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Determination of organophosphorus pesticide residues in vegetables by an enzyme inhibition method using α-naphthyl acetate esterase extracted from wheat flour*

Jun-liang WANG[1], Qing XIA[1], An-ping ZHANG[†‡1], Xiao-yan HU[2], Chun-mian LIN[1]

([1]*International Joint Research Center for Persistent Toxic Substances, College of Biological and Environmental Engineering, Zhejiang University of Technology, Hangzhou 310014, China*)

([2]*Zhejiang Province Environmental Radiation Monitoring Center, Hangzhou 310012, China*)

[†]E-mail: azhang@zjut.edu.cn

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Abstract: The widespread use of organophosphorus pesticides (OPs) poses a great threat to human health and has made the detection of OP residues in food an important task, especially in view of the fact that easy and rapid detection methods are needed. Because OPs have inhibitory effects on the activity of α-naphthyl acetate esterase (ANAE) in plants, in this work we evaluated the possibility of detecting OPs in vegetables with ANAE extracted from commercial flour. The limits of detection (LODs) obtained for methamidophos, dichlorvos, phoxim, dimethoate, and malathion in lettuce samples with crude ANAE were 0.17, 0.11, 0.11, 0.96, and 1.70 mg/kg, respectively. Based on the maximum residue limits (MRLs) for OPs in food stipulated by Chinese laws which are 0.05, 0.20, 0.05, 1.00, and 8.00 mg/kg for methamidophos, dichlorvos, phoxim, dimethoate, and malathion, respectively, the esterase inhibition method with crude ANAE had sufficient sensitivity to detect the residues of dichlorvos, dimethoate, and malathion in lettuce, but it could not be used to guarantee the safety of the same samples if methamidophos or phoxim residue was present. The sensitivity of the method was improved by the use of esterase purified by ammonium sulfate salting-out. The LODs obtained for methamidophos and phoxim with purified esterase were lower than the MRLs for these OPs in food. This is a very promising method for the detection of OP residues in vegetables using crude or purified esterase because of its cheapness, sensitivity, and convenience.

Key words: Organophosphorus pesticides (OPs), α-Naphthyl acetate esterase (ANAE), Limit of detection (LOD), Enzyme inhibition, Maximum residue levels (MRLs)

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1 Introduction

Organophosphorus pesticides (OPs) are one of the most common classes of chemicals used for the control of insects on vegetables because of their high efficacy and broad spectrum of activity. As a result, OP residues are likely to occur in vegetables, such as lettuce, celtuce, and cabbage. The inappropriate and illegal usage of OPs further increases the risk of human exposure. Therefore, it is important to determine the levels of OPs in vegetables to protect human health (de Silva *et al*., 2006; Uygun *et al*., 2007; Darko and Akoto, 2008; Zhao and Zhao, 2009).

Although chromatographic methods for the analysis of OPs in the environment and in food offer notable advantages for qualitative and quantitative

‡ Corresponding author

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analyses, they are generally time-consuming, expensive, labor-intensive, and use relatively large volumes of solvents. Recently, the acetylcholinesterase inhibition method has been successfully applied to the determination of OPs in various environmental matrices (Schulze *et al*., 2002). In most cases, the acetylcholinesterase applied in this method is extracted from the head of the fly *Musca domestica*. The flies are cultured in a tightly controlled environment that is free from pollution by any pesticides, especially OPs. The extraction procedure is complex and elaborate, and requires perfect technique and low-temperature processing. These limitations make it inconvenient to use acetylcholinesterase for the rapid determination of OPs (Li X. *et al*., 2008; Shi *et al*., 2008). An esterase extracted from plants could react with OPs and play an important role in the rapid determination of OP residues because of its low cost, easy extraction, convenient preservation, sensitivity, and accuracy (Huang *et al*., 2003; Meng *et al*., 2006; Li J.K. *et al*., 2009). As the esterase generally uses α-naphthyl acetate as its substrate, the esterase is referred to here as α-naphthyl acetate esterase (ANAE).

The present study was performed to determine the optimal parameters for OP detection using the ANAE inhibition method and to establish the method's limits of detection (LODs) for methamidophos, dichlorvos, phoxim, malathion, and dimethoate. A simple purification method, ammonium sulfate salting-out, was also evaluated by comparing the LOD values of the crude ANAE inhibition method with those of the method using purified ANAE.

