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Synergistic effect of washing and cooking on the removal of multi-classes of pesticides from various food samples

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ABSTRACT

The principal objective of this study was to investigate the effect of household processing, including washing and cooking on pesticide residue levels in various food samples. For this study, 31 food materials were selected and 44 pesticide residues were monitored using the “quick, easy, cheap, effective, rugged, and safe” QuEChERS extraction-based and liquid chromatography–tandem mass spectrometry (LC–MS/MS) methods. Eight pesticides, including acetamiprid, azoxystrobin, fenobucarb, fosthiazate, iprobenfos, lufenuron, propiconazole, and trifloxystrobin were detected in nine food samples including colored rice, glutinous rice (white rice), glutinous rice (unpolished rice), green chili, ginger, butterbur, chinamul, spinach, and perilla leaf. Results indicated that residue levels in positive food commodities declined substantially following washing and cooking. However, the residual level of acetamiprid increased in green chilis after boiling and stir-frying. In sum, household processing (washing and cooking) tended to substantially reduce or eliminate pesticide residues in a synergistic manner. These applications are necessary to protect consumers from the negative health effects of pesticide residues detected in food commodities.

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1. Introduction

Pesticide residues have been found in various raw and processed fruits and vegetables. Pesticides are widely used to protect crops from pest infestation; however, they may have a negative effect on the environment and the health of consumers (Keikotlhaile, Spanoghe, & Steurbaut, 2010). The stability of certain pesticides, and the fact that residues can remain in foods either processed or unprocessed, increases the human health hazard (Soliman, 2001). Public attention has focused on estimating the level of exposure at the point of consumption after processing (Ling et al., 2011). Cooking might alter and degrade the chemical structure of the analytes and might change the matrix in food as well (Park et al., 2011). Therefore, to evaluate the hazardous effects of pesticides

on human health, raw and cooked foodstuffs appear to be a more reasonable sample choice (Park et al., 2011).

Scientists and food processors have long been interested in the effect of commercial processing on persistence of pesticide residues in food. Elkins (1989) and Chin (1991) reported that several canning processes such as peeling, washing, juice extraction and heat processing reduce residue levels in US crops such as tomatoes, broccoli, green beans, and spinach. Additionally, Cabras and Angioni (2000) reported a reduction in organophosphates residues on grapes, wine, and their processing products. Moreover, Soliman (2001) investigated changes in the concentrations of pesticide residues on potato during washing and home preparation processes. Obviously, it is important to estimate the level of exposure at the point of consumption as well as after cooking (Ling et al., 2011).

A number of techniques have been developed to extract and clean-up pesticide residues from food products. These include liquid–liquid extraction (LLE) (Rissato, Galhiane, Knoll, & Apon, 2004; Torres, Picó, & Mañes, 1996), solid-phase extraction (SPE) (Adou, Bontoyan, & Sweeney, 2001; Juan-García, Picó, & Font, 2005;

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Obana, Okihashi, Akutsu, Kitagawa, & Hori, 2003), accelerated solvent extraction (ASE) (Adou et al., 2001), gel permeation chromatography (GPC) (Ueno et al., 2004), microwave-assisted extraction (MAE) (Barriada-Pereira et al., 2007), matrix solid-phase dispersion (MSPD) (Torres et al., 1996), and supercritical fluid extraction (SFE) (Rissato et al., 2004; Torres et al., 1996). However, most of these methods are rather complicated, consume a large volume of solvent, are labor-intensive, and are very expensive due primarily to the complexity of the analytes and matrices involved (Park et al., 2011). In 2003, Anastassiades, Lehotay, Stajnbaher, and Schenck (2003) described the “quick, easy, cheap, effective, rugged, and safe” (QuEChERS) method for multiclass, multiresidue analyses of pesticides from different food matrices with high water content. Recently, the QuEChERS method for multiple pesticides in fruits and vegetables has received the distinction as the Official Method of AOAC International (Lehotay, 2007).

