



Degradation behaviour of methamidophos and chlorpyrifos in apple juice treated with pulsed electric fields

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ABSTRACT

Apple juice (13 °Brix) spiked with methamidophos and chlorpyrifos (2–3 mg/l of each compound) was treated by pulsed electric fields (PEF), and pesticide residues were quantified by gas chromatography with flame photometric detection (GC-FPD). Results showed that electric field strength (8–20 kV/cm) and pulse number (6–26 pulses) have significant effects on the degradation of methamidophos and chlorpyrifos. PEF treatment is effective for the degradation of methamidophos and chlorpyrifos residues in apple juice, and chlorpyrifos is much more labile to PEF than methamidophos. An increase in either pulse number or electric field strength could speed the degradation of methamidophos and chlorpyrifos, and the kinetics equations and related parameters quantitatively characterized the degradation behavior of the pesticides. The exponential model better fits the experimental data for all treatments than the linear model.

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1. Introduction

Organophosphorus pesticides (OPPs) are one of the most important and widely used classes of agricultural pesticides, accounting for about 70% of the insecticides in current use in China. This widespread use poses a potential risk to human health because OPPs inhibit acetylcholinesterase and lead to the modification of cholinergic signaling (Pope, Karanth, & Liu, 2005). In addition, OPPs have been known to be cytotoxic (Giordano et al., 2007; Wagner, McMillan, & Plewa, 2005), genotoxic (Cakir & Sarikaya, 2005; Rahman, Mahboob, Danadevi, Saleha, & Grover, 2002), reproductively toxic (Kang et al., 2004) and immunotoxic (Crittenden, Carr, & Pruet, 1998; Yeh, Sung, Chang, Cheng, & Kuo, 2005). Therefore, there is a growing interest in dissipation of OPP residues in agricultural products and foods all over the world.

Concentrated apple juice (CAJ) has become an economically important food product in China, where export of CAJ accounts for nearly 50% of the world export volume. However, the application of OPPs has resulted in pesticide residues in fruit and deterioration of CAJ quality. Several methods have been demonstrated to

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be effective in the removal of pesticide residues, such as post-harvest storage (Athanasopoulos & Pappas, 2000; Pappas, Kyriakidis, & Athanasopoulos, 2003), ozone washes, and resin adsorption during processing (Karaca & Velioglu, 2007; Ong, Cash, Zabik, Siddiq, & Jones, 1996). Unfortunately, these methods can reduce nutritional and flavor qualities of the juice (Dan & Seth, 1990; Nijssen, 1991) and in the case of ozonation, may also produce by-products with higher toxicity than the original organophosphorus pesticides themselves (Hwang, Cash, & Zabik, 2002; Ikehata & El-Din, 2005). Thus, it seems to be necessary to develop innovative processing methods to decrease pesticide residues without undesirable effects. Pulsed electric field (PEF) is a novel non-thermal technology, causing few losses of flavor, color, taste or nutrients (Ayhan, Yeom, Zhang, & Min, 2001; Cortes, Esteve, & Frigola, 2008) compared to conventional thermal processing. PEF technology can inactivate microorganisms (Mosqueda-Melgar, Raybaudi-Massilia, & Martin-Belloso, 2007; Zhong et al., 2005;) and enzymes (Aguilo-Aguayo, Odriozola-Serrano, Quintao-Teixeira, & Martin-Belloso, 2008; Marsells-Fontanet & Martin-Belloso, 2007). This raises an interesting question of whether or not pesticide residues in apple juice can be dissipated by PEF treatment. This application of PEF, to the best of our knowledge, has not been reported. Methamidophos (*O,S*-dimethyl phosphoramidothioate) and chlorpyrifos [*O,O*-diethyl-*O*-(3,5,6-trichloro-2-pyridinyl) phosphorothionate] were used as representative examples in present study, because they are active ingredients in most organophosphorus formulations. The aim of

this paper is to investigate whether both methamidophos and chlorpyrifos in apple juice can be degraded by PEF.

