

Determination of the Geographic Origin of Onions between Three Main Production Areas in Japan and Other Countries by Mineral Composition

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Onions (*Allium cepa* L.) are produced in many countries and are one of the most popular vegetables in the world, thus leading to an enormous amount of international trade. It is currently important that a scientific technique be developed for determining geographic origin as a means to detect fraudulent labeling. We have therefore developed a technique based on mineral analysis and linear discriminant analysis (LDA). The onion samples used in this study were from Hokkaido, Hyogo, and Saga, which are the primary onion-growing areas in Japan, and those from countries that export onions to Japan (China, the United States, New Zealand, Thailand, Australia, and Chile). Of 309 samples, 108 were from Hokkaido, 52 were from Saga, 77 were from Hyogo, and 72 were from abroad. Fourteen elements (Na, Mg, P, Mn, Co, Ni, Cu, Zn, Rb, Sr, Mo, Cd, Cs, and Ba) in the samples were determined by frame atomic adsorption spectrometry, inductively coupled plasma optical emission spectrometry, and inductively coupled plasma mass spectrometry. The models established by LDA were used to discriminate the geographic origin between Hokkaido and abroad, Hyogo and abroad, and Saga and abroad. Ten-fold cross-validations were conducted using these models. The discrimination accuracies obtained by cross-validation between Hokkaido and abroad were 100 and 86%, respectively. Those between Hyogo and abroad were 100 and 90%, respectively. Those between Saga and abroad were 98 and 90%, respectively. In addition, it was demonstrated that the fingerprint of an element pattern from a specific production area, which a crop receives, did not easily change by the variations of fertilization, crop year, variety, soil type, and production year if appropriate elements were chosen.

KEYWORDS: Onion; geographic origin; mineral; element; composition; liner discriminant analysis; ICP

INTRODUCTION

Laws in many countries enforce the labeling of the geographic origin of agricultural products due to consumers' demands for more information regarding foods or to maintain domestic agricultural production. Meanwhile, producers, for the sake of economic advantage, have begun to advertise their brands of high-quality agricultural products by labeling their geographic origin. The prices of agricultural products can differ widely among different production areas. This fact induces fraudulent labeling, and frauds by food-related companies have been revealed. It therefore becomes important to find ways of preventing deceptive labeling of an agricultural product's

geographic origin. The development of scientific techniques to determine the geographic origin of agricultural products is needed to cope with this issue. Various techniques have been studied based on organic constituents (1, 2), mineral contents or composition (3, 4), light- (7, 8) or heavy-element (9, 10) isotope ratios, or their combination (11, 12). Of these techniques, that based on mineral composition is currently the most commonly applied because of the prevalence of inductively coupled plasma optical emission spectrometry (ICP-OES) and inductively coupled plasma mass spectrometry (ICP-MS), which can acquire a considerable amount of data simultaneously.

We have been studying methods for using this technique to determine the geographic origin of onions (*Allium cepa* L.) based on previous studies of the geographic origin of Welsh onions (4–6). Onions are produced in many countries and are one of the most popular vegetables in the world, thus leading to an enormous amount traded internationally. In Japan, fresh onions

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constitute the largest percentage of fresh vegetables imported from abroad. In 2006, about one-third of the onions consumed in Japan were imported from abroad. The common price of an imported onion is less than half that of a Japanese one. It has therefore become necessary to develop a technique for determining the onion's geographic origin. Onions are imported into Japan from China, the United States, New Zealand, Thailand, and Australia, etc. The main Japanese areas of onion production are Hokkaido, Saga, and Hyogo; their shipping volumes in 2005 were 58, 14, and 9%, respectively, comprising 81% of the total national production. We therefore targeted these three production areas in Japan.

The technique for determining the place-of-origin in terms of element composition is based on the differences of mineral compositions between onions grown in different production areas. These differences are thought to be caused by soil features (mineral composition, pH, soil type, and particle size), temperature, rainfall, and humidity, etc. It has been suggested as well that the mineral compositions of agricultural products could vary by various factors such as fertilization, climatic condition in the cultivated year, differences in soil types, history of fields, and variety—even within a single farm field. To cope with this issue, we previously investigated the range and pattern of variance of the mineral compositions of onions grown under different conditions, with a variety of variable factors including fertilization, crop year, variety, and provenance. We revealed that the differences of fertilization, crop year, and variety affected the mineral composition of onions. The variation by those factors, however, was smaller than differences among production places when judged by combination of many elements (13). In this study, it was demonstrated that the difference of mineral compositions between onions grown in Japan and other countries (China, the United States, New Zealand, Thailand, Australia, and Chile) was larger than the differences of fertilization, crop year, variety, soil type, and production year by including onion samples with differences in these factors and by choosing appropriate elements.

Chemometrics such as cluster analysis, principal component analysis, linear discriminant analysis (LDA), soft independent modeling of class analogy, and artificial neural networks are all methods frequently used to determine the geographic origin based on multielement data (1–3, 8, 14). In these techniques, LDA, which is a parametric pattern recognition method, establishes simple linear functions as a model for discrimination. LDA is one of the techniques used most in this field. The major reason for this is as follows: (i) The application software is easily available; (ii) much statistic information regarding a model and discrimination results is available; (iii) because of its simplicity, the model can be easily understood; and (iv) the model in many cases has high prediction as well as classification accuracy as compared to other techniques. Therefore, in the present study, we applied LDA for determining the geographic origin of onions in this study.

