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Effect of the Distribution of Analyte Concentration in Lot, Sample Size, and Number of Analytical Runs on Food-Testing Results

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ABSTRACT: In testing, it is necessary to obtain the correct measured values that reflect analyte concentrations in the lot. Control of the analytical performance and appropriate sampling are essential to obtain the correct values. In the present study, we estimated the distribution of the analyte concentrations in specific food product lots and examined the influence of the sample size and the number of analytical runs on the variability of the testing results. The combinations of analyte and food studied were pesticide residues in fresh vegetables, nitrate in fresh vegetables, and food additives in processed meat products. The results of our study suggested the following: an increase in the sample size beyond a certain number does not efficiently reduce the variability of the test results; the specific sample size required to maintain the variability of the testing results at an appropriate level depends on the breadth of distribution of concentrations in the lot and the precision of the analysis; and increasing the number of analytical runs was more efficient in reducing the variability of the testing results than increasing the sample size, when the breadth of distribution of concentrations in the lot was narrow enough to be comparable with the analytical precision.

KEYWORDS: *sampling, testing, variation, pesticide, food additive*

INTRODUCTION

In Japan, the Food Sanitation Act has established maximum residual levels (MRLs) for various chemical substances that may cause adverse effects on health, to regulate their intake. Testing of food items is one of the regulatory practices. In the testing of food, the average concentration of analyte in a lot or consignment (hereinafter referred to as "the lot") is compared to the MRL to decide the conformity of the lot. The average concentration in the lot (hereinafter "the lot average") is estimated from the analytical results. Therefore, the analytical results should correctly represent the lot average. To obtain accurate analytical results, the performance of analytical systems, including methods of analysis, analytical environment, and the skills of the analysts, should be properly controlled.[1] Furthermore, it is essential to take samples representing the lot.

In the actual testing procedure, multiple items are taken from a lot according to a specified sampling plan and procedure. The important premise of sampling is that the items are randomly taken from the lot.[2] The average value, which is derived from an abundance of lot average estimates obtained by repeating the random sampling many times, is expected to be consistent with the lot average. However, the individual lot average estimate is distributed in a certain pattern around the lot average. The number of items taken from a lot (i.e., sample size) is defined in the sampling plan so as to allow the breadth of the distribution of individual average estimates to fall into a proper range.

The distribution of lot average estimates depends on the sample size and the variance of analyte concentrations in the lot, that is, lot variance, and the larger the sample size is, the smaller the variance of the lot average estimates. In addition, analytical results also vary due to the analytical variance. Therefore, the breadth of distribution of the lot average estimate changes depending on the sample size, lot variance, and analytical variance. Knowledge and information on the lot variance are essential for reasonably determining the sample size to ensure the reliability of the testing results. There have been previous reports on sampling for the testing of chemical substances including pesticide residues, microbes, and mycotoxins.[3−10] However, there have been few reports evaluating the influence of both sampling and analysis on the variability of the testing results through a multifaceted approach by analysis of the concentrations of multiple analytes. In the present study, we measured the concentrations of pesticide residues and nitrate in fresh vegetables grown in a single farm field, as well as those of sodium nitrite and acesulfame potassium in processed meat products taken from the same production lot. On the basis of these analytical results, we estimated the lot variance of each food item. Furthermore, we examined the influence of sample size and the number of analytical runs on the variability of the testing results and compared the testing results obtained by analysis of individual items taken from the same lot and by analysis of a composite sample.

MATERIALS AND METHODS

Collection and Preparation of Fresh Vegetable Samples. Samples of cabbage, Chinese cabbage, Japanese radish, and spinach grown for sale were collected from a farm field in Sakuragawa city, Ibaraki prefecture. The sampling scheme in this study was as follows: The total farm field was divided into 50 areas to which the numbers 1−50 were assigned. Uniform random numbers from 1 to 50 were generated, and the primary samples were collected from the areas corresponding to the random numbers. Primary samples of cabbage, Chinese cabbage, or Japanese radish consisted of one item. Primary samples of spinach consisted of four bundles collected together. Sixteen primary samples were collected for each vegetable. One

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laboratory sample was prepared from each primary sample. Laboratory samples were prepared as follows: a cabbage was vertically divided into eight pieces from which two opposing pieces were taken to be shredded and mixed. A Chinese cabbage was vertically divided into eight pieces from which two pairs of two opposing pieces were taken. Each piece was horizontally divided into four parts of top, upper-middle, lower-middle, and bottom positions. Only one part was taken from the top, upper-middle, lower-middle, and bottom positions through the four pieces, and the resultant four parts were shredded and mixed. The root of a Japanese radish was vertically and horizontally divided into eight pieces from which two pairs of diagonally opposing two pieces were taken to be shredded and mixed. The whole leaves of a Japanese radish were shredded and mixed. Each bundle of spinach was vertically divided into four pieces from which the opposing two pieces were taken to be shredded and mixed. Four bundles of spinach were processed as above to yield an amount equal to two bundles. From each laboratory sample, a required amount was taken for an analytical sample. The concentrations of pesticide residues were measured immediately after preparing the laboratory samples. The analytical samples were stored at −20 °C until the measurement of nitrate concentrations.