2. Materials and methods

2.1. Materials

Methamidophos (>95% pure) and chlorpyrifos (98.5% pure) were purchased from the China Agricultural Environment Protection and Inspection Center (Tianjin, China). All solvents were analytical grade and obtained from Beijing Beihua Fine Chemicals Co. (Beijing, China). Acetone and acetonitrile were redistilled before use. Concentrated apple juice (CAJ) at 78 °Brix was manufactured by a local factory and diluted to 13 °Brix (similar to raw apple juice, pH 3.86) for PEF treatment. The initial concentration of methamidophos was 0.004 ± 0.001 mg/l, but no residue of chlorpyrifos was detected. Standard stock solutions (100.0 mg/l) of methamidophos and chlorpyrifos were prepared in acetone. The above stock solutions were added to the reconstituted apple juice with final pesticide concentrations of 2–3 mg/l followed by PEF treatment.

2.2. PEF Treatment of apple juice

PEF treatment was performed using a laboratory scale apparatus (jointly designed by Tsinghua University and China Agricultural University, Beijing, China), which included a high voltage pulse generator, a treatment chamber (volume (V) = 2 ml), and a peristaltic pump. A schematic diagram of the apparatus is shown in Fig. 1. Two round parallel-plate electrodes with a 3.57 mm radius were made of stainless steel, and the gap between electrodes was 5 mm. The PEF treatment parameters used were as follows: exponentially-decaying wave, 1 Hz pulse frequency, 0.5 μ F capacitor, and 10 ms pulse width. A thermocouple was attached to the exit of the chamber to monitor the post-treatment temperature. A 20 ml aliquot of apple juice was pumped through the treatment chamber under a given combination of PEF conditions. Different electric field strengths (8, 12, 16 and 20 kV/cm) and pulse numbers (6, 9, 12, 19 and 26) were used as treatments. The pulse number depends on the pulse frequency (f) and the flow rate (v , ml/s) of apple juice. The pulse number was calculated as $V \times f/v$, and total treatment time was calculated as the product of the pulse width and the pulse number. The temperature of treated apple juice was kept below 40 °C due to the short treatment time (60–

260 μ s). Each treatment was conducted in triplicate. The treated apple juice was stored at 4 °C and the pesticides were tested within one day after treatment.

2.3. GC analysis of methamidophos and chlorpyrifos in apple juice

The extraction of pesticides was carried out according to a standard method established by the Ministry of Agriculture of China (2004) with some modifications. An aliquot of apple juice (20.0 ml) was mixed with 50.0 ml of acetonitrile in a conical flask (100 ml). The mixture was shaken vigorously for 15 min and filtered through Whatman No. 1 filter paper into a conical flask (100 ml) containing 10.0 g NaCl. The sample was centrifuged at 2000 g for 5 min. A 10.0 ml portion of the upper acetonitrile layer was carefully transferred to a glass test tube and evaporated to dryness under a stream of nitrogen in a water bath at 40 °C. The residue on the wall of glass tube was redissolved in 2.0 ml of acetone and transferred to vials for GC analysis.

Methamidophos and chlorpyrifos were detected with GC-14A (Shimadzu Corporation, Kyoto, Japan) equipped with a HP-5 fused silica capillary column (30 m \times 0.53 mm, 1.5 μ m, 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 (7 min). Nitrogen carrier gas was used at the flow rate of 59.0 ml/min. Sample solution (2.0 μ l) was injected in splitless mode, and the quantification of pesticide was performed using an external standard.

2.4. Degradation kinetics

2.4.1. First order kinetics (linear model)

A general reaction rate expression for the degradation kinetics of pesticides can be written as follows (Timme, Frehse, & Laska, 1986; Ambrus & Lantos, 2002):

$$C_t = C_0 e^{-kt} \quad (1)$$

where C_0 and C_t are the concentration of pesticide before and after treatment, t is the treatment time and k is the rate constant. Defining $S = C_t/C_0$ (S is the persistent pesticide fraction) and combining into Eq. (1) gives the function of $\ln S$ versus t as

$$\ln S = -kt \quad (2)$$

In order to directly reflect the effect of pulse number on the degradation of pesticides, we used $t = 10n$ in Eq. (2) and obtained the following:

$$\ln S = -10kn \quad (3)$$

which was used for degradation kinetics of pesticides in this study. In addition, the half-life ($n_{1/2}$) of pesticide upon PEF treatment could be calculated as

$$n_{1/2} = \frac{\ln 2}{10k} \quad (4)$$

where $n_{1/2}$ is the pulse number at which the concentration of pesticide is one-half the original concentration.

