Technical Report

Effects of separate analyses of pulp and peel on pesticide residues in watermelons and muskmelons

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To evaluate the effects of sample-size reduction and separate analyses of fruit pulp and peel, residual levels of 3 pesticides in watermelons and muskmelons were determined. The pesticide residue levels in 2 types of size-reduced samples were compared. In one size-reduced sample, the pesticide residues in the pulp with the peel were analyzed (A). In the other size-reduced sample, residues in the pulp and peel were analyzed separately. Afterward, the pesticide residue levels were calculated for the whole fruit (B). The majority of the pesticide residues were found to be present in the peel. The mean percent differences ($A/B \pm$ standard deviations [SD]) of pesticide residue levels in the watermelon and muskmelon samples were $91 \pm 15\%$ and $83 \pm 11\%$, respectively. No significant effect of sample-size reduction and separate analyses of pulp and peel were found in determining the pesticide residues in large-sized fruiting vegetables. © Pesticide Science Society of Japan

Keywords: sample-size reduction, separate analysis, watermelon, muskmelon, pesticide residue.

Electronic supplementary materials: The online version of this article contains supplementary materials (Supplemental Tables S1 and S2), which are available at <http://www.jstage.jst.go.jp/browse/jpestics/>.

Introduction

International harmonization of sample processing for pesticide residue analysis is one of the most important tasks for international trade and effective utilization of residue data. Codex maximum residue limits (MRLs) are typically stated in terms of specific, whole, raw agricultural commodities as they move through international trade. According to the Food and Agriculture Organization of the United Nations (FAO) manual in 2009,¹⁾ whole samples of watermelon and muskmelon should be analyzed after the stems are removed. The Japanese test guide-

lines for pesticide residue studies in crops were recently refined to recommend separate analyses of the pulp and peel from watermelons and muskmelons.²⁾ This modification based on the international method allows the calculation of residue levels in the whole fruit based on the levels in the pulp and peel portions.

The current Japanese MRLs for watermelons and muskmelons are expressed as the levels in the edible pulp portion without the peel.³⁾ In such a situation, the compatibility between the pesticide residues from direct analysis of the whole commodity and the calculated pesticide residues from separate analyses of the pulp and peel portions gives valuable information for estimating the effect of the international harmonization. However, little data is available regarding Japanese common agricultural procedures.

Moreover, the Codex recommendation for sampling pesticide residues for compliance with MRLs specifies that when laboratory samples are prepared directly from a lot or bulk sample, it represents the conceptual sum of the laboratory samples; further, analysts should ensure accurate preparation of the analytical samples for residue analysis.⁴⁾ Furthermore, according to FAO guidelines on pesticide residue trials, because it is unacceptable to cut or divide sample units, the problem is greatest with large-sized fruits and wrapped leafy vegetables such as melons or cabbages.⁵⁾ In practice, without reducing the sample size of large-sized fruits and wrapped leafy vegetables, the homogenization process that uses a conventional-sized blender is more difficult. For example, the total weight of 6 watermelon or muskmelon samples shipped to our laboratory was often more than 10 kg. In such cases, the homogenization process when using a blender with a less than 1-L capacity would be time consuming; therefore, a large blender would be required for single-step homogenization. Reducing the size of a large sample, such as by cutting or dividing the sample units to make a more suitable amount for the homogenization process, is convenient for analytical laboratories. Nevertheless, only limited information is available regarding the effect of sample-size reduction in estimating the uncertainty of the results of pesticide residue analysis, especially for field samples prepared in accordance with Japanese common agricultural practices.^{6,7)}

On the basis of the above-mentioned requirements, this study was undertaken to estimate the effects of sample-size reduction and separate analyses of pulp and peel portions on determining pesticide residues in watermelons and muskmelons, which were selected as representatives of large-sized fruiting vegetables in Japan. Both fruits were obtained from 2 test fields in Japan that had been sprayed using 3 pesticides that widely differed in their respective physicochemical properties so that an accurate determination of the variations in pesticide residue analyses was possible. Acetamiprid, a neonicotinoid insecticide, has a relatively low $\log P_{OW}$ of 0.80 and relatively high water solu-

