Vol. 11, No. 4, 2020
ISSN 2233-4203/ e-ISSN 2093-8950
ARTICLE
www.msletters.org | Mass Spectrometry Letters

Development of a Mushroom Powder Certified Reference Material for Element Analysis

Tegegn Gizachew Betru[1,2], Yong-Hyeon Yim[1,2], and Kyoung-Seok Lee[1]*

[1]Inorganic Metrology Group, Korea Research Institute of Standards an Science (KRISS), Dajeon 34113, Korea
[2]University of Science and Technology (UST), Dajeon 34113, Korea

Received December 17, 2020; Revised December 23, 2020; Accepted December 24, 2020
First published on the web December 31, 2020; DOI: 10.5478/MSL.2020.11.4.108

Abstract : A certified reference material (CRM) for the analysis of nutrient elements in an edible mushroom (*Ganoderma lyceum*) powder has been developed (KRISS CRM 108-10-011). The mass fractions of calcium (Ca), iron (Fe), and zinc (Zn) were measured by isotope dilution inductively coupled plasma mass spectrometry (ID ICP/MS). To dissolve the fungi cell wall of mushroom consisted of chitin fibers, sample preparation method by single reaction chamber type microwave-assisted acid digestion with acid mixtures was optimized. The mean measurement results obtained from 12 sample bottles were used to assign as the certified values for the CRM and the between-bottle homogeneities were evaluated from the relative standard deviations. The certified values were metrologically traceable to the definition of the kilogram in the International System of Units (SI). This CRM is expected to be used for validation of analytical methods or quality control of measurement results in analytical laboratories when they determine the mass fractions of elements in mushroom or other similar samples.

Keywords : mushroom, CRM, nutrient element, ID ICP/MS, metrological traceability, KRISS

Introduction

Mushrooms are representative species of fungi and edible species have been consumped as popular foods in many parts of the world since ancient times.[1] Not only tatste and aroma but also various nutrients such as protein, vitamins, and minerals, make mushrooms attractive foods. Becasue of their health benefits, mushrooms also have been used as important traditional medicines. Nowadays, mushrooms are important as specialty crops worth growing for high profit in agriculture.[1]

Rich minerals in mushrooms are one of the important features for both of foods and traditional medicine. To evaluate their qualities and health benefits, accurate analyses of nutrient elements have also been important. To obtaine realiable results in testing laboratories, verification or validation of the method applied for their analytical procedure are required. One of the good approaches for method validation has been the use of certified reference material (CRM) which has a matrix as close as possible to the matrix of the sample.[2,3]

There are various CRMs for food analysis. However, for mushroom, it cannot be said that CRMs developed for agricultural products such as grains or vegetablesseems fit well for the purpose. In taxonomy, mushrooms are classified as kingdom fungi which is completely differet from kingdom plantae consisting of normal plants. The cell walls of mushrooms mainly consist of chitin fibers while those of plants contain celluose fibers. However, it is difficut to find out CRMs commercially available for the analysis of nutrient elements in mushroom matrix. To the extent of our knowledge, only one mushroom CRM was developed by researchers of Health Sciences Authority (HSA) in Singapore and they reported the certified values of mass fractions of arsenic, cadmium, and lead for hazardous element and only calcium for nutrient element.[4]

In this work, we have reported the analytical procedure for the development of a CRM for the analysis of nutrient elements in mushroom powder in accordance with ISO 17034 and ISO Guide 35: production of the candidate CRM from mushroom raw material; characteriztion and homogeneity evaluation for mass frctions of Ca, Fe, and Zn by a primary method of measurment based on ID ICP/MS; assignment of the certified values and measurement uncertaity evaluation with metrological traceability to the definition of kg of the SI.[5-7,] We carefully select the

*Reprint requests to Kyoung-Seok Lee
E-mail: kslee@kriss.re.kr

mushroom species of *Ganoderma lucidum* (known as Lingzhi or Reishi) as the CRM material because this mushroom has been used not only for food but aloso for traditional medicine. Therefore, this CRM is expected to be a good reference to improve reliability for the analyses of nutrient elements in mushroom or other similar samples in anlytical laboratories.