In general, gas chromatographic (GC) separation and detection with selective and sensitive detectors such as electron-capture detection (ECD), nitrogen-phosphorus detection (NPD) and mass spectrometry (MS) are used for detecting pesticide residues in food samples (Adou et al., 2001; Lehotay, 2007; Sannino, Bandini, & Bolzni, 1999). However, liquid chromatography (LC) coupled to MS is a versatile technique than GC for analysis of compounds that are of low volatility, high polarity, and thermal lability in nature (Venkateswarlu, Rama Mohan, Kumar, & Seshiah, 2007). Additionally, LC–MS has been used to analyze pesticide residues in fruits and vegetable (Obana et al., 2003; Pous, Ruiz, Pico, & Font, 2001). More recently, usage of LC with tandem mass spectrometry (MS–MS) detection has been increasing for pesticide residue analysis (Lehotay, 2007; Venkateswarlu et al., 2007). This technique is more efficient and suitable for analysis of residual levels in food matrices than LC–MS (Hernandez, Pozo, Sancho, Bijisma, Barreda, & Pitarch, 2006).

In this study, we determined the residues of 44 pesticides in representative Korea food samples, before washing, after washing, and after cooking, using a QuEChERS extraction-based LC–MS/MS method. All tested food samples are included in the main menus of Koreans. The samples were obtained from a large market in Seoul and were prepared by cooking rice, boiling, stir-frying, and blanching according to the cooking practices established by KHIDI (KHIDI, 2007). The data collected using this analytical method were expected to prove useful for regulating the concentration of pesticide residues in food samples and also for estimating the human health hazards posed by these residues (Park et al., 2011).

2. Experimental

2.1. Chemicals and reagents

Standards of 44 pesticides were purchased from Dr Ehrenstorfer GmbH (Augsburg, Germany) and Sigma–Aldrich (St Louis, MO, USA) (Table 1). Anhydrous magnesium sulfate (MgSO_4) and acetic acid (HAc) were obtained from Junsei Chemical Co. Ltd. (Kyoto, Japan) and Daejung Chemicals & Materials (Siheung, Republic of Korea), respectively. Primary secondary amines (PSA) and C_{18} EC were purchased from Agilent Technologies (Santa Clara, CA, USA). Sodium acetate (NaAc) and acetonitrile (ACN) of analytical grade were purchased from E. Merck (Darmstadt, Germany).

2.2. Samples

2.2.1. Sample collection

Representative food samples were selected based on data obtained from the Ministry of Health and Welfare (MW 2001, 2005, 2007). Five different diet items were chosen, including rice and rice

products, soup or stew, green vegetables, stir-frying, and kimchi. These items mostly reflect dietary intake at every meal for a Korean. To select the reliable representative food materials, the top 80% of each diet item were aligned, and all food materials were organized in a descending order. Among the aligned food materials, the top 30 were selected for each diet item. With the exception of duplicated, processed, and food of animal-origin, 31 materials were selected as representative foods and used throughout the experimental protocol (Table 2). All food samples were purchased from a market basket survey in Seoul (main center assembling) where all agricultural commodities produced in the whole country are distributed to regional retailers.

2.2.2. Sample preparation and processing

To investigate the declining pattern in pesticide residue use; all food samples were treated before washing (BW), after washing (AW), and after processing (Table 2).

- (i) Before washing: the food samples were cleaned to remove soil and impurities, divided into small parts, homogenized, and then frozen at -21°C pending analysis.
- (ii) After washing: the food samples were washed with distilled water to remove soil and impurities, divided into small parts, homogenized, and then frozen at -21°C pending analysis.
- (iii) Processing: after washing, the food samples were cooked using representative standardized techniques, such as blanching, boiling, stir-frying, and cooking rice. One or two representative recipes were selected from the Database Management System of Traditional Foods. Samples were homogenized after cooking and then stored at -21°C pending analysis.

2.3. Analytical method

2.3.1. Extraction and sample preparation

Pesticide residues were extracted according to the QuEChERS method described previously by Anastassiades et al. (2003). Food samples were extracted with 15 mL acetonitrile into a 50 mL Teflon centrifuge tube with 150 μL of acetic acid (pH 5.5–6). Subsequently, 6 g anhydrous magnesium sulfate and 1.5 g sodium acetate were added, immediately shaken for 1 min, and then the extract was centrifuged at $966\times g$ and 4°C for 5 min. Six mL of the upper layer were transferred to a 15 mL Teflon centrifuge tube containing 900 mg anhydrous magnesium sulfate and 300 mg primary secondary amine (+150 mg C_{18} for fatty food samples). The centrifuge tube was shaken for 1 min followed by centrifugation for 5 min at $966\times g$ and 4°C . One mL from the upper layer was taken, and the samples were subjected to qualitative and quantitative analysis using LC–MS/MS (Scheme 1).