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bility of 4250 mg/L.⁸⁾ Pyridaben, an acaricide, has a relatively high log P_{OW} of 6.37 and low water solubility of 0.012 mg/L.⁸⁾ Penthiopyrad, a pyrazolecarboxamide insecticide, has physico-chemical properties that are an intermediate of the other 2 pesticides, with a log P_{OW} of 3.20 and water solubility of 7.53 mg/L.⁸⁾ This investigations provided valuable information on the effects of sample-size reduction and separate analyses of pulp and peel portions on determining pesticide residues in large-sized fruiting vegetables.

Materials and Methods

1. Field experiments

In accordance with Japanese Guidelines,⁹⁾ the field experiments on watermelons and muskmelons were conducted in greenhouses of the Japan Plant Protection Association at Ibaraki and Miyazaki. Field experiment information such as field locations, variety of crops, measuring sample weights, and planting dates is summarized in Table 1. The pesticides were mixed in a tank, and spraying was performed using backpack sprayers connected to cone nozzles. After diluting Sanmite flowable (pyridaben; Nissan Chemical Industries, Ltd.) of 20% active ingredient (a.i.) by a 1:1000 dilution and mixing it with Mospilan water soluble granules 20% a.i. (acetamiprid; Nippon Soda Co., Ltd.) and Affet flowable (penthiopyrad; Mitsui Chemicals Agro, Inc.) of 20% a.i. by a 1:2000 dilution, the sprays were applied three times at approximately 7-day intervals. The pesticides were applied at 220–280 L/10 a. Samples were collected 1, 3, and 7 days after the final treatment. Each sample consisted of 6 fruits randomly taken from the test fields. The samples were stored at 3°C and shipped to our institute *via* a commercial shipping service.

Parts of the pesticide applications of this study were optimized for examination purposes, which were not in accordance with Japan's common agricultural practices. The Mospilan water-soluble granules were applied at rates higher than the maximum rates for muskmelons as indicated on the label (1:8000 dilution). The Sanmite flowable was applied more often than the maximum numbers of application (twice for watermelons and

muskmelons) as indicated on the label, whereas Affet flowable is not registered for use in watermelons.

2. Sample preparation

After measuring the sample weight, the stems of the watermelon and muskmelon samples were removed. A diagram illustrating the sample-size reduction procedure of the watermelon and muskmelon portions, which were used for analysis after removal of the stem, is shown in Fig. 1. Each unit sample was cut vertically into 6 pieces, and a pair of 2 opposite pieces from each sample was chosen as size-reduced sample A. The other pieces were divided into pulp and peel portions, collected separately, and used as size-reduced sample B. The peel and pulp sample portions were separated, which appear as white–red for watermelons and dark–pale green for muskmelons. Each prepared sample was maintained frozen at –20°C until analysis and was individually homogenized using a blender (BLIXER-5Plus or R45, Robot Coupe, MS, USA) immediately before analysis.

3. Residue analysis

Residue analysis methods were optimized for rapid analysis of the 3 pesticides in the homogenized samples of pulp with/without the peel and in the peel portion of the watermelons and muskmelons.

3.1 Chemicals and reagents

Standards for acetamiprid (purity 100%), penthiopyrad (purity 99.9%), and pyridaben (purity 100%) were purchased from Wako Pure Chemical Industries, Ltd. and Kanto Chemical (Japan). Pesticide analysis-grade acetone, acetonitrile, and toluene; HPLC-grade tetrahydrofuran; LC-MS-grade acetonitrile; and analytical-grade ammonium formate were purchased from Wako Pure Chemical Industries, Ltd.. Purified water used for the experiments was obtained using a Milli-Q system (Millipore, MA, USA).

