Chlorine dioxide treatment for the removal of pesticide residues on fresh lettuce and in aqueous solution

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Abstract

The effectiveness of chlorine dioxide (CD) to remove phorate and diazinon residues on fresh lettuce and in aqueous solution was investigated. The results indicated that CD (20 mg/L) added in tap water can significantly improve the removal of phorate and diazinon on lettuce ($p < 0.05$), as compared to tap water wash. The study in aqueous solutions suggested that addition of CD could increase the removal rates of phorate and diazinon by 40–80% and 10–20% more than that in tap water without CD, respectively, indicating CD can result in the degradation of the both pesticides. The removal of the both pesticides in aqueous solutions was influenced by concentration of CD, pH value, treatment time, initial concentration and kind of pesticides. The degradation efficiency increased with the concentration of CD and treatment time, and the least removal rates of the both pesticides were obtained in the aqueous solution at pH 4.6. Furthermore, the lower initial concentration of pesticides, the higher degradation rate would be obtained. The degradation kinetics of both pesticides were fitted to the first-order kinetics model well, and the kinetics parameters indicated that phorate was much easier to be degraded than diazinon. The degradation products of both pesticides were identified by GC-MS, phorate and diazinon were oxidized to phorate sulfoxide and phoratoxon sulfoxide, diazoxon, respectively. The present study validates the application of CD treatment as a safe and promising method for the removal of pesticides on fresh fruits and vegetables.

1. Introduction

Chlorine dioxide (CD) is a powerful oxidizing agent that can be applied in solution (Vandekinderen, Devlieghere, et al., 2009). Several studies have suggested that the use of CD could significantly decrease microbial contaminants on apples, lettuce, strawberries and ground beef (Pohlman, Stivarius, Mcelyea, Johnson, & Johnson, 2002; Rodgers, Cash, Siddiq, & Ryser, 2004; Vandekinderen, Van Camp, et al., 2009). The U.S. Environmental Protection Agency has considered CD as the first choice of the disinfectant in place of liquid chlorine (Huang, Chao, & Wang, 1994). Moreover, CD has proved to be an effective bleaching agent for the treatment of drinking water, cooling water, and wastewater (Hwang, Cash, & Zabik, 2002a; Tzanavaras, Themelis, & Kika, 2007).

More importantly, several reports have shown that CD can remove or significantly reduce pesticide residues on fresh fruits and vegetables. Hwang, Cash, and Zabik (2001) reported that only 34% and 32% of mancozeb was left in fresh apples after 5 min wash with CD (5 and 10 mg/L, respectively). CD also could effectively reduce ethylenethiourea, ametryn and isoproturon (Hwang, Cash, & Zabik, 2002b; Lopez, Mascolo, Tiravanti, & Passino, 1997), and mechanisms for methiocarb, mancozeb and ethylenethiourea degradation by chlorine dioxide were revealed (Hwang, Cash, & Zabik, 2003; Tian, Qiang, Liu, Zhang, & Dong, 2010).

In China, organophosphorus pesticides (OPPs) are one of the most important groups of widely used insecticides (Zhang et al., 2010). The application of OPPs can effectively prevent the loss of agricultural production, however, it is well known that OPPs can inhibit the activity of cholinesterases and impair nerve conduction (Pope, Karanth, & Liu, 2005), and have genotoxicity (Cakir & Sarikaya, 2005), reproductive toxicity (Kang et al., 2004) and immune toxicity (Crittenden, Carr, & Pruett, 1998). The OPPs residues in agricultural products have become a public concern. Therefore,
there is a strong need to develop methods to decrease the OPPs residue levels in agricultural products. As a representative of fresh-cut vegetable, lettuce (Lactuca sativa) is primarily consumed fresh or in salad mixes (Dupont, Mondi, Willamson, & Price, 2000). However, pesticide residues on or in the vegetable are often tested (Latif, Sherazi, & Bhanger, 2011) because OPPs are commonly applied to protect lettuce from the insect attack during growth. The objective of this study was to determine the effectiveness of CD to eliminate the residues of popular OPPs formulations, phorate and diazinon contaminated on fresh lettuce. In addition, the degradation kinetics, as well as factors influencing the removal of pesticides by CD treatment were investigated in aqueous solution.