Experimental

Chemicals and equipment

Standard solutions of Ca, Fe, and Zn with metrologically traceable to the SI were produced by gravimetry and certified by KRISS (Daejeon, Korea). Enriched isotope materials of ^{44}Ca, ^{57}Fe, and ^{68}Zn were purchased from Oak Ridge National Laboratory (Oak Ridge, TN, USA) and dissolved in 5% nitric acid. Nitric acid (~68%, electronic grade, ChemiTop Co., Ltd., Jincheon, Korea) were purifed by sub-boiling distillation system (duoPUR, Milestone SRL, Sorisole, Italy). Hydrogen peroxide (30%, Sigma Aldrich, MO, USA) and hydrofluoric acid (Merck, Germany) were used without further purification. Deionized water (resistivity > 18 MW×cm, Puris, MIRAEST Co., Ltd., Anyang-Si, Korea) was used for all solution preparation or cleaning procedures.

Production of the Refernce Mateiral

The candidate CRM was prepared in well controled environment according to a procedure developed by KRISS. Briefely, mushroom powder about 20.2 kg was purchased in a local market and spiked with standard solutions of arsenic (As), cadmium (Cd), chromium (Cr), lead (Pb), and mercury (Hg), which were planned to be characterized for the analysis of hazardous elements. But, nutrient elements in the candidate CRM in this work characterized as inherent level without further spiking. The mushroom paste was mixed thoroughly for two days using a mixer (YSM70, YOUNGSONG Co., Ltd., Seoul, Korea) with fluoropolymer coated bowl and agitator. The homogenized paste was dried by freeze-dryer (PVTFD 100R, IlShinBioBase Co., Ltd., Dongducheonsi, Korea). Then, the dried flakes were ground to powder by variable speed rotor mill ((Pulverisette 14, FRITSCH, Idar-Oberstein, Germany) at 12,000 rpm and passed through a 0.5 mm sieve. The blades and sieve ring were made of titanium. Only fine particles with sizes ranged from 50 μm to 250 μm were selectively collected by a vibrating sifter (V/SIFTER-14, Daega Powder Systems, Seoul, Korea) equipped with two different mesh nylon sieves. The collected powder was homogenized by a V-blender (Daega Powder Systems, Seoul, Korea) at 15 rpm for 10 h. All surfaces of both sifter and V-blender contacting powder were coated by fluoropolymer. The homogenized material was divided into acid-cleaned amber glass bottles (60 mL, Fisher Scientific Inc., PA, USA) with more than 10 g. Finally, the sealed bottles were sterilized by γ-ray irradiation of ^{60}Co at approximately 25 kGy (Greenpia Technologies, Yeoju, Korea) and, then, stored at room temperature without exposure to direct light.

Sample preparation

Based on microwave-assisted acid digestion, various acid mixtures of nitric acid (~68% $HNO_3(aq)$), hydrogen peroxide (~30% $H_2O_2(aq)$), hydrofluoric acid (~48% $HF(aq)$), and hydrochloric acid (~30% $HCl(aq)$) combined with different reaction temperatures of 220°C and 250°C were tested. In PTFE degestion vessels, each 0.3 g (or 0.5 g) of powder was taken. Different volume (6 mL, 7 mL, 8 mL or 9 mL) of concentrated nitiric acid and, additionally, 1 mL (or 2 mL) of hydrofluoric acid (or hydrogen peroxide or hydrochloric acid) was added to each of digestion vessels. Then, they were digested by single reaction chamber (SRC) type microwave-assisted digestion system (ultraCLAVE, Milestone SRL, Sorisole, Italy) increasing the temperature to 250°C in 60 min (30 min for 220°C) and maintaining the temperature for 30 min. The digested solutions were recovered into 50 mL pre-cleaned centrifuge tubes, which were subjected to centrifugation at 9000 RPM for 20 min by a centrifuge (Theremo Scientific Inc., USA) and, then, any residuals in the bottom of the sample solution were carefully inspected to evaluate completeness of sample dissolution. Finally, for characterization by ID ICP/MS, 8 mL nitric acid and 1 mL hydrofluoric acid for 0.3 g mushroom powder were used with digestion condition of ramping-up to 250°C for 60 min and maintianing for 30 min by a SRC microwave-assisted digestion system.

