

Fate of Some Pesticides during Brining and Cooking of Chinese Cabbage and Spinach

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Abstract Chinese cabbage and spinach applied with eight pesticides at two different rates were subjected to brining, heat-cooking, and blanching to determine residue or transfer ratios of those pesticides. Residue ratio in discarded inedible portion varied significantly, 0-94%, depending on pesticides applied, vegetable type, and cooking processes. Average reduction ratios of residues during cooking process were 78, 46, 23, 12, 10, 9, 8, and 2% in dichlorvos, diazinon, chlorpyrifos, endosulfan, EPN, cypermethrin, deltamethrin, and fenvalerate, respectively.

Key words: Chinese cabbage, spinach, pesticide residue, cooking, residue reduction

Introduction

Chemical pesticides are essential for food production, and their use will be continued for the time-being. However, mis-use or over-use of pesticides will cause contamination of food resources and increase dietary risks of consumers. Therefore, governments worldwide have established legal standards for pesticide residues, that is, maximum residue limits (MRLs). Pesticide residues in raw food materials may disappear as time elapses, or by cooking and processing. It is, therefore, necessary to understand the reduction factors of pesticide residues from farm to table in order to validate the legal MRL setting and to minimize health risk (1).

Much attention has been given to vegetables, because they are the major food items vulnerable to pesticide uses. Several studies have reported on the reduction of pesticide residues in Chinese cabbage and other vegetables during processing (2-7). Other studies on the reduction covered the cooking of rice grains and other crops (8-11). Many reports have also been published on the reduction of pesticide residues in foods (12-15). However, data on the reduction factors are not systematic and cannot be applied globally likely due to the difference in dietary habits, as pointed out by Lee and Lee (16, 17). It is, therefore, necessary to accumulate data on reduction factors applicable to the establishment of global standards and risk assessment.

This study was undertaken to accumulate pesticide residue data on Chinese cabbage and spinach in connection with brining, blanching, and heat-cooking, which are the main processes for the preparation of major leafy vegetable foods consumed in Korea and other countries.

Materials and Methods

Pesticides used Pesticide formulations applied during the growth of Chinese cabbage and spinach were chlorpyrifos, diazinon, dichlorvos, EPN, endosulfan, deltamethrin,

fenvalerate, and cypermethrin of emulsified concentrations (EC) 20, 34, 50, 45, 35, 1, 5, and 5%, respectively. MRLs were established for all tested pesticides and crops whereas some of the pesticides were not registered for the tested crops in Korea.

Application of pesticides during the growth of vegetables Chinese cabbages were cultivated in a greenhouse from April 17 to June 27, 2002 in two separate field lots according to the good agricultural practices and subjected to pesticide application. Mixed pesticide solutions, including two or three pesticides diluted to half of the recommended concentrations to prevent pesticide hazard to the crops, were sprayed onto the cabbages once a week. The vegetables were harvested 3 days after ninth and eleventh pesticide applications to obtain low and high pesticide-level samples, respectively.

Spinach was cultivated in a greenhouse from March 14 to June 12, 2003 in two separate field lots according to the good agricultural practices and subjected to pesticide application. Pesticide solutions diluted to a quarter of the recommended concentrations were sprayed onto the spinach once a week. To obtain low and high pesticide-level samples, the vegetables were harvested on the 7th and 2nd day after three applications, respectively.

Brining and heat-cooking of Chinese cabbage A head of Chinese cabbage weighing about 2.5 kg was longitudinally cut into eight portions. Two portions (equivalent to 500 g) were then soaked in 2 L of 8% saline at 20°C, after 4 hr drained on a sieve for 30 min, washed by hands in 1 L tap water for 10 sec, and drained again for 30 min. The brine-soaking resulted in 428 g brined cabbage, 1,932 mL waste brine, and 1,010 mL water-washings.

One portion of the longitudinally-cut cabbage weighing about 300 g was further cut into 5-cm pieces and cooked for 20 min with two stirrings in 1.2 L covered boiling water pot. The pot was left standing for 30 min to cool down to 60°C. After draining for 30 min, 240 g cabbage solids and 1,013 mL cooked fluid were obtained.