2. Materials and methods

2.1. Materials

Fresh lettuce (L. sativa) was purchased from a local market in Beijing, China. Phorate (O,O-Diethyl-S-ethylmercaptomethyl phosphorothioate, >94.5% pure) and diazinon ((O,O-Diethyl-O-(6-methyl-2-(1-methylthyl)-4-pyrimidinyl) phosphorothioate, >97.5% pure) were purchased from Dr. Ehrenstorfer (Augsburg, Germany). The stock solutions (1000 mg/L) of phorate and diazinon were prepared in acetone and stored in glass-stoppered flasks at -18 °C. Acetic acid, sodium acetate, disodium hydrogen phosphate, sodium dihydrogen phosphate, sodium bicarbonate, and sodium carbonate were analytical grade and obtained from Sinopharm Chemical Reagent Co. Ltd (Beijing, China). HPLC-grade acetonitrile and acetone were purchased from Fisher (Fair Lawn, New Jersey). Nylon syringe filter (13 mm × 0.22 μm) was obtained from Shanghai Anpel Scientific Instrument Co. Ltd (Shanghai, China). CD powder was supplied by Tianjin Zhang Da Technology Co. (Tianjin, China). The stock solution of CD was prepared by dissolving 13 g of CD powder into 1000 ml of water and stirred thoroughly for 10 min in a sealed beaker. The concentration of CD in solution was determined using the iodometry method (American Public Health Association, 1987, pp. 298–300).

2.2. Experimental procedure in fresh lettuce system

2.2.1. Treatment of fresh lettuce

The lettuce leaves were immersed for 5 min into water containing the both pesticides with the initial concentration of 2 and 20 mg/L, respectively. Lettuce leaves were taken out and air-dried for 12 h at room temperature (Wu, Luan, Lan, Lo, & Chan, 2007) for further treatment. The contaminated lettuce samples were divided into three groups: (a) control (no wash); (b) dynamic washing in tap water; (c) dynamic washing in aqueous CD solution at two concentrations (10 mg/L and 20 mg/L). Then lettuce samples were taken out and spin-dried at room temperature after washing for 5, 10, 15 and 20 min, respectively.

2.2.2. Pesticides extraction

The extraction of pesticides from fresh lettuce was carried out according to the standard NY/T 761-2004 established by the Ministry of Agriculture of China (2004) with some modifications. Briefly, the lettuce samples were homogenized. An aliquot (20.00 g) of lettuce homogenate was transferred into a 200 ml conical flask, and 50.00 ml of acetonitrile was added. Activated carbon (0.50 g) was added and the solution was stirred for 30 min using a magnetic stirrer. The mixture was filtered through filter paper (Whatman No.1) into a 100 ml cylinder containing 7.00 g of NaCl. The mixture was shaken vigorously for 1 min and kept for 10 min. A portion of the upper acetonitrile layer (10.00 ml) was carefully transferred to a round-bottom flask and evaporated to dryness at 40 °C in a rotary evaporator. The residues on the wall of glass tube were dissolved in 2.00 ml of acetone and were transferred to vials. The solution was filtered through a 0.22 μm organic membrane before GC analysis.

2.3. Experimental procedure in aqueous solution system

The 0.2 mol/L acetic acid/sodium acetate (pH 4.6), 0.2 mol/L disodium hydrogen phosphate/sodium dihydrogen phosphate (pH 7.0), and 0.2 mol/L sodium bicarbonate/sodium carbonate (pH 10.7) were prepared. CD stock solution was added to each pH solution to bring the final concentration to 10 or 20 mg/L. Each pH solution was spiked with the phorate and diazinon stock solution to give a final concentration of 2 and 20 mg/L. CD and pesticides solutions were mixed thoroughly using a magnetic stirrer. After reacting for 0, 5, 15 and 20 min, an aliquot (10.00 ml) of solution was transferred into a 50 ml beaker, and 10.00 ml of a 0.1 mol/L sodium thiosulfate solution was added to quench the reaction. This mixture was extracted 3 times with 10.00 ml dichloromethane. The dichloromethane layer was collected in a round-bottom flask and evaporated to dryness at 40 °C in a rotary evaporator. The residue was dissolved in 5.00 ml acetone, and filtered through a 0.22 μm organic membrane before GC analysis. The solution without the addition of CD was used as a control.