Collection and Preparation of Processed Meat Product Samples. The samples of hams and sausages were collected as follows from the production lines at the manufacturing site: One primary sample consisted of one package, and 20 primary samples were picked out at a certain interval from all of the packages in the same production lot that were constantly carried on a conveyor belt. Each primary sample was mixed and homogenized to obtain a laboratory sample. From each laboratory sample, a required amount was taken for the analysis. The analytical samples were stored at −20 °C until use.

Chemical Substances Analyzed. Cultivation records for each fresh vegetable were checked to identify pesticides that were sprayed on the field within a month before sampling, and indoxacarb and etofenprox were selected as the chemicals to be analyzed in the samples of cabbage and Chinese cabbage, respectively. Furthermore, nitrate was selected as an example of a natural constituent whose concentrations are relatively high in many vegetables. Its concentration was measured in the samples of cabbage, Chinese cabbage, spinach, and Japanese radish (leaves and root). The manufacturing method of processed meat products was examined to identify food additives used in a planned manner during their production, and sodium nitrite and acesulfame potassium were selected as the chemicals to be analyzed.

Reagents. *Analysis of Pesticide Residues.* Indoxacarb (99.8%, Wako Pure Chemical Industries, Ltd.) and etofenprox (99.9%, Hayashi Pure Chemical Ind., Ltd.) were dissolved in acetone to prepare the standard stock solutions (1000 μg/mL). ENVI-Carb/LC-NH_2 (Supelco, Inc., Bellefonte, PA) was used as the multilayer graphitized carbon/aminopropylsilylated silica gel minicolumn (500 mg/500 mg). Other reagents of pesticide residue analysis grade were purchased from Wako Pure Chemical Industries, Ltd. or Kanto Chemical Co., Inc. The standard stock solutions were diluted with the mixture of acetone/*n*-hexane (1:1) to prepare standard solutions for calibration at concentrations of 0, 0.01, 0.25, 0.5, 1.0, 2.0, 4.0, 8.0, 16.0, and 20 μg/mL.

Analysis of Nitrate. The high-performance liquid chromatography (HPLC)-grade distilled water was purchased from Kanto Chemical Co., Inc. Other special-grade reagents were purchased from Wako Pure Chemical Industries, Ltd. Potassium nitrate was dissolved in distilled water to obtain the standard stock solution (1000 μg/mL). The standard stock solution was diluted with distilled water to prepare standard solutions for calibration at concentrations of 0, 0.5, 2, 5, 10, 20, 50, and 100 μg/mL.

Analysis of Sodium Nitrite and Acesulfame Potassium. The HPLC-grade acesulfame potassium was purchased from Wako Pure Chemical Industries, Ltd. Tetra-*n*-propylammonium bromide (98%) and the HPLC-grade acetonitrile were purchased from Aldrich Ltd. and Nacalai Tesque, Inc., respectively. Other special-grade reagents were purchased from Wako Pure Chemical Industries, Ltd. Sodium nitrite was dissolved in distilled water to obtain the standard stock solution (1000 μg/mL). The standard stock solution was diluted with distilled water to prepare standard solutions for calibration at concentrations of 0, 0.01, 0.02, 0.04, 0.08, 0.12, 0.16, and 0.32 μg/mL. Acesulfame potassium was dissolved in distilled water to obtain the standard stock solution (1000 μg/mL). The standard stock solution was diluted with distilled water to prepare standard solutions for calibration at concentrations of 0, 0.2, 0.4, 1, 2, 5, and 20 μg/mL.

Equipment. The knife mill Grind Mix GM200 (Retsch Co., Ltd.) with a 1 L stainless steel receptacle was used to mix and homogenize samples. For condensing the extracted solutions during pesticide analysis, a multisample evaporator apparatus Syncore Analyst (Büchi Labortechnik AG) was used. This apparatus was equipped with the following accessories: 200 mL condensation tubes, 200 mL condensation tubes with a 0.5 mL reservoir, a 12 tube rack, a reflux module, a V-500 vacuum pump, and a V-855 vacuum controller. The 7890A GC-5970C MS (Agilent Technologies, Inc.) connected to the Agilent 7683 automatic liquid sampler was used for the analysis of indoxacarb and etofenprox. Whatman syringe filters with a pore size of 0.45 μm were used in the analysis of nitrate, and they were washed well with distilled water before use. The 2695 Alliance System connected to the 2996 Photodiode Array Detector or the 2487 Dual Absorbance Detector (Waters Corp.) was used for the analysis of nitrate and acesulfame potassium. The U-2000A spectrophotometer (Hitachi, Ltd.) was used for the analysis of sodium nitrite.