2 Materials and methods

2.1 Chemicals

Malathion (purity >99.5%) was a gift from Sinochem Ningbo Chemicals Co., Ltd. (Ningbo, China). Methamidophos (purity >99.0%) was purchased from Kefa New Technology Development Co., Ltd. (Shenyang, China). Dichlorvos, phoxim, and dimethoate (purity >99.0%) were purchased from Baoling Chemical Co., Ltd. (Nantong, China). Esterase was extracted from wheat flour (*Triticum aestivum* L.) and dissolved in a potassium phosphate buffer of pH 6.38 before use. Fast blue B salt, α-naphthyl acetate, and α-naphthol were obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Other solvents or chemicals were of high performance liquid chromatography (HPLC) or analytical grade.

2.2 Principle of OP detection by ANAE

ANAE extracted from flour can catalytically hydrolyze α-naphthyl acetate into α-naphthol, which can react with fast blue B salt to form a purple-colored diazonium dye (Fig. 1) (Marston *et al*., 2002). The enzyme's activity was determined by the linear relationship between absorbance of purple-colored diazonium dye and time. The concentration of OPs in the samples was calculated according to the percentage of inhibition of enzyme activity using the equations in Table 1.

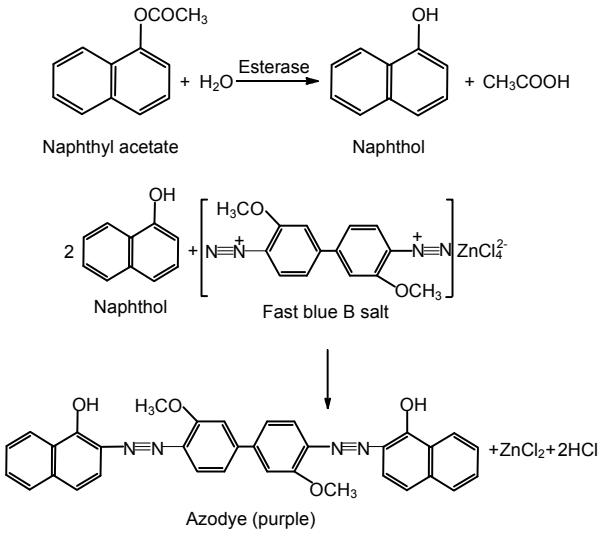

Fig. 1 Reactions involved in OP detection by the ANAE inhibition method

The percentage of inhibition of enzyme activity was calculated according to Eq. (1):

$$I=(E_0-E_1)/E_0\times 100\%, \quad (1)$$

where I is the inhibition percentage of enzyme activity, E_0 is the enzyme activity of the control group, and E_1 is the enzyme activity of the test group.

2.3 Extraction of ANAE

A total of 2 g of wheat flour was added to 20 ml of deionized water. The mixture was shaken gently for 15 min and then allowed to stand overnight at 4 °C.

It was then centrifuged for 10 min at 5000 r/min. The supernatant, representing a crude ANAE solution, was stored in a refrigerator at 4 °C until used. ANAE was purified by dialysis as follows. A measured amount of ammonium sulfate was added to the crude ANAE solution to achieve 30% saturation. The mixture was allowed to stand overnight at 4 °C and was then centrifuged for 10 min at 5000 r/min. The supernatant was collected, and ammonium sulfate was steadily added and dissolved to reach 60% saturation. After 60 min, the mixture was centrifuged for 10 min at 5000 r/min. The sediment containing enzyme was collected, dissolved in deionized water, and then transferred to a phosphate buffer at pH 6.38 using dialysis for 12 h. The operation was repeated several times until no sulfate ions were detected in the phosphate buffer. The purified ANAE solution was dried in a vacuum freeze-drier and stored at −20 °C prior to use.