2.4. Determination of pesticides by GC analysis

Phorate and diazinon were detected with GC-14A (Shimadzu Corporation, Kyoto, Japan) equipped with an HP-5 fused silica capillary column (30 m × 0.53 mm × 1.5 μm, Hewlett Packard, Avondale, USA) and flame photometric detector (FPD). The injector and detector temperatures were 250 °C and 260 °C, respectively. The temperature program was as follows: 120 °C (1 min), 10 °C/min to 240 °C (10 min). Nitrogen carrier gas, hydrogen gas and air were used at the flow rate of 90.0 ml/min, 85.0 ml/min and 120 ml/min, respectively. Sample solution (1.0 μl) was injected in splitless mode, and the quantification of pesticide was performed using an external standard method.

2.5. Degradation kinetics

The degradation kinetics of both pesticides were investigated by the first order kinetic model. A general reaction rate expression can be written as follows (Ambrus & Lantos, 2002; Chen et al., 2009):

\[ C_t = C_0 e^{-kt} \]  

Where \( C_0 \) and \( C_t \) were the concentrations of pesticides before and after CD treatment, \( k \) was the rate constant and \( t \) was the treatment time. Defining \( y = \ln \left( \frac{C_0}{C_t} \right) \) and combing into Eq. (1) got the function of \( y \) versus \( t \) as:

\[ y = kt \]  

The higher \( k \) value, the better degradation effect would be obtained.

2.6. Identification of degradation products by GC/MS analysis

The qualification analysis of potential degradation products of phorate and diazinon in aqueous solution was performed by GC/MS-QP2010 Plus (Shimadzu Corp., Kyoto, Japan) configured with a
programmed temperature vaporization (PTV) injector. The RxiTM-5ms fused silica capillary column (30 m × 0.25 mm × 0.25 μm) coated with 5% diphenyl-methylpolysiloxane stationary phase (Restek International, Bellefonte, USA) was used in GC separation. The sample solution (10.00 μl) was injected with an AS 2000 autosampler (Shimadzu Corp., Kyoto, Japan). Helium was used as the carrier gas with the flow rate of 1.75 ml/min. The GC temperature program was set as follows: 82 °C (5 min), 8 °C/min to 280 °C (1 min), then 25 °C/min to 300 °C (2 min). The mass spectrometer was operated in electron impact (EI) ionization mode at 70 eV and the temperatures of transfer line, ion source, and quadrupole were set at 200, 250 and 250 °C, respectively. Mass spectra in full-scan mode were collected at the rate of 2400 scans/min over the mass range (m/z) of 33–400 amu.

2.7. Statistical analysis

All samples were treated and analyzed in triplicate. Means and standard deviations were calculated using Origin Pro 7.5 (OriginLab, USA). The Duncan’s test was conducted to analyze the difference between various treatments, using SAS 8.0 (SAS Institute Inc., USA). A p < 0.05 was considered statistically significant.

3. Results and discussion

3.1. Performance of analysis method of phorate and diazinon

Phorate and diazinon were identified from their chromatogram and determined by comparison with authentic standards. The limit of detection (LOD) for phorate and diazinon were 0.02 and 0.05 mg/L, respectively. The linear range of the both pesticides ranged between 0.1 and 100 mg/L and the correlation coefficients of calibration curves of phorate and diazinon were 0.994 and 0.986, respectively. The average recoveries of phorate and diazinon were 97.2% and 76.4%, respectively. Thus, this method is sufficiently reliable for the analysis of the pesticides.

3.2. The effect of CD washing on removal of pesticides on fresh lettuce

The tap water with 10 mg/L CD and 20 mg/L CD were applied to wash the contaminated fresh lettuce. The concentration of pesticides declined most rapidly during the first 5 min and then changed slowly. Compared with the tap water wash, the CD treatment could

![Fig. 1. Effect of CD on the degradation of phorate residues on fresh lettuce. a, initial concentration of phorate 10 mg/L; b, initial concentration of phorate 20 mg/L.](image1)

![Fig. 2. Effect of CD on the degradation of diazinon residues on fresh lettuce. a, initial concentration of diazinon 10 mg/L; b, initial concentration of diazinon of 20 mg/L.](image2)
significantly improve the removal of pesticides on lettuce (p < 0.05) (Fig. 1). When the lettuce contaminated with 10 mg/L phorate were washed for 20 min with 10 and 20 mg/L of CD solution (Fig. 1a), the removal rates of phorate were 41.1% and 45.0% higher than that with tap water, respectively. Similarly, when the lettuce contaminated with 20 mg/L of phorate were washed for 20 min with 10 and 20 mg/L of CD solution (Fig. 1b), the removal rates of phorate were 24.1% and 50.1% after 20 min wash higher than that with tap water, respectively.

