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Degradation behavior and products of malathion and chlorpyrifos spiked in apple juice by ultrasonic treatment

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ABSTRACT

Apple juice (13 °Brix) spiked with malathion and chlorpyrifos (2–3 mg l^{-1} of each compound) was treated under different ultrasonic irradiations. Results showed that ultrasonic treatment was effective for the degradation of malathion and chlorpyrifos in apple juice, and the output power and treatment time significantly influenced the degradation of both pesticides ($p < 0.05$). The maximum degradations were achieved for malathion (41.7%) and chlorpyrifos (82.0%) after the ultrasonic treatment at 500 W for 120 min. The degradation kinetics of both pesticides were fitted to the first-order kinetics model well ($R^2 \geqslant 0.90$). The kinetics parameters indicated that chlorpyrifos was much more labile to ultrasonic treatment than malathion. Furthermore, malaoxon and chlorpyrifos oxon were identified as the degradation products of malathion and chlorpyrifos by gas chromatography–mass spectrometry (GC–MS), respectively. The oxidation pathway through the hydroxyl radical attack on the P=S bond of pesticide molecules was proposed.

1. Introduction

The organophosphorus pesticides (OPPs) are a group of very effective and widely used pesticides. It is well known that OPPs have strong inhibitory activity to cholinesterase [1], reproductive toxicity [2], cytotoxicity [3], immunotoxicity [4] and genotoxicity [5]. They eventually become the health risk to human beings in consequence of bioaccumulation through the food chain, although the use of OPPs provides benefits for increasing agricultural production. Therefore, the control of residual levels of OPPs in food has become a public concern.

The concentrated apple juice (CAJ) has been an economically important food product in China, where export of CAJ accounts for nearly 50% of the world export volume [6]. However, the application of OPPs has resulted in pesticide residues in fruit and deterioration of CAJ quality. Several methodologies have been developed for dissipation of OPPs residues in CAJ, including the post-harvest storage [7], as well as chlorine and ozone washes and resin adsorption during processing [8,9]. During the past decade, a number of studies have used the physical and chemical (sonochemistry) effects generated by acoustic cavitation for the degradation of organic pollutants [10,11]. In the case of OPPs, several results showed that parathion [12], dichlorvos [13], diazinon [14] and dimethoate [15] could be degraded by ultrasonic treatment in aqueous solution. However, the effect of ultrasonic treatment on the dissipation of pesticides in fruit juice, to the best of our knowledge, has not been reported.

In the present study, malathion (*S*-[1,2-bis(carbethoxy)ethyl]-*O*,*O*-dimethyl dithiophosphate) and chlorpyrifos (*O*,*O*-diethyl-*O*-(3,5,6-trichloro-2-pyridyl)phosphorothioate), which are often used to protect apple trees and fruits from the aphid and acarid, were chosen as representative examples. The objectives of this study are to investigate the degradation behavior and kinetics of malathion and chlorpyrifos spiked in apple juice treated by ultrasonic and to identify the degradation products of malathion and chlorpyrifos.

2. Experimental

2.1. Materials

Malathion (99.5%; CAS Registry No. 121-75-5) and chlorpyrifos (99.0%; CAS Registry No. 2921-88-2) were purchased from Dr. Ehrenstorfer (Augsburg, Germany). Acetonitrile and sodium chloride were analytical grade and obtained from Beijing Beihua Fine Chemicals Co. (Beijing, China). Acetonitrile was redistilled before use. HPLC-grade acetone was purchased from Tianjin Concord Technology Co. Ltd. (Tianjin, China). CAJ at 78 °Brix was

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manufactured by a local factory and diluted to 13 °Brix (similar to raw apple juice, pH 3.86) for ultrasonic treatment. Individual pesticide stock solutions (200–300 mg l^{-1}) were prepared in acetone and stored in glass-stoppered flasks at −18 °C. The stock solutions were added to the reconstituted apple juice with final pesticide concentrations as 2–3 mg l^{-1}.

2.2. Ultrasonic treatment

Ultrasonic treatment was performed with a high-intensity ultrasonic probe type (Ningbo Scientz Biotechnology Co. Ltd., Ningbo, China) equipped with a diameter of 6.0 mm microtip. The maximum output power and frequency are 650 W and 25 kHz, respectively. An aliquot of apple juice (100 ml) was added to a conical flask (150 ml) and irradiated through dipping the microtip in the juice 10 mm below the surface. The treatment times were set as 15, 30, 45, 60, 75, 90, 105 and 120 min and the treatment powers were set as 100, 300 and 500 W. A thermostatic circulator HX-1050 (Beijing Detianyou Technology Development Co. Ltd., Beijing, China) was used to maintain the temperature of samples at 15 °C (±2 °C). All the treated samples were stored at 4 °C for a maximum of 24 h for analysis. Each treatment was conducted in triplicate.

