Research Article

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Washing effects of limonene on pesticide residues in green peppers

Hai-Yan Lu, Yan Shen, Xing Sun, Hong Zhu and Xian-Jin Liu*

Abstract

BACKGROUND: The presence of pesticide residues in food has caused much concern. The low health risks and environmental impacts of limonene make it a very interesting solvent for use in green chemistry. Washing effects of limonene on pesticide residues of methyl chlorpyrifos, chlorothalonil, chlorpyrifos, fenpropathrin and deltamethrin were investigated in green pepper.

RESULTS: Results showed that washing with a low concentration of limonene for 5 min (where LOQ is limit of quantitation) caused 53.67%, <LOQ, 64.29%, 68.69% and 66.22% loss of the above pesticides, respectively, while corresponding values of washing with a high concentration were 84.64%, <LOQ, 90.46%, 89.00% and 89.36%, respectively. Washing with a low concentration of limonene for 10 min produced 55.90%, <LOQ, 66.19%, 72.08% and 73.25% loss, respectively, while corresponding values of washing with a high concentration were 94.42%, <LOQ, 96.58%, 92.04% and < LOQ, respectively. The reductions due to washing with tap water (for 10 min) and the emulsion with only egg yolk lecithin (at high concentration for 10 min) were 25.18 %, 37.83%, 21.84%, 20.87%, 13.86% and < LOQ, 59.70%, 54.09%, 54.76%, 54.47%, respectively.

CONCLUSION: The data indicated that washing with a low concentration of limonene for 5 min was the optimal treatment for elimination of pesticide residues in green pepper, considering effect and treatment time as well as cost.

Keywords: limonene; pesticide residues; green pepper; green washing detergent

INTRODUCTION

Food safety is an area of growing worldwide concern. The presence of harmful pesticide residues in food has caused much concern among consumers. It is important that some effective solution should be developed to tackle this situation of food safety.[1] Vegetables play important roles in human nutrition and health by providing minerals, micronutrients, vitamins and dietary fiber,[2] but residues in vegetables could affect consumers' health especially when freshly consumed. Some methods and equipment have been developed to decrease or remove pesticide residues.[3–6] Many studies have indicated that food-processing treatments such as washing, peeling or cooking could significantly reduce pesticide residues,[7–9] especially washing.[6,7,9–11]

Limonene, a major component of orange essential oil, is a natural and functional monoterpene.[12] Limonene has many physiological functions and has been used widely, including anticancer activity in medicine,[13] aromatic property in the cosmetic industry and in perfumery,[14] antimicrobial activity in the food industry and in plant protection,[15,16] etc. Recently, other applications have made use of limonene because of its ability to solubilize fats,[17] as a green solvent for Soxhlet extraction,[18] as main solvent of an ink cleaning agent[19] and as extraction solvent in the new Dean–Stark procedure.[20] Low health risks and environmental impacts of limonene make it a very interesting solvent for use in green chemistry. Meanwhile, limonene has gained acceptance in the food industry since it has been generally recognized as safe (GRAS) by the Food and Drug Administration[21] and many foods tolerate its presence.[12] All these make it possible to use limonene in the food industry as a green washing detergent, especially for the removal of pesticide residues in vegetables.

In this study, the effects of washing by limonene emulsion for different times on the main pesticide residues (organophosphates, pyrethroids and organochlorine) in green pepper were investigated. The purposes of this paper are the scientific and original use of limonene, and to find an ideal method to remove pesticide residues effectively for food safety.

MATERIALS AND METHODS

Apparatus and reagents

Apparatus

Gas chromatography with flame photometric detector (FPD) and electron capture detector (ECD) was performed on an HP6890 (Hewlett Packard, Palo Alto, CA, USA); nitrogen evaporator (Organomation Associates Inc., Berlin, MA, USA); high-speed

* *Correspondence to: Xian-Jin Liu, Key Lab of Agro-product Safety Risk Evaluation (Nanjing), Ministry of Agriculture, Jiangsu Nanjing 210014, China. E-mail: 54521955@qq.com*

Jiangsu Academy of Agricultural Sciences; Key Lab of Food Quality and Safety of Jiangsu Province – State Key Laboratory Breeding Base; Key Laboratory of Control Technology and Standard for Agro-product Safety and Quality, Ministry of Agriculture, China; Key Lab of Agro-product Safety Risk Evaluation (Nanjing), Ministry of Agriculture, Jiangsu Nanjing 210014, China

blender (Jiangsu Haimen Medical Apparatus Factory, Jiangsu, China); Xinfei refrigerator (Xinfei Electrical Co. Ltd, Henan, China); constant temperature magnetic stirrer (Shanghai Weicheng Instrument Co., Ltd, Shanghai, China); NC Ultrasonic Cleaner (Kunshan Ultrasonic Instrument Co., Ltd, Suzhou, China).