The changes in concentration of diazinon contaminated on lettuce showed the similar trends after washing with tap water and CD solution (Fig. 2). The concentrations of diazinon declined by 51% and 49% after 20 min washing with tap water for the initial

![Fig. 3](image3.jpg)  
Fig. 3. Effect of CD on the degradation of phorate in aqueous solution. a, 2 mg/L phorate, 10 mg/L CD; b, 2 mg/L phorate, 20 mg/L CD; c, 20 mg/L phorate, 10 mg/L CD; d, 20 mg/L phorate, 20 mg/L CD.

![Fig. 4](image4.jpg)  
Fig. 4. Effect of CD on the degradation of diazinon in aqueous solution. a, 2 mg/L diazinon, 10 mg/L CD; b, 2 mg/L diazinon, 20 mg/L CD; c, 20 ml diazinon, 10 mg/L CD; d, 20 ml diazinon, 20 mg/L CD.)
contaminated level of 10 and 20 mg/L, respectively. When the lettuce with 10 mg/L of diazinon was washed for 20 min with 10 and 20 mg/L CD, about 68% and 70% of diazinon was removed (Fig. 2a), whereas 56% and 60% of diazinon removed for the lettuce with 20 mg/L of diazinon under the same treatment (Fig. 2b), respectively. This suggested that the pesticide at the low initial concentration was easier to be degraded, and the treatment with higher concentration of CD declined most efficiently.

It is obvious that tap water wash also could effectively decrease the pesticides contaminated on the lettuce \((p < 0.05)\). This fact might be due to the solubility of phorate (50 mg/L, 25 °C) and diazinon (40 mg/L, 20 °C) is much larger than the contaminated level of pesticides (Kidd & James, 1991). When rinsed by dynamic tap water, the impact force of the water flow could accelerate the dissolution of pesticides in water. Moreover, CD treatment reduced the pesticide residues more significantly \((p < 0.05)\), compared with rinsing in tap water. The similar phenomenon has been demonstrated by Hwang et al. (2002a, 2002b), and the reason was proposed that CD was able to promote the degradation of pesticides.

### 3.3. The effect of CD on the removal of phorate in aqueous solution

To clarify the ability of CD to degrade pesticide residues during treatment, the further study was carried out in aqueous system with different pH values.

For the spiked level at 2 and 20 mg/L, the degradation rate of phorate in buffer solution at pH 7.0 was only about 7.7% and 5.6% after treatment for 20 min, respectively. Whereas the degradation rates of phorate (20 mg/L) increased to 11.1% and 12.3% in the buffer solutions at pH 4.6 and 10.7, respectively (Fig. 3). Therefore, pH value was an important factor influencing the degradation of phorate in water. This is in accordance with the characteristics of phorate which is degraded in alkaline water easily (U.S. Environmental Protection Agency, 1985, pp. 15–89).

Similar to the treatment on lettuce, the concentration of CD in solutions and the initial concentration of phorate influenced the degradation of phorate significantly. When the buffer solutions at pH 4.6 and 10.7 were added with 10 and 20 mg/L CD, the degradation rate of phorate increased significantly \((p < 0.05)\). Fig. 3(c and d) showed that about 40% and 82% of phorate (20 mg/L) was reduced after reaction for 20 min in solution at pH 7 with 10 and 20 mg/L CD, respectively. Especially, there was no phorate left after treatment for 5 min in the aqueous solution at pH 10.7.

### Table 1
The degradation kinetics of phorate and diazinon by first order kinetics model in aqueous solution.

<table>
<thead>
<tr>
<th>Pesticides</th>
<th>pH value</th>
<th>k value</th>
<th>(R^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phorate</td>
<td>4.6</td>
<td>0.0053 ± 0.0003</td>
<td>0.9454</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>0.0697 ± 0.0007</td>
<td>0.9316</td>
</tr>
<tr>
<td></td>
<td>10.7</td>
<td>0.0488 ± 0.0004</td>
<td>0.9520</td>
</tr>
<tr>
<td>Diazinon</td>
<td>4.6</td>
<td>0.0047 ± 0.0002</td>
<td>0.9623</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>0.0137 ± 0.0004</td>
<td>0.9476</td>
</tr>
<tr>
<td></td>
<td>10.7</td>
<td>0.0213 ± 0.0006</td>
<td>0.9113</td>
</tr>
</tbody>
</table>

Fig. 5. Mass spectra of (a) phoratoxon sulfoxide, (b) phorate sulfoxide, (c) diazoxon.
Additionally, the initial concentration of phorate significantly impacted the degradation efficiency (p < 0.05). In the solutions with initial concentration of 2 mg/L, there were no detect of phorate after treatment with 10 mg/L CD for 5 min at both pH 7 and 10.7 (Fig. 3a and b). In contrary, about 60% and 18% of phorate was remained after treatment with 10 mg/L and 20 mg/L CD for 20 min at pH 7 in the solutions with initial concentration of 20 mg/L (Fig. 3c and d).