2.4.2. Exponential model

Originally, an extension of the exponential model was proposed by Hülshager, Potel, and Niemann (1981) to describe the microbial survival fraction (S) with PEF treatment time (t) by Eq. (1)

$$\ln S = -b_t \ln(t/t_c) \quad (5)$$

where S is the survival rate of microbes (the ratio of living cell count before and after PEF treatment), b_t is the coefficient, t is treatment time, and t_c is the critical treatment time corresponding to the

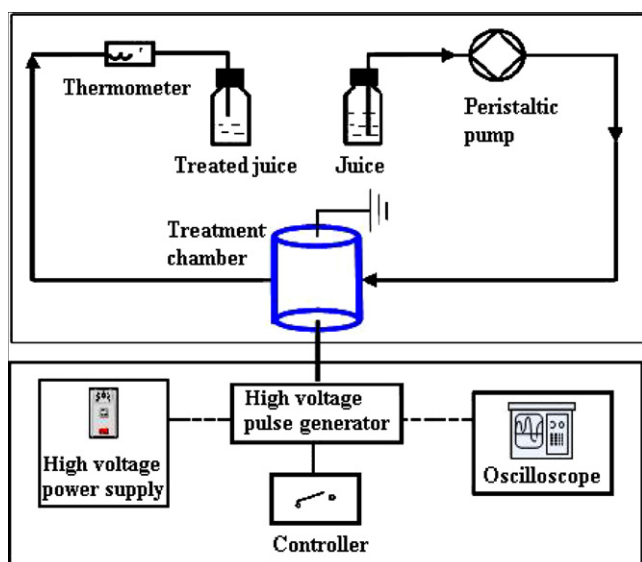


Fig. 1. Schematic diagram of the experimental PEF apparatus.

extrapolated value of t for 100% survival rate. In the present study, assuming that the degradation of pesticides would be similar to the inactivation of microbes during PEF treatment, Eq. (6) would be obtained which would describe the relationship between persistent pesticide fraction S' and treatment time t as

$$\ln S' = b'_t \ln(t/t'_c) \quad (6)$$

where b'_t is the regression coefficient and t'_c is a critical treatment time (the extrapolated value of t for 100% pesticides existence). Similarly, we introduced the pulse number n into Eq. (6) and converted it from logarithmic form to exponential form:

$$S' = \left(\frac{1}{n_c}\right)^{-b'_t} n^{-b'_t} \quad (7)$$

which was used for degradation kinetics of pesticides in this study.

2.5. Statistical analysis

Statistical analysis was performed by using Student's t -test with SPSS 15.0 software. Analysis of variance (ANOVA) was based on a significance level of $p = 0.05$.

3. Results and discussion

3.1. Determinations of methamidophos and chlorpyrifos

Methamidophos and chlorpyrifos were identified from their chromatogram and determined by comparison with authentic

standards, the GC chromatograms of pesticides in standard solution and spiked apple juice were shown in Fig. 2. The satisfactory correlation coefficients of calibration curves (over 0.98) and the linear range (0.25–10 mg/l) for both pesticides were obtained. Limits of detection (LOD) for methamidophos and chlorpyrifos were 0.005 mg/l and 0.002 mg/l, respectively. In addition, spiked recoveries ranged from 85% to 98% for methamidophos, and 89% to 104% for chlorpyrifos at various concentration levels. These levels were within the range of 60–140% for routine pesticide residue analyses recommended by Putnam, Nelson, and Clark (2003). The reproducibility of the recovery results, as indicated by relative standard deviations (RSDs), confirmed that the method is sufficiently reliable for the pesticide analysis in this study.

3.2. Effect of PEF treatment on the degradation of methamidophos and chlorpyrifos

The changes in concentrations of methamidophos and chlorpyrifos during PEF treatment were shown in Fig. 3. PEF treatment was effective in dissipating methamidophos and chlorpyrifos, and electric field strength and pulse number were the both important factors in the degradation of these compounds. The ANOVA results indicated that electric field strength and pulse number had significant effects on their degradation ($p < 0.05$), and there is a significant interaction between electric field strength and pulse number ($p < 0.05$). The increase of pulse number (6–26 pulses) could speed the degradation of methamidophos and chlorpyrifos. Under PEF treatment at 8 kV/cm for 26 pulses the persistent pesticide fraction