The study's objective was to develop a technique to determine the geographic origin of onions from Hokkaido, Saga, Hyogo, and those imported into Japan. We determined 14 elements—Na, Mg, P, Mn, Co, Ni, Cu, Zn, Rb, Sr, Mo, Cd, Cs, and Ba—in 309 onion samples, including 108 from Hokkaido, 52 from Saga, 77 from Hyogo, and 72 from abroad, by frame atomic adsorption spectrometry (FAAS), ICP-OES, and ICP-MS. We established models of LDA to discriminate production places between four production places (Hokkaido, Saga, Hyogo, and abroad), Hokkaido and abroad, Saga and abroad, and Hyogo

and abroad, and cross-validated those models to verify their effectiveness.

MATERIALS AND METHODS

Samples. Samples were 309 lots of fresh onions (*A. cepa* L.) of various varieties grown under various conditions in various places in Hokkaido, Saga, and Hyogo, Japan, and those imported into Japan in 2003–2005. There were 108 samples from Hokkaido, including onions grown under various cultivation conditions and of various varieties, 52 samples from Saga, 77 samples from Hyogo, and 72 samples from abroad. Of the onion samples from abroad, 31 were from China, 12 were from the United States, 15 were from New Zealand, five were from Thailand, four were from Australia, and five were from Chile, with importations from these countries constituting almost 100% of the onions imported into Japan.

It is very important to gather representative samples of population samples as much as possible in order to develop a reliable technique. The effectiveness and reliability of the discrimination models depends on the samples used. Therefore, the details of the samples used in this study are shown in the Supporting Information.

Ten onions in each sample lot were used for analysis. A composite sample prepared by pulverizing and homogenizing 10 onions was used as a test sample to exclude individual differences in the same lot.

Determination of Mineral Contents. Analyses were conducted at three laboratories, the Hyogo Technology Center for Agriculture, Forestry and Fishery (HTCAFF), the National Food Research Institute (NFRI), and the Center for Food Quality, Labeling and Consumer Services (CFQLCS), over a period of 3 years. Methods for determining mineral contents differed partially between these laboratories or in terms of the analytical year because of installation of new instruments and changes of instruments or analytical personnel. The methods were established so as to allow accurate determination of element concentrations by analyzing certified reference materials (CRMs) of apple leaves (SRM1515) and spinach leaves (SRM1570a) from the National Institute of Standard and Technology. As well, we prepared three kinds of reference onion samples to check the methods' performance, analyzed these samples, and checked the results in three labs each year. The three samples were prepared by pulverizing, drying, and sufficiently homogenizing onions from Hokkaido, Hyogo, and Saga, respectively.

The target part of an onion sample was the bulb, excluding the orange- or brown-colored outer skins, the top edge, and the part within 1 cm from the base. Samples were prepared by homogenizing 10 onions from the same lot. The target parts of 10 onions were evenly cut into 16 or 24 pieces along the axis, and the diagonal parts were placed in beakers. Finally, they were pulverized by a mixer with ceramic cutting blades (B400, Nihon Büchi, Tokyo, Japan). Moisture was obtained by drying 5–6 g of a pulverized sample in an Al-foil cup at 70 °C for 24 h under atmospheric pressure.

Acid digestion using open vessels was performed at HTCAFF over a period of 3 years, at NFRI in the first year, and at CFQLCS in the second and third years. The procedure was the same as that reported by Ariyama et al. (4–6) with minor modifications. The acids used were 61% HNO₃ (electronics industry grade, Kanto Kagaku, Tokyo, Japan), 60% HClO₄ (atomic absorption spectrometry grade, Kanto Kagaku, Tokyo, Japan), and 50% HF (semiconductor grade, Daikin, Osaka, Japan). Five grams of pulverized sample was weighed in a Teflon digestion vessel and digested using a household hotplate. The residue after digestion was transferred with 1% HNO₃ into 50 mL volumetric flasks into which indium was added as an internal standard to give a concentration of 5 µg/L, so that a sample solution was prepared. This series of procedures was replicated three times, and blank analyses without the test portion were also replicated three times together with the sample analyses.

Acid digestion using closed vessels was performed at NFRI in the second and third year and at CFQLCS in the first year. Five grams of pulverized sample was weighed in a Teflon digestion vessel, and 10 mL of 61% HNO₃ (electronics industry grade) and 1 mL of 50% HF (semiconductor grade) were added. The sample was left at room temperature for 30 min and digested using a microwave digestion system (Ethos Plus, Milestone General, Kanagawa, Japan). The samples

were heated to 80 °C in 5 min, held for 10 min, heated to 150 °C in 5 min, held for 10 min, heated to 200 °C in 3 min, and held for 17 min. The residue after digestion was transferred into a Teflon beaker, heated on a hotplate, and dried. The sample solution was prepared by transferring it with 1% HNO₃ into 50 mL volumetric flasks in which indium was added as an internal standard to give a concentration of 5 µg/L. This series of procedures was doubled, and blank analyses without the test portion were also doubled together with sample analyses.

Extraction with 1% HCl was performed for Na measurement at HTCAFF in the first and second years and at NFRI in the first year. Three grams of pulverized test portion was weighed into a 50 mL polypropylene bottle, and 17.3 mL of 1.15% HCl was added. It was shaken using a shaker for 1 h at room temperature and filtered for use as a sample solution. This series of procedures was repeated three times, and blank analyses without the test portion were also repeated three times together with the sample analyses.

Seven elements (Na, Mg, P, Mn, Zn, Sr, and Ba) were simultaneously determined by ICP-OES including an absolute calibration method using three instruments. Seven elements (Co, Ni, Cu, Rb, Mo, Cd, and Cs) were simultaneously determined by ICP-MS including an internal standard method using three instruments. It was confirmed that linear calibration curves consisting of three standard levels have correlation coefficients of >0.998 before sample measurements. Sodium was determined by flame atomic adsorption spectrometry (FAAS) including an absolute calibration method using two instruments unless sodium was determined with enough trueness and precision by ICP-OES. For FAAS, it was confirmed that linear calibration curves consisting of four or five standard levels have correlation coefficients of >0.998 before sample measurements. These measurement conditions are shown in **Tables 1–3**.

