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Effects of home preparation on organophosphorus pesticide residues in raw cucumber

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ABSTRACT

The effects of washing with tap water and different detergent solutions, storage at different temperatures and ultrasonic cleaning on organophosphorus pesticide (trichlorfon, dimethoate, dichlorvos, fenitrothion, and chlorpyrifos) residue levels in raw cucumber was investigated. Analysis was carried out by liquid chromatography–tandem mass spectrometry. Washing with detergent solutions proved more effective than tap water. The organophosphorus pesticides reduced from 31.1% to 98.8% after washing with detergent solutions for 20 min. Among detergent solutions, 5% sodium carbonate solution caused the greatest loss in trichlorfon and dimethoate, and 5% sodium bicarbonate solution caused the greatest loss in dichlorvos, fenitrothion and chlorpyrifos. Storage at 4 °C for 48 h caused pesticides reduction by 60.9–90.2%. Ultrasonic cleaning for 20 min lowered pesticides by 49.8–84.4%. The data indicated that home preparation is effective for the reduction of organophosphorus pesticide residues in raw cucumber and it is useful for reducing the dietary exposure.

1. Introduction

Pesticide residues in fruits and vegetables are a major concern to consumers due to their perceived negative health effects. The presence of their residues in vegetables can be a significant route to human exposure (Council Directive 90/642/EEC, 1990). It is important to estimate the level of pesticide exposure from vegetables. Knowledge of the effects of home preparation on the levels of pesticide residues in vegetables is required to reduce dietary exposure (Byrne & Pinkerton, 2004; Keikotlhaile, Spanoghe, & Steurbaut, 2010).

Cucumber, which is susceptible to insect and disease attacks, has been widely studied for the presence of pesticide residues. Many studies have reported that the main pesticide residues are organophosphorus pesticides (Mansour, Belal, Abou-Arab, & Gad, 2009; Pan, Wang, Kong, Jiang, & Qian, 2002). Although boiling, frying, roasting and blanching lead to a significant reduction of pesticide residues (Chavarri, Herrera, & Arino, 2005; EI-Behissy, King, Ahmed, & Youssef, 2001; Nagayama, 1996; Radwan, Abu-Elamayem, Shiboob, & Abdel-Aal, 2005; Randhawa, Anjum, Ahmed, & Randhawa, 2007; Soliman, 2001; Zabik, Cash, Zabik, Jones, & El-Hadidi, 2000), cucumber is most often eaten raw in salads and in cold soups.

There have been many examinations on the removal of pesticide residues from vegetables during home preparation and commercial processing. Zhang, Liu, and Hong (2007) measured the chlorpyrifos, *p,p*-DDT, cypermethrin and chlorothalonil residue levels in cabbage after washing, refrigeration and stir-frying for different times. Ling et al. (2011) studied the effects of washing and cooking on chlorpyrifos and its toxic metabolites in vegetables and showed that cooking was more effective than washing for the removal of chlorpyrifos residues. Byrne and Pinkerton (2004) investigated the effect of cooking on chlorpyrifos and 3,5,6-trichloro-2-pyridinol levels in chlorpyrifos-treated produce and showed that reduction in residues was dependent upon the commodity and cooking procedure. Reports by Cengiz, Certel, and Gocmen (2006) showed that the culinary applications were effective in reducing the dichlorvos and diazinon residue levels in cucumber.

Chlorpyrifos and dichlorvos have been studied by several investigators, but trichlorfon, dimethoate and fenitrothion residues in cucumber have been rarely studied, and so far no one has reported the influence of home preparation on five organophosphorus pesticide residues in cucumber for raw eating. In this study, home preparations including washing with different detergent solutions, storage at different temperatures and ultrasonic cleaning were applied to evaluate the effects on the organophosphorus pesticide residues in raw cucumber. This study will provide a guide for the consumer on how to remove pesticides effectively in cucumber for raw eating.

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2. Materials and methods

2.1. Plant materials and sampling

Fresh cucumbers were collected from experimental fields located in Jiangsu Academy of Agricultural Science (Nanjing, China) with no previous pesticide applications. They were maintained at 4 °C and analysed within 24 h of harvest. Before home preparation, a 10 mL 1000 μg mL^{-1} mixed pesticides standard (trichlorfon, dimethoate, dichlorvos, fenitrothion, and chlorpyrifos) stock solution was dissolved in tap water. Five hundred grams of fresh cucumber were immersed in dilution solution for 50 min until the pesticide residue levels did not increase. The contaminated cucumber was air-dried in a fume hood.

