

REVIEW

The Determination Technique of the Geographic Origin of Welsh Onions by Mineral Composition and Perspectives for the Future

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Abstract

Scientific techniques for determining the geographic origin of agricultural products have attracted considerable attention in recent years. The most popular origin-determining technique is based on the composition of inorganic elements, and the technique has been applied to Welsh onions (*Allium fistulosum* L.). The technique on Welsh onions has been developed and compiled in a manual for monitoring market merchandise earlier than other origin-determining techniques in Japan. This technique makes use of chemometric analysis on concentration ratios of 19 elements (Na, P, K, Ca, Mn, Fe, Cu, Zn, Sr, Ba, Co, Ni, Rb, Mo, Cd, Cs, La, Ce, and Tl) to Mg to determine whether the geographic origin of Welsh onions sold in Japan is Japan or China. The technique is quite practical in that it can screen geographic origin within 2 days by the analysis of inorganic elements by inductively coupled plasma optical emission spectrometry and inductively coupled plasma mass spectrometry.

Discipline: Food

Additional key words: chemometrics, inductively coupled plasma mass spectrometry, inorganic elements

Introduction

In recent years, there has been increasing interest in developing methods of scientifically determining the geographic origin of agricultural products. There are a number of reasons for this interest. First, laws enforce labeling of the geographic origin of agricultural products in many countries due to demands for more information on foods by consumers and also to maintain domestic agricultural production. Secondly, producers have begun to advertise their brands of high-quality agricultural products by labeling the geographic origin for their economic advantage or to protect their brands from deceptive labeling. Thirdly, retailers require a way of preventing accidental violation of the law caused by selling merchandise acquired from wholesalers or producers which does not match its labeling. From these points of view, the development of techniques to determine geographic origin is highly desirable for consumers, agricultural farmers, retailers and administrative authorities. Thus, the first

technique for determining the geographic origin of Welsh onions (*Allium fistulosum* L.) was developed in 2004^{4–6}. The analytical manual for Center for Food Quality, Labeling and Consumer Services to monitor merchandise in the Japanese market was compiled and published on the internet in 2005⁹. In this review, we introduce techniques for determining the geographic origin of agricultural products, review the technique on Welsh onions and describe perspectives for the future.

Determination of the geographic origin of agricultural products by scientific techniques

1. Scientific techniques for determining the geographic origin of agricultural products

Reports on scientific techniques for determining the geographic origin of agricultural products have been increasing since the 1980s. The initial focus was on processed agricultural products such as wine^{10,13,16,18}, honey²², teas^{12,17}, olive oil³, and orange juice¹⁹, while later studies examined fresh products such as potatoes¹, Welsh

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onions⁴⁻⁶, pistachios², and garlic²³, chiefly because worldwide trade in fresh agricultural products has increased year after year and the law now enforces labeling of their geographic origin. The techniques reported had been based primarily on the composition of organic constituents^{19,22}, inorganic elements^{1,2,4-6,12-14,21} and isotope ratios^{3,7,18,20} or their combination¹⁰. Recently, variety-determining techniques by DNA analysis have come to be used on various agricultural items²⁴⁻²⁶ because such techniques provide high accuracy as well as the ease and rapidity of analysis. Unfortunately it is impossible to directly determine geographic origin by DNA analysis even though the variety can be determined; thus, the same variety from different target places cannot be distinguished. The technique based on the composition of inorganic elements is the most commonly applied nowadays because the inductively coupled plasma optical emission spectrometer (ICP-OES) and inductively coupled plasma mass spectrometer (ICP-MS) have become comparatively prevalent and can acquire considerable data simultaneously.

2. The determination of geographic origin by the composition of inorganic elements

The composition of the inorganic elements of a crop is greatly affected by the features of the soil, such as its composition, pH, soil type, moisture, and organic constituents. Climatic conditions such as temperature and humidity are also thought to be other contributing factors. Therefore, this technique discriminates the origin of agricultural products based on differences in the composition of their inorganic elements caused by differences in cultivation conditions. The larger the differences between production areas, the more clearly the geographic origin can be discriminated. However, the composition of the inorganic elements of a crop may vary easily because so many factors could affect it. Although there have been some reports on correlations between the concentrations

of certain elements in crops and in extracts from soils, the correlations have not yet been shown to be sufficient to predict some concentrations in a crop based on an extract from soil or vice versa^{14,21}.

