Influences of sample homogenization time and standing time before extraction on the determination of incurred pesticide residue levels in grapes

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To estimate the influence of sample processing with a blender, we conducted a homogeneity test using a bulk sample of pre-harvest grapes. Relative standard deviations (RSDs) were calculated from the concentrations of pesticides in the portions from the top, middle, and bottom of the homogenate with fine and rough particles. The results from adequate sample processing showed that the RSDs of the residue levels of all five pesticides in the fine-particle homogenate were lower than 10%. In contrast, the results under problematic conditions such as short blending times and long standing times after blending showed higher RSDs (>15%). The RSDs of nonpolar pesticides showed greater variabilities under the problematic conditions than those of polar pesticides. Separate analyses of the precipitate and supernatant phases suggested that the distribution bias of skin particles in the homogenate has a major effect on the concentration of nonpolar pesticides because of weighing errors in the extracted portions.

Introduction

The importance of sample processing for accurate residue analysis is widely recognized, especially for evaluating and enforcing maximum residue limits, because pesticide residues in raw agricultural commodities are not uniform. Various factors requiring consideration during sample processing have been presented in several guidelines and reports. For example, it is necessary to prepare a representative sample homogenate from a sufficient sampling weight and number to provide an accurate pesticide residue level, because the distribution of pesticide residues in vegetables and fruits is not uniform among units.1-6 It is also necessary to prepare an adequate sample homogenate with fine particles to reduce the uncertainty induced by errors in weighing the extract portions analyzed and to ensure sufficient extraction efficiency for pesticide residues in the samples.6-10 It is necessary to weigh extract portions from a sample homogenate without a bias in content between the peel and pulp portions of fruits, because the majority of pesticide residues from foliage application with short pre-harvest intervals are present in the peels of fruits such as melons.11 Similarly, it is necessary to prepare a sample homogenate without a bias in content between the outer and inner leaves of wrapped leafy vegetables such as cabbage, because the majority of pesticide residues from foliage application are present in the outer leaves.12

Uncertainty in pesticide residue level measurement associated with sample processing parameters cannot be estimated by recovery tests using general spiked samples. Unfortunately, the incurred residue data is influenced not only by the sample processing but also by various other factors, such as the variability of plant cultivation and/or pesticide application; however, information on the crop field is lacking. Therefore, it is difficult to simply evaluate the uncertainty due to sample processing and that due to the physicochemical properties of the pesticides.

Under routine analysis, measurement uncertainty is evaluated only by the variability of concurrent duplicate analysis of a same-sample homogenate13 or of the re-analysis of subsamples.6 In our institute, when a resultant range in duplicate analysis is outside of the acceptable criterion, additional analysis is performed in duplicate, and then the quadruplicate analytical values are evaluated using Grubbs’ outlier test. Even if outliers are recognized and we assume that problematic sample processing is possible, it is currently difficult to estimate the reason for...
the analytical uncertainty. This is because limited information is available on latent error factors in the sample processing, such as blending time, standing time after blending, and the use or lack thereof of mixing before weighing the extract portion.

In order to prepare a sample homogenate with fine particles, we should optimize the sample processing conditions, such as blending times, depending on the test samples to be analyzed. For example, longer blending times are required for immature fruits with hard tissues than for ripe fruits with soft tissues, even for fruit from the same crop field. This is one technical difficulty in standardizing the details of each raw agricultural commodity. Furthermore, we should be careful in weighing extract portions from the sample homogenate to obtain accurate analytical results. It is necessary to mix the sample homogenate well before weighing extract portions, because the sample homogenate could separate into a supernatant and a precipitate, or floating tissues could accumulate at the top, with liquid collecting at the bottom.

In consideration of the above-mentioned sources of error, we have demonstrated a comparative homogeneity test using a bulk of a pre-harvest grape sample under both adequate sample processing (as our institute standard, with fine particles) and inadequate sample processing (as a problematic sample, with rough particles) conditions using a blender at room temperature. Grapes were selected from among the worst-case samples because they have hard and thin skins and soft pulp portions, like tomatoes.6,8 In this homogeneity test, we investigated the distribution of pesticide residues in the sample homogenate in combination with different conditions, such as blending time, standing time after blending, and the use of hand mixing for frozen–thawed samples.