Blanching and heat-cooking of spinach About 200 g

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spinach leaves were dipped into 1 L boiling water for 2 min and drained on a sieve for 30 min. The blanching resulted in 228 g spinach solids and 875 mL blanched fluids. For heat-cooking, about 200 g of spinach bunches were cut into four portions cross-wise and cooked in 1 L tap water for 15 min as with the Chinese cabbage. The heat-cooking resulted in 218 g spinach solids and 810 mL cooked fluid.

Analysis of pesticide residues The raw vegetable samples were cleaned by removing roots and spoiled leaves, and subjected to residue analysis. Solid and liquid samples obtained in duplicate runs were extracted using appropriate solvent systems and subjected to residue analyses in triplicates according to the Official Analytical Methods of Pesticides (18).

Organic solvents, reagents, and standard pesticides were of first grade purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). Adsorbants for column chromatography were active carbon (Daroco® G-60, Sigma-Aldrich), cellulose (Avicel® PH-101, Fluka, Switzerland), and florasil (60-100 mesh, Sigma-Aldrich). Instruments employed were heating plate (Barnstead Thermolyne, USA), electrical aspirator (Jeio Tech, Korea), homogenizer (Polystron®, Switzerland), rotary evaporator (Buchi, Switzerland), and nitrogen concentrator (TurboVap® LV, Zymark, USA). Gas chromatograph (HP 6890 plus, USA) equipped with an HP-5 column (30 m × 0.32 mm × 0.25 µm) was used to detect organophosphorus pesticides with a flame photometric detector, and organochlorine and synthetic pyrethroids with an electron capture detector.

Recovery test Samples for residue analysis were spiked with standard pesticides at 0.5-2.0 mg/kg of vegetable or liquid samples and subjected to routine analysis in triplicates. The recovery was above 78% in all cases and judged to be satisfactory so was not considered in the calculation of residue concentration (Table 1).

Results and Discussion

Natural contamination of pesticides in vegetables

Naturally contaminated food materials are necessary to investigate the pesticide reduction factors; however, obtaining enough samples with sufficiently high concentration of residues is very difficult. In this study, pesticides were applied during cultivation of Chinese cabbage and spinach

under good agricultural practices to achieve residues at levels of legal MRLs and concentrations appropriate for analysis. The crops were sprayed at two application rates. The concentrations of eight pesticide residues in the vegetables were analyzed and compared with legal MRLs (Table 2).

The residue levels in harvested crops vary depending on the size of crops, application method, and environmental conditions. Within the crop subjected to the same pesticide application, variability (ratio of maximum to median values) of the residue levels in field trials is known to reach 3.8-fold (19). Legal MRLs established by Codex are determined based on the highest residue level obtained from several field trials through rounding-up in accordance with the latitude concept. On the other hand, the Korea MRLs are established by applying the regulatory margin of 4.8-fold to the highest value of the field trial data. It is, therefore, very difficult to predict the residue levels of crops in relation to the application rate or frequency of pesticides, which are based on the safe use guidance of pesticides in Korea (20). In this study, by regulating the application rate, frequency, and pre-harvest interval, crop samples of low or high level of pesticide residues, which neighbor the legal MRLs and are appropriate for studies on pesticide reduction during cooking processes of food materials, could be obtained. Out of two different samples, those with residue concentrations nearer to the Korea MRLs were chosen and subjected to reduction studies. In general, employment of naturally contaminated samples rather than artificially spiked samples is recommended for reduction studies of chemical residues.

Fate of pesticides in brining process of Chinese cabbage

Chinese cabbage is an important raw commodity of Kimchi in Korea. Crop samples of natural contamination with residues near MRLs for individual pesticides were subjected to brining process of Chinese cabbage which is an essential step for the preparation of Kimchi and Korean-style cabbage salads.

Residue concentrations of raw and brined cabbages, and brine obtained after soaking and washings of brined cabbages were determined. Weights or volumes of the samples were measured, and the total amount of residues in analyzed samples were calculated. The residue ratios of samples were calculated as the total amount of residues on the basis of raw cabbage as 100, and difference from 100% was regarded as the loss during the brining process (Table 3).

Table 1. Recovery of pesticide residues added into analysis samples¹⁾

Pesticide	Recovery (%)					
	Raw cabbage	Cooking water, cabbage	Raw spinach	Cooked spinach	Cooking water, spinach	
Chlorpyrifos	94	109	100	95	96	
Cypermethrin	94	99	90	110	102	
Deltamethrin	107	98	87	114	98	
Diazinon	-	-	100	100	101	
Dichlorvos	86	90	78	102	99	
Endosulfan	84	94	92	96	97	
EPN	93	113	89	91	95	
Fenvalerate	96	107	115	115	115	

¹⁾Initial pesticide concentrations were at a level in the range of 0.5-2.0 mg/kg. Values are mean of triplicate analyses.