2.3. Extraction

The extraction of pesticides is a modification of the standard method established by Ministry of Agriculture of China [16]. 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 measuring cylinder (100 ml) containing 10.0 g sodium chloride. The mixture was centrifuged at 2000*g* for 5 min after mixing thoroughly. A portion (10.0 ml) 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 re-dissolved in 2.0 ml of acetone and transferred to vials for gas chromatography (GC) and gas chromatography–mass spectrometry (GC–MS) analysis.

2.4. GC Analysis of malathion and chlorpyrifos in apple juice

GC analysis was conducted with a Fuli GC 9790 (Fuli Analytical Instrument Co. Ltd., Zhejiang, China) equipped with a HP-5 fused silica capillary column (30 m × 0.53 mm, 1.5 μm, Hewlett Packard, Avondale, USA) and flame photometric detector operating with an optical filter selective for phosphoric compounds (passing band centered at 526 nm). Nitrogen carrier gas was used at the constant pressure of 0.05 MPa. The temperature program was as follows: initial temperature isothermal at 120 °C for 1 min, then from 120 to 240 °C at 10 °C min^{-1}, and held 7 min at 240 °C. The injector and detector temperatures were set at 250 and 260 °C, respectively. Sample solution (1.0 μl) was injected in splitless mode.

2.5. GC–MS analysis of degradation products of malathion and chlorpyrifos

Degradation products of malathion and chlorpyrifos were identified with a Shimadzu GC/MS-QP2010 Plus (Shimadzu Co., Kyoto, Japan) configured with a programmed temperature vaporization injector (Shimadzu Co., Kyoto, Japan). The sample solution was injected (10.0 μl) with an AS 2000 autosampler (Shimadzu Co., Kyoto, Japan). An Rxi™-5 ms fused silica capillary column (30 m × 0.25 mm, 0.25 μm; Restek International, Bellefonte, USA) was used in GC separation. Helium was used as the carrier gas with flow rate of 1.75 ml min^{-1}. The GC temperature program consisted of the initial temperature of 82 °C, held for 5 min, followed by the increase at 8 °C min^{-1} up to 280 °C, held for 14.25 min. The mass spectrometer was operated in electron impact (EI) ionization mode at 70 eV and the temperatures of the transfer line, ion source, and quadrupole were set as 200, 250 and 250 °C, respectively. The total ion current (TIC) chromatograms were recorded between m/z 33 and 400 at a rate of 40 scans per second. EI mass spectrum database searches were carried out in a mass spectral library (National Institute for Standard Technology (NIST), search program version 1.5, Gaithersburg, MD, USA). Simultaneously, the unspiked apple juice was used as the blank control to eliminate those peaks coming from the sample preparation procedure and chromatographic system.

2.6. Statistical analysis

Data were analyzed using the SAS 8.0 software (Statistical Analysis System Inc., Cary, NC, USA). The effects of ultrasonic power and treatment time on the degradation of malathion and chlorpyrifos in apple juice were evaluated by analysis of variance (ANOVA), followed by Duncan's test. ANOVA was based on a significance level of $p = 0.05$.

3. Results and discussion

3.1. Determination and recovery of malathion and chlorpyrifos

The quantification of malathion and chlorpyrifos was performed by GC through the external standard method. The good linearity was obtained in the concentration range of 0.25–5.00 mg l^{-1} for both pesticides ($R^2 > 0.98$). Limits of detection (LOD) were calculated as 0.008 mg l^{-1} for malathion and 0.005 mg l^{-1} for chlorpyrifos by using a signal-to-noise ratio of 3. In addition, the recoveries of method ranged from 84% to 103% for malathion and 86% to 103% for chlorpyrifos at various concentration levels, which were within the range of 60–140% for routine pesticide residue analyses recommended by Putnam et al. [17]. The relative standard deviations (RSDs) were from 5.1% to 13.6% for malathion and 3.4% to 12.8% for chlorpyrifos, the values being within the accepted range for residue determinations.