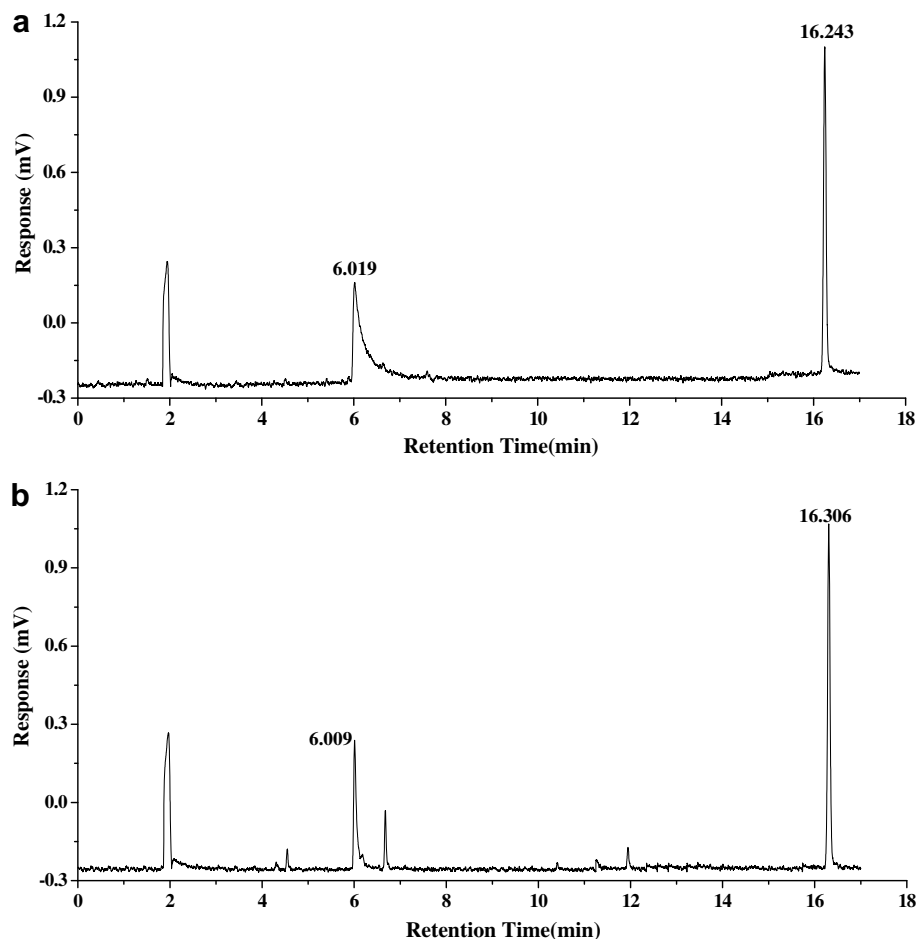


Fig. 2. GC chromatogram of methamidophos and chlorpyrifos. a: standard solution of methamidophos ($R_t = 6.019$) and chlorpyrifos ($R_t = 16.243$) (concentration of methamidophos and chlorpyrifos is 2.20 mg/L and 2.50 mg/L, respectively); b: fortified apple juice, methamidophos ($R_t = 6.009$), chlorpyrifos ($R_t = 16.306$).