2.3.2. LC–MS/MS analysis

The LC–MS/MS analysis was performed using a Waters Alliance 2695 separation Module (Waters Corp., Milford, MA, USA) and a Micromass Quattro Micro triple quadrupole tandem mass spectrophotometer (Waters). The chromatographic separation was carried out on a SunFire C_{18} column (2.1 mm i.d. \times 150 mm, 3.5 μm particle size, Waters). The mobile phase consisted of distilled water with 0.1% formic acid (v/v, solvent A) and acetonitrile with 0.1% formic acid (v/v, solvent B). For analysis, A linear mobile phase gradient program was used as following: 5% solvent B (0 min), 75% solvent B at 1 min, 95% solvent B at 20 min, and 5% solvent B at 30 min. The flow rate, injection volume, and column temperature were set to 0.2 mL/min, 20 μL , and 35°C , respectively.

MS/MS detection was conducted in the positive electrospray ionization mode using multiple reaction monitoring with two mass

Table 1

Name, retention times (Rt), and multiple reaction monitoring (MRM) transitions of the tested pesticides for LC–MS/MS analysis.

No	Compound	Rt (min)	MW (m/z)	Precursor ion	Collision energy 1 (v)	Product ion 1	Collision energy 2 (v)	Product ion 2
1	Acetamiprid	8.94	222.1	223	18	125.9	30	90
2	Anilofos	13.44	367	368	14	198.9	14	171
3	Azoxystrobin	11.17	403.1	404	13	372	30	329
4	Boscalid	11.67	342.2	343	20	307	20	140
5	Cadusafos	14.25	270.3	271	13	158.9	13	131
6	Carbendazim	9.52	191.1	192	18	160	28	132
7	Carbofuran	10.07	221.2	222	13	165	13	123
8	Chlorfluazuron	17.07	540.0	540	20	382.8	20	158
9	Diethofencarb	11.35	267.3	268	20	180	20	226
10	Difenoconazole	13.16	405.1	406	23	251	23	337
11	Dimethomorph	10.69	387	388	22	301	22	165
12	Ethaboxam	9.66	320.1	321	25	183	25	154
13	Etoxazole	17.85	359.2	360	26	140.9	26	177
14	Fenobucarb	11.32	207	208	8	152	13	95
15	Fenpyroximate	17.71	421.3	422	15	366	33	138
16	Flufenoxuron	16.07	488.1	489	19	157.9	19	306
17	Fluquinconazole	11.83	375	376	20	348.9	20	307
18	Fosthiazate	10.23	283	284	12	227.9	12	104
19	Iprobenfos	12.27	288.1	289	10	204.9	19	91
20	Kresoxim-methyl	13.04	313.1	314	11	222	11	282
21	Lufenuron	15.17	509.9	511	20	157.9	20	328
22	Mepanipyrim	12.25	223.1	224	30	105.9	30	143
23	Metabenzthiazuron	10.09	221.1	222	16	165	32	150
24	Metaxyl	10.37	279.3	280	14	220	14	192
25	Methomyl	8.47	162.1	163	10	87.7	10	106
26	Methoxyfenozide	11.99	368.2	369	10	313	17	149
27	Napropamide	12.12	271.3	272	17	129	17	171
28	Oxadiazone	16.77	346.1	347	15	305	15	222
29	Pencycuron	14.09	328.3	329	22	124.9	22	218
30	Propamocarb	3.45	188.2	189	17	101.8	17	144
31	Propiconazole	12.78	341.1	342	25	158.8	25	69
32	Pyraclostrobin	13.68	387.1	388	13	194	25	163
33	Pyridaben	19.69	364.2	365	13	309	26	147
34	Pyrimethanil	10.69	199.1	200	33	81.8	33	107
35	Spiromesifen	19.29	370.2	371	20	273	25	255
36	Tebuconazole	11.99	307.1	308	20	69.7	20	125
37	Tebufenozide	12.66	352.2	353	8	297	8	133
38	Teflubenzuron	14.17	380.1	381	15	157.9	15	141
39	Thiacloprid	9.22	252	253	19	125.9	19	186
40	Thiamethoxam	8.47	291	292	12	211	22	181
41	Thifluzamide	12.53	528	529	30	488.9	30	168
42	Thiophanate-methyl	9.5	342.1	343	20	150.9	20	160
43	Tricyclazole	8.94	189.1	190	30	135.9	30	163
44	Trifloxystrobin	14.5	408.1	409	16	185.9	16	206