Standard stock solutions (200 mg/L) of each pesticide were separately prepared using acetonitrile. Portions of each stock solution were diluted with an acetonitrile/water solution to make standard solutions in the range of 0.1–4 µg/L to prepare a cali-

Table 1. Data obtained from the field experiments

Field location	Variety	Sample weight, min–max ^{a)}	Planting date ^{b)}
Watermelon			
Ibaraki	Benikodama V	1.47–1.57 kg (80:20–82:18)	Sep 27 to Oct 18, 2010
Miyazaki	Hitorijime HM	1.22–1.39 kg (75:25–77:23)	Nov 14 to Dec 5, 2010
Muskmelon			
Ibaraki	Quincy	1.52–1.70 kg (88:12–92:8)	Oct 17 to Nov 7, 2010
Miyazaki	Earls seine aki-fuyu II	1.67–1.83 kg (91:9–92:8)	Nov 28 to Dec 19, 2010

^{a)}Mean sample weight from 6 units in each sampling point and weight ratio of pulp and peel portions in size-reduced sample B (pulp:peel, in parentheses). ^{b)}From the first pesticide application date to the sampling date.

Fig. 1. Sample-size reduction procedure of the watermelon and muskmelon samples that were used for analyses after removal of the stems.

bration curve.

3.2 Extraction

Each homogenized sample was analyzed in duplicate as follows: a portion (20 g when using pulp with/without peel and 10 g when using peel samples) of the sample was weighed into an Erlenmeyer flask and extracted using 100 mL of acetonitrile by shaking for 30 min using a reciprocal shaker. The mixture was then filtered by vacuum suction, and the residual cake was washed using 50 mL of acetonitrile. The filtrates were combined and made up to 200 mL by using acetonitrile.

3.3 Cleanup

A 2 mL aliquot of the acetonitrile extract (as 0.2 g of pulp with/without peel or 0.1 g of peel samples) was cleaned using solid-phase extraction with a styrene-divinylbenzene cartridge (1 g/6 mL, InertSep PLS-2; GL Sciences, Japan; conditioned with acetonitrile and water). Eight-mL of water was added to the acetonitrile extract, and the mixture was loaded onto the cartridge. The cartridge was washed using 10 mL of acetonitrile:water (1:4, v/v), and 15 mL tetrahydrofuran was passed through the cartridge; the eluate was evaporated to dryness using a rotary evaporator and nitrogen blowdown apparatus.

The residue was dissolved and diluted using 10 mL of acetonitrile:toluene (3:1, v/v) and loaded onto a graphite carbon black cartridge (500 mg/6 mL, Supelclean ENVI-Carb; Sigma-Aldrich, MO, USA; conditioned with the acetonitrile:toluene mixture). An additional 20 mL of the acetonitrile:toluene mixture was passed twice through the cartridge. All of the eluate was collected in a round-bottom flask and evaporated to dryness using a rotary evaporator and nitrogen blowdown apparatus, after which the residue was dissolved and diluted using a suitable volume (10–400 mL for pulp with/without peel or 5–200 mL for peel samples) of acetonitrile:water (4:1, v/v). An aliquot (10 μ L) of each diluted test solution was injected into the liquid chromatography-tandem mass spectrometry (LC-MS/MS) system.

3.4 LC-MS/MS analysis

Each amount of pesticide in the injected solution was determined using linear regression analysis of each standard calibration curve by comparing the peak area to each concentration of the pesticide in the sample. An LC-MS/MS (Model 1290 Infinity Pumping System; Agilent, CA, USA; MS/MS, Model 6460 Triple Quadrupole Tandem Mass Spectrometer; Agilent) equipped with an electrospray interface operating in a positive ion mode was used. Data were processed using Agilent Mass Hunter (version B03.01).