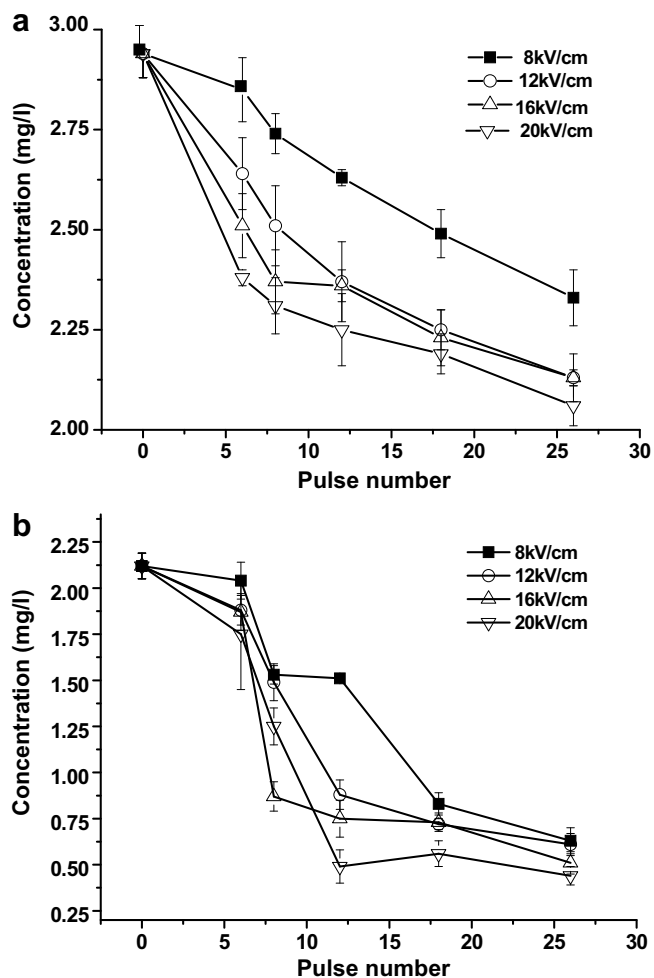


Fig. 3. Concentration changes of pesticides in apple juice under different PEF treatments. a: methamidophos; b: chlorpyrifos.

of methamidophos and chlorpyrifos were 27.7% and 70.3% lower than that at 8 kV/cm for 6 pulses, respectively. Similarly, the degradation of both pesticides was accelerated with an increase of electric field strength. The persistent pesticide fraction of methamidophos and chlorpyrifos under treatment at 8 kV/cm for 26 pulses were 9.1% and 9.0% higher than that treated at 20 kV/cm for 26 pulses, respectively. It could be explained that a pulsed electric field stemming from the application of higher voltage increases the vibration and rotation of polar molecules, subsequently facilitating the degradation of pesticides. Furthermore, the degradation behavior of methamidophos and chlorpyrifos appeared to differ (Fig. 3). The concentration of methamidophos decreased slowly as the pulse number increased. In contrast, the concentration of chlorpyrifos did not show a significant decrease when less than 6 pulses was applied, but a sharp decline occurred from 6 pulses, and then the degradation rate declined. However, the trends were similar for each pesticide in different field strengths. Therefore, it is necessary to determine the degradation kinetic equations and parameters based on experimental data, in order to describe degradation behaviors of methamidophos and chlorpyrifos in apple juice treated by PEF.

3.3. Degradation kinetics of methamidophos and chlorpyrifos

The first order kinetics equation (linear model) was fitted with changes in concentrations of methamidophos and chlorpyrifos with pulse number, and their plots are shown in Fig. 4. Degradation equations and deduced parameters for different treatments are shown in

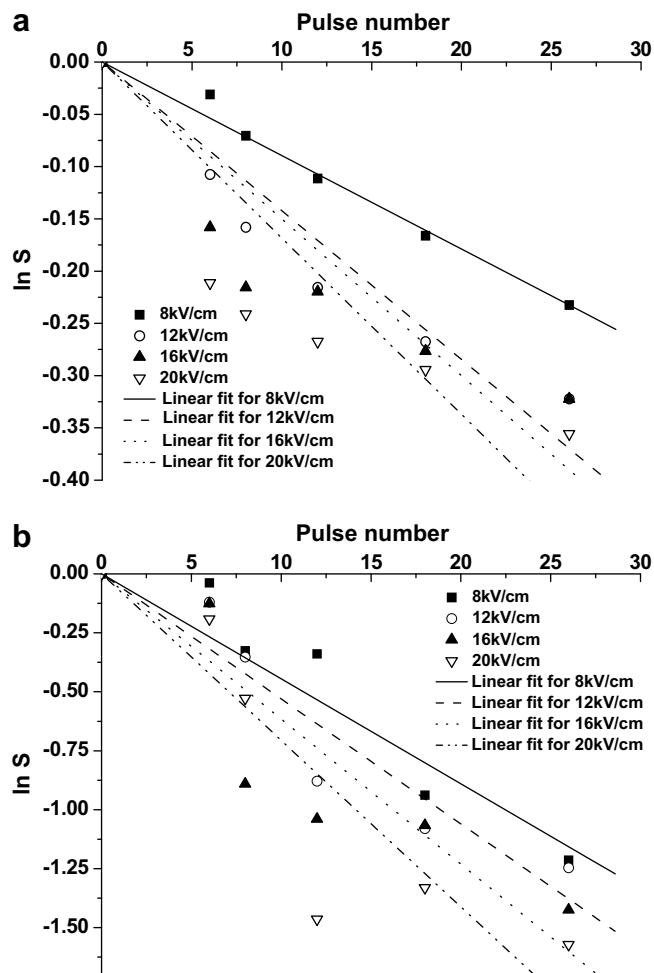


Fig. 4. Degradation kinetics of pesticides fitted to the linear model. a: methamidophos; b: chlorpyrifos.