Statistical Analysis. Mineral contents were calculated on a dry weight basis by correction of moisture content. Mineral data above and at the detection limit were used for statistical analyses. The Lilliefors test as a normality test was carried out. The difference in means between onions from Japan and those from abroad was evaluated using the Mann–Whitney test as a nonparametric test because most elements did not show normal distributions. LDA with forward or backward stepwise regression was performed by setting *p*-in and *p*-out values at 0.05 with the same weighing of each group to choose effective elements and to decrease the number of elements for classification functions. Forward or backward stepwise regression was selected, and *p*-in and *p*-out values were set so that the highest classification accuracy could be obtained when each factor was moved. These statistical analyses were carried out using the application software Statistica Pro 06J (StatSoft Japan, Tokyo, Japan).

Discrimination models established by LDA were models for four-group discrimination (Hokkaido, Hyogo, Saga, and abroad) and those between Hokkaido and abroad, Hyogo and abroad, and Saga and abroad. In this software, the coefficients of canonical variates were derived on the basis of the maximization of the correlation between canonical variates in what was called canonical correlation analysis. Actually, these coefficients were equivalent to coefficients of canonical discriminant functions that defined a hyperplane that separated each group. The functions were referred to as canonical functions and, in this paper, were used for figuring the plots of scores. Functions, referred to as group classification functions in this software, were also derived at the same time. These were discrimination models or linear discriminant functions that were introduced for each group; a case will be classified into the group for which the score was largest (15).

An established discrimination model was necessary to evaluate the efficiency of discrimination in unknown samples. We performed 10-fold cross-validations on all models established for that purpose. It was carried out 10 times to establish a model, which consisted of the same variables, excluding randomly the tenth of whole sample data, and to predict the geographic origin of samples excluded using the established model so that all samples were excluded from a model and predicted just one time. Finally, a discrimination model was evaluated by summing the accuracies of all predictions, although the models established using whole data and excluding the tenth samples had slightly different coefficients and constants in each discriminant function. These differences were little, and the models established using

Table 1. Operating Conditions for ICP-OES Instruments

ICP-ES1		
Shimadzu: ICPS-7000, version 2		
lab and year of analysis	HTCAFF first–third	
Plasma Condition		
RF power	1.0 kW	
plasma gas	8.0 L/min	
auxiliary gas	0.6 L/min	
nebulizer gas	0.6 L/min	
solution uptake rate	1 mL/min	
Signal Acquisition		
integration time	5 s	
integration	three times	
element	wavelength (nm)	observation direction
Na	589.592	axial
P	213.620	axial
Mg	285.213	axial
Mn	257.610	axial
Zn	213.856	axial
Sr	407.771	axial
Ba	455.404	axial
ICP-ES2		
Varian: Vista Pro		
lab and year of analysis	NFRI first–third	
Plasma Condition		
RF power	1.2 kW	
plasma gas	15 L/min	
auxiliary gas	1.5 L/min	
nebulizer gas	0.75 MPa	
solution uptake rate	2 mL/min	
Signal Acquisition		
integration time	3 s/peak	
integration	three times	
element	wavelength (nm)	observation direction
Na	589.592	axial
P	213.618	axial
Mg	285.213	axial
Mn	257.610	axial
Zn	213.857	axial
Sr	407.771	axial
Ba	493.408	axial
ICP-ES3		
Thermo Electron: IRIS Advantage		
lab and year of analysis	CFQLCS first–third	
Plasma Condition		
RF power	1.15 kW	
plasma gas	14 L/min	
auxiliary gas	0.5 L/min	
nebulizer gas	0.22 MPa	
solution uptake rate	1.4 mL/min	
Signal Acquisition		
integration time	20 s	
axial	10 s	
radial	10 s	
integration	one time	
element	wavelength (nm)	observation direction
Na	589.592	radial
P	213.618	axial
Mg	279.553	radial
Mn	257.610	axial
Zn	213.856	axial
Sr	407.771	radial
Ba	455.403	radial

whole data considered that these have discrimination accuracies of at least the same levels as those by cross-validations.

RESULTS AND DISCUSSION

Analyses of CRMs and Reference Onion Samples. Fourteen elements in the CRMs of apple leaves and spinach leaves

Table 2. Operating Conditions for ICP-MS Instruments

ICP-MS1	
JOEL: JMS-PLASMAX2 lab and year of analysis	HTCAFF and NFRI first
Plasma Condition	
RF power	1.3 kW
plasma gas	14.0 L/min
auxiliary gas	0.7 L/min
nebulizer gas	0.96 L/min
integration	3 times
resolution	500
element	<i>m/z</i>
Cu	63
Cd	111
In	115
Cs	133
resolution	3000
element	<i>m/z</i>
Co	59
Ni	60
Rb	85
Mo	98
ICP-MS2	
Thermo Electron: Element2 lab and year of analysis	NFRI second–third
Plasma Condition	
RF power	1.2 kW
plasma gas	16 L/min
auxiliary gas	0.8 L/min
nebulizer gas	1.1 L/min
solution uptake rate by using PFA microflow	0.05 mL/min
Signal Acquisition	
integration time	0.15 s/peak
integration	nine times
resolution	500
element	<i>m/z</i>
Cu	63
Cd	111
In	115
Cs	133
resolution	3000
element	<i>m/z</i>
Co	59
Ni	60
Rb	85
Mo	98
ICP-MS3	
HP4500: Agilent Technologies lab and year of analysis	HTCAFF second–third and CFQLCS first–third
Plasma Condition	
RF power	1.4 kW
plasma gas	14.2 L/min
auxiliary gas	0.97 L/min
nebulizer gas	1.22 L/min
solution uptake rate	0.7 mL/min
Signal Acquisition	
data point	3 points/peak
integration time	0.1 s/point
integration	three times
element	<i>m/z</i>
Ni	60
Cu	63
Rb	85
Mo	98
Cd	111
In	115
Cs	133

