Assessing Washing Methods for Reduction of Pesticide Residues in Tomatoes and Other Produce Using LC/MS and GC/MS

Application Note

Food

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Abstract

Various washing protocols have been assessed for efficacy using Agilent LC/MS and GC/MS instrumentation to measure pesticide residues in tomatoes and other produce. Washing procedures in water and various solutions, with and without sonication, were evaluated in the laboratory. In general, the effect of sonication depended on the washing treatment and on the pesticide. A separate experiment measured pesticide residues in contaminated samples before and after being washed in a pilot plant flume, which resulted in reductions of 40 to 90% after one minute in water at room temperature.

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Introduction

Because of the expansion of worldwide trade, more foods are being imported into the United States. There are safety concerns of these commodities due to reports of the presence of banned antimicrobial agents in imported farm-raised seafood, melamine in milk products, and pesticides in produce [1].

Pesticides can affect the nervous, endocrine, immune, and reproductive systems. Infants, young children, the unborn, and the elderly are more susceptible to pesticide poisoning. Therefore, it is desirable to reduce the levels of pesticide residue in foods to lower the exposure and risk to human health [1].

Washing with water or detergent is important to remove pesticide residues in fruits and vegetables. The efficiency of the washing treatments on pesticide removal depends on the washing solution, the chemical properties of the pesticide, the surface area, the nature of the food, the length of time the pesticide is in contact with the food, and the formulation and application method of the pesticide. Usually, the pesticide is lodged in the outer wax-like layers and then moves to the inside, making washing and removal of the pesticides less effective [2].

This application note describes a published study conducted to determine the effectiveness of washing and sanitizing treatments alone or in combination with ultrasound for removing pesticide residues from tomatoes [2]. This was the first in-depth study of washing and sonication treatments used for reduction of pesticide levels in tomatoes. A commercial-scale produce washing operation in the pilot plant at the Institute for Food Safety and Health (IFSH), Illinois Institute of Technology was also used to assess the efficacy of a water wash for removal of pesticides in select contaminated produce (tomatoes, apples, green peppers, peaches, oranges, and lemons).

Experimental

Reagents and standards

Supplies and chemicals, including cherry tomatoes, were obtained and prepared as described [2]. Nine pesticide standards (acephate, malathion, carbaryl, bifenthrin, cypermethrin, cyhalothrin, permethrin, chlorothalonil, and imidacloprid) were purchased from Sigma-Aldrich, and solutions containing a mixture of these standards were prepared as described.

Instruments

This study was conducted using both LC/MS and GC/MS instrumentation. Three pesticides not amenable to GC analysis were analyzed using an Agilent 1260 Infinity LC system coupled to an Agilent 6460 Triple Quadrupole LC/MS System equipped with Jet Stream Technology. The other pesticides were analyzed using an Agilent 7890A GC coupled to an Agilent 5975C Series GC/MSD. The instrument run conditions are shown in Tables 1 and 2.

Sample preparation

Tomatoes were dipped in standard pesticide solution for 5 or 30 seconds, and then dried at ambient temperature. Various washing protocols were used to wash the tomatoes, which were then extracted using the QuEChERS extraction procedure according to the AOAC Official Method 2007.1, as described [2].

Table 1. LC/MS/MS Run Conditions

LC run conditions	
Column	Agilent ZORBAX Extend-C18, 2.1 × 100 mm, 1.8 µm particles (p/n 728700-902)
Column temperature	40 °C
Injection volume	5 µL
Mobile phase	A = 10 mM ammonium acetate in water v/v B = methanol
Linear gradient	5% B for 0.5 minutes 5% B for 12 minutes 98% B for 15 minutes 100%B for 16 minutes Then 10% B to 100% B over 25 minutes at constant flow Hold at 100% B for 10 minutes
Flow rate	0.3 mL/min

MS conditions	
Ion mode	Positive electrospray ionization
Nebulizer pressure	40 psig
Drying gas flow rate	10 L/min
Drying gas temperature	325 °C
Sheath gas flow rate	11 L/min
Sheath gas temperature	350 °C
Nozzle voltage	4,000 V
Capillary voltage	1,000 V