transitions. The product ion with the strongest intensity and the product ion with the lowest intensity were used as the quantifier and qualifier ions, respectively, for the two mass transitions (Table 1). Capillary voltage, source temperature, and desolvation temperature were set at 3.2 kV, 150 °C, and 350 °C, respectively, for MS. The cone and desolvation gas were ultra-pure nitrogen set at 30 and 500 L/h.

Validation studies were carried out with 44 pesticide standards. Six concentration levels ranging between the LOQ and 50× the LOQ were used for calibration curve, and the correlation coefficients (r^2) were >0.986. The limit of detection (LOD), determined with a signal-to-noise ratio (S/N) ≥ 3 , ranged between 0.0003 and 0.0010 mg/kg and the limit of quantification (LOQ), determined with a signal-to-noise ratio (S/N) ≥ 10 , ranged from 0.0078 to 0.0237 mg/kg.

3. Results and discussion

Kaushik, Satya, and Naik (2009) reported a reduction in pesticide residue levels in foodstuffs due to processing technique, with the exception of foodstuffs that underwent concentration, such as juicing of fruits and pressing or extraction of oil from vegetable seeds. The authors suggested that the effects of food processing on

pesticide residue levels may be influenced by the physical location of the pesticide residue as well as the physico-chemical properties of the pesticide such as solubility, volatility, hydrolytic rate constants, water-octanol partition coefficient, and thermal degradation. In the current study, the effect of household processing, including washing and cooking on the removal of multi-classes of pesticides from various food samples was studied.

3.1. Pesticide residues in food samples

Among the tested 44 pesticides in 31 representative food samples, eight pesticides were detected before washing and five were detected after washing and cooking (Fig. 1 and Table 3). The percent detected and quantified samples were 29.03 (9/31 treatment), 16.13 (5/31 treatment), and 13.16% (5/38 treatment), respectively. Acetamiprid, azoxystrobin, fenobucarb, fosthiazate, iprobenfos, lufenuron, propiconazole, and trifloxystrobin were detected in food samples, including colored rice, glutinous rice (white rice), glutinous rice (unpolished rice), green chilis, ginger, butterbur, chinamul, spinach, and perilla leaf (Table 3). The detected pesticides are commonly used in agricultural crops in the Republic of Korea (KFDA, 2012). Notably, the residue levels in positive food samples (before washing) were below the maximum

Table 2
Various food samples and processing.

No	Food samples	Processing	No	Food samples	Processing
1	White rice	Bw, ^a Aw, ^b Cooked rice	17	Onion	Bw, Aw, Boiling, Stir-frying
2	Unpolished rice	Bw, Aw, Cooked rice	18	Garlic	Bw, Aw, Boiling
3	Colored rice	Bw, Aw, Cooked rice	19	Carrot	Bw, Aw, Boiling, Stir-frying
4	Glutinous rice (white rice)	Bw, Aw, Cooked rice	20	Potato	Bw, Aw, Boiling, Stir-frying
5	Glutinous rice (unpolished rice)	Bw, Aw, Cooked rice	21	Ginger	Bw, Aw, Stir-frying
6	Glutinous foxtail millet	Bw, Aw, Cooked rice	22	Mushroom	Bw, Aw, Stir-frying
7	Barley	Bw, Aw, Cooked rice	23	Bracken	Bw, Aw, Boiling, Stir-frying
8	Sorghum	Bw, Aw, Cooked rice	24	Burdock	Bw, Aw, Boiling
9	Red pepper	Bw, Aw, Boiling	25	Butterbur	Bw, Aw, Boiling
10	Green chili	Bw, Aw, Boiling, Stir-frying	26	Chinamul	Bw, Aw, Blanching
11	Chinese cabbage	Bw, Aw, Boiling	27	Spinach	Bw, Aw, Blanching
12	Cabbage	Bw, Aw, Boiling	28	Perilla leaf	Bw, Aw, Boiling
13	Leek	Bw, Aw, Boiling	29	Bean sprout	Bw, Aw, Blanching, Boiling
14	Young pumpkin	Bw, Aw, Boiling, Stir-frying	30	Red bean	Bw, Aw, Boiling
15	Eggplant	Bw, Aw, stir-frying	31	Soybean	Bw, Aw, Boiling
16	Radish	Bw, Aw, Boiling			

^a Before washing.