Table 3. Operating Conditions for FAAS Instruments

FAAS1	
Varian: SpectrAA55B lab and year of analysis	HTCAFF first–second
Flame Condition	
flame type	air–C ₂ H ₂
fuel flow rate	1.5 L/min
air flow rate	3.5 L/min
burner type	100 mm
integration	three times
element	wavelength
Na	589.0 nm
FAAS2	
Nippon Jarrel-Ash: SOLAAR969 lab and year of analysis	NFRI first
Flame Condition	
flame type	air–C ₂ H ₂
fuel flow rate	1.2 L/min
burner type	50 mm
integration	three times
element	wavelength
Na	589.0 nm

were analyzed by three labs in order to verify the methods that can obtain accurate element concentrations. **Table 4** shows the results regarding apple leaves and detection limits. The detection limits were calculated from three times of standard deviations obtained by seven or more blank analyses on each analytical method, and the highest value was set as the detection limits of the total, which are shown in **Table 4**. These results were generally in agreement with certified values as well as with each lab's results except for those of Cd. The cause that the analytical results of Cd had biases was thought to be due to the low concentration, i.e., 0.013 $\mu\text{g/g}$. Analytical results of Cd regarding spinach leaves ranged from 2.44 to 2.98 $\mu\text{g/g}$ (results not shown) and showed sufficient agreements with 2.89 $\mu\text{g/g}$ of a certified value. Analytical results of other elements regarding spinach leaves also showed sufficient agreements with certified values (results not shown). We deemed the trueness of analysis sufficient to obtain data for determining the geographic origin of onions. Fourteen elements in three reference onion samples were analyzed by three labs every year to verify the performance of the methods used by the three labs.

Table 5 shows the results and the estimations of analytical errors on reference onion samples of Hokkaido. **Table 6** shows the estimations of analytical errors on reference onion samples of Hyogo and Saga. We estimated analytical errors on a temporary basis since analytical data were limited as three laboratories, three annual analyses, and three repeat analyses. Many of the relative standard deviations of intralaboratory repeatability (RSD_r), the relative standard deviations of intralaboratory reproducibility on the analytical year (RSD_y), and the relative standard deviations of interlaboratory reproducibility (RSD_R) were less than the predicted RSD_R by the Horwitz equation (16, 17). However, several in these RSD on Na, Mg, and P were larger than the predicted RSD_R ; namely, the analyses of these elements tended to be large analytical errors on the basis of the concentration. The reminder that the analytical value accidentally had a bias could affect its result because of the limited number of data. RSD_y values of several elements were larger than RSD_R . This was because the methods including the acid digestion and the instruments were changed in annual analyses at the same laboratories. A guideline on food analysis by Codex (18) sets a criterion that the HorRat value (16) would be less than two. The HorRat value is the ratio of the observed

Table 4. Analytical Results of 14 Elements in CRM: Apple Leaves (NIST SRM1515) by Three Labs and Detection Limits

laboratory	digestion ^a	element concentration ± standard deviation (μg/g) (n = 3)													
		Na	Mg	P	Mn	Co	Ni	Cu	Zn	Rb	Sr	Mo	Cd	Cs	Ba
HTCAFF	open	FAAS1 ^b	OES1 ^b	OES1	OES1	MS1 ^b	MS1	MS1	OES1	MS1	OES1	MS1	MS1	MS1	OES1
	open	ND	2810 ± 31	1470 ± 27	53.3 ± 0.5	0.12 ± 0.02	0.91 ± 0.06	5.4 ± 0.2	11 ± 0.3	10.6 ± 0.3	27.5 ± 0.1	0.12 ± 0.01	0.024 ± 0.005	0.008 ± 0.001	48.2 ± 0.6
	open	FAAS2	OES2	OES2	OES2	MS1	MS1	MS1	OES2	MS1	OES2	MS1	MS1	MS1	OES2
NFRI	open	30 ± 0.6	2850 ± 17	1620 ± 24	51.6 ± 0.05	0.10 ± 0.008	0.88 ± 0.13	5.4 ± 0.05	13 ± 0.2	9.1 ± 1.5	27.1 ± 0.2	0.08 ± 0.01	0.022 ± 0.002	0.006 ± 0.006	49.3 ± 0.3
	closed	OES2	OES2	OES2	OES2	MS2	MS2	MS2	OES2	MS2	OES2	MS2	MS2	MS2	OES2
	open	ND	2680 ± 17	1620 ± 19	52.4 ± 0.2	0.08 ± 0.004	0.88 ± 0.04	5.7 ± 0.3	12 ± 0.0	9.8 ± 0.5	25.1 ± 0.1	0.09 ± 0.004	0.014 ± 0.0006	0.006 ± 0.0005	47.5 ± 0.2
CFQLCS	open	OES3	OES3	OES3	OES3	MS3	MS3	MS3	OES3	MS3	OES3	MS3	MS3	MS3	OES3
	certified value	ND	2660 ± 29	1510 ± 18	50.5 ± 0.5	0.09 ± 0.003	0.80 ± 0.05	5.1 ± 0.2	11 ± 0.1	9.1 ± 0.1	26.0 ± 0.3	0.09 ± 0.004	0.011 ± 0.001	0.006 ± 0.0005	46.0 ± 0.4
	detection limit	24 ± 1.2	2710 ± 8	1590 ± 11	54 ± 3	0.09 ^c	0.91 ± 0.12	5.6 ± 0.24	13 ± 0.3	10.2 ± 1.5	25 ± 2	0.094 ± 0.013	0.013 ± 0.002	0.006 ± 0.0005	49 ± 2
		30	23	27	0.1	0.01	0.27	1.7	5	0.08	0.10	0.02	0.003	0.005	0.5