3.2. Effect of ultrasonic irradiation on the degradation of malathion and chlorpyrifos

The changes in concentrations of malathion and chlorpyrifos during ultrasonic treatment were shown in Fig. 1. The ANOVA results indicated that the degradation of both pesticides increased significantly with the ultrasonic power and treatment time ($p < 0.05$). The degradation rates of malathion and chlorpyrifos after the treatment at 500 W for 15 min were 2.8 and 2.3 times greater than that of treated at 100 W for 15 min, respectively. Similar results have been reported in previous research in which the degradation rate of dichlorvos treated at 161 W was higher than that of treated at 86 W in aqueous solution under ultrasonic treatment for 30 min [13]. Similarly, the degradation rates of malathion and chlorpyrifos after the treatment at 100 W for 120 min were 4.0 and 3.8 times as compared with that at 100 W for 15 min, respectively. Among all treatments, the maximum degradation rate for malathion (41.7%) and chlorpyrifos (82.0%) were achieved after the ultrasonic treatment at 500 W for 120 min. Some evidence supported the positive effect of increased output power and treatment time on the degradation of both pesticides [15,18]. On one hand, more energy was provided to the samples when output power elevated, resulting in the more rapid occurrence of the formation and collapse of cavitation bubble. The higher concentration of hydroxyl radical formed correspondingly and reacted with more pesticides

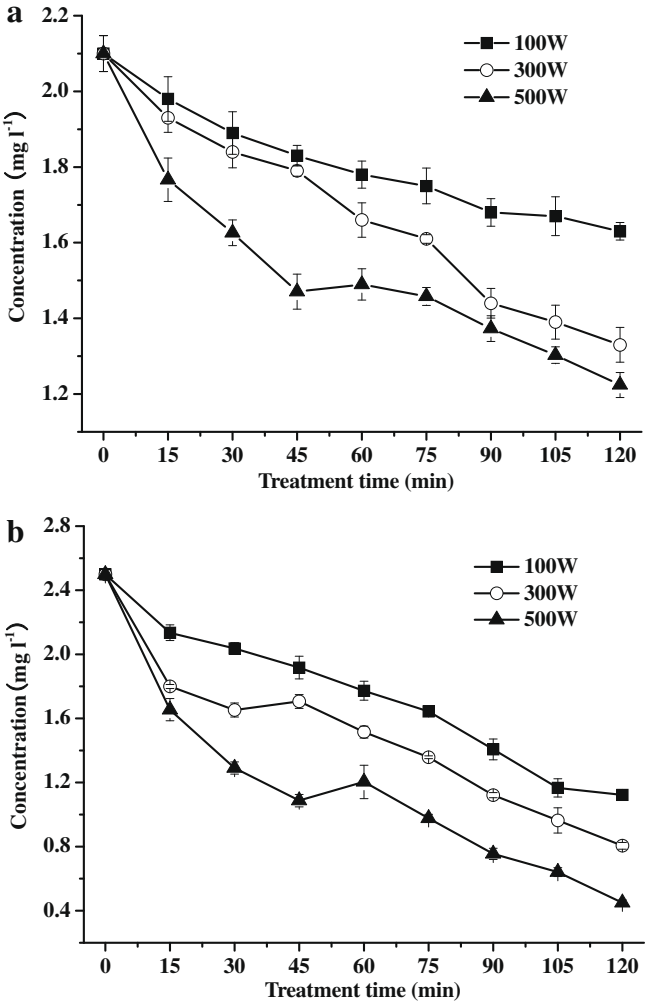

Fig. 1. The changes of concentration of malathion (a) and chlorpyrifos (b) in apple juice under different ultrasonic conditions.