Table 2. Application and residue levels of pesticides in test vegetables

Pesticide	Concern level	Chinese cabbage			Spinach		
		Appl. rate (kg ai/ha)	Residue concn (mg/kg)	Korea MRL (mg/kg)	Appl. rate (kg ai/ha)	Residue concn (mg/kg)	Korea MRL (mg/kg)
Chlorpyrifos	Low	0.98	0.126	1.0	0.51	3.45 ¹⁾	
	High	1.19	0.561 ¹⁾		0.72	10.10	0.01
Cypermethrin	Low	0.24	0.176	5.0	0.13	1.32	
	High	0.30	0.195 ¹⁾		0.17	2.61 ¹⁾	2.0
Deltamethrin	Low	0.48	0.058	0.5	0.026	0.49	
	High	0.60	0.074 ¹⁾		0.07	0.59 ¹⁾	0.5
Diazinon	Low	1.6	0.125 ¹⁾	0.1	0.87	2.79 ¹⁾	
	High	2.0	0.613		1.2	7.66	0.1
Dichlorvos	Low	2.4	0.062	0.3	1.3	0.12	
	High	3.0	0.284 ¹⁾		3.0	0.94 ¹⁾	0.3
Endosulfan	Low	1.7	0.148	2.0	0.92	0.43 ¹⁾	
	High	2.1	0.249 ¹⁾		1.2	17.0	1.0
E P N	Low	2.2	0.138 ¹⁾	0.1	1.7	6.82 ¹⁾	
	High	2.7	1.611		1.6	11.0	0.2
Fenvalerate	Low	0.24	0.298	1.0	0.13	0.70 ¹⁾	
	High	0.30	0.358 ¹⁾		0.07	4.44	0.5

¹⁾Samples having residues nearer to legal MRLs were subjected to cooking test.

Table 3. Residue ratio in brining and washing of Chinese cabbage

Pesticide	Residue concn (mg/kg)	Residue ratio (%)				Loss in brining (%)
		Raw cabbage	Brined cabbage	Soaking brine	Washings after brining	
Chlorpyrifos	0.56	100	95	2	1	2
Cypermethrin	0.20	100	112	10	7	0
Deltamethrin	0.07	100	112	5	2	0
Diazinon	0.13	100	74	2	3	21
Dichlorvos	0.28	100	55	16	7	22
Endosulfan	0.25	100	98	1	1	0
E P N	0.14	100	106	12	1	0
Fenvalerate	0.36	100	105	7	4	0

The residue ratios of chlorpyrifos, cypermethrin, deltamethrin, endosulfan, EPN, and fenvalerate in the brined cabbage were almost 100%, whereas those of diazinon and dichlorvos were 60-70%. The transfer ratio of pesticides into the soaking brine or washings after brining was around 10% maximum in all pesticides. The loss of pesticides during the brining process was around 20% in diazinon and dichlorvos, whereas the loss was not observed in other pesticides, which could be due to the relatively high water solubility and degradability of these two pesticides.

Results of our study suggest that the removal ratios of residues are different depending on the pesticide constituents. Removal ratios during the brining processes of Chinese cabbage were about 30-40% in diazinon and dichlorvos, whereas about 10% in chlorpyrifos, cypermethrin, deltamethrin, endosulfan, EPN, and fenvalerate, because only brined cabbages are consumed, while soaking brine or washings are discarded. The residue-containing brined cabbages are directly taken up by consumers in the form of Kimchi, a fact which should be taken into consideration in the safety assurance of fermented Kimchi.

Reports on the fate of pesticides in Kimchi fermentation are quite limited. Lee & Lee (3) reported that 30% of EPN residues are removed during brining, whereas Han *et al.* (5) reported that 86-100% of dichlorvos, diazinon, and methidathion were removed; however, comparison of these reports with the present data is difficult, because the results greatly vary depending on the residue level, contamination phase (natural or artificial), freshness of cabbage, and brining conditions.