The field experiments involved five pesticides with greatly varied physicochemical properties. Acetamiprid is a neonicotinoid insecticide that has a relatively low log P<sub>OW</sub> of 0.80 and a relatively high water solubility of 4280 mg/L.14 Bifenthrin is a synthetic pyrethroid insecticide that has a relatively high log P<sub>OW</sub> of >6 and a relatively low water solubility of <0.001 mg/L.14 The other three pesticides, methidathion (DMTP), malathion, and tebuconazole, are two organic phosphorus insecticides and a fungicide, respectively, with intermediate physicochemical properties of log P<sub>OW</sub> and water solubility (log P<sub>OW</sub> of 2.2–3.7 and water solubilities of 36–200 mg/L).14 These pesticides were sprayed twice via tank-mix application with an interval of seven days, which was a consistent test condition, except for the dilution factor, thus allowing comparison of the influences of the physicochemical properties of the pesticides. These investigations provided valuable information for estimating the factors affecting sample processing by blender for determination of the residue levels of sprayed pesticides in grape samples.

Materials and Methods

1. Field experiments
Field experiments were supervised by us and performed by the Japan Plant Protection Association in accordance with the Organization for Economic Co-operation and Development Guideline.15 The field experiment on grapes (a seedless variety: Delaware) was conducted in an in-house vineyard (field area, ca. 600 m<sup>2</sup>; planting rate, 10 vines/10 a) in Yamanashi, Japan. Thirty-one-year grapevines were placed on a horizontal shelf with a maximum height of 1.8 m. After diluting with water, the pesticides were sprayed twice, on July 25 and August 1, 2016, at a rate of 388 L/10a per application. The sprays were administered with a tank-mix combination of Mospiran<sup>®</sup> water-soluble granules for acetamiprid 20% a.i. (Nippon Soda Co., Ltd., Japan), Spracide<sup>®</sup> wettable powder as DMTP 36% a.i. (Kumiai Chemical Industry Co., Ltd., Japan), Malathion<sup>®</sup> emulsifiable concentrate as malathion 50% a.i. (Sumitomo Chemical Co., Ltd., Japan), Only-one<sup>®</sup> flowable concentrate as tebuconazole 20% a.i. (Bayer CropScience, Germany), and Talstar<sup>®</sup> wettable powder as bifenthrin 2% a.i. (Nissan Chemical Industries, Ltd., Japan) using a back-carried sprayer (MSB1500Li, Maruyama Mfg., Inc., Japan) connected to a cone nozzle (NND-DS-5S, Yamaho Industry Co., Ltd., Japan). Twenty-four bunches of grapes were randomly collected from the treatment plot of the vineyard on the day after the final application. Additionally, thirty-three bunches of grapes were randomly collected from an untreated plot of the same vineyard as a control sample. The samples were shipped to our institute via a commercial shipping service and were maintained at a standard temperature of 3°C.

This integrated application condition was specialized for the research objective of estimating the influence of pesticide physicochemical properties; the interval from the final application to harvesting (PHI) is shorter than the PHI specified by the manufacturer for each formulation. Therefore, the test samples are expected to have higher pesticide residue levels than the maximum residue concentration permitted under normal Japanese agricultural practice.

2. Preparation of samples
After measuring the total weight of the harvested grape samples, the stems were removed. The control sample of the grape berry was blended for 3 min using a BLIXER-5Plus (Robot Coupe, USA) equipped with a vertical cutter until no obvious particles remained. In this comminution process, small portions of the homogenate were spread on petri dishes to observe the particle sizes after 10, 20, 30, 60, 120, and 180 sec of blending.