3. Statistical analysis for determining geographic origin based on the composition of inorganic elements

The technique for determining the geographic origin based on the composition of inorganic elements requires a significant amount of data on many inorganic elements in many samples because it is usually extremely difficult to determine origin using only one or two elements. Multivariate data are analyzed statistically and classified into target groups by chemometric pattern recognition methods (Table 1). Unsupervised pattern recognition methods are used to simply recognize differences among obtained data, while supervised pattern recognition methods are more appropriate to establish a technique for determining geographic origin because these pattern recognition methods give clearer discrimination results. Models for discrimination are established by applying these methods on the obtained multivariate analytical data. An unknown sample is discriminated by applying the analytical data of the sample to the models. Supervised pattern recognition methods are classified to non-parametric methods, which do not hypothesize statistical distributions, and parametric methods, which do hypothesize such distributions. The former do not require a lot of data to establish models for discrimination, but it cannot be estimated statistically how correctly unknown samples are classified nor can any statistical information be acquired. The latter requires considerable data to establish models for discrimination, but it can be estimated statistically how correctly unknown samples are classified, and statistical information is also obtained. Therefore parametric methods are more commonly used to establish models for discrimination. Linear discriminant

Table 1. Typical chemometric techniques used to determine geographic origin

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| 1. Unsupervised pattern recognition method (conducting exploratory analysis and classification using only explanatory variable data) Principal Component Analysis (PCA), Cluster Analysis |
| 2. Supervised pattern recognition method (conducting analysis and classification by selecting and weighting explanatory variables to classify the target groups) |
| 2-1 Non-parametric method (does not hypothesize statistical distribution; applicable with only little sample data) K-Nearest Neighbors (KNN), Artificial Neural Networks (ANN) |
| 2-2 Parametric method (hypothesizes statistical distribution; provides considerable statistical information) Linear Discriminant Analysis (LDA), Soft Independent Modeling of Class Analogy (SIMCA) |

analysis (LDA) and soft independent modeling of class analogy (SIMCA) as parametric methods in supervised pattern recognition methods were used to establish the present models for determining the geographic origin of Welsh onions. These are methods that have frequently been used in origin-determining studies^{1,2,10,13,22}.

4. Scheme to develop an origin-determining technique

Figure 1 shows our proposed scheme for developing an origin-determining technique. First, we establish an adequate analytical method by which target elements can be accurately determined. The method must be validated by analyzing certified reference materials or recovery tests. The method validation in the present study was carried out using two kinds of certified reference materials from the National Institute of Standards and Analysis (NIST): Spinach Leaves (SRM 1570a) and Apple Leaves (SRM 1515), in the case of Welsh onions. Secondly, an appropriate number of samples must be acquired from various farm fields in target production areas. Samples should be acquired from different areas to reflect their market share, though such acquisition is the most difficult task. In the present study, 220 authentic samples of Welsh onions were acquired. Thirdly, the acquired samples are analyzed according to the established method. Fourth, chemometric analyses using the obtained multivariate data are carried out, and finally, the origin-determining technique is developed using the models

established by the chemometric analyses. The LDA and SIMCA models were established in the case of Welsh onions.

5. Positioning of the scientific origin-determining technique for surveillance

Some scientific origin-determining techniques are already used in customs inspections and border protection upon import or to survey products on the market. Unfortunately, it is difficult to develop a 100% accurate method for determining geographic origin, and the techniques, which have been developed, usually cannot avoid a certain number of mistakes. Thus, the practical application of these techniques is used primarily for screening. In the event that a suspicious sample is identified, the truth of the labeling is confirmed by investigating invoices and tracking the flow of the sample from receipt to sale. It remains essential to develop simple and rapid techniques for screening various agricultural products.

Determination of the geographic origin of Welsh onions

1. Background

The amounts of fresh vegetables imported to Japan have increased in recent years, particularly the import of fresh Welsh onions. In 2001, a provisional safeguard for Welsh onions was applied against China. On the other