Table 2. GC/MSD Run Conditions

GC run conditions	
Analytical column	Agilent HP-5MS UI 15 m × 0.25 mm, 0.25 µm (p/n 19091S-431)
Inlet temperature	250 °C
Injection volume	1 µL
Injection mode	Pulsed splitless
Liner	2-mm dimpled single taper deactivated splitless liner (p/n 5190-2296)
Flow	Constant pressure mode, 4 psi at purged ultimate union
Oven program	70 °C, hold for 1 minute 50 °C/min to 150 °C 6 °C/min to 200 °C 16 °C/min to 280 °C Hold at 280 °C for 5 minutes
Carrier gas	Helium
Run time	20.9333 minutes, plus a 4-minute post run for backflush
Transfer line temperature	280 °C
MS conditions	
Acquisition mode	Electron ionization, SIM with four time segments
Solvent delay	3.2 minutes
Gain factor	2.00
Extractor source temperature	300 °C
Quadrupole temperature	200 °C

Statistical analysis

Averages and standard deviations from sample studies and linear regressions for calibration curves were determined using Microsoft Excel 2010. Pesticide concentrations from GC/MS and LC/MS/MS analyses were determined using Agilent Enhanced ChemStation E.02.00.493 and Agilent MassHunter Quantitative Software for Triple Quad B.03.01 (B2065). Statistical analysis was performed with one-way analysis of variance (ANOVA) followed by Fisher's test with-the significance set at P values of < 0.05 for temperature studies and two-way ANOVA for pesticide washing treatments with the significance set at P values of < 0.05 using Minitab statistical software.

Analysis parameters

The acquisition parameters used to perform LC/MS/MS and GC/MS analyses are shown in Tables 3 and 4.

Table 3. LC/MS/MS Analysis Parameters for Pesticides in Tomato Extracts

Pesticide	Formula	RT* (min)	Nominal mass	Precursor ion (*m/z*)	Product ion(s) (*m/z*)	Fragmentor voltage (eV)	Collision energy (V)
Acephate	$C_4H_{10}NO_3P_5$	3.6	183.18	184	142.9, 49.1	45	4, 20
Imidacloprid	$C_9H_{10}CN_5O_2$	6.7	255.05	256.1	209, 175.10	80	10, 10
Carbaryl	$C_{12}H_{11}NO_2$	9.9	201.079	202	145, 127	60	5, 30

*RT, retention time

Table 4. GC/MS Analysis Parameters for Pesticides in Tomato Extracts

Pesticide	Formula	RT (min)*	Nominal mass	Target ion (*m/z*)	Qualifier ion 1 (*m/z*)	Qualifier ion 2 (*m/z*)
Acephate	$C_4H_{10}NO_3P_5$	3.922	136	94	95	96
Chlorothalonil	$C_8C_{14}N_2$	7.556	265.9	266	264	268
Carbaryl	$C_{12}H_{11}NO_2$	8.577	201.079	144.1	115.1	116.1
Malathion	$C_{10}H_{19}O_6PS_2$	9.594	330.04	125	93	173
Bifenthrin	$C_{23}H_{22}C_1F_3O_2$	14.558	422.88	181.2	165.1	166.1
l-Cyhalothrin	$C_{23}H_{19}ClF_3NO_3$	15.322	449.9	181	197	208
Permethrin	$C_{12}H_{20}O_3$	15.918	391.29	165.1	181.2	166.1
Cypermethrin	$C_{22}H_{19}C_{12}NO_3$	16.526	416.3	163	181	165

*RT, retention time

Results and Discussion

Pesticide dipping time

Two different dipping times (5 and 30 seconds) in the pesticide solution were evaluated for reproducible contamination of tomatoes with pesticides for subsequent washing studies. Both the 5- and the 30-second dipping times provided a homogenous level of pesticides contaminating tomato surfaces, enabling consistent reduction of pesticides after the water washing treatments, as shown by the error bars in Figure 1. All subsequent tomato washing and sonication experiments were performed using the shorter dipping time (5 seconds). This dipping time closely modeled the time of spraying used in commercial and residential applications of pesticides. However, dipping the tomatoes in a pesticide solution allowed consistent application, unlike spraying, in which not all pesticides were likely to be absorbed on the surface of tomatoes. This process facilitated more reproducible and accurate results.

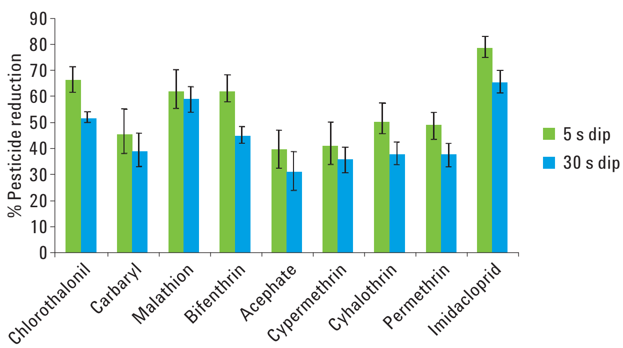

Figure 1. Comparison of percentages of pesticide reduction on tomatoes after 5- and 30-second dipping times in pesticides and then washing. All bar graphs represent the averages ± standard deviations of three trials.