The pre-harvest sample of grape berries (3.1 kg) was randomly divided into two samples. One was blended for 3 min, with no obvious remaining particles, to prepare an adequate homogenate as the standard fine-particle sample; the other sample was blended for 1 min, with obvious particles remaining, to prepare an inadequate homogenate as the problematic rough-particle sample.

3. Reagents
Analytical standards (purity≥98%) of acetamiprid, DMTP, malathion, tebuconazole, and bifenthrin were purchased
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from Hayashi Pure Chemical (Japan) and FUJIFILM Wako Pure Chemical (Japan). Pesticide analysis-grade or liquid chromatography-mass spectrometry (LC-MS)-grade acetonitrile and analytical-grade ammonium acetate were purchased from FUJIFILM Wako Pure Chemical. Water used for the experiments was purified using a PURELAB Flex System (Veolia Water Solutions & Technologies, France). Standard stock solutions (200 mg/L) of each pesticide were separately prepared with acetonitrile. Aliquots of each stock solution were mixed and diluted with an acetonitrile–water solution to form standard solutions in the concentration range 2.5–100 µg/L to prepare calibration curves.

4. Extraction
Twenty grams of the sample was placed in an Erlenmeyer flask and shaken with 100 mL acetonitrile for 30 min using a reciprocal shaker. The mixture was stirred by a disperser for 1 min, and then the disperser was washed with 20 mL acetonitrile. The mixture was shaken again for 30 min using the reciprocal shaker. The mixture was filtered by vacuum suction, and the residual cake was washed with 50 mL of acetonitrile before filtering again. The filtrates were combined, and acetonitrile was added until the volume reached 200 mL.

5. Cleanup
One aliquot (1 mL as 0.1 g of the original sample) of the acetonitrile extract was transferred into an eggplant-shaped flask and concentrated using an evaporator to remove the organic solvent. The residue was dissolved in 3 mL of acetonitrile/water (2:8, v/v) and loaded onto a styrene-divinyl-benzene copolymer cartridge (InertSep PLS-2, 500 mg/6 mL; GL Sciences, Japan), which was previously conditioned with 5 mL of each acetonitrile and water. The flasks were washed with 5 mL of mixed acetonitrile/water (2:8, v/v), and the washings were loaded into the cartridge. The cartridge was eluted through 15 mL acetonitrile, the eluate was evaporated in a 40 °C water bath, and the residue was dried under a gentle stream of nitrogen. The residue was dissolved in a suitable volume of acetonitrile/water (1:1, v/v), and then the test solution was injected into a liquid chromatography-tandem mass spectrometry (LC-MS/MS) system.

6. Determination
The level of each pesticide in the injected solution was determined by a linear regression analysis of each standard calibration curve and by comparing the peak area to each concentration in the sample. An LC-MS/MS (Model 1290 Infinity Pumping System; MS/MS, Model 6460 Triple Quadrupole Tandem Mass Spectrometer; Agilent Technologies, USA) equipped with an electrospray interface operating in positive-ion mode was used. The data was processed using Agilent MassHunter (version B03.01). LC separation was performed using a ZORBAX Eclipse Plus C18 column (2.1 mm×50 mm, 1.8 µm; Agilent Technologies) at 40°C.

Acetonitrile and 5 mmol/L ammonium acetate aqueous solution were used in the mobile phase at a flow rate of 0.3 mL/min. For the gradient-elution analysis, the ratios of acetonitrile and the buffer solution were as follows: 15:85 (2 min hold) – 6 min –95:5. The MS parameters were as follows: capillary voltage, 3500 V; nebulizer gas, 45 psi; drying gas, 81 L/min (300°C); and sheath gas, 8 L/min (200°C). For bifenthrin determination, the pump was set under the same conditions described above except for the gradient program. The ratios of acetonitrile and the buffer solution were as follows: 75:25 – 4 min – 95:5 (1 min hold). The MS parameters were set under the same conditions described above except for the capillary voltage, 5000 V; drying gas, 5 L/min (300°C); and sheath gas, 5 L/min (200°C).