3.4. The effect of CD on the removal of diazinon in aqueous solution

Some research indicated that diazinon in acidic water was less stable than in neutral and alkaline water (Kouloumbos & Tsipi, 2003; Ku & Chang, 1998). In solution at pH 4.6, the diazinon with initial concentration of 2 and 20 mg/L decreased by 10% and 9.2% after treatment for 20 min, respectively, whereas the concentration of diazinon only decreased by 5.6% and 4.7% in solution at pH 10.7. Differently, the degradation of diazinon in neutral and alkaline water were better than that in acidic condition when CD were added to the aqueous solutions (Fig. 4), indicating CD can significantly improve the degradation of diazinon (p < 0.05). Furthermore, the degradation rate of diazinon increased with the concentration of CD. When the aqueous solution of diazinon (20 mg/L) at pH 10.7 were treated for 20 min with 10 mg/L CD, the degradation rate was less than 20%, while, the degradation rate was almost raised to 40% when treated by 20 mg/L CD (Fig. 4c, d). However, more than 80% of diazinon (20 mg/L) still remained in the solution (pH 4.6) after treatment for 20 min with both CD concentrations of 10 and 20 mg/L and there was no significant difference between the solutions with 2 and 20 mg/L of initial concentration of diazinon under the same CD treatment (p > 0.05).

3.5. Removal of kinetic of phorate and diazinon in aqueous solution

The degradation kinetics of phorate and diazinon by first order kinetics model in aqueous solution were conducted in the present study. When the aqueous solution with concentration of 20 mg/L was treated using 20 mg/L CD, all the regression coefficients ($R^2$) values were more than 0.85 except for phorate at pH 10.7 (Table 1), indicating that the first order kinetic model could be used to describe the degradation behavior of the both pesticides. The rate constant $k$ can reflect the influence of pH on the degradation rate of pesticides. Specifically, the $k$ values in neutral and basic conditions were larger than the ones in acidic condition, suggesting that solutions with higher pH were beneficial to the action of CD on the pesticides. In the solutions at pH 7.0 and 10.7, the $k$ values of phorate were significantly larger than that of diazinon under the same treatment (Table 1). This means that phorate is easier to degrade than diazinon.

3.6. Identification of degradation products of phorate and diazinon

The mechanisms for phorate and diazinon degradation by CD were proposed on the basis of the identified degradation products. The major degradation products of phorate and diazinon (10 mg/L) treated by 20 mg/L CD for 20 min were identified by matching their GC–MS retention times and MS spectra with those of authentic standards. Results showed that the oxon analogs of phorate and diazinon were found to be the primary degradation products. Phorate and diazinon were oxidized to phorate sulfoxide and phorateoxon sulfoxide, diazoxon, respectively. Mass spectra of phorate sulfoxide, phorateoxon sulfoxide and diazoxon were shown in Fig. 5. The main differences between these and their parent compounds are the substitution of sulfur or hydrogen by oxygen in the P=S bond and S–H bond, which is agreement with study of Druzina and Stegu (2007).

4. Conclusions

The present study demonstrated that CD treatment was a promising process for removal of phorate and diazinon on fresh lettuce and in aqueous solution. The addition of CD could significantly increase the degradation rates of both pesticides compared to tap water wash (p < 0.05). Degradation rates of phorate and diazinon in aqueous solutions were influenced by concentration of CD, pH value, treatment time, initial concentration of pesticides. The lower initial concentration of pesticides, the higher concentration of CD and treatment time, better degradation effect would be obtained. And CD treatments at neutral and alkaline buffer were more effective for removing the both pesticides than in acidic condition. The degradation kinetics of both pesticides could be described by the first-order kinetics model, and phorate was shown to be much more labile to CD treatment than diazinon. Degradation products of phorate and diazinon were determined by GC–MS, the main differences between these and their parent compounds are the substitution of sulfur or hydrogen by oxygen in the P=S bond and S–H bond.

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References