Table 1. All regression coefficients (R^2) are greater than 0.75, demonstrating that the degradation of pesticides could follow the general degradation pathway of OPPs such as hydrolysis and oxidation (Pehkonen & Zhang, 2002). Furthermore, the values of R^2 varied with field strength, and were larger than 0.9 in treatments at 8 kV and 12 kV, suggesting that degradation of pesticides in apple juice treated at lower electric field strength followed the first order kinetic model better than that at higher PEF strength. This might be explained by the significant effect of the high field strength on the degradation of pesticide, but the effect of PEF on pesticide degradation was not considered in the linear model. Actually, the degradation

Table 1
Linear model for the degradation kinetics of methamidophos and chlorpyrifos

Field strength (kV/cm)	Regression equation	(R^2)	p value	k (ms^{-1})	$n_{1/2}$
Methamidophos					
8	$\ln S = -0.009n$	(0.99)	0.000	0.9×10^{-2}	77.9
12	$\ln S = -0.014n$	(0.94)	0.001	1.4×10^{-2}	49.5
16	$\ln S = -0.015n$	(0.84)	0.022	1.5×10^{-2}	46.2
20	$\ln S = -0.017n$	(0.77)	0.061	1.7×10^{-2}	40.8
Chlorpyrifos					
8	$\ln S = -0.044n$	(0.93)	0.001	4.4×10^{-2}	15.6
12	$\ln S = -0.053n$	(0.90)	0.000	5.3×10^{-2}	13.1
16	$\ln S = -0.062n$	(0.80)	0.001	6.2×10^{-2}	11.3
20	$\ln S = -0.071n$	(0.79)	0.001	7.1×10^{-2}	9.8

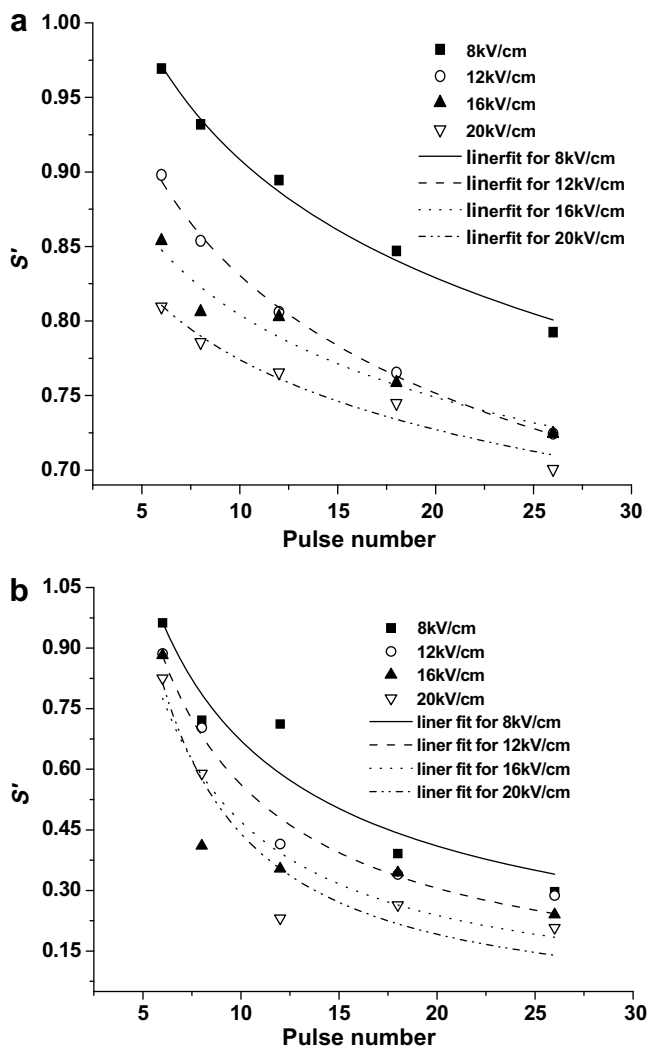


Fig. 5. Degradation kinetics of pesticides fitted to the exponential kinetic model. a: methamidophos; b: chlorpyrifos.

rate constant k was ascending, but value of $n_{1/2}$ was descending as the field strength was increased. These results further confirm the significance of PEF parameters.