2.4 Extraction of OPs from vegetables

The extraction of OPs followed the modified procedure of GB/T 18630-2002. Muddy water was gently removed from samples of lettuce. Methamidophos, dichlorvos, phoxim, malathion, or dimethoate solutions of various concentrations were sprayed on the samples. After standing overnight, the samples were cut into 1 cm×1 cm pieces and immersed in a flask containing acetone for 5 min. Active carbon was added to eliminate interference from chlorophyll. The mixture was filtered to obtain an acetone solution containing OPs, and the solvent was evaporated using a rotary evaporator (R-210, Buchi Laboratory Equipment, Flawil, Switzerland). The residues were dissolved by the mixed solution of phosphate buffer and acetone (99/1, v/v) before detection. Meanwhile, pesticide-free lettuce samples were prepared in the same manner to serve as controls.

2.5 Enzyme inhibition assay

The method described by Silva Filho *et al*. (2004) was used to determine the LOD of the ANAE inhibition method. Briefly, solutions of lettuce extracts (20 μl) with various OP concentrations that could inhibit an enzyme activity of 10%–90% were added to 500 μl centrifuge tubes. Next, 180 μl of ANAE solution was added to each tube. The enzyme-inhibitor solution was incubated at 35 °C for 30 min. Control samples were prepared with 20 μl of pesticide-free vegetable extract in place of the test solution. Enzyme activity was spectrophotometrically determined using a Bio-Rad model 680 microplate reader (Bio-Rad Laboratories, Hercules, CA, USA) at 35 °C (Ellman *et al*., 1961). A 230-μl aliquot of solution consisting of fast blue B salt and α-naphthyl acetate was added to the wells of a 96-well microtiter plate. After the incubation of the enzyme-inhibitor (or control) solution for 30 min, a 20-μl aliquot was drawn from the centrifuge tubes and added to the wells. The rate of hydrolysis of α-naphthyl acetate was monitored at 550 nm at 60-s intervals for 30 min following the addition of the enzyme-inhibitor (or control) solution. All of the above tests and measurements were repeated four times.

2.6 Validation of the method

Six lettuce samples from the field were used to validate the method. One of these samples did not receive any pesticide and served as the control. The other five samples were treated in the field using the five pesticides examined in this study. The initial concentrations of OPs for this treatment were approximately 0.1 mg/kg for methamidophos, 1.0 mg/kg for dichlorvos, 0.3 mg/kg for phoxim, 1.0 mg/kg for dimethoate, and 3.0 mg/kg for malathion. The treated samples were taken to the laboratory after 10 d of aging in the field and were analyzed using the purified ANAE inhibition method described above.

The accuracy of the method was confirmed by capillary gas chromatography with a nitrogen/phosphorus detector (GC-NPD, Agilent 6890, Agilent Technologies Inc., Santa Clara, USA) for methamidophos, dichlorvos, malathion, and dimethoate and by HPLC with an ultraviolet detector (HPLC-UV, JASCO LC-2000, JASCO, Tokyo, Japan) for phoxim. The analyses followed published procedures (Rastrelli *et al*., 2002; Anastassiades *et al*., 2003; Hamscher *et al*., 2007; Wang *et al*., 2008; Lv *et al*., 2009). In brief, the homogenized lettuce sample (10 g), ethyl acetate (20 g), and anhydrous sodium sulfate (15 g) were mixed thoroughly in a 50-ml centrifuge tube wrapped in silver paper using a vortex mixer, and were shaken for 30 min by reciprocating shakers at room temperature. The extraction and residues were separated by filtering through Whatman No. 1 filter paper. The filtrate containing OPs was

concentrated to 0.5 ml in a rotary evaporator, and was further purified by a solid phase extraction cartridge. The eluate in hexane from the column was collected and concentrated to 1 ml, 1 μl of which was injected into the GC-NPD for methamidophos, dichlorvos, malathion, and dimethoate analyses. With respect to phoxim, the concentrated eluate was completely dried under a gentle stream of nitrogen, and redissolved in 1 ml of acetonitrile.