^a Acid digestion methods: open means, open vessel digestion, and closed means digestion by closed system. ^b Instrument measurement methods: OES means measurement by ICP-OES and MS means measurement by ICP-MS. Details of each method are shown in Figure 1. ^c Not certified information.

Table 5. Analytical Results of 14 Elements in Reference Onion Sample of Hokkaido Conducted for 3 Years by Three Labs and Estimations of Analytical Errors on Those

lab	year of analysis	element concentration (μg/g) ± standard deviation (n = 3)													
		Na	Mg	P	Mn	Co	Ni	Cu	Zn	Rb	Sr	Mo	Cd	Cs	Ba
HTCAFF	first	180 ± 3	1190 ± 27	4280 ± 100	12.4 ± 0.3		ND	3.7 ± 0.3	13 ± 1	26.0 ± 2.7	8.3 ± 0.3	0.09 ± 0.02	0.050 ± 0.022	0.035 ± 0.002	2.6 ± 0.1
	second	180 ± 3	1100 ± 21	4520 ± 67	10.9 ± 0.2	0.017 ± 0.001	ND	3.6 ± 0.2	14 ± 1	25.3 ± 0.7	7.3 ± 0.3	0.11 ± 0.01	0.049 ± 0.001	0.031 ± 0.002	2.3 ± 0.1
	third	160 ± 4	990 ± 40	4510 ± 240	9.7 ± 0.8	0.015 ± 0.001	0.37 ± 0.11	3.9 ± 0.4	9 ± 1	24.0 ± 0.1	6.9 ± 0.3	0.09 ± 0.003	0.041 ± 0.001	0.025 ± 0.001	2.3 ± 0.2
NFRI	first	170 ± 2	1170 ± 26	4540 ± 120	12.5 ± 0.6	^a	ND	3.8 ± 0.4	14 ± 0.6	26.7 ± 1.6	6.9 ± 0.2	0.12 ± 0.02	0.040 ± 0.008	0.029 ± 0.002	2.8 ± 0.4
	second	200 ± 3	1120 ± 14	4840 ± 390	12.9 ± 0.2	0.014 ± 0.001	ND	3.9 ± 0.1	14 ± 0.4	24.1 ± 2.2	7.0 ± 0.1	0.10 ± 0.01	0.048 ± 0.004	0.023 ± 0.001	2.4 ± 0.04
	third	200 ± 8	1220 ± 4	4780 ± 38	13.1 ± 0.1	0.012 ± 0.001	ND	3.2 ± 0.1	13 ± 0.4	20.4 ± 0.4	8.2 ± 0.1	0.09 ± 0.004	0.034 ± 0.001	0.022 ± 0.0005	2.7 ± 0.01
CFQLCS	first	190 ± 3	1140 ± 28	4630 ± 35	12.2 ± 0.2	0.009 ± 0.005	ND	4.0 ± 0.6	14 ± 0.1	22.2 ± 1.3	7.7 ± 0.3	0.11 ± 0.002	0.043 ± 0.003	0.025 ± 0.0001	2.9 ± 0.3
	second	170 ± 2	1150 ± 6	4510 ± 77	12.1 ± 0.1	0.014 ± 0.001	ND	3.7 ± 0.2	13 ± 1	23.5 ± 0.5	7.4 ± 0.03	0.09 ± 0.003	0.035 ± 0.001	0.024 ± 0.0001	2.8 ± 0.04
	third	170 ± 3	1150 ± 7	4310 ± 55	12.3 ± 0.1	0.012 ± 0.001	0.35 ± 0.04	3.2 ± 0.02	13 ± 0.3	21.5 ± 0.01	7.5 ± 0.03	0.09 ± 0.003	0.035 ± 0.001	0.024 ± 0.0001	2.8 ± 0.03
grand mean (μg/g)	181	1136	4547	12.0	0.014		3.7	13	23.7	7.3	0.10	0.040	0.027	2.6	
RSD _i (%) ^c	2.2	2.0	3.7	3.1	13.6		7.8	5.1	5.8	2.7	11.2	22.7	4.8	6.1	
RSD _y (%) ^d	6.8	5.6	16.4	6.7	16.4		9.2	12.3	8.5	8.0	12.6	18.9	13.0	6.1	
RSD _R (%) ^e	4.8	3.6	3.3	7.8	16.8		1.4	7.8	6.3	1.2	3.0	19.2	18.4	7.8	
predicted RSD _R (%)	7.3	5.5	4.5	11.0	30.5		13.2	10.8	9.9	11.8	22.6	25.9	27.7	13.8	
HorRat values ^g	0.7	0.6	0.7	0.7	0.5		0.1	0.7	0.6	0.1	0.1	0.7	0.7	0.6	
RSD _t (%) ^h	8.4	6.8	5.2	10.4	24.7		10.3	14.8	11.1	8.2	14.5	30.0	22.7	10.5	

^a The analytical result was not accepted because of instrumental trouble. ^b Analysis was not conducted. ^c Relative standard deviation of intralaboratory repeatability. ^d Relative standard deviation of intralaboratory reproducibility on the analytical year. ^e Relative standard deviation of interlaboratory reproducibility. ^f Predicted RSD_R = 2 × (grand mean/10⁶)^{-0.1505}. ^g HorRat value = RSD_R/predicted RSD_R. ^h Total relative standard deviation = √(RSD_t²/3 + RSD_y² + RSD_R²).