Standards

Methyl chlorpyrifos, chlorothalonil, chlorpyrifos, fenpropathrin and deltamethrin were purchased from the Institute for the Control of Agrochemicals, Ministry of Agriculture, China. Mixed pesticide standard stock solutions (1000 mg L^{-1}) were prepared in acetone and stored at 4 °C. The maximum residue levels (MRLs) of five pesticides in pepper used in our case according to data from www.foodmate.net are 0.5, 5, 0.5, 0.5 and 0.2 mg kg^{-1}, respectively; Limonene was purchased from Damas-beta Company (purity 95%, Shanghai, China); egg yolk lecithin was obtained from the Institute for the Control of Agrochemicals, Ministry of Agriculture, China.

Reagents

Acetone, acetonitrile, *n*-hexane, methanol, sodium chloride and anhydrous sodium sulfate were of analytical grade, purchased from Kermel Chemical Reagent Co. Ltd (Tianjin, China). 0.2 μm SCAA-104 membranes and 500 mg florisil SPE cartridges were obtained from Anpel Scientific Instrument Co. Ltd (Shanghai, China).

Experimental design

Preparation of polluted green peppers

The mixed pesticides standard (methyl chlorpyrifos, chlorothalonil, chlorpyrifos, fenpropathrin, deltamethrin) stock solution (15 mL, 1000 mg L^{-1}) was dissolved in 3.0 L tap water. Pesticide-free green peppers were soaked in the polluted tap water for 4 min and then air-dried under room conditions.

Preparation of limonene emulsion

Limonene was emulsified in order to improve its solubility. The emulsification method was according to previously reported methods[22] with slight changes. Egg yolk lecithin was used as surfactant. Initially, the organic phase – limonene and egg yolk lecithin – was mixed at a weight ratio of 25:2 and stirred magnetically for 50 min. Then, the aqueous phase (distilled water) was added to the organic phase at a weight ratio of 4:1. The mixture was agitated for 20 min on a magnetic stirrer. The solution then underwent sonification using ultrasound for 8 min with 70% amplitude. The emulsion was stored at 4 °C until use.

Experimental design

Six treatments (including a control) were set up, with three replicates of each. The treatments were as follows:

- *treatment* ***I***: polluted green peppers without washing;
- *treatment* ***II***: polluted green peppers washed with tap water;
- *treatment* ***III***: polluted green peppers washed with a low concentration of limonene emulsion (6 mL limonene emulsion was dissolved in 3.0 L tap water);
- *treatment* ***IV***: polluted green peppers washed with a high concentration of limonene emulsion (12 mL limonene emulsion was dissolved in 3.0 L tap water);
- *treatment* ***V***: polluted green peppers washed with a low concentration of emulsion with only egg yolk lecithin (6 mL emulsion was dissolved in 3.0 L tap water);
- *treatment* ***VI***: polluted green peppers washed with a high concentration of emulsion with only egg yolk lecithin (12 mL emulsion was dissolved in 3.0 L tap water).

Dry polluted green peppers were soaked in different solutions for 5 or 10 min, respectively, and then air-dried under room conditions and prepared for analysis of pesticide residues.

Analysis of pesticide residues

Extraction

Each whole and unwashed green pepper sample was homogenized in a blender. A 25 g minced sample was taken and transferred to a 100 mL conical flask, 50 mL acetonitrile was added, the mixture was homogenized for 2 min, and then 6 g sodium chloride was added. After shaking, the liquid phase layer was allowed to separate and two 10 mL samples were taken: one for the detection of organophosphorus insecticides and the other for the detection of pyrethroids and chlorothalonil.

Purification

The 10 mL sample of acetonitrile phase was evaporated to dryness in a nitrogen evaporator in a water bath at 60 (±1) °C. The residue was made up to 5 mL with *n*-hexane, cleaned up using a florisil disposable cartridge previously activated with acetone + *n*-hexane (1 + 9 by volume, 5 mL), then conditioned with 5 mL *n*-hexane. The active ingredients were recovered in a 15 mL polypropylene centrifuge tube by eluting the cartridge with acetone + *n*-hexane (1 + 9 by volume, 10 mL). The solution was evaporated to dryness in a nitrogen evaporator in a water bath at 60 (±1) °C, the residue was dissolved in 2 mL *n*-hexane and the solution was analyzed by gas chromatography with different detectors (organophosphorus with FPD, pyrethroid and chlorothalonil with ECD).