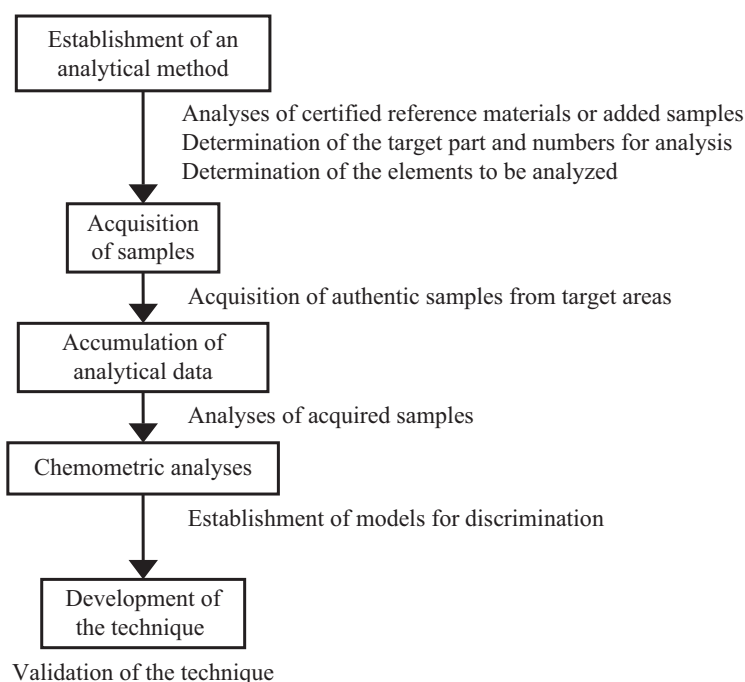


Fig. 1. Scheme for developing an origin-determining technique of agricultural products by analysis of inorganic elements



Fig. 2. Production areas (Shandong, Shanghai and Fujian) of Welsh onions imported into Japan

hand, a regulation requiring that geographic origin shall be stated on all perishable foods had already been enacted in Japan in 2000. Under these circumstances, techniques for determining the geographic origin of Welsh onions were developed. The Welsh onion is a popular vegetable in Japan and is mainly produced in East Asia. At the time of writing, most fresh Welsh onions imported into Japan are grown in Shandong, Shanghai and Fujian in China (Fig. 2). The present technique is able to determine whether a Welsh onion originated in Japan or China because an imported agricultural product must be labeled with the name of the producing country.

2. Technique based on 20 elements⁵

This technique determines the geographic origin of a Welsh onion by determining the concentrations of 20 elements: Na, P, K, Ca, Mg, Mn, Fe, Cu, Zn, Sr, Ba, Co, Ni, Rb, Mo, Cd, Cs, La, Ce, and Tl. The target part is the lower 10 cm of a Welsh onion (Fig. 3), which is cut and pulverized. About 2.5 g of pulverized test samples are taken in Teflon vessels. The test portion is digested by open-vessel digestion on a hotplate with HNO_3 and HClO_4 , or by closed-vessel digestion using a Microwave system with HNO_3 . Digested residues are transferred with 1% HNO_3 into a 25-mL volumetric flask, into which indium was added as an internal standard. The concentrations of the 20 elements in the prepared solutions are determined with ICP-OES and ICP-MS. The geographic origin of a Welsh onion is determined by analyzing the results of 20 elements by LDA and SIMCA. This series of procedures can be performed in 3 days.

Ninety-five percent of 103 Welsh onion samples from Japan or China were classified correctly using the LDA model; 94% of another 89 samples were predicted correctly. Ten models were established in SIMCA using 103 samples. Ninety-six percent of a total of 192 samples including the 103 samples used for modeling were predicted correctly. In discrimination by combined LDA and SIMCA, none of the 81 Japanese samples were judged to be Chinese, and only 8 of 111 Chinese samples were judged to be Japanese.

Using this method, it is possible to determine whether a Welsh onion sold in Japan is Japanese or Chinese, but the process needs 3 days. The technique described in the following section was developed in order to discriminate geographic origin more rapidly.

3. Technique based on the concentration ratios of 19 elements to Mg⁶

This technique determines the geographic origin of a Welsh onion based on the concentration ratios of 19 elements, Na, P, K, Ca, Mn, Fe, Cu, Zn, Sr, Ba, Co, Ni, Rb, Mo, Cd, Cs, La, Ce, and Tl, to Mg (hereinafter referred to as 19-element/Mg composition). Using these ratios allows for the simplification of sample preparation, and the geographic origin of a Welsh onion can be determined within 2 days. The lower 10 cm of the onion is cut and pulverized together with approximately the same amount of superpure water using a mixer with ceramic blades (B400, Büchi, Tokyo Japan). About 5 g of pulverized test sample are taken in Teflon vessels. Acid digestion is carried out by open-vessel digestion on a hotplate with HNO_3 and HClO_4 , or by closed-vessel digestion using a Microwave system with HNO_3 . Indium as an internal standard is added to digested residue in the vessels and 1% HNO_3 is added to achieve a total volume of c.a. 25 mL. The digested residues are dissolved by heating and prepared into sample solutions by filtering with membrane filters. Twenty elements in the prepared solutions were determined with ICP-OES and ICP-MS. The geographic origin of a Welsh onion is determined by analyzing the results of 19-element/Mg obtained by LDA and SIMCA. This series of procedures can be performed in 2 days.