LC separation was performed using an Atlantis dC18 column (150 $\times$ 2.1 mm, 5 μ m; Waters, MA, USA) at 40°C. Acetonitrile and a 5-mmol/L ammonium formate aqueous solution were used as the mobile phase at a flow rate of 0.2 mL/min. In gradient elution analysis, the initial mobile phase of 30% acetonitrile increased linearly to 95% in 4 min. The retention times of acetamiprid, penthiopyrad, and pyridaben were 4.0, 6.4, and 8.0 min, respectively. The MS parameters were as follows: capillary voltage, 4000 V; nebulizer gas, 45 psi; drying gas, 5 L/min

(300°C); and fragmentor voltage, 100 V. Nitrogen was used as the collision gas for acetamiprid, penthiopyrad and pyridaben at 15, 10, and 20 V, respectively. Precursor ions of acetamiprid, penthiopyrad, and pyridaben were selected for *m/z* 223, 360 and 365, respectively. Product ions of acetamiprid, penthiopyrad and pyridaben for *m/z* 126, 276, and 147 respectively, were detected in multiple-reaction monitoring mode.

3.5 Effects of sample-size reduction and separate analyses

The percent difference (A/B) and the coefficient of variations (C.V.) for the whole fruits were calculated based on the residue levels of the size-reduced samples A and B, which were used as indexes to estimate the effect of sample-size reduction and separate analyses of pulp and peel portions of the watermelons and muskmelons. The undetected level in the pulp without the peel (<0.01 mg/kg) was replaced using the limit of quantification in the calculation to express the pesticide residue level of the whole fruit.¹⁾ The A/B value was calculated from the measured residue levels in size-reduced sample A divided by B. The C.V. value was calculated from the absolute value of the difference between size-reduced samples A and B divided by the overall mean of the 2 samples.

In this study, the following assumptions were made: the measured pesticide residue level in sample A in the pulp-with-peel portion would express the effect of the sample-size reduction. Further, the calculated pesticide residue level in the whole fruits from the separate analysis of sample B would express the combined effects of the sample-size reduction and separate analyses of the pulp and peel portions. The overall mean residue levels of samples A and B express the theoretical pesticide residue level in the whole fruit as though analyzed directly without sample-size reduction and separate analyses. Throughout this study, 2 variability factors such as the effects of sample-size reduction and separate analyses of the pulp and peel portions were investigated using 3 pesticides to determine residues in watermelon and muskmelon samples.

Results

1. Validity of the analytical method

Results of the recovery tests in watermelons and muskmelons, which were used to verify the residue analytical method applied in this study, are summarized in Supplemental Table S1. The accuracy and precision of the analytical method was confirmed by recovery tests on 3 portions (pulp with/without peel and peel) after spiking pesticides at 3 doses from the lowest limit of quantification of 0.01 mg/kg to more than the highest residue level of 5 mg/kg. The mean recoveries of spiked samples in quintuplicate (total: 54 sets) ranged from 75 to 104% (relative SDs, $\leq$ 11.0%). The specificity of the analytical method was confirmed by analyzing duplicate blank samples, which were obtained from each field sample. No interference peak was observed around the retention time of each pesticide in the chromatograms of blank samples.

Accurate and consistent instrument performance was ensured using additional recovery samples (quality control [QC] samples

spiked using 0.1 mg/kg of pesticides) and blank samples and by running a control after every 20 samples. All recoveries of the 14 spiked QC samples were within the acceptable range (70–120%). No interference peak was observed around the retention time of each investigated pesticide in the chromatograms of the 14 blank QC samples (Supplemental Table S2).

Portions of the homogenized sample spiked at 0.5 mg/kg of each pesticide and were stored for a period ranging from 62 to 127 days at -20°C . All recoveries from the stability samples were within the acceptable range (more than 70%, Supplemental Table S2).²⁾ From the results described in this section, the residue analysis methods applied to this study were confirmed to provide adequate datasets, by which the effects of sample-size reduction and separate analyses of pulp and peel portions in determining pesticide residue levels were evaluated.