Gas chromatographic analysis

1. FPD: J&W Scientific (Folsom, CA, USA) DB-17 column, 30 m × 0.53 mm × 0.25 μm; detector 270 °C; injector 250 °C; oven 80 °C for 1 min, 80–250 °C at 10 °C min^{-1}, 250 °C maintained for 2 min; carrier (nitrogen) 35 mL min^{-1}; injection volume 1 μL.
2. ECD: cyanopropyl phenyl polysiloxane column, 30 m × 0.32 mm × 0.25 μm; detector 300 °C; injector 250 °C; oven 80 °C for 0.5 min, 80–120 °C at 7 °C min^{-1}, 120–280 °C at 10 °C min^{-1}, 280 °C maintained for 10 min; carrier (nitrogen) 50 mL min^{-1}; injection volume 1 μL.

The pesticide residues in green peppers were qualitatively determined by retention time and quantitatively determined by peak-area external standards.

RESULTS AND DISCUSSION

Accuracy and precision of methods used in this paper

Various standards of pesticides (0.05–10 mg L^{-1}) were prepared using green pepper matrix and injected into the gas chromatograph under the conditions stated above ('Analysis of pesticide residues'). The retention times, linear equations, correlation coefficients (R^2) and limits of quantification (LOQ) of the five pesticides are presented in Tables 1 and 2. The results

Table 1. Related parameters of used methods

Pesticide	Retention time (min)	Linear equation	R^2	LOQ (mg kg^{-1})
Methyl Chlorpyrifos	17.963	$y = 16772.617x + 1770.907$	0.9991	0.20
Chlorothalonil	18.781	$y = 9481.401x - 996.995$	0.9993	0.02
Chlorpyrifos	18.818	$y = 17215.145x + 1802.609$	0.9993	0.02
Fenpropathrin	20.284	$y = 10386.339x + 398.660$	0.9999	0.02
Deltamethrin	25.962	$y = 28229.082x + 2396.980$	0.9995	0.02

Table 2. Recovery and precision of five pesticides in green pepper at three spike levels

Pesticide	Spike level (mg kg^{-1})	Found (mg kg^{-1})	Recovery (%)	RSD (%)
Methyl chlorpyrifos	1	0.827 ± 0.073	82.73	8.85
	0.5	0.482 ± 0.019	96.36	3.92
	0.05	0.042 ± 0.003	83.59	6.66
Chlorothalonil	10	9.222 ± 0.505	92.22	5.48
	5	4.739 ± 0.206	94.78	4.35
	0.5	0.454 ± 0.024	90.71	5.21
Chlorpyrifos	1	0.863 ± 0.029	86.27	3.37
	0.5	0.471 ± 0.038	94.22	8.06
	0.05	0.048 ± 0.004	95.36	7.88
Fenpropathrin	1	0.879 ± 0.066	87.89	7.54
	0.5	0.456 ± 0.043	91.25	9.39
	0.05	0.045 ± 0.004	89.82	9.28
Deltamethrin	0.4	0.318 ± 0.020	79.47	6.22
	0.2	0.173 ± 0.011	86.60	6.30
	0.02	0.018 ± 0.001	88.37	5.14

indicated that under the methods used correlation coefficients were all above 0.9991, while detection limits (setting a signal-to-noise ratio of 3) were all under MRLs, and average recoveries ranged from 79.47% to 96.36%, with a maximum relative standard deviation (RSD) of 9.39%. The methods used in this paper could meet the requirements for the detection of pesticide residues in green pepper.

Effects of washing on the removal of pesticide residue in green peppers

Residues of the five pesticides in green pepper under different treatments are summarized in Table 3. The results showed that the washing process including tap water, as well as different concentrations of limonene emulsion and emulsion with only egg yolk lecithin, had an effect in reducing pesticides.

Among these washing methods, the most effective was washing with limonene emulsion (low concentration with 53.67–73.25% reduction, high concentration with 84.64–97.96% reduction), followed by emulsion with only egg yolk lecithin (low concentration with 19.37–28.20% reduction, high concentration with 43.92–59.70% reduction), and the least effective was tap water (with 10.32–37.83% reduction). The reductions with tap water were close to those shown by low concentration of emulsion with only egg yolk lecithin, while they were much lower compared with high-concentration ones. Between washing times, 10 min (with 13.86–96.58% reduction) was more effective than 5 min (with 10.32–90.46% reduction). The difference due to washing methods was greater than that due to treatment times. Washing by limonene emulsion, regardless of high concentration or low concentration, 10 min or 5 min, could reduce the pesticide residues to a value below MRL.

