0.00a	3.14ab	5.04a	5.20a	5.26a	5.08a	4.91ab	5.81a	5.29a	6.42ab
	Water/ozone/pH 7	0.00a	3.24ab	4.01ab	4.83a	5.29ab	4.82a	5.57a	5.64a	5.73a	5.46ab
	Water/ozone/pH 9	0.00a	3.32ab	4.84ab	4.88a	4.99ab	4.77a	5.17a	6.12a	5.87a	6.51a

Note: Values within a column followed by the same letter do not differ significantly ( $P \geq 0.05$ ) for each characteristic available.

quantitation limit for chlorothalonil were 0.017 and 0.050 mg kg<sup>-1</sup>, respectively.

The LOQ is far below the MRLs established by the Brazilian Sanitary Surveillance Agency (ANVISA, 0.1 mg kg<sup>-1</sup>) and the Codex Alimentarius (0.3 mg kg<sup>-1</sup>). For the analysis of the pesticide residue, the analytical procedure should be able to recover, at each concentration level, 70 to 120% on average, with a CV < 20% (Brasil, 2011). Because the values obtained are within this range, the recovery and CV for chlorothalonil are suitable.

### Saturation of the aqueous solutions with ozone

The saturation time and the concentration of the saturated solution are inversely proportional to the pH values of the buffer solutions (Table 1).

The ozone concentrations in aqueous solution were in accordance with those obtained by Elovitz et al. (2000) for ozonation experiments in the pH 6–9 range. These authors demonstrated an increase in ozone depletion rates with increasing solution pH.

### Removal of chlorothalonil residues

After saturation of the aqueous solution with ozone, the potato samples were immersed in a washing tank for 60 min. Then, the potatoes were triturated and subjected to the SLE/LTP-GC/ECD validated method.

The potatoes treated with aqueous ozone solutions showed higher removal of fungicide than those treated with non-ozonized solutions (Figure 2). The removal of chlorothalonil residues was statistically equal for washing with pure water, buffer solution with pH 4, buffer solution at pH 7 and buffer solution with pH 9, and for the water/O<sub>3</sub>, water/O<sub>3</sub>/pH 4, water/O<sub>3</sub>/pH 7, and water/O<sub>3</sub>/pH 9 washes.

The highest chlorothalonil residue removal in potatoes was 75.6 ± 1.0% with treatment with aqueous ozone solutions without pH adjustment (~6.2 mg kg<sup>-1</sup> to ~1.5 mg kg<sup>-1</sup>). In the treatments with aqueous ozone solutions, the average removal was 72.7 ± 4.0%. In the no-ozone treatments, the average removal of chlorothalonil residues in potatoes was 35.7 ± 11.8%. The results showed that the wash treatments with aqueous ozone

solutions, regardless of pH, were more effective (chlorothalonil significantly higher removals) than the no ozone wash treatments.

Kusvuran et al. (2012) evaluated the removal of chlorothalonil residues by ozonation. In their studies, the removal efficiency of chlorothalonil residues with ozone was dependent on the matrix (lemon, orange, and grapefruit). The same treatment with ozone removed 92% of the chlorothalonil residue in lemon, 100% in orange, and 44% in grapefruit. In the literature, there are no pesticide residue removal studies on potatoes using ozone. High rates of chlorothalonil removal were obtained by Chen et al. (2013) after treating two vegetables (Chinese white cabbage and green-stem bok choy) with ozone. Cleaning for 15 min with pump recirculation removed 53% of the chlorothalonil. When the ozone production rate was 250 mg h<sup>-1</sup>, the removal efficiency was 55%. When the ozone production rate was 500 mg h<sup>-1</sup>, the removal efficiency was 77%, an increase of 24% compared to pump recirculation only. After ozone treatment, the pesticide residuals met the Standards for Pesticide Residue Limits in Foods.

The study of pesticide degradation in potatoes was of scientific and practical significance to safeguard humans from the consumption of pesticide-contaminated vegetable.

### Quality of potatoes

The samples subjected to different washing procedures were stored at room temperature in the dark. Periodically for 24 days, potato quality parameters were evaluated. Significant changes were observed in the amounts of titratable acidity ( $F_{3236} = 22.82$  and  $P = 0.0000$ ), pH ( $F_{3236} = 22.00$  and  $P = 0.0000$ ) and total color difference ( $F_{3796} = 15.07$  and  $P = 0.0000$ ) between the potatoes washed with pure water and those washed in buffered solutions at pH 4, 7, and 9 without ozone. The quality of potatoes washed with aqueous solutions saturated with ozone was not significantly altered in comparison with potatoes washed with pure water (titratable acidity:  $F_{4295} = 1.37$  and  $P = 0.2435$ ; pH:  $F_{4295} = 2.00$  and  $P = 0.0803$ ; total color difference:  $F_{4965} = 1.21$  and  $P = 0.3027$ ). Table 2 shows the average values of titratable acidity, pH, and total color difference ( $\Delta E$ ) during storage.

Beltrán et al. (2005) evaluated the exposure of potato strips to ozone and showed that after 14 days of storage, there was no evidence of browning in fresh-cut potatoes dipped in ozonated water and stored under vacuum. These treatments maintained the initial texture and aroma. However, the use of ozonated water alone was not effective in reducing the total microbial populations.

## CONCLUSION

Washing potatoes with aqueous ozone solutions (0.43–0.91 mg L<sup>-1</sup>), regardless of the pH (4, 7, and 9), increased the removal of chlorothalonil residues without changing the characteristics of the product.

## DECLARATION OF CONFLICTING INTERESTS

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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