The chromatographic conditions were as follows. For GC, an HP-5 capillary column (30 m, 0.25 mm i.d., 0.25 μm film thickness, Agilent Technologies Inc., Santa Clara, USA) was used. The oven temperature program had an initial temperature of 60 °C, held for 1 min, then rising by 12 °C/min to 140 °C and then by 8 °C/min to 280 °C, held for 10 min. The carrier gas was nitrogen at 1 ml/min, the make-up gas was nitrogen at 9 ml/min, the auxiliary gas was air at 60 ml/min, and the combustion gas was hydrogen at 3 ml/min. Injector and detector temperatures were 250 and 300 °C, respectively. For HPLC, an analytical reversed-phase C_{18} column (250 mm×4.6 mm, Hangzhou Puhui, Co., Ltd., China) was used. The mobile phase was a mixture of methanol and water (70/30, v/v), the flow rate was 0.8 ml/min, and the detection wavelength was 280 nm. The recoveries for all OPs were higher than 80%, and the relative standard deviations (SDs) were lower than 10%. The LOD was 0.01 mg/kg for phoxim, and was in the range of 0.001–0.005 mg/kg for methamidophos, dichlorvos, dimethoate, and malathion.

3 Results and discussion

3.1 Determination of the dilution ratio of crude ANAE

The crude ANAE contained large amounts of protein and caused the rapid generation of large amounts of hydrolytic products of α-naphthyl acetate. As a result, the measurement of the subsequent colorimetric reaction using the spectrophotometer was very difficult. Therefore, the dilution of the crude ANAE solution was very important for the exact determination of OP residues with the crude ANAE inhibition method. The effect of the dilution ratio on the production rate of azo dye is shown in Fig. 2.

The absorbance change per minute was inversely related to the increase in the dilution ratio. This result was consistent with the general principle that the rate of an enzymatic reaction is directly proportional to the concentration of enzyme in the limited range. In addition, it was found that the enzymatic reaction quickly reached equilibrium and could not be precisely measured on a spectrophotometer at dilution ratios of 3 and 5. The range of absorbance changes that could be exactly determined was from 0.04 to 0.07 U/min. It can be concluded from Fig. 2 that a dilution ratio of 10 was optimum for the measurement of the colorimetric reaction catalyzed by ANAE.

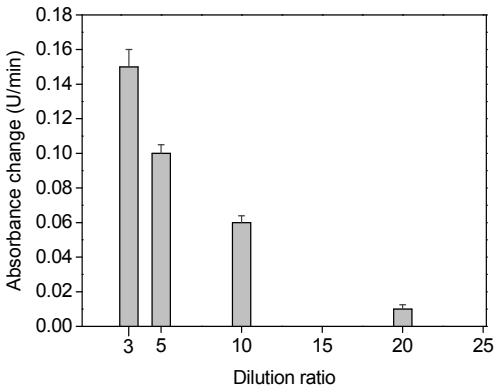

Fig. 2 Effect of dilution ratio of crude ANAE on reaction rate
Values are expressed as mean±SD (n=4)

3.2 Effects of temperature and pH on the activity of ANAE

Temperature and pH were the two key factors for the enzymatic reaction and had important effects on the sensitivity and the accuracy of the enzyme inhibition method. To determine optimal temperature and pH values for the method, we investigated the effects of temperature and pH on the activity of ANAE. These effects were measured by the absorbance change per minute (Figs. 3 and 4). The appropriate temperature and pH values for the enzymatic reaction were 35 °C and 6.38, respectively. Under these conditions, the rates of formation of azo dye reached their maximum values. These values were consistent with the ranges of temperature and pH required by general enzymatic reactions.

3.3 LOD of ANAE inhibition method for OPs

The inhibition percentage for crude enzyme activity at various concentrations of the five OPs (methamidophos, dichlorvos, phoxim, malathion, and

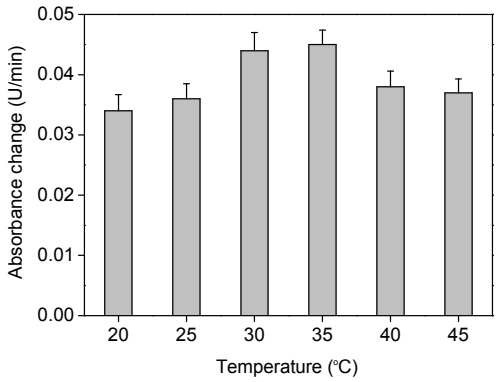

Fig. 3 Effect of temperature on reaction rate
Values are expressed as mean±SD (n=4)