2. Sample information on watermelons and muskmelons

Mean weights of whole fruits ranged from 1.22 to 1.57 kg for watermelon samples, and 1.52 to 1.83 kg for muskmelon samples (Table 1). The varieties of cultivated plants in this study were empirically selected as small types of watermelons and muskmelons with net-like skin, which were expected to have high res-

idue levels. The weight ratio of peel portions to the whole fruits ranged from 18 to 25% for watermelon and 8 to 12% for muskmelon samples. The thicknesses of the peel portions of the watermelon and muskmelon samples were approximately 4–5 mm and approximately 1–2 mm, respectively.

3. Measured residue data

The results of the residue data and the calculated parameters in watermelon and muskmelon samples are summarized in Tables 2 and 3, respectively. The measured mean residue levels of acetamiprid, penthiopyrad, and pyridaben in the pulp-with-peel portions of the watermelon samples (size-reduced sample A) ranged from 0.06 to 0.09, 0.04 to 0.07 and 0.10 to 0.17 mg/kg, respectively. Similarly, the measured mean residue levels of the 3 pesticides in the pulp-with-peel portions of the muskmelon samples ranged from 0.18 to 0.36, 0.12 to 0.28, and 0.16 to 0.33 mg/kg, respectively. The residue levels in the muskmelon samples were obviously higher than those in the watermelon samples, even though all tests were conducted after the same pesticide application. It was assumed that the rugged surface of the muskmelon's skin (net-skinned types) was conducive to high residue levels as compared with the smooth surface of the water-

Table 2. Effect of sample-size reduction and separate analyses of pulp and peel portions on pesticide residue levels in watermelons

Pesticide; field	PHI	Sample A ^{a)} (mg/kg)	Sample B (mg/kg)			A/B (C.V.) ^{c)} (%)
			Pulp	Peel	Whole ^{b)}	
Acetamiprid;						
Ibaraki	1 day	0.07	0.02	0.29	0.07	100 (—)
	3 days	0.08	0.02	0.25	0.07	114 (13)
	7 days	0.07	0.04	0.35	0.10	70 (35)
Miyazaki	1 day	0.06	0.01	0.20	0.06	100 (—)
	3 days	0.09	0.02	0.34	0.10	90 (11)
	7 days	0.06	0.02	0.24	0.07	86 (15)
Penthiopyrad;						
Ibaraki	1 day	0.06	<0.01	0.28	0.06	100 (—)
	3 days	0.06	<0.01	0.27	0.06	100 (—)
	7 days	0.04	<0.01	0.23	0.05	80 (22)
Miyazaki	1 day	0.07	<0.01	0.24	0.07	100 (—)
	3 days	0.05	<0.01	0.23	0.07	71 (33)
	7 days	0.04	<0.01	0.19	0.05	80 (22)
Pyridaben;						
Ibaraki	1 day	0.16	<0.01	0.78	0.16	100 (—)
	3 days	0.16	<0.01	0.61	0.13	123 (21)
	7 days	0.10	<0.01	0.72	0.14	71 (33)
Miyazaki	1 day	0.17	<0.01	0.67	0.18	94 (6)
	3 days	0.14	<0.01	0.70	0.18	78 (25)
	7 days	0.12	<0.01	0.56	0.14	86 (15)

PHI: preharvest interval after the final pesticide application. ^{a)}Mean of the measured residue levels from the size-reduced sample A (pulp with peel sample) in duplicate analysis. ^{b)}Calculated residue levels from the mean of the measured residue levels from the size-reduced sample B in duplicate separate analyses of pulp and peel portions. Then, the non-detect value in pulp without peel portion (<0.01 mg/kg) was replaced by the limit of quantification in the calculation to express the residue level of the whole fruit. ^{c)}The percent difference (A/B) was calculated from the residue levels in the size-reduced sample A divided by B. Coefficient of variation (C.V., in parentheses) was calculated from the absolute value of the percent difference between the size-reduced samples A and B divided by their overall mean.

melon skin.