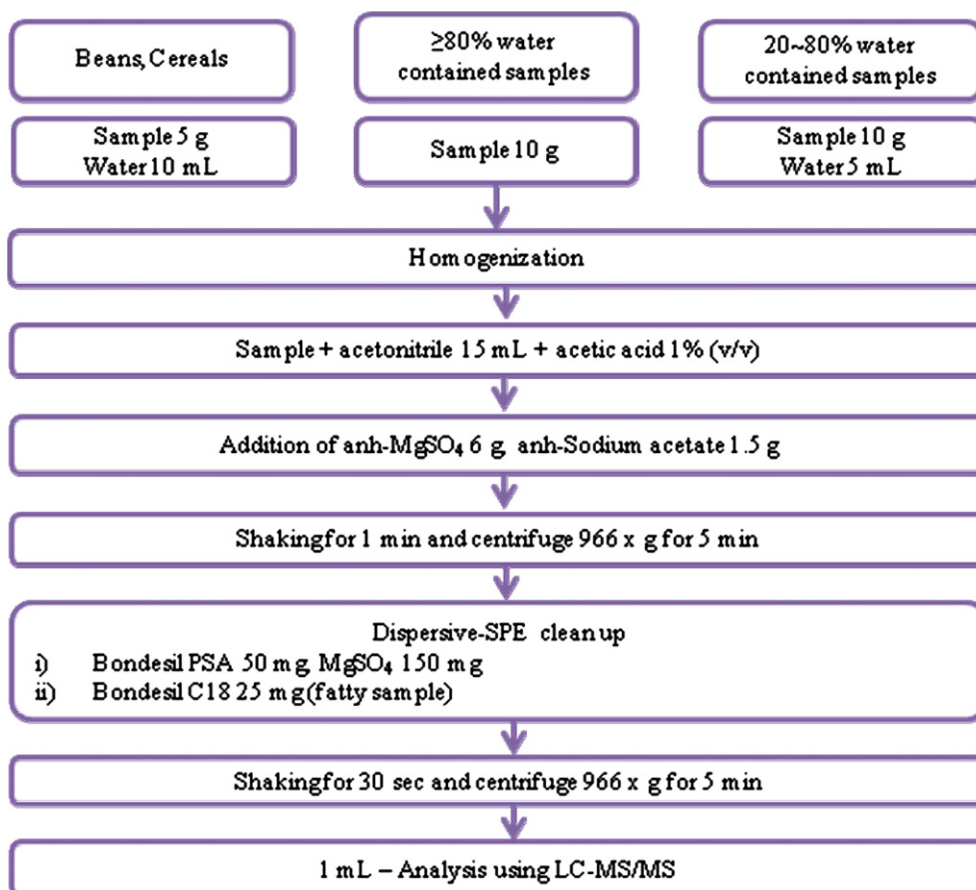
^b After washing.

residue limit (MRL) established by the Korea Food and Drug Administration (KFDA, 2011) (Table 3).

3.2. Effect of washing

In the present study, considerable reductions of 19–100% were observed during the food washing process (Fig. 2). The residues of propiconazole, trifloxystrobin, fosthiazate, lufenuron, and azoxystrobin were completely removed from the food samples. This

means that they washed off easily by removing the loosely attached pesticide at the surface. Chavarri, Herrera, and Arino (2005) found that chlorpyrifos, cypermethrin, and ethylenebisdithiocarbamates residue levels decreased with washing of asparagus by 24%, 35%, and 52%, respectively. We also suggest that there is no correlation between water solubility and the level of degradation, as the high and the low water soluble pesticides (iprobenfos and fenobucarb in colored rice, propiconazole and fenobucarb in glutinous rice) have a controversial pattern (Fig. 2). Our findings are consistent with



Scheme 1. Flowchart of the quick, easy, cheap, effective, rugged, and safe (QuEChERS) extraction method.