Analytical Methods for Pesticides. The analytical methods conformed to "Multiresidue Method for Determination of Pesticides Etc. in Farm Products by GC-MS" promulgated by the Department of Food Safety, Ministry of Health, Labour and Welfare (MHLW) of Japan.[11] Procedures for extraction and purification were in accordance with the "Methods for Fruits, Vegetables, Herbs, Tea Leaves and Hops".

Analytical Methods for Nitrate in Fresh Vegetables. The analytical method was in accordance with that described by Matsuda et al.[12]

Analytical Methods for Sodium Nitrite. The analytical method was in accordance with the methods promulgated by MHLW of Japan.[13]

Analytical Methods of Acesulfame Potassium. The analytical method was in accordance with the methods notified by MHLW of Japan.[14]

Calculation Models. If items were randomly taken from a lot to prepare a sample and analyzed individually to obtain analyte concentration, the average of analyte concentrations, that is, the sample average, has a distribution depending on the lot variance (V_L) and the sample size. Assuming that there is no bias in the analytical procedure, the subsample is truly representative, and there are no other sources of error, the average of many sample averages is identical to the lot average, and the variance of many sample averages (V_S) varies depending on V_L and the sample size, n. When the lot is tested, the sample average is available after analysis, and the variance of the observed sample average (V_{obs}) is affected by the analytical variance (V_A).

The relationships among these variances are as follows

$$V_S = V_L/n \quad (1)$$

$$V_{obs} = V_L/n + V_A/m \quad (2)$$

where m is the number of analytical runs.

RESULTS AND DISCUSSION

Normality of Distribution of the Concentration of Each Analyte. The goodness-of-fit of the data to a normal distribution was tested by the Kolmogorov test. The results of the test showed that the goodness-of-fit to the normal distribution was not rejected in all combinations of analyte and food item ($\alpha = 0.05$). Although the detection power of the Kolmogorov test is not high, it was assumed that the analyte concentration in the lot follows a normal distribution.

Table 1. ANOVA of the Analytical Results of Pesticide Residues, Nitrate, Nitrite, and Acesulfame Potassium

analyte	commodity	sample size (n)	unit	average	SD		RSD (%)	
					SD_L	SD_A	RSD_L	RSD_A
indoxacarb	cabbage	16	ng/g	32.4	16.5	3.4	51.0	10.5
etofenprox	Chinese cabbage	16	ng/g	26.8	18.1	2.7	67.5	10.0
	cabbage	16	μg/g	1264	248	11.8	19.6	0.9
	Chinese cabbage	16	μg/g	1753	250	27.9	14.3	1.6
nitrate	spinach	16	μg/g	1221	309	14.4	25.3	1.2
	Japanese radish leaf	16	μg/g	4612	837	66.1	18.2	1.4
	Japanese radish root	16	μg/g	2492	412	54.8	16.5	2.2
nitrite	sausage	20	μg/g	20.6	1.2	0.6	5.9	2.9
	ham	20	μg/g	10.4	3.9	0.2	37.2	2.4
acesulfame potassium	sausage	20	μg/g	8.6	0.5	0.5	6.0	6.3
	ham	20	μg/g	17.3	1.7	0.8	9.9	4.6

Estimation of V_A and V_L. Two analytical samples taken from each laboratory sample were analyzed in parallel. Data sets for fresh vegetables consisted of 32 (16 × 2) analytical results of concentration of the pesticides or nitrate. Data sets for processed meat products consisted of 40 (20 × 2) analytical results of concentration of sodium nitrite and acesulfame potassium. Each data set was analyzed by one-way analysis of variance (ANOVA), and the total variation of the analytical results was divided into the variation among the primary samples and the variation of analysis. The former is the estimate of V_L, and the latter is the estimate of V_A. The results are shown as standard deviations (SD_L and SD_A) and relative standard deviations (RSD_L and RSD_A) in Table 1. The average concentrations of the analytes contained in the primary samples that were used to calculate RSD are also shown in Table 1.