The MS detection was set to multiple-reaction monitoring mode. The injection volume was set at 2 µL. The fragmentor voltages, collision energies, and monitoring ions are summarized in Table 1. The precursor ions of acetamiprid, DMTP, and tebuconazole were [M+H] + ions at satisfactory sensitivity. The precursor ions of malathion and bifenthrin were [M+NH4] + because of the difficulty of detecting protonated ions in our optimizing process.

7. Validity of the analytical method
The accuracy and precision of the analytical method were evaluated using recovery tests on pesticides at 0.01 (equivalent to the levels at the limit of quantification), 1, and 10 mg/kg. The mean recoveries of spiked samples in five replicates ranged from 77 to 108%, with a relative standard deviation (RSD) of ≤8%. The specificity of the analytical method was evaluated by analyzing duplicate control samples, which were obtained from the same grape vineyard. No interference peak was observed around the retention times of any of the pesticides on the chromatograms from the control sample.

<table>
<thead>
<tr>
<th>Pesticide (Code)</th>
<th>Molecular weight</th>
<th>log P_{OW}</th>
<th>Precursor ion (m/z)</th>
<th>Product ion (m/z)</th>
<th>Fragmentor voltage (V)</th>
<th>Collision energy (eV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetamiprid (A)</td>
<td>222.7</td>
<td>0.8</td>
<td>222.9</td>
<td>126.0</td>
<td>100</td>
<td>20</td>
</tr>
<tr>
<td>DMTP (D)</td>
<td>302.3</td>
<td>2.2</td>
<td>303.0</td>
<td>145.0</td>
<td>75</td>
<td>16</td>
</tr>
<tr>
<td>Malathion (M)</td>
<td>330.4</td>
<td>2.89</td>
<td>348.0</td>
<td>127.4</td>
<td>50</td>
<td>10</td>
</tr>
<tr>
<td>Tebuconazole (T)</td>
<td>307.8</td>
<td>3.7</td>
<td>308.0</td>
<td>70.0</td>
<td>140</td>
<td>20</td>
</tr>
<tr>
<td>Bifenthrin (B)</td>
<td>422.9</td>
<td>&gt;6.6</td>
<td>440.0</td>
<td>181.1</td>
<td>50</td>
<td>8</td>
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</tbody>
</table>
8. Test system
The grape homogenate samples with fine and rough particles were placed in separate glass containers (diameter 10.3 cm×height 16.5 cm). In order to evaluate the variability of pesticide residue levels in the test samples, homogeneity testing was performed on three portions from the top, middle, and bottom for the grape homogenate samples with both fine and rough particles, as obtained using the various test conditions described below. The test portions were weighed carefully to avoid mixing using a glass pipette with a tip diameter of approximately 8 mm. The pesticide residue concentrations were analyzed immediately after blending and after 30 and 120 min of standing after blending.

After mixing well by hand-stirring, both grape homogenate samples, with fine and rough particles, were frozen in a freezer at −25°C. The frozen samples were placed in a cold room at 4°C overnight and thawed. The concentrations of pesticide residues in the thawed homogenate samples were analyzed immediately without mixing and then after mixing well by hand-stirring.

After mixing well by hand-stirring, 20 g portions of the thawed homogenate samples were weighed into Teflon-coated centrifuge tubes. After centrifugation at 2000×g for 10 min, supernatant and precipitate phases (soluble and insoluble phases) were separated and collected, respectively. The pesticide residue concentrations in both phases were analyzed separately.

9. Calculations
The pesticide concentrations were determined in duplicate for each test sample. To evaluate homogeneity, the RSD was calculated from the mean concentrations of pesticides in the portions from the top, middle, and bottom of each test sample. In addition, the coefficient of variation of range (CV_R) was calculated in duplicate analysis for every determination of the test sample according to the following equation

\[ CV_R = \frac{\text{range in duplicate analysis/mean concentration}}{100} \times 0.89 \]

When the concentration range in the duplicate analysis is higher than twice the limit of qualification (0.01 mg/kg), the acceptability criterion for the range in duplicate analysis is defined as that yielding a CV_R of \( \leq 10\% \).