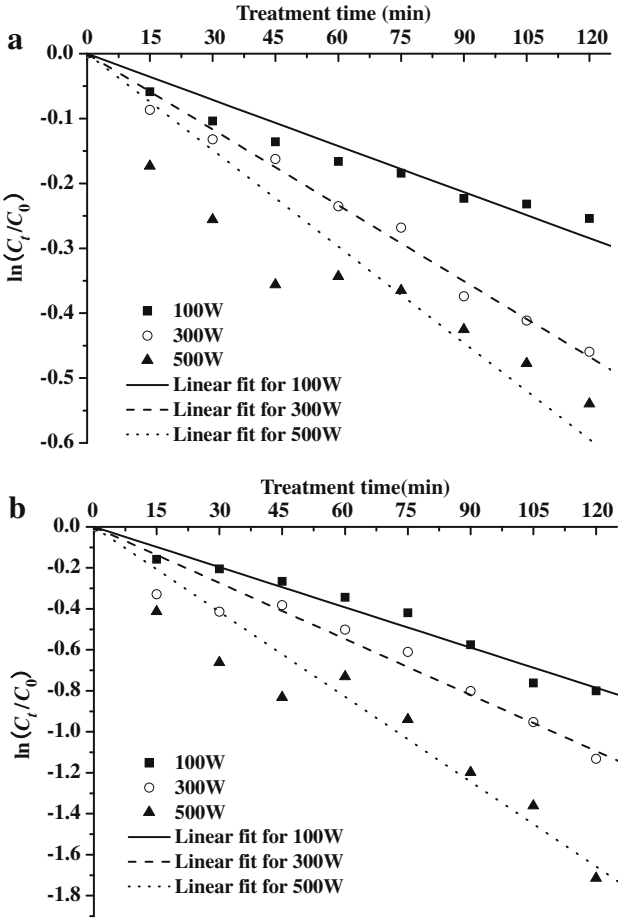

Fig. 2. The first-order kinetics model of malathion (a) and chlorpyrifos (b).

in samples [18]. On the other hand, due to the ultrasonic secondary mechanical effects such as stirring and oscillation when the output power and treatment time enhanced, mixing of samples was improved and the degradation of pesticides was accelerated [15].

Interestingly, the concentration of malathion declined more slowly than that of chlorpyrifos under the same treatment conditions. The degradation rate of chlorpyrifos (48.4%) was 2.1 times higher than that of malathion (22.6%) after the treatment at 500 W for 30 min. This indicated that malathion was more resistant to ultrasonic treatment than chlorpyrifos. Therefore, it is necessary to determine the degradation kinetics to describe the degradation behavior of both pesticides.

3.3. Degradation kinetics of malathion and chlorpyrifos

The plots of $\ln(C_t/C_0)$ vs. irradiation time for both pesticides were given in Fig. 2, and the deduced parameters for different treatments were listed in Table 1. The linear relationship between $\ln(C_t/C_0)$ and irradiation time indicated that the degradation followed a first-order kinetics well ($R^2 \geqslant 0.9$). This was in accordance with the description in degradation kinetics of dichlorvos by ultrasonic treatment in aqueous solution [13]. Moreover, there was a similar increase of k values and a similar decline of $t_{1/2}$ values for both pesticides when the output power was enhanced from 100 to 500 W. For example, the k values at 500 W were twice as large as that at 100 W, and the $t_{1/2}$ values at 500 W were half of that at 100 W. In addition, the difference in the values of kinetics parameters between both pesticides was significant. The k values of chlorpyrifos were 2.7–3.6 times higher than those of malathion, indicating that chlorpyrifos is much more labile to ultrasonic treatment than malathion. This could be explained by the Henry's constant of chlorpyrifos and malathion. Henry's constant characterizes the relative amount of a substrate that will enter the vapor bubbles, and it has been demonstrated that the compounds with higher Henry's constants are more prone to diffusing into the cavitation bubbles and degraded more rapidly compared with the compounds with lower Henry's constant under the same sonication condition [19]. Therefore, chlorpyrifos (Henry's constant is 6.76×10^{-1} Pa m^3 mol^{-1}) degrades more rapidly than malathion (Henry's constant is 1.21×10^{-2} Pa m^3 mol^{-1}) [20]. In addition,

Table 1
The first-order kinetic model fitted to the degradation of malathion and chlorpyrifos and deduced parameters.

Power (W)	Regression equation	R^2	p Value	k (min^{-1})	$t_{1/2}$ (min)
Malathion					
100	$\ln(C_t/C_0) = -0.0024\ t$	0.96	<0.0001	0.0024 ± 0.0001	292.5
300	$\ln(C_t/C_0) = -0.0039\ t$	0.99	<0.0001	0.0039 ± 0.0001	178.2
500	$\ln(C_t/C_0) = -0.0050\ t$	0.90	0.0006	0.0050 ± 0.0004	138.6
Chlorpyrifos					
100	$\ln(C_t/C_0) = -0.0065\ t$	0.97	<0.0001	0.0065 ± 0.0002	105.9
300	$\ln(C_t/C_0) = -0.0091\ t$	0.95	<0.0001	0.0091 ± 0.0004	76.0
500	$\ln(C_t/C_0) = -0.0138\ t$	0.94	<0.0001	0.0138 ± 0.0007	50.2

the chemical structures of both pesticides were considered to be related to the degradation behavior. This also has been proposed in the biodegradability of organophosphorus pesticides in soil [21] and in water [22].