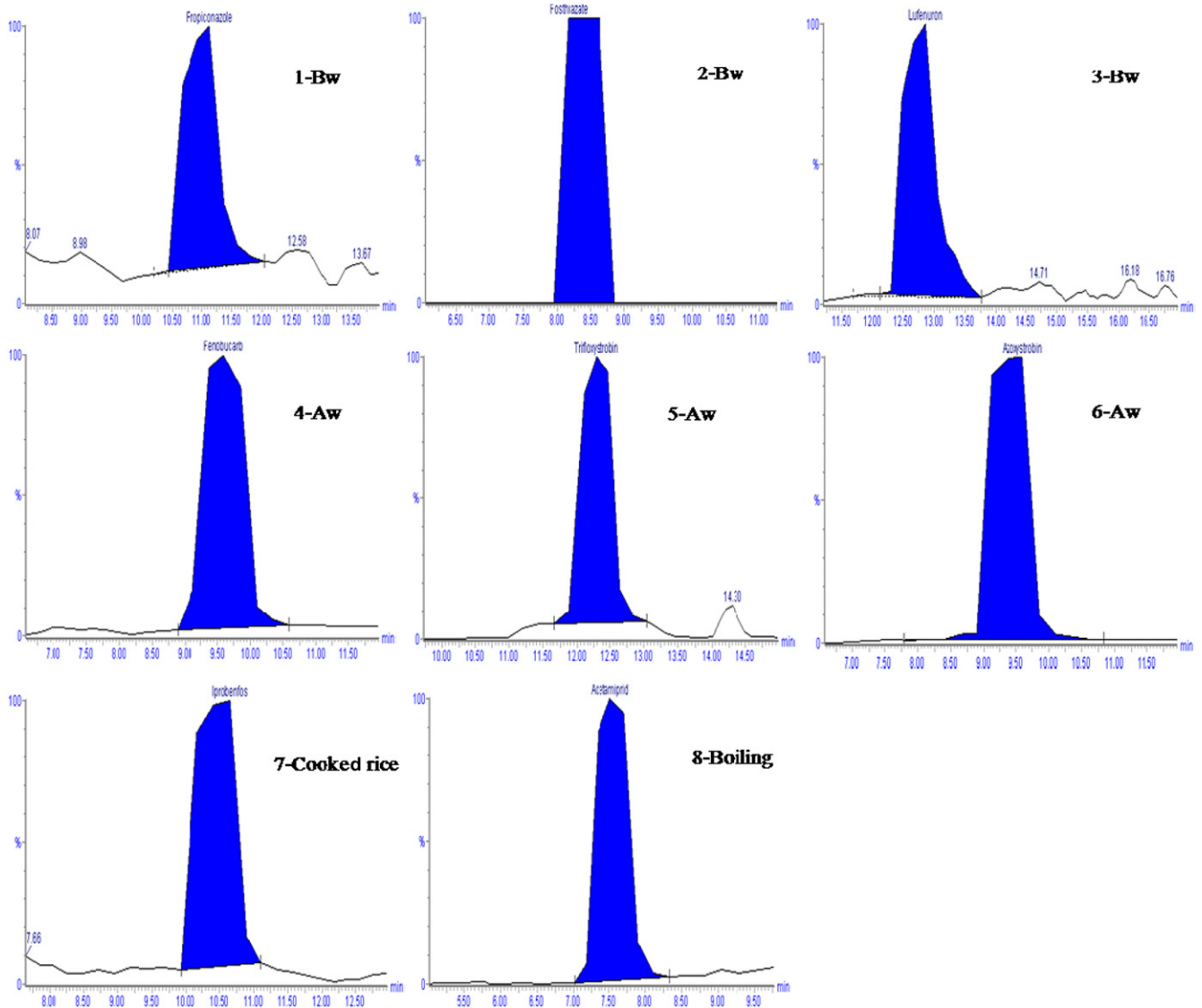


Fig. 1. Multiple reaction monitoring (MRM) of detected pesticides in food samples. 1-Propiconazole residue before washing in glutinous rice (white rice); 2-fosthiazate residue in ginger before washing; 3-lufenuron residue in butterbur before washing; 4-fenobucarb residue in glutinous rice (unpolished rice) after washing; 5-trifloxystrobin residue in green chilis after washing; 6-azoxystrobin residue in chinamul after boiling; 7-iprobenfos residue in colored rice after cooking; and 8-acetamidol residue in boiled green chilis.