Ninety-seven percent of 101 Welsh onion samples originating from Japan or China used for LDA modeling were classified correctly using the LDA model. Figure 4 shows the plots of 3 canonical functions established by LDA on these 101 training samples. The patterns of element concentrations in Welsh onions were different not only between China and Japan but also differed among the 3 areas of origin in China. Ninety-three percent of a further 119 samples were predicted correctly by this



Fig. 3. The target part for analysis of Welsh onion

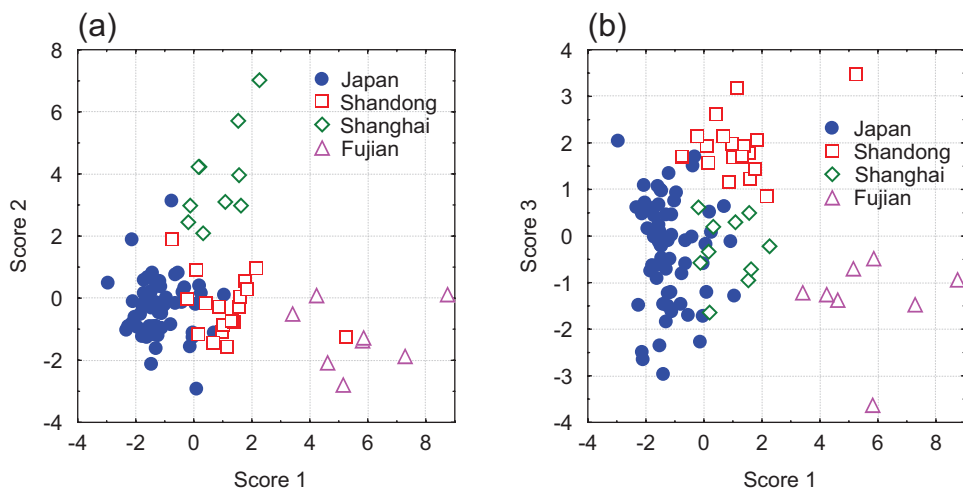


Fig. 4. Plots of score 1 versus score 2 (a) and score 1 versus score 3 (b) of 3 canonical functions established by LDA on 101 training samples⁶

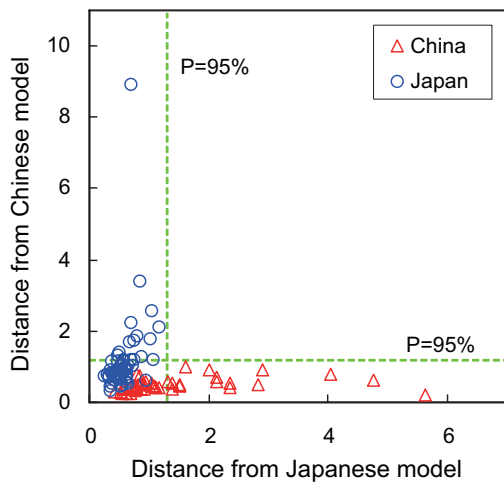


Fig. 5. Coomans plot on 60 Japanese and 41 Chinese training samples by SIMCA based on 19-element/Mg

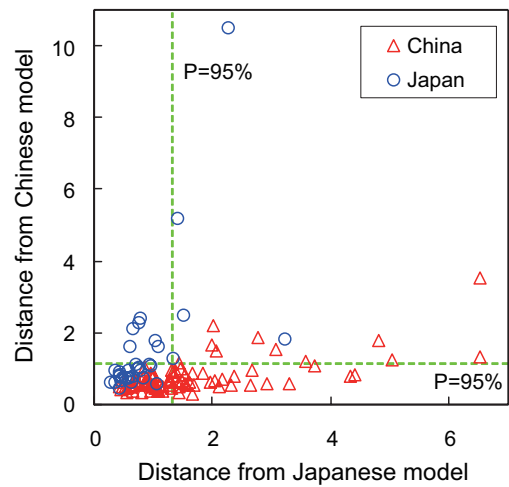


Fig. 6. Coomans plot on prediction of 34 Japanese and 85 Chinese samples by SIMCA based on 19-element/Mg

model. Ten models were established in SIMCA using the data from the 101 samples. Ninety-two percent of 220 samples including the 101 samples used for modeling were predicted correctly. Figures 5 and 6 show plots of training and prediction samples, respectively, by SIMCA models. These plots were referred to Coomans plots showing distances of samples from each class. Although many of the samples belong to both areas of Japan and China in the Coomans plots, classification by distances from both classes could be done. In discrimination by combined LDA and SIMCA, all 94 Japanese samples were correctly judged to be Japanese, and only 17 of 126 Chinese samples were judged to be Japanese.