3.4. Identification of degradation products of malathion and chlorpyrifos

The apple juice after the ultrasonic treatment at 500 W for 120 min was analyzed with GC–MS and the TIC chromatogram was shown in Fig. 3. Positively confirmed by the NIST Mass Spectral Library with high spectrographic fit (>89%), malaoxon and chlorpyrifos oxon were found as the products of malathion and chlorpyrifos, respectively. Simultaneously, this result was confirmed by the mass spectra of malaoxon and chlorpyrifos oxon (Fig. 4 and Fig. 5). In the mass spectra of malaoxon (Fig. 4), the fragment ion at m/z 268 was formed by the successive losses of ethoxy group and H from the molecular ion at m/z 314 (the response was low and not labeled). Next, loss of CO from the fragment ion at m/z 268 yielded fragment ion at m/z 240. In

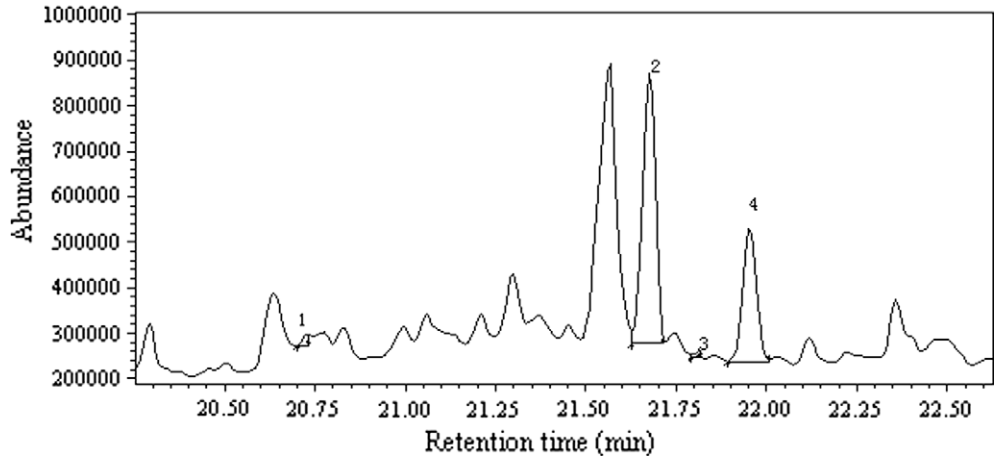

Fig. 3. TIC chromatogram of both pesticides and their degradation products in apple juice treated by ultrasonic at 500 W for 120 min malaoxon (1), malathion (2), chlorpyrifos oxon (3) and chlorpyrifos (4).