Results and Discussion

1. Visual observation and weight measurement
The photographs in Fig. 1 show changes in the comminution state of the grape berry sample over the blending time. As the blending time increases, the comminution process is observed, in which the particles of grape skin become finer. Based on this visual observation and our standard sample processing procedure, we selected a standard grape homogenate with fine particles (<2 mm length) formed after a blending time of 3 min. For contrast, we selected a problematic grape homogenate with rough particles (∼10 mm length) obtained after 1 min of blending, in which visually obvious skin particles were observed.

As the standing time after blending increased, the appearance of the grape homogenate changed dramatically. After standing for 30 min, the insoluble tissues in the homogenate floated up, and the color of the sample became nonuniform. After standing for 120 min, a distinct liquid layer appeared at the bottom of the homogenate. Based on this visual observation, the homogeneity test was conducted at three time points: immediately after blending and upon standing for 30 and 120 min after blending.

In Fig. 2, the changes in the weight percentage of the precipitate obtained by centrifugation are shown for the top, middle, and bottom portions of the grape homogenate as a function of the standing time after blending. Immediately after blending,
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no significant difference appears in the weight percentages of the precipitate from the top, middle, and bottom portions of the grape homogenate (38–39%). As the standing time increases, the weight percentages of precipitate are increased in the top portion and decreased in the bottom portion. The differences in weight percentages of the precipitate after standing for 120 min are +12% in the top portion and −13% in the bottom portion. The weight percentages of the precipitate from the middle portion remain almost constant regardless of the standing time (38–39%). The results of visual observation and weight measurement indicate that the content of insoluble tissues, such as skins, increased in the top portion and decreased in the bottom portion as the standing time increased.

2. Individual residue data

The residue levels of pesticides in the grape homogenate with fine particles, analyzed immediately after blending (standard condition), were 1.92±0.04 mg/kg for acetamiprid, 1.84±0.07 mg/kg for DMTP, 4.00±0.18 mg/kg for malathion, 1.94±0.05 mg/kg for tebuconazole, and 0.24±0.02 mg/kg for bifenthrin (overall means ± standard deviation, n=3). These resi-

Fig. 2. Weight percentage of precipitate (insoluble tissues) phase by centrifugation in top, middle, and bottom portions of grape homogenates at 0, 30, and 120 min standing after blending.

Fig. 3. Relative concentrations of pesticides in the top (○), middle (□), and bottom (△) portions from grape homogenate samples with fine particles (F) and rough particles (R) at 0, 30, and 120 min standing after blending. Pesticides are plotted in order of their polarity (see Table 1 for pesticide codes). Relative concentrations are calculated according to the overall mean concentration of each pesticide in the grape homogenate with fine particles immediately after the blending (0 min standing).
due levels corresponded to the applied amounts of each pesticide formulation. To compare the behaviors of various pesticides on the same scale, the concentration relative to the overall mean concentration of each pesticide under the standard conditions is used in this study.

The individual relative concentrations of pesticides in the top, middle, and bottom portions of the grape homogenate samples are plotted in Fig. 3 for 0, 30, and 120 min of standing after blending. As the standing time after blending increases, the variability of the residue levels in the sample homogenate obviously increases. Additionally, the variability of the problematic sample with rough particles tends to be greater than that of the standard sample with fine particles. The relative concentration of pesticides with a long standing time after blending tends to increase in the order of bottom, middle, and then top portions. The variabilities of the nonpolar pesticides show larger ranges as the standing time increases. As the variability in fine particle sample was observed even with the polar pesticide acetamiprid, it is necessary to be careful during sample processing for all kinds of pesticides.

Despite the results in the duplicate analysis of the same portions, the residue levels are widely varied in the middle portion of the homogenate, with rough particles measured after 120 min of post-blending standing. In contrast, no significant difference in residue levels appears in the duplicate analysis of the bottom portion. In the results of duplicate analysis of the obvious liquid phase in the bottom portion, the determined residue levels of the pesticides are lower than the average value, and their range is narrow.