The cabbage samples used in this experiment were sprayed with water once daily during cultivation to simulate the natural rainfall or watering, and were harvested 3 days after the last pesticide application to maintain a safe preharvest interval. The residues on the surface were weathered naturally, resulting in the low removal ratio as compared with other reports, thereby assuring reliability of data, because the pesticide contamination was simulated naturally and at reasonably high level.

Heat-cooking of Chinese cabbage Thin soup made of Chinese cabbage and seasonings is widely consumed by Koreans. The naturally contaminated cabbage was cooked

according to the house-hold practice, and the residue concentrations of the solid cabbage and fluid were analyzed, and the residue ratios were calculated as mentioned in the previous section (Table 4).

The residue ratios of cypermethrin, deltamethrin, and fenvalerate in the heat-cooked cabbage were above 100%, the transfer ratio into the fluid or loss during cooking being zero, suggesting that these pesticides do not decompose or vaporize during cooking. The residue ratios of diazinon and dichlorvos in the cooked cabbage and the fluid were 10-20%, the loss during cooking being 80-90%. The removal ratios of these two pesticides during heat-cooking were the highest among the tested pesticides, and higher than the results obtained with brining. The fate of pesticides during heat-cooking is thus dependent on the transfer extent into the fluid (water-solubility), transfer acceleration by higher temperature, vaporization tendency, and heat stability of the chemicals. Diazinon and dichlorvos are more water-soluble than other pesticides, and dichlorvos has a high vapor pressure; thus 20-min cooking would be sufficient to remove most of the pesticides. However, chlorpyrifos, endosulfan, and EPN showed different results, likely due to their physico-chemical properties.

Lee & Lee (3) reported that the removal ratio of EPN from artificially contaminated Chinese cabbage was 30% during washing-cooking, while only 5% through heat-cooking. Initial residue concentration obtained through the previous study was very high at 53 mg/kg, whereas, in the present study, was 0.14 mg/kg, near the legal MRL of 0.1 mg/kg. Park *et al.* (4) reported that the residue ratios of diazinon, chlorpyrifos and EPN were 9, 5, and 42%, respectively, in 10-min cooked Chinese cabbage, the removal of diazinon and chlorpyrifos being much higher ratio than observed in the present study.

Results of this study on naturally contaminated Chinese cabbage showed the residue/transfer ratios of pesticides during heat-cooking as follows. Diazinon and dichlorvos with high transfer ratios gave distribution of 10% in cooked cabbage, 10% in cooked fluid, and 80% by disappearance. The synthetic pyrethroids, cypermethrin, deltamethrin, and fenvalerate, with negligible transfer ratios showed 100% residue in cooked cabbage. Endosulfan, a persistent organochlorine pesticide, showed a distribution of 70 and 10%, and EPN 80 and 20% in cooked cabbage and cooked fluid, respectively, while chlorpyrifos 30% in cooked cabbage and 70% loss in cooking. Thus, caution should be taken not to disregard the residue in the cooking fluid in

determining the removal ratio, because it is also consumed.

Blanching of spinach Spinach is an important raw commodity in the preparation of side dishes such as vegetable soup and salad in Korea. Crop samples naturally contaminated with pesticides were subjected to blanching process, which is an essential step in the preparation of Korean-style seasoned salad, *namulmuchim*. The naturally contaminated spinach was blanched according to the house-hold practices, and the residue concentrations of the solid spinach and blanching fluid were analyzed, and the residue ratios calculated as mentioned in the previous section (Table 5).

Residue ratios of pesticides differed depending on the kind of pesticides. Chlorpyrifos and fenvalerate remained at 100% in the blanched spinach, probably due to their stability. On the other hand, the residue ratios of cypermethrin, deltamethrin, diazinon, endosulfan, and EPN in blanched spinach were 80-90%, whereas only 6% of dichlorvos remained. The partitions of pesticides into blanched spinach, blanching water, and blanching loss were 75, 19, and 6% in diazinon, and 6, 22, and 72% in dichlorvos, respectively. The higher ratios in blanching water and blanching loss in the case of dichlorvos as compared with diazinon could be due to its lower water-solubility and vapor pressure. The partitions were 90, 3, and 7% in endosulfan, and 82, 4, and 14% in EPN, suggesting water solubility and vapor pressure differed from those of the synthetic pyrethroids.

Farrow *et al.* (21) reported that 60% of diazinon was removed during blanching of spinach. The present study showed a removal of only 25% of diazinon, which might be due to the omission of washing process after blanching. The present study for blanching of spinach employed only 2-min heating, deleting the after-washing process with cold water. Thus, caution must be taken when applying these data for commercial blanching.