Table 3
Residue levels during processing expressed as mg/kg (mean ± standard deviation of three replicates).

No	Food sample	Compound	Household processing						MRL
			Bw	Aw	Cooked rice	Boiling	Stir-frying	Blanching	
1	Colored rice	Iprobenfos	0.005 ± 0.0015	0.004 ± 0.0009	0.003 ± 0.0002	–	–	–	0.2
		Fenobucarb	0.008 ± 0.0030	0.005 ± 0.0017	N.D	–	–	–	0.5
2	Glutinous rice (white rice)	Fenobucarb	0.015 ± 0.0080	0.013 ± 0.0097	N.D	–	–	–	0.5
		Propiconazole	0.014 ± 0.0044	N.D	N.D	–	–	–	0.1
3	Glutinous rice (unpolished rice)	Fenobucarb	0.024 ± 0.0139	0.015 ± 0.0049	0.004 ± 0.0013	–	–	–	0.5
		Acetamidol	0.015 ± 0.0058	0.008 ± 0.0028	–	0.018 ± 0.0073	0.029 ± 0.0128	–	2.0
4	Green chili	Trifloxystrobin	0.064 ± 0.0409	0.051 ± 0.0307	–	0.023 ± 0.0108	0.030 ± 0.0157	–	2.0
		Trifloxystrobin	0.005 ± 0.0008	N.D	–	–	N.D	–	*
5	Ginger	Fosthiazate	0.003 ± 0.0001	N.D	–	–	N.D	–	*
		Lufenuron	0.010 ± 0.0051	N.D	–	N.D	–	–	*
6	Butterbur	Lufenuron	0.010 ± 0.0051	N.D	–	N.D	–	–	*
7	Chinamul	Azoxystrobin	0.306 ± 0.2196	0.111 ± 0.0700	–	–	–	0.100 ± 0.0678	20
8	Spinach	Azoxystrobin	0.004 ± 0.0012	N.D	–	–	–	N.D	20
9	Perilla leaf	Azoxystrobin	2.580 ± 1.7856	N.D	–	N.D	–	–	20

N.D., not detected.

*MRLs have not established yet by the KFDA (2011).

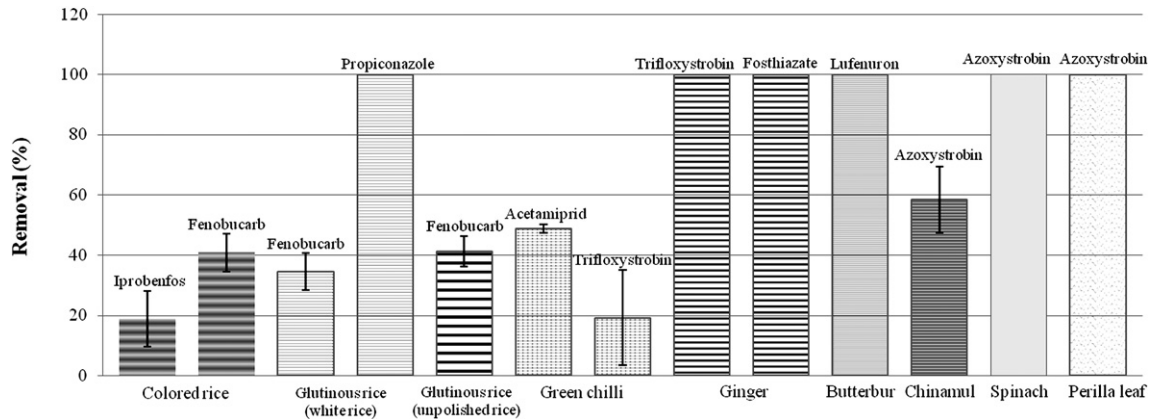


Fig. 2. Effect of washing on the percentage of removed pesticide from various food samples.