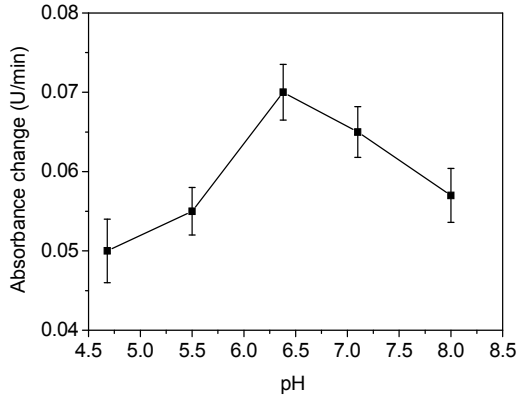

Fig. 4 Effect of pH on reaction rate
Values are expressed as mean±SD (n=4)

dimethoate) was calculated using Eq. (1). The relationships between the inhibition percentage and the concentrations of OPs are shown in Table 1. The inhibition percentage of enzyme activity was enhanced by the increase in pesticide concentration. The relationship between the concentrations of OPs and enzyme activity was in accordance with the typical dose-effect curve. The dynamic ranges of crude esterase assay spanned 0.12–30.67, 0.08–2.97, 0.01–6.74, 0.51–50.83, and 0.97–107.18 mg/kg for methamidophos, dichlorvos, phoxim, dimethoate, and malathion, respectively. In this study, the concentration of OPs that produced 10% inhibition of enzyme activity was taken as the LOD (Jeanty and Marty, 1998). The LOD values for methamidophos, dichlorvos, phoxim, malathion, and dimethoate using the ANAE inhibition method ranged from 0.11 to 1.70 mg/kg (Table 1). The average recoveries (the ratios of the concentration determined by the enzyme inhibition assay to the concentration spiked on samples) of these five OPs were above 90%. The detection sensitivity of the ANAE inhibition method differed significantly for different OPs. This finding might be related to the selective recognition of the enzyme to different OP configurations. The LOD values for dichlorvos, dimethoate, and malathion with crude ANAE were lower than the standard for the maximum residue limits (MRLs) for pesticides in food (GB 2763-2005), whereas the method did not exhibit sufficient sensitivity for detecting methamidophos and phoxim and thus cannot be used to determine whether the residues of methamidophos and phoxim in lettuce samples satisfy food safety requirements.

Purification of the enzyme generally improved the sensitivity of the enzyme inhibition method for detecting pesticide residues. The ammonium sulfate salting-out procedure is a common, simple, and widely-used method for the purification of enzymes. To satisfy the basic requirements for detecting OP residues in lettuce by the ANAE inhibition method, the ammonium sulfate salting-out procedure was used to purify crude enzyme extracted from wheat flour (*Triticum aestivum* L.). The dynamic ranges of purified esterase assay spanned 0.03−42.18, 0.01−9.72, and 0.75−138.73 mg/kg for methamidophos, phoxim, and malathion, respectively. The LOD values for methamidophos, phoxim, and malathion with the purified enzyme were 0.044, 0.020, and 1.020 mg/kg, respectively (Table 2). The LOD values for the purified enzyme were significantly lower than the corresponding values for the crude enzyme.

Table 1 LOD values of the crude ANAE inhibition method for OPs

OP	Regression equation	R^2	LOD (mg/kg)	MRL (mg/kg)	Recovery (%)
Methamidophos	I=16.3ln$C$+39.2	0.99	0.17	0.05	92.7
Dichlorvos	I=25.8ln$C$+66.9	0.93	0.11	0.20	103.9
Phoxim	I=2.2ln$C$+14.8	0.97	0.11	0.05	90.7
Dimethoate	I=12.6ln$C$+10.5	0.95	0.96	1.00	95.5
Malathion	I=16.9ln$C$+1.0	0.99	1.70	8.00	109.8

MRL: maximum residue limits for pesticides in food (GB2763-2005); I: inhibition percentage of enzyme activity; C: OP concentration

Table 2 LOD values of the dialysis ANAE inhibition method for OPs

OP	Regression equation	R^2	LOD (mg/kg)	Recovery (%)
Methamidophos	I=12.4ln$C$+48.6	0.99	0.044	95.7
Phoxim	I=7.3ln$C$+38.4	0.96	0.020	97.2
Malathion	I=16.3ln$C$+9.6	0.99	1.020	98.6

I: inhibition percentage of enzyme activity; C: OP concentration

The purified-enzyme method could completely satisfy the requirements for the detection of OP residues in lettuce. It would be useful to investigate the further purification of the target protein. Progress in this area may greatly improve the sensitivity of the OP determination method investigated in this study.