Table 6. Estimation of Analytical Errors on Analytical Results of 14 Elements in Reference Onion Samples of Hyogo and Saga conducted for 3 Years by Three Labs

	Na	Mg	P	Mn	Co	Ni	Cu	Zn	Rb	Sr	Mo	Cd	Cs	Ba
Sample of Hyogo														
grand mean ($\mu\text{g/g}$)	76	784	2851	18.7	0.033	ND	2.7	12	2.2	4.8	0.22	0.045	ND	0.8
RSD _r (%) ^a	10.8	3.4	3.3	1.6	14.9		9.6	2.4	5.7	1.8	6.8	9.8		14.8
RSD _y (%) ^b	8.8	7.1	3.8	3.0	12.1		9.5	3.3	12.1	11.2	15.2	15.5		11.5
RSD _R (%) ^c	5.0	1.1	5.0	4.0	9.2		4.9	9.9	2.5	11.6	2.7	10.4		19.5
predicted RSD _R (%) ^d	8.3	5.9	4.8	10.3	26.7		13.8	10.9	14.2	12.7	20.1	25.5		16.4
HorRat value ^e	0.6	0.2	1.0	0.4	0.3		0.4	0.9	0.2	0.9	0.1	0.4		1.2
RSD _T (%) ^f	11.9	7.5	6.5	5.0	17.4		12.0	10.5	12.8	16.2	16.0	19.5		24.2
Sample of Saga														
grand mean ($\mu\text{g/g}$)	158	799	2530	8.6	0.013	ND	ND	8	1.1	4.7	0.15	0.040	ND	ND
RSD _r (%) ^a	3.7	3.0	4.3	2.6	16.4			2.9	2.6	4.9	6.5	9.6		
RSD _y (%) ^b	9.9	6.7	6.4	8.7	21.4			5.0	13.8	10.9	8.6	14.7		
RSD _R (%) ^c	6.4	2.0	6.2	8.0	29.2			16.8	9.9	1.2	9.4	10.3		
predicted RSD _R (%) ^d	7.5	5.9	4.9	11.6	30.9			11.7	15.7	12.7	21.4	26.0		
HorRat value ^e	0.9	0.3	1.3	0.7	0.9			1.4	0.6	0.1	0.4	0.4		
RSD _T (%) ^f	12.0	7.2	9.2	11.9	37.4			17.6	17.0	11.3	13.3	18.8		

^a Relative standard deviation of intralaboratory repeatability. ^b Relative standard deviation of intralaboratory reproducibility on the analytical year. ^c Relative standard deviation of interlaboratory reproducibility. ^d Predicted RSD_R = $2 \times (\text{grand mean}/10^6)^{-0.1505}$. ^e HorRat value = RSD_R/predicted RSD_R. ^f Total relative standard deviation = $\sqrt{(\text{RSD}_r^2/3 + \text{RSD}_y^2 + \text{RSD}_R^2)}$.

Table 7. Element Concentrations on a Dry Weight Basis in Onions from Four Production Areas

	average \pm standard deviation ($\mu\text{g/g}$)			
	Hokkaido (<i>n</i> = 108)	Hyogo (<i>n</i> = 77)	Saga (<i>n</i> = 52)	abroad (<i>n</i> = 72)
Na	116 \pm 87	107 \pm 45	137 \pm 76	343 \pm 221
Mg	925 \pm 104	798 \pm 131	861 \pm 135	948 \pm 209
P	3120 \pm 498	3071 \pm 573	2530 \pm 423	3006 \pm 601
Mn	11.4 \pm 2.5	15.7 \pm 7.7	14.7 \pm 5.6	15.4 \pm 11.0
Co	0.011 \pm 0.010	0.031 \pm 0.026	0.032 \pm 0.042	0.038 \pm 0.049
Ni	0.34 \pm 1.12	0.36 \pm 0.51	0.43 \pm 0.34	0.48 \pm 0.61
Cu	3.4 \pm 1.1	2.5 \pm 1.3	2.8 \pm 0.9	3.9 \pm 1.3
Zn	12.8 \pm 3.0	17.9 \pm 7.2	12.6 \pm 3.6	14.5 \pm 5.1
Rb	12.3 \pm 8.4	3.0 \pm 1.2	1.9 \pm 1.2	7.7 \pm 8.6
Sr	5.0 \pm 1.7	4.8 \pm 1.5	6.3 \pm 2.1	13.2 \pm 5.9
Mo	0.058 \pm 0.066	0.208 \pm 0.225	0.045 \pm 0.086	0.159 \pm 0.218
Cd	0.060 \pm 0.040	0.125 \pm 0.116	0.195 \pm 0.206	0.149 \pm 0.150
Cs	0.015 \pm 0.022	0.001 \pm 0.003	0.000 \pm 0.001	0.017 \pm 0.040
Ba	2.0 \pm 1.5	0.6 \pm 0.6	1.4 \pm 2.8	4.0 \pm 3.1

RSD_R to the predicted RSD_R by the Horvitz equation. HorRat values obtained were calculated as less than 1.4 and satisfied the criterion. The total relative standard deviations (RSD_T) were calculated as $\sqrt{(\text{RSD}_r^2/3 + \text{RSD}_y^2 + \text{RSD}_R^2)}$ by taking account of three repeat analysis. RSD_T means the analytical error estimated on this analytical method and has a different value depending on the concentration. All RSD_T were sufficiently low values, which were less than two times of the predicted RSD_R. The analytical errors obtained were sufficiently low.