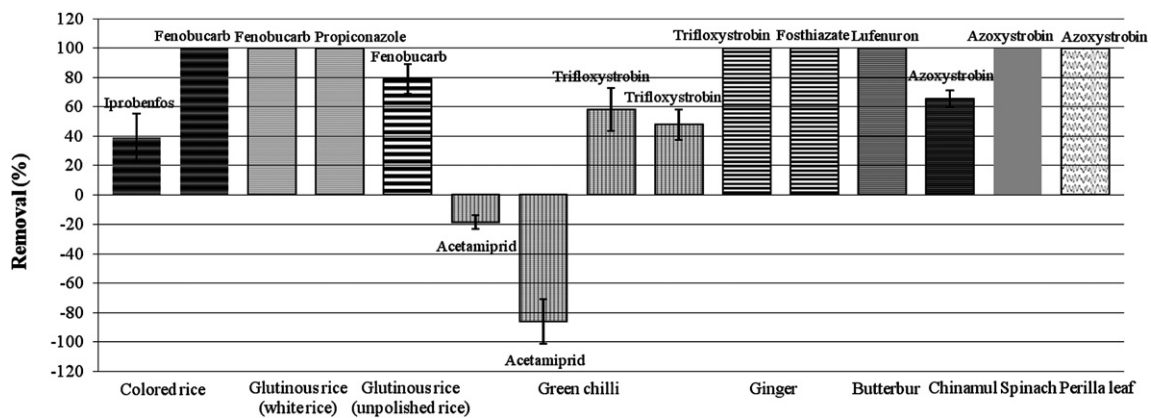


Fig. 3. Effect of washing and cooking on the percentage of removed pesticide from various food samples.

those reported by Cabras et al. (1997) who showed that decreases in olive pesticide residues after washing are not correlated with pesticide water solubility. Similarly, Cabras et al., 1998 and Walter, Arsenault, Pylypiw, and Mattina (2000) concluded that pesticide water solubility is not an important factor for removing pesticide residues. Atif Randhawa, Muhammad Anjum, Ahmed, and Saqib Randhawa, 2007 found that chlorpyrifos residue levels could be dissolved or physically dislodged from raw agricultural products by washing. Their findings could work for crops such as tomato and eggplant, as most pesticide residues exist on thick and smooth surfaces and can be easily removed. In contrast, pesticide residues can be translocated internally (e.g., cucumber and garlic sprouts); thus, washing would not reduce pesticide residues very easily (Krol, Arsenault, Pylypiw, & Mattina, 2000). Therefore, the status of the pesticide residue affects the removal of pesticide residues from vegetables (Ling et al., 2011). For example, removing pesticide residues by washing depends on the age of the chemicals (Guardia-Rubio, Ayora-Canada, & Ruiz-Medina, 2007). Those authors found that it was easier to wash off the pesticide residue from olives 1 day after spraying than after 1 week.

3.3. Effect of washing and cooking

The effect of washing and cooking on the residual pattern of pesticides is shown in Fig. 3. Except for acetamiprid in the green chili sample, the decline ranged from 39% to 100%. Open systems may result in water loss during heating due to evaporation, thereby concentrating the pesticide residues if they are not destroyed by heating, which could explain the high residues of acetamiprid after

cooking (Keikothaile et al., 2010). The boiling treatment exhibited a significant effect on pesticide residue degradation in the treated food samples. Complete pesticide removal (100%) was observed in colored rice, glutinous rice (white rice), ginger, butterbur, spinach, and perilla leaf by cooking, boiling, stir-frying, and blanching. The results for baking, boiling, canning, and juicing indicated a possibility of both an increase and a reduction. Processes that normally occur during cooking are volatilization, hydrolysis, and thermal breakdown (Abou-Arab, 1999; Balinova, Mladenova, & Shtereva, 2006). These results may be influenced by the physico-chemical properties of the pesticides. Abou-Arab (1999) found that home canning reduces organophosphates more than organochlorine pesticide residue levels.

4. Conclusion

In the present study, the effects of washing and cooking on the residual pattern of 44 pesticides in 31 food samples were evaluated. Eight pesticides were detected in nine food samples and most of the detected pesticide residues were below the MRLs. No correlations was observed between water solubility and the level of degradation. We recommend that a combination of washing and cooking should effectively remove most of the residues on/in food samples.

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