3.4 Validation of the method

The concentrations of OPs determined by the ANAE inhibition method and the chromatographic method in field-treated lettuce samples are shown in Table 3.

Table 3 Concentrations of OPs in field-treated lettuce samples determined by the ANAE inhibition method and the chromatographic method

OP	OP concentration (mg/kg)*	
	ANAE inhibition method	Chromatographic method
Control	BLD	BLD
Methamidophos	0.044±0.004	0.030±0.002
Dichlorvos	0.150±0.020	0.100±0.003
Phoxim	0.040±0.003	0.020±0.001
Dimethoate	0.580±0.030	0.410±0.010
Malathion	1.500±0.090	0.970±0.040

BLD: below limit of detection; * Data are expressed as mean±SD (n=4)

No OPs were detected in the control by either method. All concentrations of OPs detected in the samples of field-treated lettuce were below the MRLs and higher than the LODs of the ANAE inhibition method. The concentration of each OP, as determined by the ANAE inhibition method, was higher than that determined by the traditional chromatographic method. However, the qualitative results of the two methods were the same. This result indicated that the ANAE inhibition method was sufficiently accurate to evaluate whether the OP residues in vegetables exceeded the MRL. Thus, the method can be used as a convenient test to assure the safety of vegetables that are being marketed and to reduce the exposure of the population to OPs.

4 Conclusions

In this study, the ANAE inhibition method was used to determine the residues of five OPs in lettuce samples, and the LODs of the method were determined. As the results show, the LOD values of the crude ANAE inhibition method were lower than the MRLs for dichlorvos, dimethoate, and malathion and higher than the MRLs for methamidophos and phoxim in vegetables. If the enzyme was purified by the ammonium sulfate salting-out procedure, the detection sensitivity could be improved to satisfy the food safety test requirements for detecting methamidophos and phoxim in vegetables. The results of the validation study indicated that the purified ANAE inhibition method could be used to evaluate OP residues in vegetables and to ensure the safety of vegetables for human health. Future studies should focus on the purification of ANAE, the structural analysis of the target enzyme, and the exploration of the inhibition mechanism to obtain better sensitivity and accuracy for the detection of OPs in food.

References

Anastassiades, M., Lehotay, S.J., Stajnbaher, D., Schenck, F.J., 2003. Fast and easy multiresidue method employing acetonitrile extraction/partitioning and "dispersive solid-phase extraction" for the determination of pesticide residues in produce. *J. AOAC Int.*, **86**(2):412-431.

Darko, G., Akoto, O., 2008. Dietary intake of organophosphorus pesticide residues through vegetables from Kumasi, Ghana. *Food Chem. Toxicol.*, **46**(12):3703-3706. [doi:10.1016/j.fct.2008.09.049]

de Silva, H.J., Samarawickrema, N.A., Wickremasinghe, A.R., 2006. Toxicity due to organophosphorus compounds: what about chronic exposure? *Trans. R. Soc. Trop. Med. Hyg.*, **100**(9):803-806. [doi:10.1016/j.trstmh.2006.05.001]

Ellman, G.L., Courtney, K.D., Andres, V., Featherstone, R.M., 1961. A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochem. Pharmacol.*, **7**(2): 88-95. [doi:10.1016/0006-2952(61)90145-9]

GB/T 18630-2002. Method for Simple Determination of Organophosphorus and Carbamate Pesticide Residues in Vegetables—Enzyme Inhibition Method. General

Administration of Quality Supervision, Inspection and Quarantine, China.

GB 2763-2005. Maximum Residue Limits for Pesticides in Food. Ministry of Health of the People's Republic of China, Standardization Administration, China.