3. Evaluation of RSD values

Figure 4 shows the results of the homogeneity test as the RSD values of pesticide residue concentrations in the top, middle, and bottom portions of the grape homogenate samples. For the standard homogenate with fine particles immediately after blending, the analytical homogeneity result shows that the RSD values of residue concentrations for all five pesticides are <10%. This result confirms the good homogeneous state obtained using the standard sample processing procedure applied in this study. On the other hand, for the problematic homogenate with rough particles...
particles immediately after blending, the analytical homogeneity result shows that the RSD values of residue concentrations of all five pesticides are >10%. Despite being immediately analyzed without standing, the RSD value of acetamiprid is 16%, outside the acceptable range (≤15%).

The RSD values of the problematic sample with rough particles are generally larger than those of the standard sample with fine particles. Additionally, the RSD values clearly increase as the standing time after blending increases. At a standing time of 30 min, the RSD values from the problematic homogenate with rough particles are 16–57%, which exceed those from the sample homogenate with fine particles of 7–16%. At a standing time of 120 min, the RSD values from both homogenate samples, with fine and rough particles, vary from 24% for acetamiprid to 93% for bifenthrin. The RSD values of the nonpolar pesticides are generally larger than those of the polar pesticides.

4. Effect of hand-mixing on the frozen–thawed homogenate

Figure 5 shows the relative concentrations of pesticides in the top, middle, and bottom portions of the grape homogenates with and without hand-mixing, which were thawed after 30 days of storage in a freezer. The appearance of the frozen–thawed homogenate was nonuniform, despite the sample being thoroughly stirred before freezer storage. The insoluble tissues in the homogenate floated up, and a distinct liquid layer appeared at the bottom. After mixing well by hand, the state of the frozen–thawed homogenate was visually confirmed to return to a homogeneity state similar to the initial sample immediately after blending.

Without mixing, the analytical results of the samples vary greatly after the freeze–thaw process. The relative concentrations of pesticides increase in the order of bottom, middle, and top portions of the homogenate. The variabilities of nonpolar pesticides are broader than those of the polar pesticides. The highest residue level of bifenthrin in the top portion of the homogenate with rough particles is 22 times higher than the lowest residue level in the bottom portion. The highest and lowest concentrations of bifenthrin relative to the overall mean concentration under the standard condition are 292% from the top portion of the homogenate with rough particles and 13% from the bottom portion, respectively. These tendencies are similar to the results after a long post-blending standing time. Except for the above, there are no significant differences between the results from the two homogenate samples with fine and rough particles.

After mixing well by hand, the variability of the relative concentrations of pesticides in the frozen–thawed homogenate is dramatically reduced, approaching the variability observed in the initial sample immediately after blending. The highest and lowest concentrations relative to the overall mean concentration under the standard condition are 156% for bifenthrin from the top portion of the homogenate with fine particles and 87% for malathion from the bottom portion of the homogenate with
rough particles, respectively. The overall mean concentration relative to the overall mean concentration under the standard condition ranges from 108% for DMTP in the homogenate with fine particles to 126% for bifenthrin. This result confirms the stability of the pesticides investigated during this study.

Figure 6 shows the results of homogeneity testing as the RSD values of pesticide residue concentrations in the top, middle, and bottom portions from the frozen–thawed homogenate with and without hand-mixing. The RSD values of the nonpolar pesticides are generally greater than those of the polar pesticides, as with the results observed for a long standing time after blending. The analytical results for the homogeneity of both test samples with fine and rough particles after mixing thoroughly by hand are obviously reduced. The RSD values of the residue concentrations of all five pesticides, with and without mixing, are >27%, all outside the acceptable range (≤15%), and the maximum value is 94% for bifenthrin from the homogenate with rough particles. The RSD values of the nonpolar pesticides are generally greater than those of the polar pesticides, as with the results observed for a long standing time after blending. The analytical results for the homogeneity of both test samples with fine and rough particles after mixing thoroughly by hand are obviously reduced. The RSD values of the residue concentrations of all five pesticides are ≤21%; only the RSD of 21% for bifenthrin in the homogenate with fine particles is out of the acceptable range.