Heat-cooking of spinach The naturally contaminated spinach used in the blanching test was cooked by heating according to the house-hold practice, and the residue concentrations of the solid spinach and cooking fluid were analyzed (Table 6).

Residue ratios of cooked spinach appeared to be similar to those of the blanched spinach except in cases of chlorpyrifos and diazinon. This is understandable from the fact that the regular heat-cooking requires a longer

Table 4. Residue ratio in cooking of Chinese cabbage

Pesticide	Residue concn (mg/kg)	Residue ratio (%)			Loss in cooking (%)
		Raw cabbage	Cooked cabbage	Cooking water	
Chlorpyrifos	0.56	100	27	2	71
Cypermethrin	0.20	100	119	1	0
Deltamethrin	0.07	100	112	0	0
Diazinon	0.13	100	11	9	80
Dichlorvos	0.28	100	3	6	91
Endosulfan	0.25	100	74	9	17
E P N	0.14	100	107	25	0
Fenvalerate	0.36	100	119	0	0

Table 5. Residue ratio in blanching of spinach

Pesticide	Residue concn (mg/kg)	Residue ratio (%)			Loss in blanching (%)
		Raw spinach	Blanched spinach	Blanching water	
Chlorpyrifos	3.5	100	108	2	0
Cypermethrin	2.6	100	87	0	13
Deltamethrin	0.6	100	83	0	17
Diazinon	2.8	100	75	19	6
Dichlorvos	0.9	100	6	22	72
Endosulfan	0.4	100	90	3	7
E P N	6.8	100	82	4	14
Fenvalerate	0.7	100	108	0	0

Table 6. Residue ratio in heat-cooking of spinach

Pesticide	Residue concn (mg/kg)	Residue ratio (%)			Loss in cooking (%)
		Raw spinach	Cooked spinach	Cooking water	
Chlorpyrifos	3.5	100	84	1	15
Cypermethrin	2.6	100	92	0	8
Deltamethrin	0.6	100	90	0	10
Diazinon	2.8	100	42	7	51
Dichlorvos	0.9	100	5	14	81
Endosulfan	0.4	100	80	2	18
EPN	6.8	100	83	6	11
Fenvalerate	0.7	100	110	0	0

Table 7. Disappearance (%) of pesticide residues in cooking of two vegetables

Pesticides	Chinese cabbage		Spinach		Average removal
	Brining-washing	Cooking	Blanching	Cooking	
Chlorpyrifos	5	71	2	15	23
Cypermethrin	13	0	13	8	9
Deltamethrin	6	0	17	10	8
Diazinon	26	80	25	51	46
Dichlorvos	45	91	94	81	78
Endosulfan	2	17	10	18	12
EPN	11	0	18	11	10
Fenvalerate	9	0	0	0	2

Data are % of residues which are discarded in cooking processes, out of total in the raw materials.

Edible and inedible portions were regarded as follows. An edible portion in brining of Chinese cabbage is brined cabbage; its inedible portions are soaking brine, washings after brining, loss in brining. Edible portions in cooking of Chinese cabbage are cooked cabbage and cooking water; its inedible portion is loss in cooking. An edible portion in blanching of spinach is blanched spinach; its inedible portions are blanching water and loss in blanching. Edible portions in cooking of spinach are cooked spinach and cooking water; its inedible portion is loss in cooking.

Residues in edible and inedible portions were regarded as 100%.

heating-time than blanching.

The residue ratios of pesticides in heat-cooked spinach were: 100% in fenvalerate, the loss being negligible; 80-90% in chlorpyrifos, cypermethrin, deltamethrin, endosulfan, and EPN; 40% in diazinon; and 5% in dichlorvos.

Table 7 shows the % disappearance of pesticide residues in the raw vegetables during the cooking processes. The residue ratios in the discarded inedible portion varied significantly, 0-94%, depending on the pesticides applied, vegetable type, and cooking processes. The average reductions of residues in cooking processes were 78, 46, 23, 12, 10, 9, 8, and 2% in dichlorvos, diazinon, chlorpyrifos, endosulfan, EPN, cypermethrin, deltamethrin, and fenvalerate, respectively. Residues in the edible portions which will be taken up by consumers, were in the range of 6-100% of initially present amounts depending on the kinds of pesticides

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