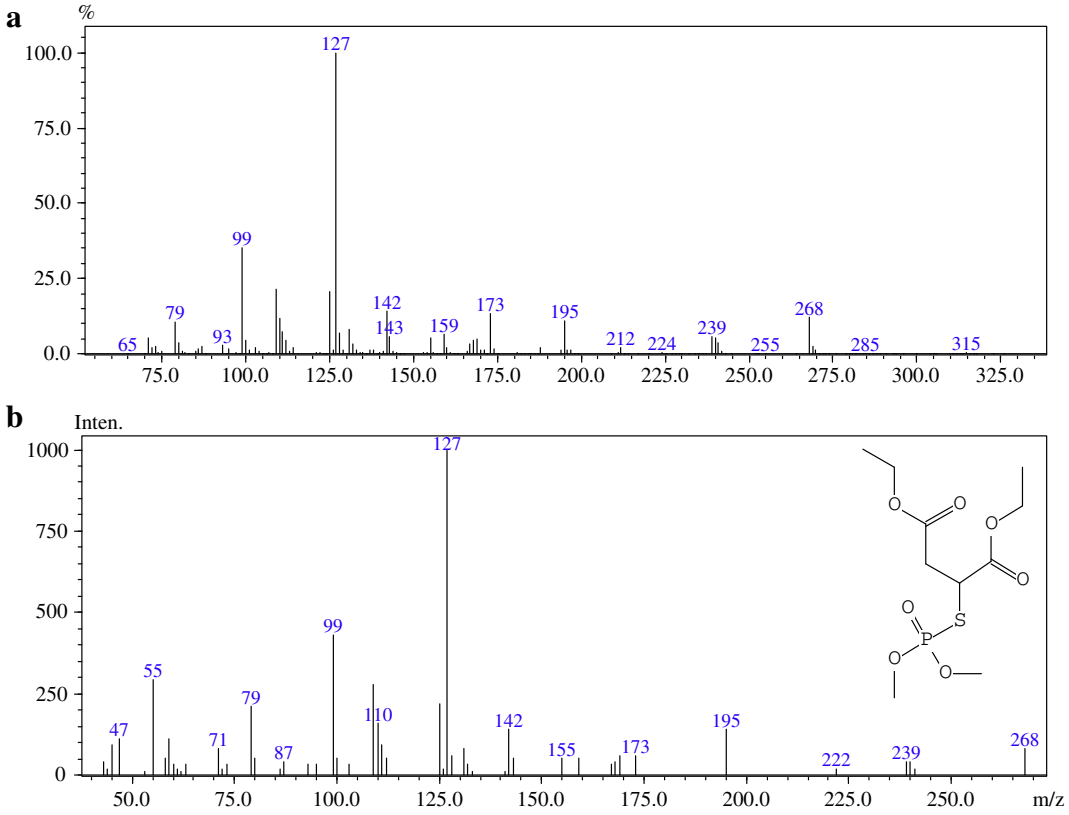

Fig. 4. GC/MS spectra of malaoxon obtained from apple juice treated by ultrasonic irradiation at 500 W for 120 min (a) and NIST library (b).

addition, the cleavage of the C–S bond of the molecular ion afforded the ions at *m*/*z* 173 and *m*/*z* 142, which was in accordance with the explanation on the mass spectra of malathion with GC–MS in negative chemical ionization performed by Amendola et al. [23]. Furthermore, fragment ion at *m*/*z* 173 yielded ethyl oxonium ion at *m*/*z* 127 by the successive losses of C_2H_4 and H_2O, intramolecular cyclization and rearrangement. Subsequently, the fragment ion at *m*/*z* 99 was formed by the loss of C_2H_4 from the fragment ion at *m*/*z* 127. For the mass spectra of chlorpyrifos oxon (Fig. 5), molecular ion at *m*/*z* 335 was observed. The fragment ion at *m*/*z* 298 was corresponded to $[M-Cl]^+$, and fragment ions at *m*/*z* 270 and 242 occurred due to the loss of one and two ethylene from the fragment ion at *m*/*z* 298, respectively. The fragment ions at *m*/*z* 109 and 81 were produced from the cleavage of P–O bond and corresponded to the side chain released from the ring of the fragment ions at *m*/*z* 270 and 242, respectively. In addition, the fragment ion at *m*/*z* 197 was yielded from the molecular ion by the loss of the side chain. The ion at *m*/*z* 29 was the base peak and it was the response of ethylene molecules released from the ion of *m*/*z* 298.