5. Separate analyses of precipitate and supernatant phases obtained from centrifugation

To investigate the pesticide distributions in the grape homogenate, additional analyses were performed separately for the precipitate and supernatant phases (insoluble and soluble phases) obtained via centrifugation of the middle portions of the test samples. The residues of acetamiprid, the most polar pesticide investigated, are 60% and 40% in the precipitate and aqueous phases, respectively (see Fig. 7). Meanwhile, the residues of bifenthrin, the most nonpolar pesticide investigated, are only detected in the precipitate phase (>99%), with none found in the supernatant phase (<1%). Thus, most of the bifenthrin residues exist in insoluble tissues, such as skins. The residue distributions of the other three pesticides are intermediate relative to the results for acetamiprid and bifenthrin. The correlation between the pesticide distribution ratios in the precipitate phase and their log P<sub>OW</sub> values showed good agreement (R<sup>2</sup>=0.9235, y=7.465x+59.085, Supplemental Fig. S1). These results suggest that the distribution bias of insoluble tissues in the grape homogenate has a more significant effect on the nonpolar pesticides because of weighing errors in the extract portion than it does on the polar pesticides. No significant difference was observed in the distribution patterns in the precipitate and supernatant phases between the results from the two samples with fine and rough particles.

From this distribution pattern of pesticide residues, as well as the visual observation and the weight change of the sample homogenate after long standing times and freeze–thaw processing, it was confirmed that the residue levels of pesticides increased in the order of the top, middle, and bottom portions, caused by a bias of the contents of soluble and insoluble tissues in the weighing of the extract portions. The residue levels of pesticides in the top portion of the homogenate became higher because the content of insoluble tissues, such as skins, was higher; meanwhile, the residue levels of pesticides in the bottom portions became lower because the content of insoluble tissues was lower.

6. Evaluation range in duplicate analysis

The CV<sub>R</sub> values in duplicate analysis for every determination of the test samples are summarized in Table 2. All of the CV<sub>R</sub> values in duplicate analysis for every determination of the test samples are summarized in Table 2. All of the CV<sub>R</sub> values in duplicate analysis for every determination of the test samples are summarized in Table 2.
values for the standard grape homogenate with fine particles analyzed immediately after blending and those analyzed after the freeze–thaw process with hand-mixing are ≤10%, within the acceptable range (≤10%). However, some of the CV R values for the other test samples are outside the acceptable range. The determination ratios for problematic sample processing range from 13% (2/15 for the homogenate with fine particles analyzed at 30 min of standing after blending) to 53% (8/15 for the homogenate with rough particles analyzed at 30 or 120 min of standing after blending). When the range result from duplicate analysis was outside the acceptability criterion, an additional analysis was performed in duplicate. This result suggests that the ability to identify problematic sample processing by evaluating duplicate analyses is limited, but the method is effective for determining errors associated with the sample processing, including the weighing of extract portions from the sample homogenate.

Conclusion

Although further investigations should be performed for a variety of raw agricultural commodities, this homogeneity test using a bulk of pre-harvest grape samples provides valuable basic knowledge about homogeneity uncertainty associated with sample processing. The results of this study indicate that an adequate sample homogenate with fine particles is needed to provide accurate analytical results. The results from the inadequate homogenate with rough particles obtained under problematic conditions provide us with some points for consideration with regard to sample processing. Separate analyses of the precipitate and supernatant phases, which were obtained via centrifugation of the homogenated grape sample, suggested that the distribution bias of skin particles in the homogenate has a greater effect on the nonpolar pesticides because of weighing errors in the extract portion. That is, the RSD values of pesticide residues vary with the physicochemical properties of the pesticides; nonpolar pesticides typically show greater variabilities under problematic processing conditions such as long standing times after blending or without thorough mixing before weighing.

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References