The separate analyses were performed on the pulp and peel portions from size-reduced sample B as described in Fig. 1. In the pulp-without-peel portions, penhiopyrad and pyridaben residues were not detected in any of the watermelon and muskmelon pulp samples. Acetamiprid residue was not detected in the pulp portions of the muskmelon samples from the Miyazaki field; acetamiprid residues were the only ones detected in all of the watermelon and muskmelon samples from the Ibaraki field, even though some of the pesticides were applied in amounts greater than the maximum doses suggested on the labels or greater than the maximum recommended numbers of application. It is well known that the neonicotinoid insecticides, including acetamiprid, have penetration and translocation abilities.^{10,11)} However, the pesticide residue levels in the pulp portions in this study were less than 0.04 mg/kg of acetamiprid in the watermelon from the Ibaraki field.

In contrast, most of the pesticide residues were present in the peel portions of watermelons and muskmelons. The mean residues of 3 kinds of pesticides in the peel portions of size-reduced sample B ranged from 0.19 to 0.78 mg/kg for watermelon and 1.78 to 3.82 mg/kg for muskmelon. Because of the low pesticide

residue levels in the pulp-without-peel portions of the watermelon and muskmelon samples, it is impossible to estimate the relationship of pesticide residues between the pulp and peel portions.

4. Effect of sample-size reduction and separate analyses

The calculated pesticide residue levels in the whole fruits based on the separate analyses of pulp and peel portions of size-reduced sample B and the A/B and C.V. values from the size-reduced samples A and B are summarized in the right columns of Tables 2 and 3. The calculated residue levels of acetamiprid, penhiopyrad, and pyridaben in whole watermelons from separate analyses of size-reduced sample B ranged from 0.06 to 0.10, 0.05 to 0.07, and 0.13 to 0.18 mg/kg, respectively. Similarly, the calculated residue levels of the 3 pesticides in whole muskmelons from separate analyses ranged from 0.19 to 0.38, 0.17 to 0.38, and 0.20 to 0.45 mg/kg, respectively.

The A/B values of acetamiprid, penhiopyrad, and pyridaben in the watermelon and muskmelon samples ranged from 70 to 123% and from 62 to 100% with the mean values ($\pm$ S.D.) $91 \pm 15\%$ and $83 \pm 11\%$, respectively. The C.V. values of the 3 pesticides in the watermelon and muskmelon samples were $<35\%$

Table 3. Effect of sample-size reduction and separate analyses pulp and peel portions for pesticide residue levels in muskmelons

Pesticide; field	PHI	Sample A ^{a)} (mg/kg)	Sample B (mg/kg)			A/B (C.V.) ^{c)} (%)
			Pulp	Peel	Whole ^{b)}	
Acetamiprid;						
Ibaraki	1 day	0.36	0.01	3.11	0.38	95 (5)
	3 days	0.36	0.02	3.11	0.36	100 (—)
	7 days	0.31	0.03	3.82	0.33	94 (6)
Miyazaki	1 day	0.22	<0.01	2.88	0.24	92 (9)
	3 days	0.18	<0.01	2.44	0.23	78 (24)
	7 days	0.19	<0.01	2.04	0.19	100 (—)
Penthiopyrad;						
Ibaraki	1 day	0.28	<0.01	3.12	0.38	74 (30)
	3 days	0.26	<0.01	2.66	0.30	87 (14)
	7 days	0.26	<0.01	3.51	0.29	90 (11)
Miyazaki	1 day	0.19	<0.01	2.68	0.22	86 (15)
	3 days	0.14	<0.01	2.29	0.22	64 (44)
	7 days	0.12	<0.01	1.78	0.17	69 (36)
Pyridaben;						
Ibaraki	1 day	0.33	<0.01	3.64	0.45	73 (31)
	3 days	0.24	<0.01	2.50	0.28	86 (15)
	7 days	0.20	<0.01	2.89	0.24	83 (18)
Miyazaki	1 day	0.22	<0.01	3.41	0.28	79 (24)
	3 days	0.16	<0.01	2.84	0.26	62 (48)
	7 days	0.16	<0.01	2.14	0.20	80 (22)