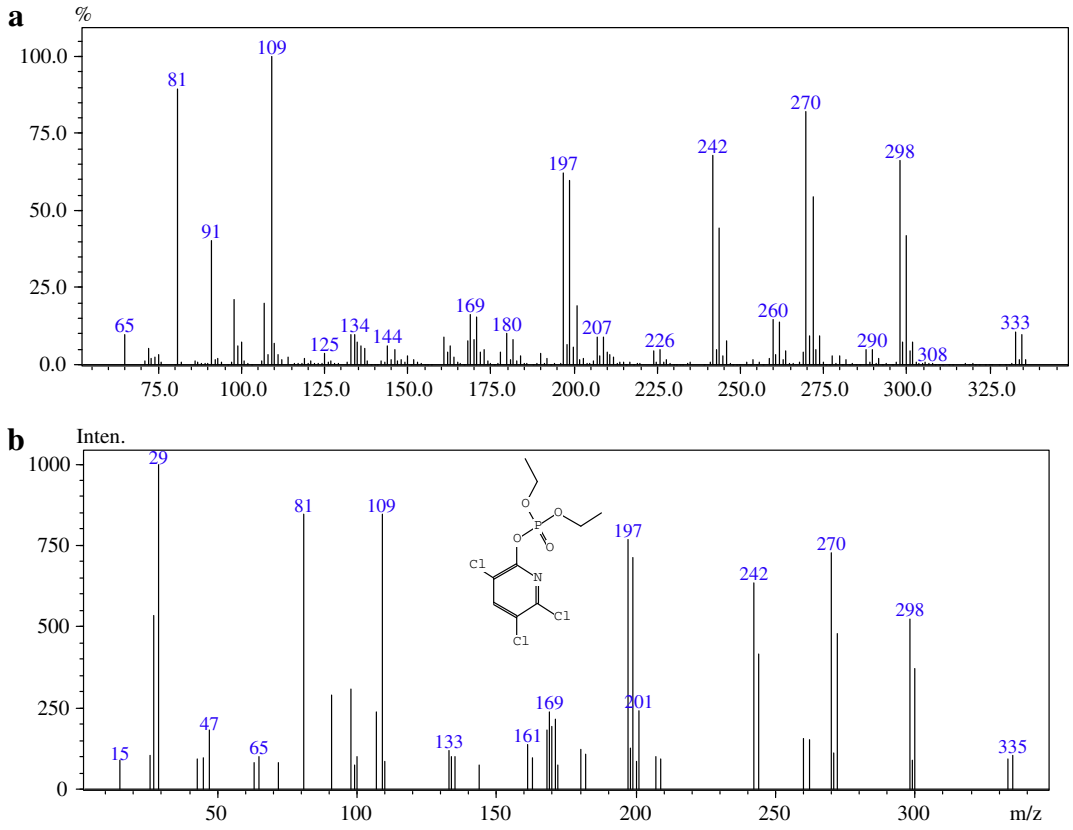

Fig. 5. GC/MS spectra of chlorpyrifos oxon obtained from apple juice (a) treated by ultrasonic irradiation at 500 W for 120 min and NIST library (b).

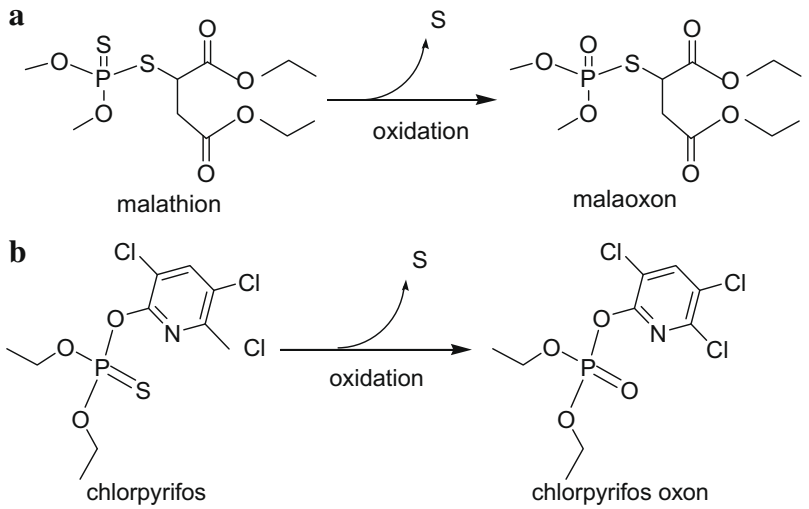

Fig. 6. Ultrasonic oxidation pathways of malathion (a) and chlorpyrifos (b).

Based upon the identification of degradation products of malathion and chlorpyrifos, the ultrasonic oxidation pathway of pesticides were assumed in Fig. 6. It has been proposed that ultrasound-induced reactions in aqueous solutions involve free-radical mechanisms due to the formation of hydroxyl radicals [24]. Furthermore, due to both pesticides with the low vapor pressure (5.3×10^{-3} Pa for malathion at 30 °C and 2.7×10^{-3} Pa at 25 °C for chlorpyrifos [20], both pesticides could be mainly present in the gas–liquid interface of the cavitation bubbles or in the bulk of the solution, where hydroxyl radical reaction was predominant during ultrasonic irradiation [25], thus the oxidative desulfuration of both pesticides promoted by hydroxyl radical would be more likely to occur. Also remarkable is the fact that the hydrolysis products of malathion and chlorpyrifos were not found in the present study, whereas the hydrolysis reactions of compounds during ultrasonic treatment were reported in some references [12,26]. The reason is probably that they were not extracted from samples by acetonitrile due to their strong polarity. Thus, the sample preparation procedure should be improved in future.