Characterization by ID ICP/MS

Measurement procedures for characterization of mass fractions of elements in a candidate CRM by applying ID ICP/MS were described in detail previously.[8,9] To obtain

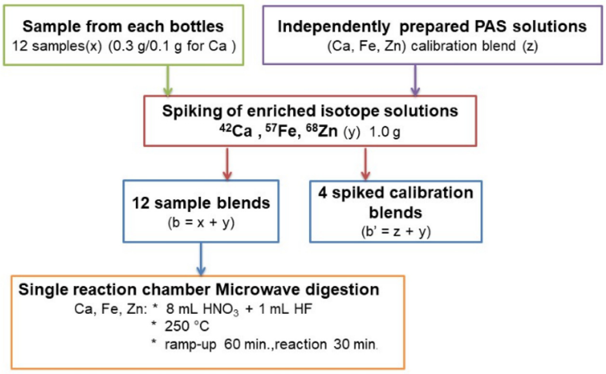

Figure 1. Schematic diagram for sample preparataion for Ca, Fe, and Zn in mushroom powder by ID ICP/MS, where x, y, and z denoted for sample, enriched isotope, and standard solution, respectively.

accurate mass fractions by ID ICP/MS in which calibration strategy of one point exact matching has been used, preliminary anlyses were carried out to determine mass fractions of elements in the candidate mushroom RM. Then, based on the preliminary results, optimal isotope ratios considering measurement uncertainty propagation, dilution factors of elemental stadnard solutions and enriched isotope solutions, and amount of these diluted solutions to be used were calculated. In a PTFE digestion vessel, 0.3 g (0.1 g for Ca) of mushroom powder was taken from each of the selected 12 bottles of the candidate RM batch and enriched isotope solution with calculated amount and concentration was spiked to each sample. Then, microwave-assisted acid digestion was performed according to the sample preparation procedure developed in this work. The sample preparation for characterization by ID ICP/MS is summarized in Figure 1.

For dry-mass correction, three subsamples of 0.5 g were taken from each of 12 corresponding bottles simultaneously with taking the samples for ID ICP/MS and placed in desiccators with P_2O_5 as drying reagent. The dry-msss correction factor was obtained from the mass changes after drying for one week.

Isotope ratios of ^{44}Ca/^{42}Ca, ^{56}Fe/^{57}Fe, ^{66}Zn/^{68}Zn in the isotope diluted sample solutions (sample blends) and the isotope diluted standard solutions (calibration blends) were measured using a double focusing magnetic sector type ICP/MS (Element 2, Thermo Scientific Inc., Bremen, Germany). Because of hydrofluoric acid in the sample solutions, sample introduction parts made of PFA and platitum were applied. To avoid polyatomic interferences, isotope ratios were measured at medium resolutoin of ca. 4000 (Table 1).

Table 1. Experimental parameters for ICP/MS measurements for Ca, Fe, and Zn in mushroom powder.

Experimental parameter	Specification or operating conditions
Instrument	ICP-SFMS (Element 2, Thermo-scientific Inc., Bremen, Germany)
Cones	Nickel, H-type skimmer
Spray chamber	Cyclonic (PFA, 50 mL)
Nebulizer	Microconcentric (PFA, 400 µL/min)
Injector	Platinum, 1.0 mm diameter
Gas flow	15 L/min (Cool gas), 0.8 mL/min (Auxiliary gas), 0.8 L/min (sample gas)
RF power	1230 W
Resolution	~4000 (medium resolution mode)

Determination of mass fractions

The mass fractions were determined according to the model equation as follows:[9-11]

$$w_x = w_z \cdot \frac{m_{y,xy} m_z}{f \cdot m_x m_{y,yz}} \cdot \frac{R_y - R_{xy}}{R_{xy} - R_x} \cdot \frac{R_{yz} - R_z}{R_y - R_{yz}} \cdot \frac{\Sigma R_{xi}}{\Sigma R_{zi}} - w_b \qquad (1)$$

where the subscript x, y, and z represent sample for analysis, enriched isotope material, standard solution with mass fraction as certified value, respectively. Sample blend (xy) and calibration blend (yz) are prepared by spiking of the enriched isotope material (y) to the sample (x) and the standard solution (z). The variables, w, m, and R, are the mass fraction, mass, and isotope ratios of those represented by the subscripts, respectively. f is the dry-mass correction factor. SR_{xi} and SR_{zi} denote the sum of isotope abundance ratios in the sample (x) and in the standard solution (z), respectively. Then, the mass fraction of the sample, w_x, can be obtained by substraction of mass fraction of the procedural blank, w_b. Based on the model equation, the measurement uncertainties were estimated following the ISO Guide to the Expression of Uncertainty in Measurement, which is described in detail elsewhere.[12]