Using this method, it is possible to determine within 2 days (16 working hours) whether a Welsh onion sold in Japan is Japanese or Chinese.

Perspectives for the future

1. Necessity of validation

Any newly developed analytical method requires method validation; harmonized collaborative validation, multiple-laboratory validation or single-laboratory validation. Harmonized collaborative validation is the most desired of these, however, it requires considerable time and effort. The protocol¹⁵ requires valid data obtained on a minimum of 5 kinds of samples at each of a minimum of 8 laboratories in this validation. Conducting harmonized collaborative validation poses a number of problems, however, because of the limited number of laboratories which have the necessary equipment, particularly an ICP-MS and HClO₄-usable draft chamber or a microwave digestion system, in the case of Welsh onions. If harmonized collaborative validation cannot be conducted, multiple-laboratory validation with fewer laboratories could be conducted, or as a final option, single-laboratory validation, which should be carried out in any case as a basic step of method development. Single-laboratory validation includes analyses of certified reference materials with similar matrix and element concentrations in the target sample or recovery test. To date, little harmonized collaborative or multiple-laboratory validation has been performed on techniques for determining geographic origin by inorganic elements. Nevertheless, conducting these validations is demanded in order to implement the technique in customs inspections, import control or surveillance of merchandise on the market.

2. Technique based on the heavy elements isotope ratio

At present, the technique based on the composition of inorganic elements is the most commonly applied to

determine the geographic origin of agricultural products, particularly in Japan. However, it takes considerable time to develop the technique because considerable analytical data are required. Additionally, a certain number of discrimination errors cannot be avoided, and constant updating of the discrimination model is also necessary.

Another effective technique for determining geographic origin is that based on the heavy elements isotope ratio. Techniques using Sr (⁸⁷Sr, ⁸⁶Sr)²⁰ and Pb (²⁰⁴Pb, ²⁰⁶Pb, ²⁰⁷Pb, ²⁰⁸Pb)⁷ isotope ratios have already been reported for brown rice²⁰ and wine⁷. These elements vary their isotope ratios depending on the age of the bedrock¹¹. The isotope ratios of these heavy elements in plants reflect those of soils which are generated by the weathering of minerals in the bedrock because the isotope ratios in plants and those of extracts from the soil are almost identical²⁷ and the isotopes are not fractionated in plants⁸. Therefore, if the isotope ratios of extracts from soils are different enough between target areas, this technique can be an effective tool. Furthermore, it is possible that a geographic origin can be traced from the isotope ratios of a crop and vice versa. However, there remain some problems with this technique as well. The thermal ionization mass spectrometer (TIMS) or multicollector inductively coupled plasma mass spectrometer (MC-ICP-MS), which are expensive and require strict maintenance and measurement conditions and manipulative skills, must be used to measure an isotope ratio at high precision because the differences between isotope ratios are small between different geographical areas. For the same reason, careful and time-consuming pretreatment of samples is necessary; MC-ICP-MS does not require as strict pretreatment of samples as TIMS. However, isotope ratios can be measured in only a small number of laboratories at present because laboratories with MC-ICP-MS are limited.

3. Expansion of target items by the technique based on the composition of inorganic elements

The origin-determining technique based on inorganic composition is the most popular technique and is used at many laboratories due to its ease of implementation. However, these techniques which have already developed remain limited in terms of the number of agricultural products to which they can be applied; it is desirable to further develop them for application to more and more products. The analytical method using multielements in foods has already become popular, and information on efficient elements for origin-determining has also been accumulating. Therefore, we have now entered a phase in which it is possible to expand target items of the origin-determining technique based on the composition

of inorganic elements in order to achieve and maintain fair trade of foods. In addition, it is desirable to regularly update discrimination models of developed techniques by keeping and obtaining analytical data to maintain the reliability of these discrimination models.

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