2.2. Home preparation of raw cucumber

2.2.1. Washing

Spiked cucumber was soaked in different concentrations of sodium chloride, acetic acid, sodium carbonate and sodium bicarbonate solutions for 5, 10 and 20 min, respectively. The treated cucumber was air-dried in a fume hood and then analysed.

2.2.2. Storage

Spiked cucumber was stored at 4 °C and room temperature (25 °C) in the dark for 12, 24 or 48 h and then analysed.

Ultrasonic cleaning: Spiked cucumber was soaked in water and cleaned by in an ultrasonic bath for 5, 10 or 20 min, then analysed.

2.3. Sample extraction and cleanup

Cucumber (20 g) was blended and transferred to a 100-mL conical flask; 40 mL of acetone were added and the flask shaken for 30 min. The homogenate was filtered. After the addition of 4 g of sodium chloride to the filtrate, the liquid phase layer was allowed to separate and 10 mL of acetone extracts were taken and evaporated to dryness. The residue was then subjected to Florisil SPE and eluted with 20 mL of acetone and *n*-hexane (4:1, V/V). The eluate was evaporated to dryness and dissolved in methane to 1 mL. After filtration through a 0.2-μm membrane, the solution was analysed by LC–MS/MS.

2.4. LC–MS/MS analysis

LC–MS/MS was conducted with an Agilent 1200 Series HPLC (Agilent, Santa Clara, CA) and Agilent 6410A triple quadrupole mass spectrometer with Agilent G1948B electrospray ionisation (ESI). All data were acquired employing Agilent 6410 Quantitative Analysis data processing software. Chromatographic separation was achieved by gradient elution using an Agilent Zorbax SB-C18 column (150 × 2.1 mm, 5 μm). Five microlitres of filtrate were injected into the LC-MS/MS. The mobile phase consisted of water (elution solvent A) and methanol (elution solvent B). At a flow rate of 0.3 mL/min, the gradient programme was as follows: 0–6 min, 50–90% B; 6–12 min, 90–55% B. The detector was operated in positive ESI mode using multiple reaction monitoring mode (MRM). Gas flow was 10 L/min and temperature was 350 °C; nebuliser pressure was 25 psi; gas capillary voltage was 4000 V, source temperature was set at 100 °C. In order to achieve the highest sensitivity, the fragmentor voltage and the collision energy were optimised. A summary of the transitions monitored (Chung & Chan, 2010), the fragmentor voltage and collision energy parameters for each compound are given in Table 1.

2.5. Recovery studies

Various standards of pesticides (10–2000 ng/mL) were prepared and injected into the LC–MS/MS under the conditions stated in Section 2.4. The limits of detection (LOD) were below 0.003 mg/kg for the five pesticides listed above.

The method was optimised by recovery studies before the determination of pesticides in samples. The same extraction procedure and HPLC–MS/MS conditions as applied for sample analyses were used for recovery studies. From five replications spiked at levels of 0.01–1 mg kg^{-1}, average recoveries ranged from 75.5% to 98.0%, with a maximum relative standard deviation (RSD) of 9.0%.

3. Results and discussion

3.1. Effects of washing on the removal of pesticide residues in cucumber

The effects of washing by tap water and detergent solutions for 5, 10 or 20 min on pesticide residues in cucumber are shown in Table 2. Washing with tap water, as well as detergent solutions had an effect in reducing trichlorfon, dimethoate, dichlorvos, fenitrothion and chlorpyrifos. Among these washing methods, the washing by tap water for 20 min proved the least effective, showing 53.7%, 32.6%, 52.4%, 26.7% and 62.9% reduction in the above pesticides, respectively, which was in agreement with Zhang et al. (2007) and Abou-Arab (1999).

Among detergent solutions, 5% sodium carbonate solution caused the greatest loss in trichlorfon and dimethoate, 97.6% and 78.3%, respectively; 5% sodium bicarbonate solution caused the greatest loss in dichlorvos, fenitrothion and chlorpyrifos, 98.8%, 66.7% and 85.2%, respectively. Hence, washing with sodium carbonate and sodium bicarbonate solution is a suitable method to remove organophosphorus pesticides. Acetic acid solution was also suitable for removing dichlorvos. There was a gradual increase

Table 1
Mass spectral transitions monitored and instrumental parameters.