The analytical results of Ni on three reference standards were not detectable. Thus, we estimated the analytical errors from results of spinach leaves of which the certified value is 2.14 \pm 0.10 $\mu\text{g/g}$. RSD_r, RSD_R, the predicted RSD_R, HorRat value, and RSD_T were calculated as 1.9, 4.2, 14.6, 0.3, and 4.4%, respectively. These analytical errors were sufficiently low. Therefore, we deemed the analytical method of mineral in this study sufficiently accurate to obtain data for determining the geographic origin of onions.

Analyses of Onion Samples. Table 7 shows the concentrations of 14 elements on a dry weight basis in onions from four production areas. Onions from abroad had larger variations in element concentrations than did those of the Japanese production areas because onions from abroad originated from a variety of

Table 8. Percentages Correctly Discriminated in Classification and Cross-Validation by the Model for Four-Group Discrimination

	no. of samples	classification (%)	cross-validation (%)
Hokkaido	108	88	86
Hyogo	77	84	87
Saga	52	94	85
abroad	72	82	81
total	309	87	85

countries. The only elements that had normal distributions at a 5% significance level by the Lilliefors test were Mg and P in Japanese onions and Mg and Zn in onions from abroad. Hence, the Mann–Whitney test as a nonparametric test was carried out to evaluate the difference in means between onions from the three production areas in Japan and those from abroad. Elements that were significant at a 1% level were Na, Mg, Co, Cu, Sr, Cs, and Ba, and any of these showed higher concentrations in imported onions than in domestically grown ones. The reason for this is not clear, but it might be because of the climate with a lot of rainfalls, Andisols, which are major soil in Japan and have been formed in volcanic ash, or both of those caused by elution from soil or less uptake of minerals in Japan. Concentration compositions among onions from the three Japanese production areas were also different.

Four-Group Discrimination. We established a model of LDA for discriminating geographic origins (Hokkaido, Hyogo, Saga, and abroad) using 309 sample data. This model consisted of 12 elements (Na, Mg, P, Co, Cu, Zn, Rb, Sr, Mo, Cd, Cs, and Ba). The classification of these samples was 87% accurate in total, and the prediction by cross-validation was 85% accurate in total (Table 8). Although the discrimination result was not high, the nonpreferred errors of cross-validation, in which Japanese onions are predicted to imported ones, occurred in only three of 237 Japanese samples (1.3%).

Figure 1 shows the plots of scores of the first vs second (a) and the first vs third (b) canonical functions. These are the projected figures of plots in a three-dimensional coordinate. The plots of onions from Hokkaido had a narrow distribution, although the onions are diversified in regard to cultivation condition and varieties. On the other hand, the plots of onions from abroad had a broader distribution than those from others

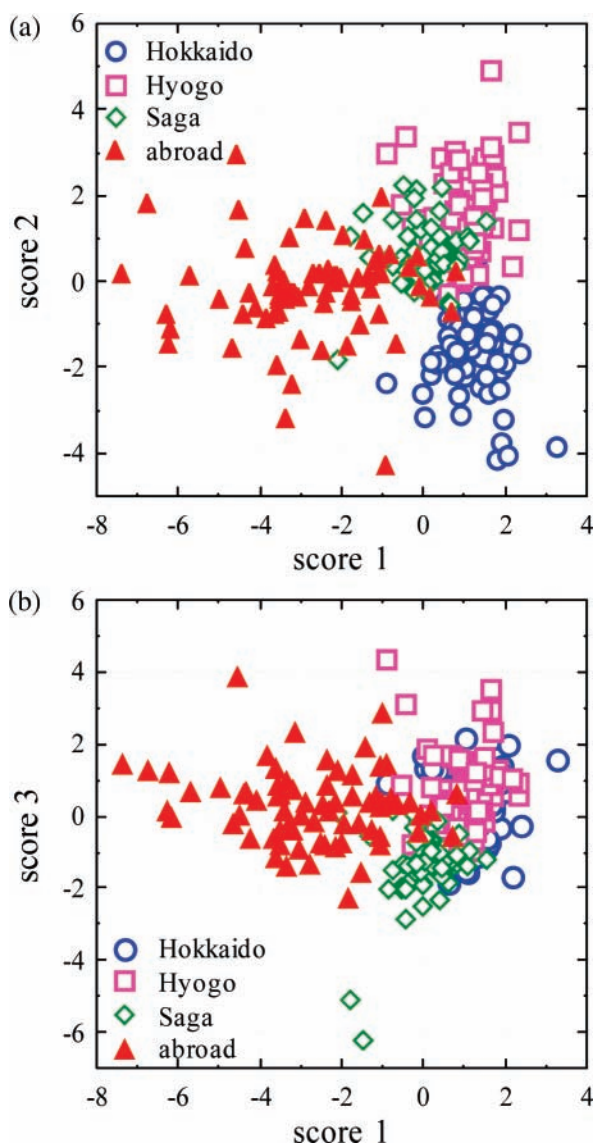


Figure 1. Plots of score 1 vs score 2 (a) and score 1 vs score 3 (b) of 309 samples by LDA model for four-group discrimination.

in Japan. The overlap between onions from Japan and those from abroad was less than those between onions from different production areas in Japan. This shows the large difference in element composition between onions from Japan and those from abroad.