The effects of washing using the same method for the same time on the removal of residues varied with the pesticide type. All washing treatments resulted in the residue of chlorothalonil being far below the MRL, and even below the LOQ (washing by limonene emulsion and emulsion with only egg yolk lecithin). The losses of organophosphorous (methyl chlorpyrifos and chlorpyrifos) were higher than those of pyrethroid (fenpropathrin and deltamethrin) when washing with tap water, while opposite effects were obtained when washing with limonene emulsion. These results may be related to the solubility of pesticides, and limonene emulsion was effective in dissolving lipophilic pesticides. This ability of limonene was consistent with the property reported before.[17–20] The residue of all pesticides tested was reduced to a safe level by limonene emulsion.

Reductions of chlorpyrifos and chlorothalonil washing by tap water were similar to that observed by Zhang *et al.*[6] who evaluated effects of home preparation on pesticide residues in cabbage, but reductions were much higher after washing with limonene emulsion in this paper than after washing with NaCl solution and acetic acid solution. The reduction of deltamethrin was also much more effective after washing with limonene emulsion than another method reported previously.[23]

Washing is the most common form of processing and is a preliminary step in both household and commercial preparation. Pesticide residues are removed with reasonable efficiency by varied types of washing processes. This was proved by many research studies in tomatoes,[7] apples,[9] cucumbers[10] and grapes.[11] In this paper, limonene indicated good potential for the removal of pesticide residues in vegetables. From these

Table 3. Effect of different treatments on removal of pesticide residues in green pepper

		Treat time			
		5 min		10 min	
Pesticide	Treatment	Found (mg kg^{-1}) (mean ± SD)	Reduction (%)	Found (mg kg^{-1}) (mean ± SD)	Reduction (%)
Methyl chlorpyrifos	I	0.987 ± 0.109			
	II	0.799 ± 0.049	19.14	0.739 ± 0.040	25.18
	III	0.458 ± 0.038	53.67	0.435 ± 0.037	55.90
	IV	0.152 ± 0.009	84.64	0.055 ± 0.004	94.42
	V	0.761 ± 0.009	22.94	0.709 ± 0.010	28.20
	VI	0.505 ± 0.012	48.86	0.398 ± 0.004	59.70
Chlorothalonil	I	0.826 ± 0.010			
	II	0.558 ± 0.015	32.42	0.514 ± 0.014	37.83
	III	<0.200	—	<0.200	—
	IV	<0.200	—	<0.200	—
	V	<0.200	—	<0.200	—
	VI	<0.200	—	<0.200	—
Chlorpyrifos	I	0.971 ± 0.114			
	II	0.794 ± 0.032	18.23	0.759 ± 0.052	21.84
	III	0.347 ± 0.022	64.29	0.328 ± 0.018	66.19
	IV	0.093 ± 0.003	90.46	0.033 ± 0.005	96.58
	V	0.762 ± 0.009	21.56	0.704 ± 0.007	27.53
	VI	0.514 ± 0.013	47.09	0.446 ± 0.010	54.09
Fenpropathrin	I	0.676 ± 0.048			
	II	0.584 ± 0.016	13.70	0.535 ± 0.021	20.87
	III	0.212 ± 0.006	68.69	0.189 ± 0.013	72.08
	IV	0.074 ± 0.006	89.00	0.054 ± 0.009	92.04
	V	0.540 ± 0.007	20.09	0.497 ± 0.008	26.55
	VI	0.379 ± 0.006	43.92	0.306 ± 0.008	54.76
Deltamethrin	I	0.470 ± 0.044			
	II	0.422 ± 0.027	10.32	0.405 ± 0.012	13.86
	III	0.159 ± 0.005	66.22	0.126 ± 0.008	73.25
	IV	0.050 ± 0.009	89.36	<0.020	—
	V	0.379 ± 0.002	19.37	0.338 ± 0.009	28.09
	VI	0.242 ± 0.006	48.51	0.214 ± 0.012	54.47

results, it should be recommended that fruits and vegetables are washed with water or other solutions before raw eating or cooking.

With demands from consumers to find alternatives to chemical-based washing detergents for food application, limonene is potentially an ideal alternative. More studies on primary food are required to assess the changes in organoleptic properties after the application of limonene, although it has been reported that some components including limonene were found to be neutral and have no effect on aroma quality.[24] The other factor that must be considered with the use of limonene on food is that it can play a role in the formation of secondary aerosols and form pollutants.[25] In order to ensure food safety and reduce the negative impact to a minimum, accurate concentration tests need to be carried out to find the lowest concentration of limonene that has the greatest effect.

CONCLUSION

Washing is effective in decreasing the intake of pesticide residues from vegetables, especially washing with limonene emulsion. Limonene has been generally recognized as safe (GRAS) by the FDA, so it is feasible to use limonene in the food industry as a green washing detergent. Meanwhile, it is suggested that citizens should wash vegetables carefully before eating it to minimize harm.

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