Effect of washing temperature

Washing with water is a common practice for reducing pesticide residues from the surface of produce in both household and commercial preparations. In general, washing tomatoes in 22 °C water produced reduction levels for most pesticides that were significantly different ($P < 0.05$) from the results obtained by washing in 5 and 10 °C water (Figure 2). While washing tomatoes in 5 °C water produced reductions in pesticide levels similar to those obtained when washing in 10 °C water, the greatest reductions occurred when tomatoes were washed at ambient temperature (22 °C, Figure 2). Diffusion of the pesticides from the surface of the tomato was accelerated by rinsing the tomatoes at the higher temperature.

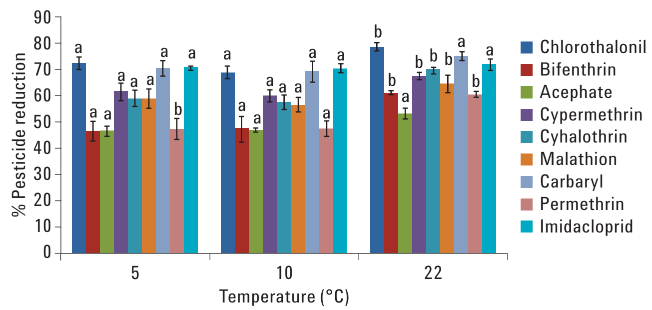

Figure 2. Comparison of percentages of pesticide reduction in tomatoes washed in water at 5, 10, and 22 °C. All bar graphs represent the averages ± standard deviations of three trials. Bars representing the same pesticide residue but having different letters at different temperatures show significantly different results ($P < 0.05$) as determined by one-way ANOVA.

Effect of washing protocol

Batches of tomatoes contaminated by the dipping procedure were washed in water, sodium hypochlorite (80 mg/mL, pH 7), peroxyacetic acid (80 mg/mL), and Tween 20 (0.1%) at 10 °C. Sonication was then added to determine any improvement in efficacy of removal of pesticides from the surface of tomatoes.

This study showed that the effect of sonication was dependent on the washing treatment as well as the pesticide (Figure 3). The reductions in pesticide levels obtained from the washing treatments were subjected to two-way ANOVA. In general, there were no statistically significant differences ($P > 0.05$) in pesticide reduction levels observed when tomatoes were washed with and without sonication using the different washing treatments (Figure 3). In particular, sonication produced no significant differences in reduction of cypermethrin, cyhalothrin, and malathion levels when added to washing with sodium hypochlorite ($P > 0.05$). The same result was observed for acephate and imidacloprid ($P > 0.05$) when using peroxyacetic acid with and without sonication. (Figure 3). In addition, rinsing tomatoes with peroxyacetic acid only, or with peroxyacetic acid and sonication did not result in statistically significant reductions of carbaryl and malathion levels on tomatoes (Figure 3).

Only water washing showed a significant improvement after sonication. For example, permethrin showed a significant difference in reduction level ($P < 0.05$) upon sonication in water, versus water washing only (Figure 3).