4. Conclusions

The present study demonstrated that ultrasonic irradiation is a promising process for the removal of malathion and chlorpyrifos from apple juice, and the ultrasonic power and treatment time have significant effects on their degradation ($p < 0.05$). The degradation kinetics of both pesticides could be described by the first-order kinetics model, but chlorpyrifos is shown to be much more labile to ultrasonic irradiation than malathion. Malaoxon and chlorpyrifos oxon were identified as the main degradation products of malathion and chlorpyrifos, and the oxidation pathway through the hydroxyl radical attack on the P═S bond of pesticide molecules was proposed.

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References

[1] C. Pope, S. Karanth, J. Liu, Environ. Toxicol. Phar. 19 (2005) 433–446.
[2] B. Piña-Guzmán, M.J. Solís-Heredia, B. Quintanilla-Vega, Appl. Pharm. 202 (2005) 189–198.
[3] G. Giordano, Z. Afsharinejad, M. Guizzetti, A. Vitalone, T.G. Kavanagh, L.G. Costa, Toxicol. Appl. Pharm. 219 (2007) 181–189.
[4] M.I. Girón-Pérez, A. Santerre, F. Gonzalez-Jaime, J. Casas-Solis, M. Hernández-Coronado, J. Peregrina-Sandoval, A. Takemura, G. Zaitseva, Fish Shellfish Immun. 23 (2007) 760–769.
[5] S. Cakir, R. Sarikaya, Food Chem. Toxicol. 43 (2005) 443–450.
[6] F. Chen, L. Zeng, Y. Zhang, X. Liao, Y. Ge, X. Hu, L. Jiang, Food Chem. 112 (2009) 956–961.
[7] C. Pappas, N.V. Kyriakidis, P.E. Athanasopoulos, Food Addit. Contamin. 20 (2003) 375–379.
[8] H. Karaca, Y.S. Velioglu, Food Rev. Int. 23 (2007) 91–106.
[9] K.C. Ong, J.N. Cash, M.J. Zabik, M. Siddiq, A.L. Jones, Food Chem. 55 (1996) 153–160.
[10] K. Okitsu, M. Ashokkumar, F. Grieser, J. Phys. Chem. B 109 (2005) 20673–20675.
[11] K. Vinodgopal, M. Ashokkumar, F. Grieser, J. Phys. Chem. B 105 (2001) 3338–3342.
[12] A. Kotronarou, G. Mill, M.R. Hoffmann, Environ. Sci. Technol. 26 (1992) 1460–1462.
[13] J.D. Schramm, I. Hua, Water Res. 35 (2001) 665–674.
[14] M.A. Matouq, Z.A. Al-Anber, T. Tagawa, S. Aljbour, M. Al-Shannag, Ultrason. Sonochem. 15 (2008) 869–874.
[15] Y.N. Liu, D. Jin, X.P. Lu, P.F. Han, Ultrason. Sonochem. 15 (2008) 755–760.
[16] Multi-residue analysis of organophosphorus, organochlorine, pyrethroid and carbamate insecticides in fruits and vegetables, Ministry of Agriculture of China, Chinese standard NY/T, 761-2004, 2004 (in Chinese).
[17] R.A. Putnam, J.O. Nelson, J.M. Clark, J. Agric. Food Chem. 51 (2003) 170–176.
[18] M. Dükkancı, G. Gündüz, Ultrason. Sonochem. 13 (2006) 517–522.
[19] Z. Wu, B. Ondruschka, J. Phys. Chem. A 109 (2005) 6521–6526.
[20] C.D.S. Tomlin, The Pesticide Manual: A World Compendium, 14th ed., British Crop Protection Council Publications, Farnham, UK, 2006.
[21] M.E. Sánchez, I.B. Estrada, O. Martínez, J. Martín-Villacorta, A. Aller, A. Morán, Chemosphere 57 (2004) 673–679.
[22] C.C.D. Yao, W.R. Haag, Water Res. 25 (1991) 761–773.
[23] L. Amendola, F. Botrè, A.S. Carollo, D. Longo, L. Zoccolillo, Anal. Chim. Acta 461 (2002) 97–108.
[24] D. Drijvers, H.V. Langenhove, M. Beckers, Water Res. 33 (1999) 1187–1194.
[25] T. Kondo, K.C. Murali, P. Ricsz, Radiat. Res. 118 (1989) 211–229.
[26] J. Kang, Environ. Sci. Technol. 32 (1998) 3194–3199.