Similarly, the exponential model was fitted with the changes in concentrations of methamidophos and chlorpyrifos with pulse number, and the plots were shown in Fig. 5, which degradation kinetic equations and related parameters for both methamidophos and chlorpyrifos were documented in Table 2. Results showed that all values of R^2 in methamidophos were larger than 0.95, and values of R^2 in chlorpyrifos were larger than 0.90 except the treatment at 16 kV/cm. In comparison to the linear model, the exponential model better describes degradation kinetics of both pesticides, presumably because the role of PEF in the degradation of pesticides was included in the exponential model (Hülshleger et al., 1981). Simultaneously, values of $-b_t$ and n_c in the exponential model were used to characterize the degradation behavior of pesticides during PEF treatment. On the one hand, values of n_c of both pesticides declined with increase of electric field strength (except the treatment at 20 kV/cm for chlorpyrifos), indicating value of n_c could reflect the effect of PEF on the degradation of pesticides. The value of n_c of methamidophos was much lower than that of chlorpyrifos for a given PEF treatment condition, which could be a result of different degradation pathways of the pesticides. This is in agreement with previous findings, where different dissipation

Table 2

Exponential model for the degradation kinetics of methamidophos and chlorpyrifos

Field strength (kV/cm)	Regression equation	(R^2)	p value	$-b_t$	n_c
<i>Methamidophos</i>					
8	$S' = (\frac{1}{4.8})^{0.13} n^{0.13}$	(0.99)	0.000	0.13	4.8
12	$S' = (\frac{1}{2.8})^{0.14} n^{0.14}$	(1.00)	0.000	0.14	2.8
16	$S' = (\frac{1}{1.2})^{0.10} n^{0.10}$	(0.95)	0.005	0.10	1.2
20	$S' = (\frac{1}{0.6})^{0.09} n^{0.09}$	(0.96)	0.003	0.09	0.6
<i>Chlorpyrifos</i>					
8	$S' = (\frac{1}{5.7})^{0.72} n^{0.72}$	(0.92)	0.008	0.72	5.7
12	$S' = (\frac{1}{5.2})^{0.88} n^{0.88}$	(0.98)	0.002	0.88	5.2
16	$S' = (\frac{1}{4.6})^{0.99} n^{0.99}$	(0.79)	0.018	0.99	4.6
20	$S' = (\frac{1}{5.0})^{1.2} n^{1.2}$	(0.93)	0.007	1.20	5.0

kinetics have been suggested for different pesticides (Branko, & Matej, 2007; Badawy, Ghaly, & Gad-Allah, 2006). On the other hand, the changes in values of $-b_t$ with the increase of electric field strength were different for each pesticide. The value of $-b_t$ of methamidophos dropped slightly, whereas the value of $-b_t$ of chlorpyrifos increased rapidly. This implies that the value of $-b_t$ is related to the nature of the pesticide. Apparently, the value of $-b_t$ of methamidophos was much lower than that of chlorpyrifos for a given PEF condition, namely, a steeper decline was seen in the degradation rate curve of chlorpyrifos. Therefore, chlorpyrifos degrades more easily than methamidophos during PEF treatment. Similarly, the differences in values of k and $n_{1/2}$ in the linear model between methamidophos and chlorpyrifos were also observed in Table 1. The k of chlorpyrifos were 3.8–4.9 fold higher than those of methamidophos, suggesting that chlorpyrifos is much more labile to PEF than methamidophos. The difference in degradation kinetics between the two pesticides might due to their different chemical structures. However, further identification of the degradation products of pesticides is required to determine whether or not the degradation of pesticides by PEF treatment is similar to the known pathway.

4. Conclusions

PEF treatment is effective for the degradation of methamidophos and chlorpyrifos residues in apple juice, with chlorpyrifos is shown to be much more labile to PEF than methamidophos. The applied field strength and pulse number have significant influence on the degradation behavior of these pesticides. The kinetics equation and related parameters quantitatively characterized the degradation of pesticide. Only at low electric field strengths did the linear model fit the experimental data, while the exponential model was more suitable for the describing degradation kinetics in all treatments.

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