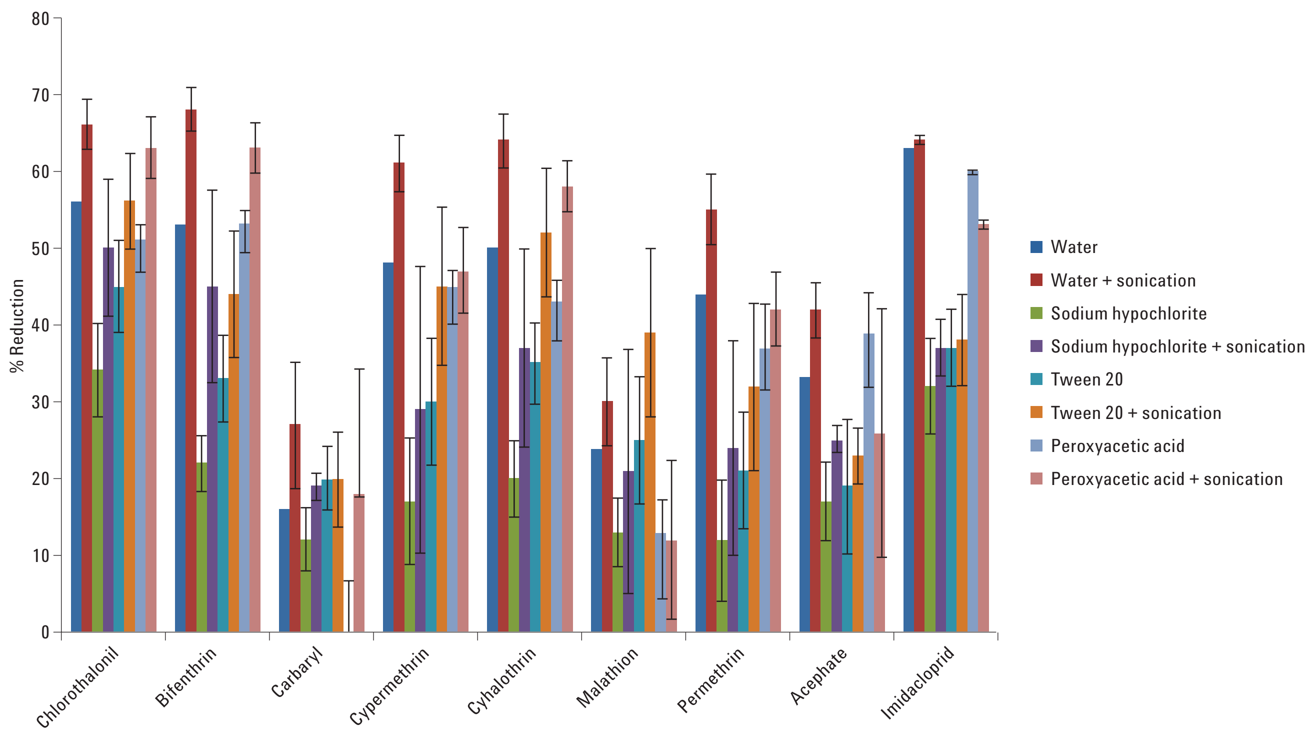

Figure 3. Effects of washing treatments and sonication on pesticide reduction in tomatoes. All bar graphs represent the averages ± standard deviations of three trials.

Effectiveness of commercial washing

Triplicate random samples of each 50 kg lot of each of several types of produce were taken before washing, homogenized, extracted, and analyzed for pesticides. Water washing of the same batch for 1 minute at room temperature in the flume and a subsequent repeat of the sampling, extraction, and analysis after washing resulted in approximately 40 to 90% reduction of the levels of the pesticides originally detected on the produce (Table 5).

References

1. F. Gale and J. C. Buzby. "Imports from China and food safety issues. U.S. Department of Agriculture, Economic Research Service, *Economic Information Bulletin* **52**, 1–37 (2009).
2. F. Al-Taher, Y. Chen, P. Wylie, J. Cappozzo. "Reduction of pesticide residues in tomatoes and other produce." *J. Food Prot*. **76**, 510-515 (2013).

Table 5. Effect of Water Washing on Pesticide Residue in Select Produce Items in the IFSH Biocontainment Pilot Plant

		Before washing		After washing			
Produce	**Pesticide detected**	**Mean conc.†**	**SD**	**Mean conc.†**	**SD***	**% Pesticide reduction**	**SD***
Bell peppers	Imidacloprid	0.087	0.016	0.025	0.003	71.20	3.05
	Chlorpyrifos	0.077	0.018	0.044	0.001	43.14	2.14
Red Delicious apples	Thiabendazole	0.863	0.032	0.42	0.025	50.97	7.20
	Diphenylamine	1.67	0.18	0.19	0.06	88.80	3.52
Fuji apples	Pyrimethanil	1.83	0.03	1.09	0.03	40.36	1.67
	Thiabendazole	0.035	0.005	0.018	0.001	49.04	1.67
	Diphenylamine	0.380	0.04	0.2	0.02	46.90	9.80
Peaches	Fludioxonil	0.90	0.09	0.25	0.019	71.63	2.13
Oranges	Imazalil	2.06	0.04	0.729	0.022	64.71	1.07
	Thiabendazole	1.05	0.043	0.23	0.014	78.05	1.31
Lemons	Imazalil	3.16	0.17	1.84	0.014	41.68	0.43

Experiment performed in triplicate

†Parts per million (ppm)

*Standard deviation

Conclusions

Washing with water and various chemical solutions for domestic and commercial appcations is necessary to decrease the pesticide residues from produce. Using LC/MS/MS and GC/MS to accurately assess the effectiveness of washing can help optimize both commercial and in-home washing procedures to minimize pesticide exposure from produce sources.

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