The average concentration of indoxacarb in cabbage was 32.4 ng/g, and that of etofenprox in Chinese cabbage was 26.8 ng/g. The RSD_L of the pesticides in both vegetables was estimated to be larger than 50%, suggesting a large dispersion in the lot. RSD_A estimates were about 10%. The average concentration of nitrate in cabbage, Chinese cabbage, spinach, and Japanese radish (leaves and root) ranged from 1200 to 4600 μg/g, and the RSD_L ranged from 14.3 to 25.3%, suggesting a smaller dispersion than that for pesticide residues in fresh vegetables. RSD_A estimates ranged from 0.9 to 2.2%, which is much smaller than those of pesticides. The average concentrations of sodium nitrite and acesulfame potassium in sausage were 20.6 and 8.6 μg/g, respectively. The RSD_L of sodium nitrite and acesulfame potassium in sausage was 5.9 and 6.0%, respectively. The RSD_L of sodium nitrite and acesulfame potassium in ham was estimated to be 37.2 and 9.9%, respectively, larger than those in sausage. RSD_A estimates ranged from 2.4 to 6.3% through the combination of food additives and processed meat products.

Relationship between the RSD_A and the Analyte Concentration Level. The relationship between the average concentration of each analyte and the RSD_A is presented in Figure 1. The concentration levels of various analytes observed in the present study were divided into the following three levels: 0.01, 10, and 1000 μg/g. The average concentration of nitrate, which is a natural constituent of fresh vegetables, is approximately 10^5-fold higher than that of pesticides remaining in fresh vegetables. The average concentration of food additives was intermediate between that of pesticides and nitrate. The RSD_A of these samples decreased as the average concentration increased.

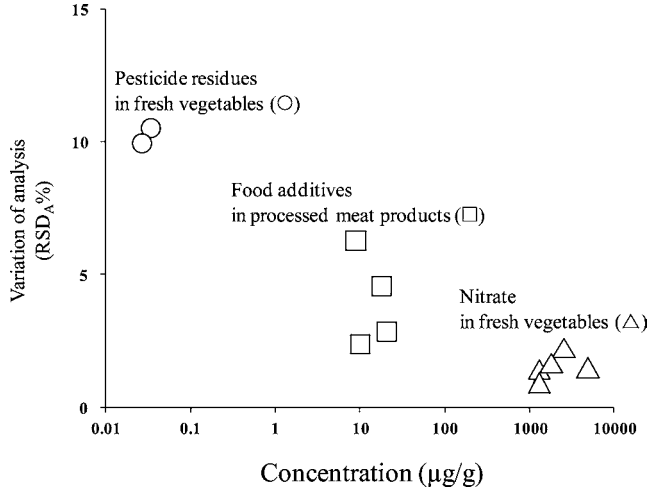

Figure 1. Relationship between the average concentration of pesticide residues, nitrate, and food additives and their RSD_A.

Relationship between the RSD_L and the Analyte Concentration Level. Figure 2 shows the relationship between the average concentration of each analyte and the RSD_L. In contrast with the relationship between the average concentration and the RSD_A (Figure 1), the RSD_L varied independently of the average analyte concentration. The RSD_L

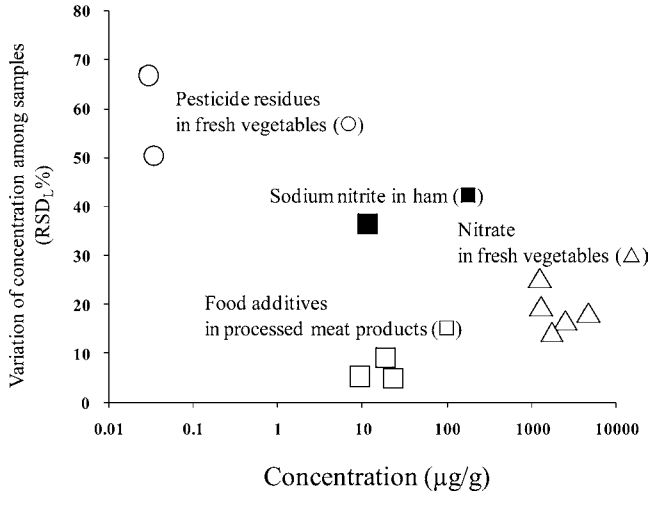

Figure 2. Relationship between the average concentration of pesticide residues, nitrate, and food additives and their RSD_L.