Analyte	Retention time (min)	Q1 mass (*m*/*z*)	Q3 mass (*m*/*z*)	Fragmentor voltage	Collision energy
Trichlorfon	2.66	257.1	221.0	110	4
			127.0[a]	110	7
Dimethoate	2.85	230.2	199.0[a]	90	5
			171.0	90	9
Dichlorvos	3.82	221.2	145.1	135	5
			109.1[a]	135	10
Fenitrothion	6.70	278.1	246.1	135	10
			125.1[a]	135	20
Chlorpyrifos	9.75	350.1	198.0	110	15
			97.1[a]	110	30

[a] Quantification ion.

Table 2
Effect of washing on pesticide residues in cucumber.

Pesticide	Treatment	Time					
		5 min		10 min		20 min	
		Concentration[a]	Reduction (%)	Concentration[a]	Reduction (%)	Concentration[a]	Reduction (%)
Trichlorfon	Spiked samples	0.41 ± 0.034					
	Tap water	0.32 ± 0.045	21.9	0.26 ± 0.058	36.6	0.19 ± 0.011	53.7
	NaCl solution (2%)	0.31 ± 0.036	24.4	0.22 ± 0.041	46.3	0.15 ± 0.032	63.4
	NaCl solution (5%)	0.25 ± 0.053	39.0	0.20 ± 0.026	51.2	0.14 ± 0.022	65.8
	CH_3COOH solution (2%)	0.27 ± 0.055	34.1	0.18 ± 0.051	56.1	0.13 ± 0.043	68.3
	CH_3COOH solution (5%)	0.20 ± 0.043	51.2	0.16 ± 0.052	60.9	0.12 ± 0.061	70.7
	Na_2CO_3 solution (2%)	0.11 ± 0.035	73.2	0.06 ± 0.021	85.4	0.05 ± 0.036	87.8
	Na_2CO_3 solution (5%)	0.04 ± 0.056	90.2	0.03 ± 0.031	92.7	0.01 ± 0.037	97.6
	$NaHCO_3$ solution (2%)	0.19 ± 0.056	53.7	0.11 ± 0.037	73.2	0.07 ± 0.014	82.9
	$NaHCO_3$ solution (5%)	0.15 ± 0.034	63.4	0.09 ± 0.028	78.0	0.06 ± 0.029	85.4
Dimethoate	Spiked samples	0.46 ± 0.025					
	Tap water	0.39 ± 0.034	15.2	0.36 ± 0.016	21.7	0.31 ± 0.017	32.6
	NaCl solution (2%)	0.27 ± 0.025	41.3	0.24 ± 0.025	47.8	0.23 ± 0.022	50.0
	NaCl solution (5%)	0.27 ± 0.034	41.3	0.21 ± 0.015	54.3	0.16 ± 0.025	65.2
	CH_3COOH solution (2%)	0.38 ± 0.054	17.4	0.33 ± 0.052	28.3	0.29 ± 0.014	36.9
	CH_3COOH solution (5%)	0.32 ± 0.053	30.4	0.30 ± 0.015	34.8	0.25 ± 0.051	45.6
	Na_2CO_3 solution (2%)	0.21 ± 0.041	54.3	0.20 ± 0.052	56.5	0.18 ± 0.051	60.9
	Na_2CO_3 solution (5%)	0.19 ± 0.057	58.7	0.11 ± 0.033	76.1	0.10 ± 0.024	78.3
	$NaHCO_3$ solution (2%)	0.20 ± 0.019	56.5	0.19 ± 0.026	58.7	0.16 ± 0.019	65.2
	$NaHCO_3$ solution (5%)	0.17 ± 0.046	63.0	0.14 ± 0.052	69.6	0.11 ± 0.050	76.1
Dichlorvos	Spiked samples	0.84 ± 0.053					
	Tap water	0.72 ± 0.024	14.3	0.65 ± 0.054	22.6	0.40 ± 0.065	52.4
	NaCl solution (2%)	0.31 ± 0.016	63.1	0.25 ± 0.053	70.2	0.21 ± 0.024	75.0
	NaCl solution (5%)	0.27 ± 0.035	67.8	0.24 ± 0.053	71.4	0.19 ± 0.041	77.4
	CH_3COOH solution (2%)	0.09 ± 0.043	89.3	0.07 ± 0.051	91.7	0.02 ± 0.056	97.6
	CH_3COOH solution (5%)	0.04 ± 0.076	95.2	0.03 ± 0.056	96.4	0.01 ± 0.031	98.8
	Na_2CO_3 solution (2%)	0.67 ± 0.034	20.2	0.59 ± 0.035	29.8	0.38 ± 0.046	54.8
	Na_2CO_3 solution (5%)	0.63 ± 0.034	25.0	0.41 ± 0.025	51.2	0.34 ± 0.046	59.5
	$NaHCO_3$ solution (2%)	0.03 ± 0.031	96.4	0.03 ± 0.056	96.4	0.01 ± 0.034	98.8
	$NaHCO_3$ solution (5%)	0.02 ± 0.054	97.6	0.01 ± 0.016	98.8	0.01 ± 0.038	98.8
Fenitrothion	Spiked samples	0.45 ± 0.019					
	Tap water	0.38 ± 0.014	13.3	0.35 ± 0.052	22.2	0.33 ± 0.022	26.7
	NaCl solution (2%)	0.39 ± 0.046	15.6	0.32 ± 0.041	28.9	0.31 ± 0.013	31.1
	NaCl solution (5%)	0.38 ± 0.066	15.6	0.28 ± 0.049	37.8	0.22 ± 0.035	51.1
	CH_3COOH solution (2%)	0.33 ± 0.064	26.7	0.28 ± 0.053	37.8	0.23 ± 0.026	48.9
	CH_3COOH solution (5%)	0.31 ± 0.017	31.1	0.25 ± 0.023	44.4	0.21 ± 0.056	53.3
	Na_2CO_3 solution (2%)	0.36 ± 0.016	20.0	0.34 ± 0.034	24.4	0.26 ± 0.056	42.2
	Na_2CO_3 solution (5%)	0.32 ± 0.053	28.9	0.27 ± 0.050	40.0	0.18 ± 0.053	60.0
	$NaHCO_3$ solution (2%)	0.28 ± 0.071	37.8	0.22 ± 0.064	51.1	0.21 ± 0.043	53.3
	$NaHCO_3$ solution (5%)	0.27 ± 0.056	40.0	0.21 ± 0.068	53.3	0.15 ± 0.016	66.7
Chlorpyrifos	Spiked samples	0.81 ± 0.065					
	Tap water	0.38 ± 0.046	53.1	0.33 ± 0.032	59.2	0.30 ± 0.043	62.9
	NaCl solution (2%)	0.36 ± 0.033	55.6	0.32 ± 0.011	60.5	0.27 ± 0.054	66.7
	NaCl solution (5%)	0.35 ± 0.046	56.8	0.30 ± 0.035	63.0	0.25 ± 0.026	69.1
	CH_3COOH solution (2%)	0.37 ± 0.023	54.3	0.30 ± 0.031	63.0	0.28 ± 0.024	65.4
	CH_3COOH solution (5%)	0.34 ± 0.034	58.0	0.29 ± 0.052	64.2	0.27 ± 0.016	66.7
	Na_2CO_3 solution (2%)	0.32 ± 0.055	60.4	0.31 ± 0.043	61.7	0.29 ± 0.058	64.2
	Na_2CO_3 solution (5%)	0.28 ± 0.054	65.4	0.27 ± 0.055	66.7	0.24 ± 0.053	70.3
	$NaHCO_3$ solution (2%)	0.20 ± 0.024	75.3	0.18 ± 0.073	77.8	0.15 ± 0.065	81.5
	$NaHCO_3$ solution (5%)	0.16 ± 0.055	80.2	0.15 ± 0.055	81.5	0.12 ± 0.023	85.2