Results and Discussion

Sample preparation

To characterize accurately mass fractions of elements in the candidate CRM by applying ID ICP/MS, it is critical to reach an dynamic equilibrium between inherent isotopes in the sample and spiked isotopes during sample preparation. This can be achieved through obtaining homogeneous solution after complete digestion of the powder sample. But mushroom contains a lot of chitin fibers which are difficult to dissolve by typical acid digestion consisting of concentrated nitric acid or chloric acid for food or bilogical matrices. Moreover, without hydrofluoric acid, precipitate was also observed, implying that the mushroom powder contains silica which is insoluble in nitric acid or hydrochloric acid. At the reaction temperature of 220°C which is a temperature to be reached by typical closed-vessel microwave-assisted digestion system for food matrices, 2 mL of hydrofluoric acid to 0.3 g sample with even large amount of concentrated nitric acid was not able to make sample solution completely clear for 30 min

Table 2. Certified results for mass fractions of Ca, Fe, and Zn in mushroom powder CRM (KRISS CRM 108-011).

Elements	Certified Values (mg/kg)	Expanded uncertainty, *U* (mg/kg)	Relative expanded uncertainty (%)	Coverage factor, *k* (95% level of confidence)	Homogeneity (%)
Ca	3301	186	5.65	2.0	2.13
Fe	716	19	2.63	2.0	0.87
Zn	49.3	1.7	3.50	2.0	0.86

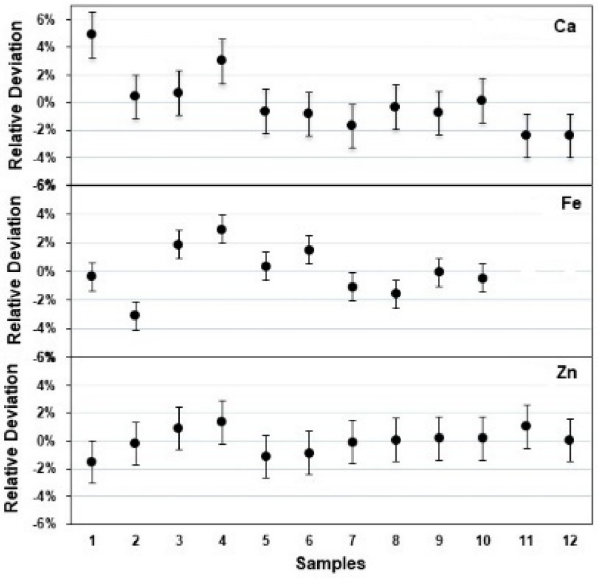

Figure 2. The measurement results of individual samples of the selected bottles from the single batch of the CRM. The error bars represent the combined uncertainties of uncertainty from systematic effects and repeatability of the measurement results.

reaction time. Therefore, for complete dissolution of mushroom powder, it is strongly recommended to apply the sample preparation procedure optimized in this work where 8 mL nitric acid and 1 mL hydrofluoric acid corresponding to 0.3 g mushroom powder were used with digestion condition of ramping-up to 250°C for 60 min and maintianing for 30 min.

Certified results

The certified results were summarized in Table 2. The certified values and their associated expanded uncertainties at 95% level of confidence for mass fractions of Ca, Fe, and Zn in mushroom powder CRM were (3301 ± 186) mg/kg, (716 ± 19) mg/kg, and (49.3 ± 1.7) mg/kg, respectively, where the coverage factor, *k*, were 2.0 for all as shown in Table 2. For Ca and Zn, the certified value was determined from the mean of 12 mass fractions of 12 selected bottles characterized by ID ICP/MS, while the certfied value of Fe was assgined from the mean of only 10 mass fractions among 12 measurement results after excluding two outliers. The corresponding relative expnaded uncertainties were 5.65%, 2.63%, and 3.50%, respectively.