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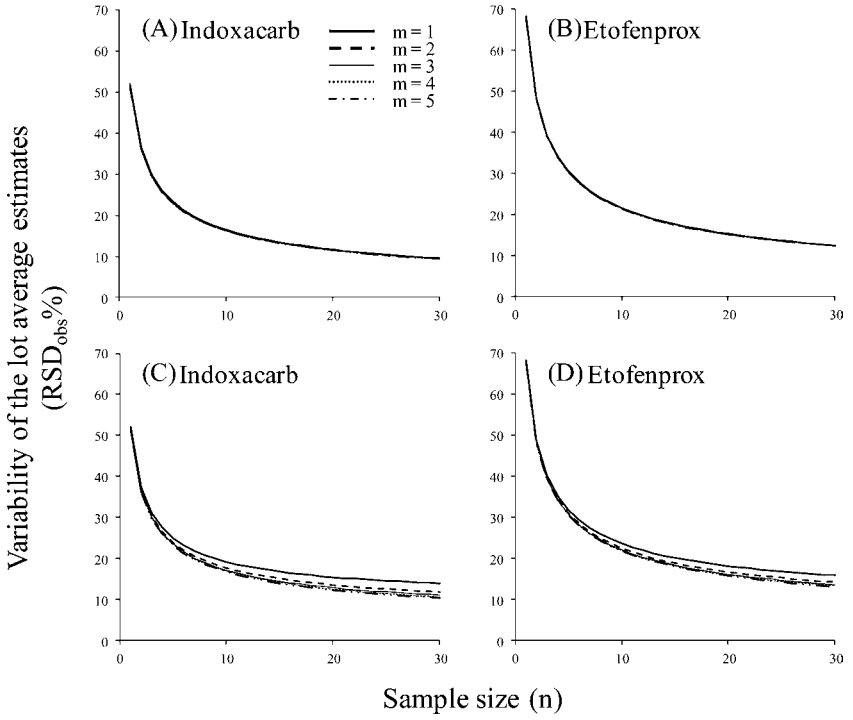

Figure 3. Influence of the sample size and number of analytical runs on the lot average estimates for pesticide residues from the analytical result(s) of individual items (A and B) or a composite sample (C and D).

of food additives, except nitrite in ham, was smaller than those of nitrate in fresh vegetables, although the average concentration of food additives is 100-fold lower. The RSD_L of sodium nitrite in ham was much higher than the others. The difference in RSD_L between nitrite in ham and sausage was attributable to the presence or absence of the product homogenization process after the addition of food additives. Sausages are produced by shaping a mixture of the raw material meat and additives after sufficient agitating and mixing. On the other hand, the homogenization process is not included in the production procedures of ham, and food additives are added to the raw material meat through an injector. The quality control in the production of ham is intended to ensure that the average concentrations of food additives are maintained at target concentrations in products of the same lot and that these concentrations in different production lots are within certain ranges. Therefore, the homogenization process is considered to contribute to the small RSD_L of food additives in sausage, and the lack of this process leads to the large RSD_L in ham. On the basis of the above results and discussion, it is strongly suggested that the RSD_L are dependent not only on the concentration levels of the chemicals but also on various other factors including the production methods of the food items, the origin of the analyte, and the sensitivity of each analyte to environmental factors.

Influence of Sample Size and Number of Analytical Runs on the Variability of Testing Results. In testing, the conformity of the lot to the standard was decided by comparing the lot average estimate obtained by sampling and analysis with the standard value. The primary testing result is expressed dichotomously as "conforming" or "not conforming". Meanwhile, the analytical result, which is obtained through the specified testing procedure consistent with the declared sampling and analytical methods, can be also referred to as the "testing result"; in the present study, the term "testing result" is used.

The lot average is estimated from the analytical result(s) for individual items forming a sample or from a composite sample prepared by mixing individual items. When individual items are analyzed, the lot average estimate is the average of individual analytical results, but when the composite sample is analyzed, the lot average estimate is the analytical result of the composite sample. However, the concentrations of the analyte in the individual item are not the same and vary according to the concentration distribution of the lot. Therefore, the lot average estimate, that is, the testing result, differs for every sampling.

If the random sampling were infinitely repeated, the average of the analyte concentration in the samples would be identical to the lot average. In this case, the SD of the testing results is determined by the lot variance and the sample size. The analysis also contributes to the SD in the testing result, and the SD in the testing result would be influenced by the analytical variance and the number of analytical runs. The SD of the testing result, SD_{obs}, is predicted by eq 2. Two cases are assumed in the testing.

When n items are taken from a lot and each item is analyzed by m runs:

$$SD_{obs} = \sqrt{\frac{SD_L^2}{n} + \frac{SD_A^2}{nm}} \quad (3)$$

In another case, n items are taken from a lot and mixed to a composite sample, and the composite sample is analyzed by m runs:

$$SD_{obs} = \sqrt{\frac{SD_L^2}{n} + \frac{SD_A^2}{m}} \quad (4)$$

The relationship among the SD of the testing result, the sample size n, and the number of analytical runs m was

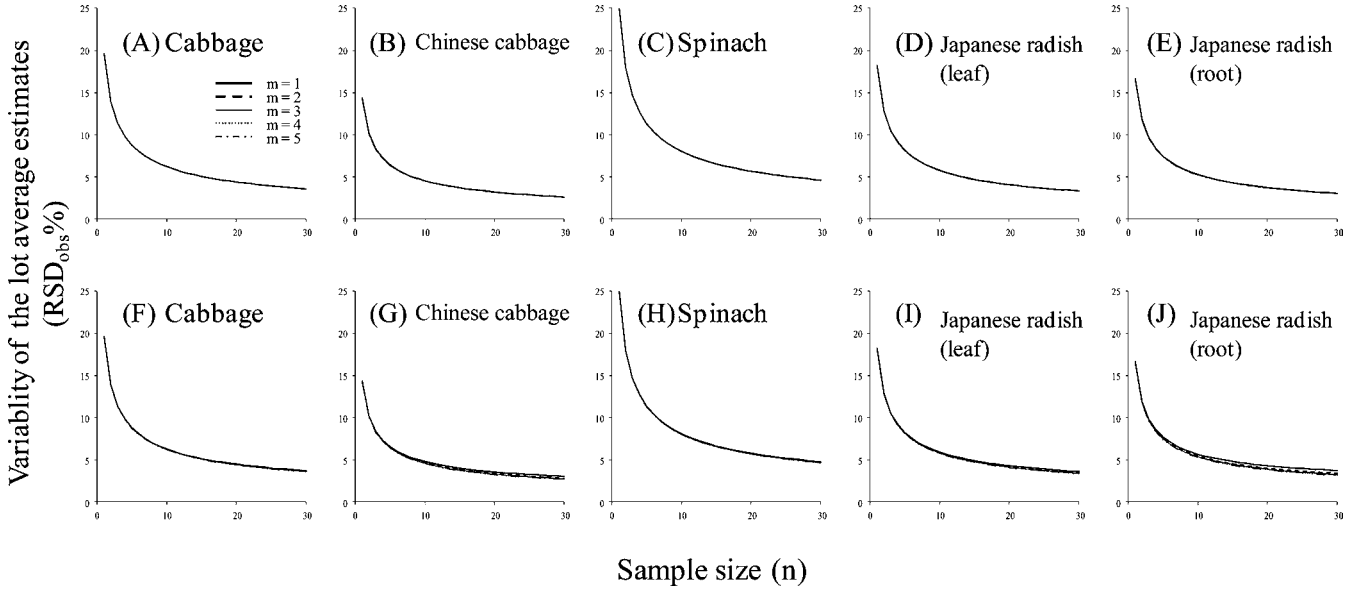

Figure 4. Influence of the sample size and number of analytical runs on the lot average estimates for nitrate from the analytical result(s) of individual items (A–E) or a composite sample (F–J).

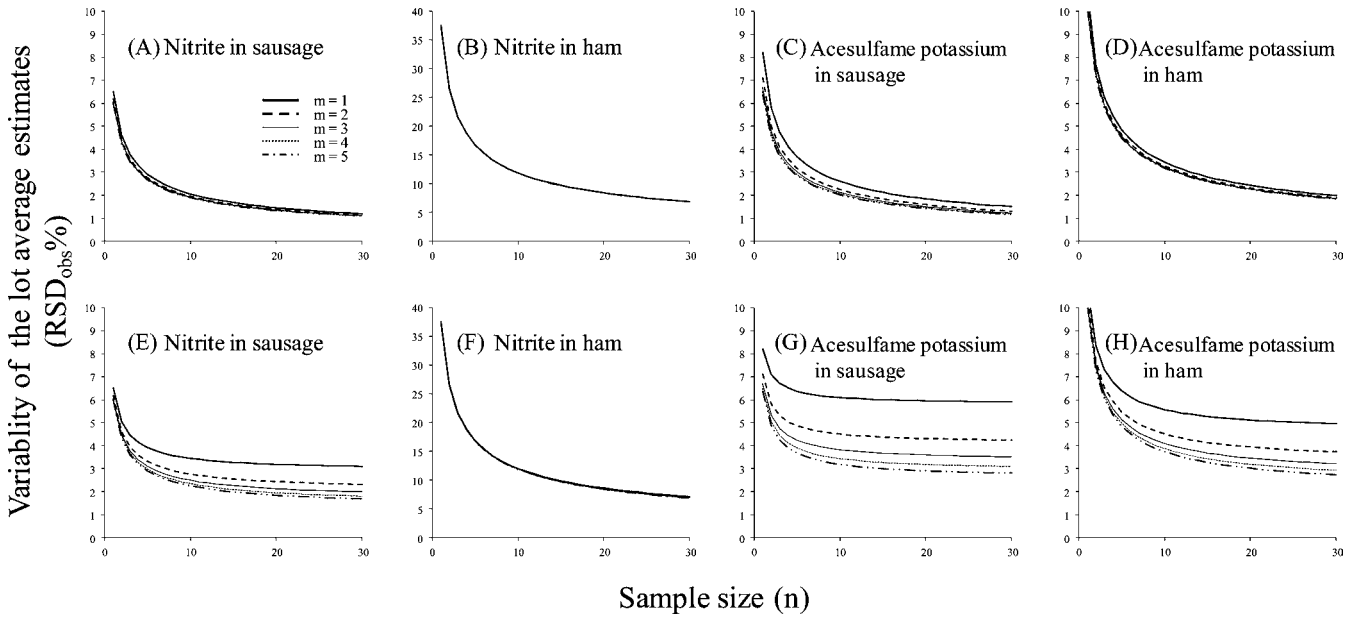

Figure 5. Influence of the sample size and number of analytical runs on the lot average estimates for nitrites and acesulfame potassium from the analytical result(s) of individual items (A–D) or a composite sample (E–H).

evaluated by substituting the SD of sample and analysis in Table 1 for SD_L and SD_A in the above equations, respectively. The results of pesticide residues are shown in Figure 3. Five curves are plotted in each figure by assigning the numbers 1–5 to *m*, the number of analytical runs, in the equations. For both of the evaluation results on etofenprox and indoxacarb assuming individual analysis, five curves were overlaid to form a single curve (Figure 3A,B). The SD_L is 5-fold or more larger than the SD_A, and the contribution of the second term of eqs 3 and 4 is negligibly small. These results indicate that the variability in the testing results can not be efficiently reduced by increasing the number of analytical runs for each item when the SD_L is dominant, as seen in the case of the two pesticides in the present study. Meanwhile, the evaluation results assuming the analysis of the composite sample confirmed that increasing the number of analytical runs has a certain degree of effect on reducing the variability of the testing results; such an effect became clear after the sample size reached a certain size where the magnitude of the first term of eq 4 diminished. It also should be noted that a larger sample size did not necessarily result in smaller variability in the testing results (Figure 3C,D). This is because SD_{obs} as a whole gradually comes close to $SD_A/\sqrt{m}$ as *n* increases, since *n* is included in all terms of eqs 3 and 4 (thus these terms come close to 0 as *n* increases), except for the second term of eq 4. For food items containing indoxacarb and etofenprox at the same concentration levels as in the present study, increasing the sample size to 20 or more would not efficiently reduce the SD_{obs} to a level that matches the effort of increased sample size, if the testing is performed by the analysis of a composite sample with only one analytical run. To

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efficiently reduce the SD_{obs}, at least two analytical runs are required for the composite sample prepared from more than 10 items. If the testing on the concentration of indoxacarb is performed by analysis of a composite sample made with 10 items, the RSD_{obs} from one and two analytical runs will be 19.2 and 17.7%, respectively. When the testing is performed by individual analysis, the SD_{obs} is expected to decrease with increasing sample size, but the effect of increase in the number of analytical runs will be small. Therefore, the RSD_{obs} is expected to be reduced effectively by selecting a sample size that is suitable for analysis of the composite sample and analyzing the sample once. If testing of indoxacarb is performed by individual analysis with a sample size of 5 and 10, the RSD_{obs} will be 23.3 and 16.4%, respectively.

The influence of the sample size and the number of analytical runs on the RSD_{obs} of nitrate concentration in each fresh vegetable is shown in Figure 4. The ratios of SD_L to SD_A of nitrate in fresh vegetables were larger than for pesticides residue. Therefore, the contribution of the first term in eq 4 is dominant, and the behaviors of variability of the RSD_{obs} are similar to pesticide residue; as a consequence, the graphs are similar for analysis of both individual items and the composite sample.

The influence of sample size and the number of analytical runs on the RSD_{obs} of sodium nitrite and acesulfame potassium concentrations in processed meat products are shown in Figure 5. The SD_L of nitrite in ham is remarkably larger than the SD_A, and the graphs are similar to those for the testing results for pesticide residue and nitrate. The ratios of SD_L to SD_A in other food additives are small, and for acesulfame potassium in sausage, they are almost equal. The results for these food additives suggest that analyzing a composite sample consisting of few items many times would reduce the RSD_{obs} efficiently rather than increasing the sample size. In the testing of acesulfame potassium in sausage, the effect was remarkable. Fixing the sample size to 5, the RSD_{obs} of one analysis of individual items will be 3.7%, and the RSD_{obs} of one and three analyses of the composite samples will be 6.4 and 4.2%, respectively. Furthermore, when the sample size is increased to 10, the RSD_{obs} of one analysis of a composite sample will be 6.1%. A comparison of the above results clearly shows that increasing the sample size is not an effective measure to reduce the RSD_{obs} when the SD_L is small enough to be comparable to the SD_A.

Conclusion. The concentrations of pesticide residues and nitrate in fresh vegetables and food additives in processed meat products were measured after preparing the samples collected from a specified lot. Variations in concentration in the lot, V_L, and the dispersion of measurements due to analytical procedures, V_A, were estimated from the measured values. On the basis of the estimated variance, the influences of the sample size and number of analytical runs on the variability in the lot average estimates (i.e., the testing results) were evaluated. In addition, it was shown that the magnitude of variability of the testing results, RSD_{obs}, could be substantially changed depending on the V_L, the sample size, V_A, and the number of analytical runs. Specifically, when the V_L/V_A ratio was large, the increase in the sample size of the composite sample beyond a certain number did not efficiently reduce the RSD_{obs}. It was also shown that the specific sample size, which was required to maintain the RSD_{obs} at an appropriate level to reduce the possibility of making an incorrect assessment, was dependent on the V_L and the V_A. Furthermore, increasing the number of analytical runs was found to be more efficient in reducing the RSD_{obs} than increasing the sample size when the V_L/V_A ratio is small.

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Notes

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ABBREVIATIONS USED

V_L, the variance in the concentrations in the lot (the lot variance); V_A, the dispersion of measurements due to analytical procedures (the analytical variance); V_S, the variance of multiple sample averages; SD_L, standard deviation of the concentration in the lot; SD_A, standard deviation of measurements due to analytical procedures; V_{obs}, the variance of the observed sample average; SD_{obs}, estimate of V_{obs} (SD of the testing result)

REFERENCES

(1) The Codex Alimentarius Commission. *Guidelines for the Assessment of the Competence of Testing Laboratories Involved in the Import and Export Control of Food*; CAC/GL27; 1997.

(2) The Codex Alimentarius Commission. *General Guidelines on Sampling*; CAC/GL50; 2004.

(3) Ambrus, Á.; Latos, J. Evaluation of the studies on decline of pesticide residues. *J. Agric. Food Chem.* **2002**, *50*, 4846−4851.

(4) Ambrus, Á.; Soboleva, E. Contribution of sampling to the variability of pesticide residue data. *J. AOAC Int.* **2004**, *87*, 1368−1379.

(5) Guillier, L.; Thébault, A.; Gauchard, F.; Pommepuy, M.; Guignard, A.; Malle, P. A risk-based sampling plan for monitoring of histamine in fish products. *J. Food Prot.* **2011**, *74*, 302−310.

(6) Fujita, M.; Yajima, T.; Iijima, K.; Sato, K. Comparison of the variability in the levels of pesticide residue observed in Japanese cabbage and grape units. *J. Agric. Food Chem.* **2012**, *60*, 1516−1521.

(7) Whitaker, T. B.; Dickens, J. W.; Monroe, R. J. Variability of aflatoxin test results. *J. AOCS* **1974**, *51*, 214−218.

(8) Whitaker, T. B.; Hagler, W. M., Jr.; Giesbrecht, F. G.; Johansson, A. S. Sampling, sample preparation, and analytical variability associated with testing wheat for deoxynivalenol. *J. AOAC Int.* **1974**, *51*, 214−218.

(9) Brera, C.; De Santis, B.; Prantera, E.; Debegnach, F.; Pannunzi, E.; Fasano, F.; Berdini, C.; Slate, A. B.; Miraglia, M.; Whitaker, T. B. Effect of sample size in the evaluation of "in-field" sampling plans for aflatoxin B(1) determination in corn. *J. Agric. Food Chem.* **2010**, *58*, 8481−8489.

(10) McClure, F. D.; Lee, J. K. Determination of operating characteristic, retesting, and testing amount probabilities associated with testing for the presence of *Salmonella* in foods. *J. AOAC Int.* **2011**, *94*, 327−334.

(11) The Department of Food Safety, Ministry of Health, Labour and Welfare of Japan. *Analytical Methods for the Residual Constituents of Pesticides, Feed Additives, And Animal Drugs in Food Items*; Notification No. 0124001; January 24, 2005 (in Japanese).

(12) Matsuda, R.; Watanabe, T.; Ikarashi, A.; Shiramasa, Y.; Maitani, T. Estimation of the daily intake of nitrate based on analysis of the total diet samples (in Japanese). *Shokuhin Eiseigaku Zasshi (J. Food Hyg. Soc. Jpn.)* **2009**, *50*, 29−33.

(13) The Department of Food Safety, Ministry of Health, Labour and Welfare of Japan. *Analytical Methods for Meat, Whale Meat, And Fish Paste Product*; Notification No. 54; March 17, 1993 (in Japanese).
(14) The Department of Food Safety, Ministry of Health, Labour and Welfare of Japan. *Analytical Methods for Acesulfame Potassium in Food Items*; Notification No. 59; December 28, 2001 (in Japanese).