[a] Concentrations are given as means (mg kg^{-1}) ±Standard error.

in the reduction of pesticide levels due to the increase of concentration of detergent solution in the treatment at the same treatment time. A gradual reduction was also observed when increasing the treatment time at the same concentration.

Radwan et al. (2005) showed that washing with acetic acid solution gave high percent removal of profenofos residues in pepper. Zohair (2001) reported that organophosphorus pesticides (pirimiphos-methyl, malathion, and profenofos) were eliminated more effectively by acidic, neutral and alkaline solutions than organochlorine pesticides. They concluded that washing by acidic, neutral and alkaline solutions is effective in the removal of organophosphorus pesticides, and the reduction of organophosphorus pesticides depends on the kind and concentration of solutions.

It has been recommended that cucumber should be washed with tap water and/or detergent solutions carefully to decrease the intake of pesticide residues before raw eating (Kaushik, Satya, & Naik, 2009).

3.2. Effects of storage on the removal of pesticide residues in cucumber

The effects of storage at 4 and 25 °C for 12, 24 or 48 h on pesticide residues in cucumber are summarised in Table 3. The reductions of pesticides stored at 4 °C for 48 h were 60.9%, 80.4%, 83.3%, 66.7% and 70.4% for trichlorfon, dimethoate, dichlorvos, fenitrothion and chlorpyrifos, respectively. The reductions in these pesticides stored at 25 °C for 48 h were 90.2%, 82.6%, 97.6%, 88.9%

Table 3
Effect of storage on pesticide residues in cucumber.