PHI: preharvest interval after the final pesticide application. ^{a)}Mean of the measured residue levels from the size-reduced sample A (pulp with peel sample) in duplicate analysis. ^{b)}Calculated residue levels from the mean of the measured residue levels from the size-reduced sample B in duplicate separate analyses of pulp and peel portions. Then, the non-detect value in pulp without peel (<0.01 mg/kg) was replaced by the limit of quantification in the calculation to express the residue level of the whole fruit. ^{c)}The percent difference (A/B) was calculated from the residue levels in the size-reduced sample A divided by B. Coefficient of variation (C.V., in parentheses) was calculated from the absolute value of the percent difference between the size-reduced samples A and B divided by their overall mean.

and 48%, respectively.

Discussion

Separate analyses of the pulp and peel portions are useful in determining the precise dietary exposure from pesticide residue in an agricultural commodity.¹²⁾ The results of this study clearly show the effect of peeling watermelons and muskmelons, as most pesticide residues were present in the inedible portion of the peel (≥ 0.19 mg/kg), as shown in Tables 2 and 3. In contrast, the residues in the edible portion of pulp were equal to or less than trace levels (≤ 0.04 mg/kg).

Evaluating the decline of pesticide residues as a function of the preharvest intervals (PHI) is one of the important tasks of crop field trials for estimating the highest residues.¹³⁾ On separate fruit-part analyses, the weight ratio of the pulp and peel portions plays an important role in calculating the pesticide residue levels in the whole fruit. For example, the acetamiprid residue levels in Ibaraki muskmelons increased to 0.01, 0.02, and 0.03 mg/kg in the pulp and to 3.11, 3.11, and 3.82 mg/kg in the peel as a function of PHI. In contrast, the calculated acetamiprid residue levels in the whole fruits decreased to 0.38, 0.36, and 0.33 mg/kg as a function of the PHI (Table 3). This reverse phenomenon manifests conversion factors of the weight ratio of pulp and peel portions at PHI of 1, 3, and 7 days of 88:12, 89:11, and 92:8, respectively.

Regarding the effect of sample-size reduction, the boxplots of the A/B values are shown in Fig. 2, which shows the range of A/B values (SD) of all pesticides in the watermelon and muskmelon samples of each field. The statistical results from the Mann–Whitney *U*-test at a 5% significance level ($n_1, n_2 = 36, 36$, $z = 0.87441$, $p = 0.381895$) indicate that no significant statistical difference was observed between the pesticide residue levels in size-reduced samples A and B of watermelons and muskmelons.

Unfortunately, it is impossible to compare directly the reference values of the same pesticide residues with the other reported data; furthermore, experimental conditions and their calculation methods are different in this study. The measured maximum C.V. values of the three pesticides in this study were 35% in watermelons and 48% in muskmelons, which were similar in range to the C.V. obtained from thiophanate-methyl residues in jackfruit (C.V.: 17%), and iprodione and pirimiphos-methyl in cucumbers (C.V.: 17–21%) reported by Omeroglu.⁷⁾ Furthermore, these measured C.V. values in this study were similar in range from 10 to 23% to the predicted uncertainty in pesticide residue levels with sampling size ($n=6$) as the relative standard deviation.¹⁴⁾

The importance of the sample preparation process has been recognized, and the international guidelines recommend that the analyst carefully prepare the laboratory analytical samples.^{4,5)} The distribution of the preharvest pesticide residues in raw agricultural commodities is not uniform and is influenced by many factors, such as the physicochemical properties of the pesticide, application directions, PHI, agricultural conditions, weather, sampling procedures, and growth rates.^{15–19)} Therefore, analysts

Fig. 2. Boxplots of the percent difference of the pesticide residue levels between size-reduced samples A and B (A/B, $n=9$). The lines from the bottom to the top indicate the range from the minimum to the maximum A/B values of each pesticide, respectively, and the band of the box indicates their standard deviations.

must strive to remove any uncertain factors that might influence their pesticide residue results. On the basis of the limited results in this study, it was considered that sample-size reduction and separate analyses of pulp and peel portions have no significant effect on determining the pesticide residues in watermelon and muskmelon samples.

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References

- 1) Food and Agriculture Organization of the United Nations: "FAO Plant Production and Protection Paper 197, Submission and Evaluation of Pesticide Residues Data for the Estimation of Maximum Residue levels in Food and Feed," Rome, Italy, 2009.
- 2) Notification of Ministry of Agriculture, Forestry and Fisheries of Japan, No. 3986, Oct 10, 2001; (Final amendments were made on Apr 1, 2011).
- 3) Notification of Ministry of Health, Labor and Welfare of Japan, No. 499, Nov 29, 2005.
- 4) The Codex Alimentarius Commission: "Recommended Methods of Sampling for the Determination of Pesticide Residues for Compliance with MRLs, CAC/GL 33–1999," Rome, Italy, 1999.
- 5) Food and Agriculture Organization of the United Nations: "Guidelines on Pesticide Residue Trials to provide Data for the Registration of Pesticides and the Establishment of Maximum Residue Limits," Rome, Italy, 1986.
- 6) Á. Ámbrus, A. Zentai, J. Sali and I. Ficzer: *Accredit. Qual. Assur.* **16**, 3–11 (2011).
- 7) P. Y. Omeroglu, Á. Ámbrus, D. Boyacioglu and E. S. Majzik: *Food Addit. Contam. Part A* **30**, 116–126 (2013).
- 8) C. D. S. Tomlin (eds.): "A World Compendium, The Pesticide Manual, 14th edition," British Crop Production Council, Hampshire, U.K., 2006.

- 9) Agricultural Production Bureau, Ministry of Agriculture, Forestry and Fisheries of Japan: "The guidelines related to the study reports for the registration application of pesticide, Appendix to Director General Notification, No. 12–Nousan–8147," Nov 24, 2000.
- 10) A. Buchholz and R. Nauen: *Pest Manag. Sci.* **58**, 10–16 (2002).
- 11) M. Fujita, T. Yajima, K. Iijima and K. Sato: *J. Agric. Food Chem.* **60**, 1516–1521 (2012).
- 12) The Organization for Economic Co-operation and Development: "OECD Test Guideline No. 508, Magnitude of the Pesticide Residues in Processed Commodities," Paris, France, 2008.
- 13) The Organization for Economic Co-operation and Development: "OECD Test Guideline No. 509, Crop Field Trial," Paris, France, 2009.
- 14) M. Fujita, T. Yajima, K. Iijima and K. Sato: *J. Agric. Food Chem.* **60**, 4457–4464 (2012).
- 15) A. A. Del Real, A. Valverde-Garcia and F. Camacho-Ferre: *J. Agric. Food Chem.* **47**, 3355–3358 (1999).
- 16) Á. Ámbrus and J. Lantos: *J. Agric. Food Chem.* **50**, 4846–4851 (2002).
- 17) J. W. Travis, W. A. Skroch and T. B. Sutton: *Plant Dis.* **71**, 606–612 (1987).
- 18) M. Tanigawa and Y. Kunimoto: *J. Pestic. Sci.* **25**, 223–227 (2000) (in Japanese).
- 19) M. Fujita, T. Yajima, H. Hamano, M. Sakasai, K. Iijima and K. Sato: *J. Pestic. Sci.* **37**, 183–189 (2012) (in Japanese).