Discrimination between Hokkaido and Abroad. We established a model of LDA for discriminating between onions from Hokkaido and those from abroad using 180 sample data. This model consisted of seven elements (Na, P, Mn, Sr, Mo, Cd, and Ba). The classifications of onions from Hokkaido and those from abroad were 100 and 89% accurate, respectively, and the predictions between onions from Hokkaido and those from abroad by cross-validation were 100 and 86% accurate, respectively (**Table 9**). The onions from Hokkaido used for this study included onions, which were from various production areas, 12 varieties, grown under various fertilization conditions, and grown in farm fields of various soil types and crop years ranging from the first to more than the 30th (the years onions are cultivated continuously in a single field) (see the Supporting Information). However, the nonpreferred errors of cross-validation, in which Hokkaido's onions were predicted to imported ones, were not obtained in 108 Hokkaido's samples. This result shows the fingerprint of element pattern from a

Table 9. Percentages Correctly Discriminated in Classification and Cross-Validation by the Model between Hokkaido and Abroad

	no. of samples	classification (%)	cross-validation (%)
Hokkaido	108	100	100
abroad	72	89	86
total	180	96	94

Table 10. Percentages Correctly Discriminated in Classification and Cross-Validation by the Model between Hyogo and Abroad

	no. of samples	classification (%)	cross-validation (%)
Hyogo	77	100	100
abroad	72	93	90
total	149	97	95

Table 11. Percentages Correctly Discriminated in Classification and Cross-Validation by the Model between Saga and Abroad

	no. of samples	classification (%)	cross-validation (%)
Saga	52	98	98
abroad	72	92	90
total	124	94	94

specific production area, which a crop receives, does not easily change if appropriate elements are chosen. Instead, the discrimination accuracy of onions from abroad was not high. This was thought to be due to the large variation of element composition of the onions originating in various countries.

Discrimination between Hyogo and Abroad. We established a model of LDA for discriminating between onions from Hyogo and those from abroad using 149 sample data. This model consisted of eight elements (Na, P, Mn, Zn, Sr, Cd, Cs, and Ba). The classifications of onions from Hyogo and abroad were 100 and 93% accurate, respectively, and the predictions of onions from Hyogo and those from abroad by cross-validation were 100 and 90% accurate, respectively (**Table 10**). The nonpreferred errors of cross-validation, in which Hyogo's onions are predicted to imported ones, were not obtained in 77 of Hyogo's samples. In this model as well, the discrimination accuracy of onions from abroad was not high. This was thought to be due to the same reason as that related to the model between Hokkaido and abroad.

Discrimination between Saga and Abroad. We established a model of LDA for discriminating between onions from Saga and those from abroad using 124 sample data. This model consisted of eight elements (Na, Mg, P, Mn, Rb, Sr, Mo, and Ba). The classifications of onions from Saga and abroad were 98 and 92% accurate, respectively, and the predictions of onions from Saga and abroad by cross-validation were 98 and 90% accurate, respectively (**Table 11**). The nonpreferred errors of cross-validation, in which Saga's onions are predicted to imported ones, occurred in only one of 52 of Saga's samples (2%). In this model as well, the discrimination accuracy of onions from abroad was not high. This was thought to be due to the same reason as those related to the other two models.

Application to Monitoring of Geographic Origin Labeling. Monitoring of the labeling of geographic origin has been conducted in Japan by mineral analyses regarding agricultural products such as seasoned pickled Japanese plums, garlic, ginger, carrots, and Welsh onions, although some of the monitoring techniques used have not been as complete as those proposed in this paper because of their lower sample numbers and the lack of validation of their discrimination models.

Unfortunately, it is difficult to develop a 100% accurate technique for determining geographic origin, and the techniques already developed usually cannot avoid a certain number of mistakes. The dominant reason for this is that discrimination models are practically unable to gather the element data of agricultural products from all of the farm fields in target production areas. As well, agricultural production areas can shift as the year passes, particularly those from abroad. These factors make the development of an accurate technique difficult. Monitoring using a technique based on mineral composition, therefore, is conducted as a screening inspection of merchandise on the market, and in the event that a suspicious label is identified, the truth can be confirmed by investigating invoices and tracking the flow of the sample from receipt to sale.

The discrimination models between Hokkaido and abroad, Hyogo and abroad, and Saga and abroad achieved excellent discrimination accuracies related to Japanese onions grown in Hokkaido, Hyogo, and Saga, i.e., 98–100%, although those related to onions from abroad ranged from 86 to 90%. This demonstrates that the technique with these models is preferable for use as a screening technique. Therefore, this technique is applicable to monitoring inspection in Japan, with the ability to determine geographic origin using data of 11 elements (Na, Mg, P, Mn, Zn, Rb, Sr, Mo, Cd, Cs, and Ba) obtained by ICP-OES and ICP-MS within 2 days (16 work hours).

ABBREVIATIONS USED

CFQLCS, Center for Food Quality, Labeling and Consumer Services; CRM, certified reference material; FAAS, flame atomic adsorption spectrometry; HTCAFF, Hyogo Technology Center for Agriculture, Forestry and Fishery; ICP-MS, inductively coupled plasma mass spectrometry; ICP-OES, inductively coupled plasma optical emission spectrometry; LDA, linear discriminant analysis; NFRI, National Food Research Institute; RSD_r, relative standard deviation of intralaboratory repeatability; RSD_R, relative standard deviation of interlaboratory reproducibility; RSD_T, total relative standard deviation; RSD_y, relative standard deviation of intralaboratory reproducibility on the analytical year.

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Supporting Information Available: Details of onion samples used for this study. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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