Hamscher, G., Priess, B., Nau, H., 2007. Determination of phoxim residues in eggs by using high-performance liquid chromatography diode array detection after treatment of stocked housing facilities for the poultry red mite (*Dermanyssus gallinae*). *Anal. Chim. Acta*, **586**(1-2): 330-335. [doi:10.1016/j.aca.2006.09.041]

Huang, Z.Y., Yuan, Y., Lv, Y.Z., 2003. Comparison of inhibiting ache and plant-esterase activity for organophosphorus pesticide residues in vegetables. *Food Sci.*, **24**(8): 135-137 (in Chinese).

Jeanty, G., Marty, J.L., 1998. Detection of paraoxon by continuous flow system based enzyme sensor. *Biosens. Bioelectron.*, **13**(2):213-218. [doi:10.1016/S0956-5663(97)00035-3]

Li, J.K., Zhou,Y.L., Wen,Y.X., Wang, J.H., Hu, Q.H., 2009. Studies on the purification and characterization of soybean esterase, and its sensitivity to organophosphate and carbamate pesticides. *Agric. Sci. China*, **8**(4):455-463. [doi:10.1016/S1671-2927(08)60232-1]

Li, X., Wang, Y., Zhang, J., Mang, Y., Wang, S., 2008. Purification and characterization of acetylcholinesterase (AChE) from musca domestica. *Chin. J. Anal. Lab.*, **27**(3):87-90 (in Chinese).

Lv, Z.L., Gao, L.L., Gao, H.B., Hou, Z.X., Zhang, B.L., 2009. Improved determination of phoxim residue in stored wheat by HPLC with DAD. *J. Food Sci.*, **74**(5):37-41. [doi:10.1111/j.1750-3841.2009.01157.x]

Marston, A., Kissling, J., Hostettmann, K., 2002. A rapid TLC bioautographic method for the detection of acetylcholinesterase and butyrylcholinesterase inhibitors in plants. *Phytochem. Anal.*, **13**(1):51-54. [doi:10.1002/pca.623]

Meng, L., Weng, X., Liu, C.J., 2006. Current status of research on using plant esterases for rapid determination of organophosphate pesticide residues. *Agrochemicals*, **45**(5): 306-308 (in Chinese).

Rastrelli, L., Totaro, K., Simone, F.D., 2002. Determination of organophosphorus pesticide residues in Cilento (Campania, Italy) virgin olive oil by capillary gas chromatography. *Food Chem.*, **79**(3):303-305. [doi:10.1016/S0308-8146(02)00143-7]

Schulze, H., Schmid, R.D., Bachmann, T.T., 2002. Rapid detection of neurotoxic insecticides in food using disposable acetylcholinesterase-biosensors and simple solvent extraction. *Anal. Bioanal. Chem.*, **372**(2):268-272. [doi:10.1007/s00216-001-1137-2]

Shi, M.A., Yuan, J.Z., Wu, J., Zhuang, P.J., Tang, Z.H., 2008. Studies on the kinetics of acetylcholinesterase in the resisiant and susceptible strains of housefly (*Musca domestica*). *Insect Sci.*, **8**(1):30-38. [doi:10.1111/j.1744-7917.2001.tb00432.x]

Silva Filho, M.V., Oliveira, M.M., Salles, J.B., Bastos, V.L., Cassano, V.P., Bastos, J.C., 2004. Methyl-paraoxon comparative inhibition kinetics for acetylcholinesterases from brain of neotropical fishes. *Toxicol. Lett.*, **153**(2): 247-254. [doi:10.1016/j.toxlet.2004.04.026]

Uygun, U., Ozkara, R., Ozbey, A., Koksel, H., 2007. Residue levels of malathion and fenitrothion and their metabolites in postharvest treated barley during storage and malting. *Food Chem.*, **100**(3):1165-1169. [doi:10.1016/j.foodchem.2005.10.063]

Wang, L.G., Liang, Y.C., Jiang, X., 2008. Analysis of eight organophosphorus pesticide residues in fresh vegetables retailed in agricultural product markets of Nanjing, China. *Bull. Environ. Contam. Toxicol.*, **81**(4):377-382. [doi:10.1007/s00128-008-9498-2]

Zhao, J.H., Zhao, D.G., 2009. Transient expression of organophosphorus hydrolase to enhance the degrading activity of tomato fruit on coumaphos. *J. Zhejiang Univ.-Sci. B (Biomed. & Biotechnol.)*, **10**(2):142-146. [doi:10.1631/jzus.B0820251]