Therefore, considering the typical interlaboratory reproducibility (or standard deviation) of measurement results predicted by the Horwits model, the certified valuse and their associated meausrement uncertainties for each element are well fitted for the intended use of the CRM.[13]

The measurement results of individual samples of the selected bottles charactrerized by ID ICP/MS using eq. (1) were shown in Figure 2. The homogeneities of the CRM for Ca, Fe, and Zn were 2.13%, 0.87%, and 0.86%, respectively, which were estimated from the relative standard deviation of 12 mass fractions for 12 selected bottles (for Fe, 10 values for 10 bottles). Even though the relative standard deviation of the mass fractions contains not only the contribution of sample inhomogeneity, but also that of measurement uceratinty from the analytical procedure, it can be a good estimator of the upper limit of inhomogeneity of the CRM.

Table 3. Major sources of measurement uncertainty of the certified values for the mushroom powder CRM.

Element	Contributions for measurement uncertainty (relative contribution, %)
Ca	Between bottle random variation (2.13%), Mass measurement of sample (0.33%)
Fe	Between-bottle random variation (0.87%), Isotope ratios of the sample and the standard solutions (0.81%)
Zn	Between-bottle random variation (0.86%), Isotope ratios of the sample and the standard solutions (1.26%)

The major contributions for measurment uncertainty of each of the certified values were evaluated in Table 3. For Fe and Zn, the contribution from the isotope ratios of the sample and the standard solutions, which were calculated from the reference values of representative isotopic abundances, so called “natural abundance”, were significant as well as that from the between-bottle random variations.[14] For Ca, mass measurement of sample also contributes 0.33% from systematic effects becasue lower masses of 0.1 g were taken.

Conclusions

A CRM for the analysis of nutrient elements in an edible mushroom powder has been developed (KRISS CRM 108-10-011). A primary method of measurement based on ID ICP/MS was developed with optimized sample preparation procedure. Applying ID ICP/MS, the certified values of the mass fractions of Ca, Fe, and Zn were assigned and their assoicated measurement uncertainties were evaluated. The certified results were metrologically traceable to the definition of kg in the SI. This CRM can be used for the validation of analytical methods or quality control of measurements in analytical laboratories in the fields of both foods and traditional medicines when they determine the mass fractions of elements in mushroom and similar samples.

Acknowledgments

This work was supported by KRISS under the project

"Establishing Measurement Standards for Inorganic Analysis" (grant no. GP2020-0003-03) and by "Development of test samples for proficiency assessment of testing laboratories authorized by MFDS (I)" (grand no.IP2019-0019). Authors acknowledge Ms. Youngran Lim and Mr. Myung Chul Lim for the production of the candidate CRM.

References

1. Boa, E., *Wild Edible Fungi: A Global Overview of their Use and Importance to People*, Food and Agriculture Organization of the United Nations, New York, **2004**, pp 1-3, 41-49.
2. ISO and IEC, *General requirements for the competence of testing and calibration laboratories*, ISO/IEC 17025:2017, Geneva, Switzerland, **2017**.
3. ISO, *Reference materials -- Good practice in using reference materials,* ISO Guide 33:2015, Geneva, Switzerland, **2015**.
4. Chew, G.; Sim, L. P.; Ng, S. Y.; Ding, Y.; Shin, R. Y. C.; Lee, T. K., *Food Chemistry*, **2016**, 190, 293, DOI: 10.1016/j.foodchem.2015.05.071.
5. ISO, *General requirements for the competence of reference material producers*, ISO 17034:2016, Geneva, Switzerland, **2016**.
6. ISO, *Reference materials — Guidance for characterization and assessment of homogeneity and stability*, ISO Guide 35:2017, Geneva, Switzerland, **2017**.
7. International Bureau of Weights and Measures (BIPM), *The International System of Units (SI)*, 8th edition, Paris, France, **2006**.
8. Kim, S. H.; Lim, Y.; Hwang, E.; Yim, Y.-H., *Anal. Methods* **2016**, *8*, 796, DOI: 10.1039/C5AY02040A.
9. Choi, J.; Hwang, E.; Yim, Y.-H.; Jo, H. M.; Lim, Y.; Kim, T. K.; Lee, K.-S., *Bull. Korean Chem. Soc.* **2017**, *38*, 211, DOI: 10.1002/bkcs.11066.
10. Vogl, J., *J. Anal. At. Spectrom.*, **2007**, 22, 475, DOI: 10.1039/B614612K.
11. Watters, R. L.; