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Analytical Methods

Determination of trace elements in soybean by X-ray fluorescence analysis and its application to identification of their production areas

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ABSTRACT

Trace elemental analysis of soybeans was performed using X-ray fluorescence (XRF) analysis in order to characterise the geographical origins of the beans. By optimising the measurement conditions of an energy-dispersive XRF spectrometer equipped with three-dimensional polarisation optics, determination of trace elements at the sub-$\mu g\ g^{-1}$ level in soybean samples was accomplished. Forty-six samples were analysed. Results showed that there were some differences between the trace element contents, reflecting a difference in their geographical origins. A statistical analysis showed that the concentrations of eight elements (Mg, P, Cl, K, Mn, Cu, Br, and Ba) are good parameters for constructing a discriminant function for geographical origin. In conclusion, we were able to accurately distinguish between domestic and imported soybeans. The present work demonstrates that XRF is useful as a rapid and simple tool for provenance analyses of agricultural products.

1. Introduction

It has recently been reported that unscrupulous producers have been mislabelling the geographical origins of their food products. It is well known that the Japanese are dependent on imported products for most of their food. Japan's food self-sufficiency rate ratio is approximately 40%, which is the lowest among major industrially advanced countries. In particular, the self-sufficiency ratio for grains such as wheat and soybean has declined, reaching 25% in 2000 (Fujita, 2003, chap. 2).

Because the prices of agricultural products can vary greatly according to their geographical origins, regulations, for example, in both Japan and abroad that require labelling of the geographical origins of all perishable foods were enacted in 2000. However, since the legislation was passed numerous instances of mislabelling has been detected, further deepening consumer concerns regarding food safety. Therefore, the development of techniques to scientifically determine the geographical origins of agricultural products has been requested to guarantee food safety (Horita, 2008). These techniques are required to prevent the deceptive labelling of provenance with good certainty.

Numerous approaches to this provenance analysis have already been reported. These methods are based on trace element signatures (Camargo, Resnizky, Marchevcky, & Luco, 2010; Franke, Hadorn, Bosset, Gremaud, & Kreuzer, 2008; Galgano, Favati, Caruso, Scarpa, & Palma, 2008; Pilgrim, Watling, & Grice, 2010), organic constituents like free amino acids (Campo et al., 2009), isotope ratios of light or heavy elements isotope ratios (Fortunato et al., 2004; Franke et al., 2008; Pilgrim et al., 2010), or some combination of the above. Of these approaches, the one based on trace element compositions is currently the most commonly applied because trace element signatures are transferred from the environment during crop cultivation and from the underlying geology and soil composition. Consequently, trace element signatures have the potential to uniquely identify the provenance of an enormous range of naturally occurring foodstuffs. Considerable research has already been performed to determine food origins based on trace element analysis using inductively coupled plasma atomic emission spectrometry (ICP-AES) and inductively coupled plasma mass spectrometry (ICP-MS), specially for garlic (Camargo et al., 2010), beef (Franke et al., 2008) and wine (Galgano et al., 2008). The accuracy of discrimination isn't clearly defined and most previous papers have reported accuracies of approximately 70–100%.

Commonly, trace elements in food have been determined using ICP-AES/MS, which allows for the simultaneous analysis of various trace elements. However, the preparation of samples for ICP-AES/MS includes acid digestion, which requires a chemical laboratory with a liquid-waste treatment facility, making this method difficult to use for daily quality checks in foodstuff markets. Moreover, the

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analysis requires the hiring of a specialist who knows how to handle the samples and the required chemicals and equipment. Therefore, reliable results can only be obtained by analysts who are skilled in these techniques. In contrast, sample preparation for XRF requires preparation of a disk from a powdered sample, which does not require a specialist or any chemical laboratory. In addition, XRF is not as highly dependent on operator skill as ICP-AES/MS, and it provides highly reproducible results. Therefore, XRF would potentially be useful in daily spot checks as a part of quality control procedures at the market. However, the conventional XRF spectrometer has much lower sensitivity at the μg g^{-1} level compared to ICP-AES/MS (Vandecasteele, & Block, 1993). It has generally been thought that the XRF technique is not suitable for quantitative analysis of trace elements in agricultural products. In this study, an energy-dispersive XRF spectrometer equipped with three-dimensional polarisation optics was used. The instrument was also equipped with a Gd anode X-ray tube operating at a high voltage of 100 kV, which is suitable for analysis of trace heavy elements with a selection of various secondary targets (Mizuhira & Iwase, 2003). Utilising these advantages of the XRF spectrometer, we attempted to develop a useful analytical technique for quality control of food commodities that require routine measurements. In our previous research, we have developed a quantification technique for trace elements in food at sub-μg g^{-1} levels that was found to be suitable for provenance analyses of spinach leaves (Yanada, Hokura, Matsuda, Mizuhira, & Nakai, 2007), wheat flour (Otaka, Yanada, Hokura, Matsuda, & Nakai, 2009), and coffee beans (Akamine, Otaka, Hokura, & Nakai, 2010). The aim of the present study was to develop a rapid and simple technique for identifying the place of origin of soybeans by applying XRF to the determination of trace elements in soybeans. Although many types of processed food products such as soy sauce, tofu, and some healthy foods are made from soybeans, the self-sufficiency rate for soybeans in Japan is approximately 25%. Most of the beans are imported from foreign countries such as the USA, Canada, and China (Ministry of Agriculture, Forestry and Fisheries of Japan, 2010). Furthermore, there is a significant difference between the price of domestic soybeans (grown in Japan) and soybeans imported from elsewhere. However, there have been no reports about developing a technique for the provenance analysis of soybeans. Although a method for provenance analysis of black soybeans, which are similar to soybeans, has been previously developed, trace element concentrations in this method are obtained using a complicated analytical technique utilising ICP-AES/MS (Homura, Suzuki, Kosaka, Horita, & Yasui, 2006). Therefore, it is worthwhile to develop a rapid and simple technique for identifying soybean origins through the use of XRF. We have developed a technique for determining trace elements in soybeans by using an energy-dispersive XRF spectrometer equipped with three-dimensional polarisation optics. The measurement conditions for the XRF were optimised, focusing on the speed of analysis as well as high sensitivity and accuracy. Finally, a discriminant function was constructed by statistical analysis of the obtained trace element concentrations to distinguish between soybean samples cultivated in Japan and those imported from abroad.

2. Materials and methods

2.1. Samples

The samples consisted of 46 different lots of soybeans. Twenty-three of the samples had been grown in various places in Japan, and the other 23 were imported into Japan. The major soybean-producing area in Japan is Hokkaido. Out of the 23 imported soybean samples, eight were from the USA, seven from Canada, seven from China, and one was a mixed soybean sample from two countries (the USA and Canada). All samples were unprocessed. Natural soybean samples were used while developing the sample preparation procedures and optimising the XRF measurement conditions. The quantitative analysis was performed with eight reference materials with matrices similar to those of the soybean: four standard reference materials supplied by the National Institute of Standards and Technology (NIST), SRM 1267a Wheat Flour, SRM 1570a Spinach Leaves, SRM 1515 Apple Leaves, and SRM 1573a Tomato Leaves, and four certified reference materials supplied by the Japanese National Institute for Environmental Studies (NIES), CRM 7501 and 7502a White Rice Flour and CRM No. 10-a and 10-b Unpolished Rice Flour.

2.2. Instruments

The spectrometer used in this study was an Epsilon 5 (PANalytical, The Netherlands), an energy-dispersive XRF spectrometer equipped with three-dimensional polarisation optics. The optical system of the Epsilon 5 involves a three-dimensional polarisation geometric arrangement among the X-ray tubes, a secondary target, the sample, and the detector, defined by three orthogonal axes (Bisgard, Laursen, & Nielsen, 1981). With this geometry, a significant reduction of the background in the X-ray fluorescent spectrum can be achieved. As such, trace element analyses of food and plant materials, which include mainly light elements (C, N, H, and O), are feasible in the low μg g^{-1} range (Margui, Queralt, & Hidalgo, 2009).

2.3. Sample preparation procedure

In XRF, the intensity of the measured X-ray radiation is influenced by variations in the physical sample characteristics, including particle size, homogeneity, and surface conditions (Honma, 2005). Normally foodstuff samples are dried in an oven, as in the cases of spinach leaves (Yanada et al., 2007) and wheat flour (Otaka et al., 2009). Like coffee beans, soybeans contain a significant amount of oil (Akamine et al., 2010). We therefore used a freeze-dry pretreatment. For easy handling, soybean samples were washed thoroughly with ultra pure water to remove surface dust and were freeze-dried at −20 °C for 24 h. The samples were then ground into a fine powder by a ball mill (Retsch MM400, Germany) for approximately 1 min. A zirconium jar (25 mL) and ball (20 mm diameter) were used to avoid contamination. Grinding resulted in a uniform grain size of approximately $\leqslant 50$ μm. A previous study (Margui et al., 2009) for plant samples showed that when the grain size is $\leqslant 710$ μm, the measured X-ray intensity becomes constant. Therefore, the aforementioned grinding time was considered sufficient for XRF analysis. Fixed amounts of samples were weighed and pressed at 98.1 MN m^{-2} for 3 min to produce compact pellets of 20 mm diameter, which is suitable for the detection area of the spectrometer, i.e., ~13–15 mm in diameter. After the pellet was positioned on a plastic cup with a polycarbonate film (2.5 μm in thickness, Mylar®), the cup was set on the spectrometer sample holders.

Food materials are mainly made up of C, H, N, and O, which are almost transparent to X-rays. Thus, the intensities of heavy trace elements in food matrices are affected by differences in sample thicknesses. It is therefore recommended that pellets be prepared having infinite thicknesses. In this work, nine pellets with different thicknesses (~2–15 mm) were prepared to determine an infinite thickness.

2.4. Experimental conditions

Experimental conditions were optimised using actual soybean samples to accurately determine the trace elements present in