Pesticide	Temperature (°C)	Time					
		12 h		24 h		48 h	
		Concentration[a]	Reduction (%)	Concentration[a]	Reduction (%)	Concentration[a]	Reduction (%)
Trichlorfon	4	0.33 ± 0.034	19.5	0.28 ± 0.046	31.7	0.16 ± 0.054	60.9
	25	0.22 ± 0.021	46.3	0.16 ± 0.034	61.0	0.04 ± 0.046	90.2
Dimethoate	4	0.17 ± 0.021	63.0	0.13 ± 0.026	71.7	0.09 ± 0.045	80.4
	25	0.14 ± 0.023	69.6	0.10 ± 0.043	78.3	0.08 ± 0.057	82.6
Dichlorvos	4	0.21 ± 0.041	75.0	0.20 ± 0.033	76.2	0.14 ± 0.046	83.3
	25	0.10 ± 0.053	88.1	0.05 ± 0.032	94.0	0.02 ± 0.058	97.6
Fenitrothion	4	0.24 ± 0.023	46.7	0.18 ± 0.016	60.0	0.15 ± 0.023	66.7
	25	0.09 ± 0.042	80.0	0.08 ± 0.026	82.2	0.05 ± 0.045	88.9
Chlorpyrifos	4	0.35 ± 0.035	56.8	0.31 ± 0.048	61.7	0.24 ± 0.053	70.4
	25	0.35 ± 0.025	56.8	0.26 ± 0.034	67.9	0.23 ± 0.036	71.6

[a] Concentrations are given as means (mg kg^{-1}) ±Standard error.

Table 4
Effect of ultrasonic treatment on pesticide residues in cucumber.

Pesticide	Time					
	5 min		10 min		20 min	
	Concentration[a]	Reduction (%)	Concentration[a]	Reduction (%)	Concentration[a]	Reduction (%)
Trichlorfon	0.26 ± 0.045	36.6	0.16 ± 0.046	61.0	0.07 ± 0.025	82.9
Dimethoate	0.26 ± 0.052	43.5	0.24 ± 0.013	47.8	0.22 ± 0.033	52.2
Dichlorvos	0.70 ± 0.023	16.7	0.66 ± 0.043	21.4	0.59 ± 0.013	49.8
Fenitrothion	0.12 ± 0.056	73.3	0.10 ± 0.045	77.8	0.07 ± 0.025	84.4
Chlorpyrifos	0.38 ± 0.064	53.1	0.33 ± 0.056	59.3	0.30 ± 0.023	63.0

[a] Concentrations are given as means(mg kg^{-1}) ±Standard error.

and 71.6%, respectively. The increase in storage temperature had a great influence on pesticides reduction except for chlorpyrifos and dimethoate. The results agree with Cengiz et al. (2006) who reported that dichlorvos residues were effectively reduced by storage. From our results, it should be concluded that storage has an effect in reducing pesticides. Pesticide reduction gradually increased with increased temperature rising and time, especially for trichlorfon.

3.3. Effects of ultrasonic cleaning on the removal of pesticide residues in cucumber

Effects of ultrasonic cleaning for 5, 10, 20 min on pesticide residues in cucumber are presented in Table 4. The ultrasonic waves in the water cause cavitation; rapid formation and violent collapse of micron-sized bubbles in a liquid medium, cause tiny implosions, which provide the cleaning power. The study of ultrasonic cleaning on the reduction of pesticides in vegetables has been rarely studied.

As shown in Table 4, pesticides reduction increased with time. Ultrasonic cleaning for 20 min caused greater reduction for trichlorfon, dimethoate and especially fenitrothion than washing with tap water; the reductions were 82.9%, 52.5% and 84.4%, respectively. The reductions of chlorpyrifos and dichlorvos using ultrasonic cleaning were no different from those obtained by washing with tap water.

4. Conclusion

This study studied the effects of home preparation on organophosphorus pesticide residues in cucumber for raw eating. Although home preparation without cooking was not sufficient for the complete removal of pesticide residues, it removes a large proportion of the pesticide residues present in raw cucumber. Washing with different detergent solutions, storage and ultrasonic cleaning all lowered the organophosphorus pesticide levels to some degree. It is suggested that raw cucumber should be treated with a combination of home preparation methods. However, the change in nutrient content as a result of storage and ultrasonic cleaning should also be considered. There is a need to address the balance between food quality and safety in future studies.

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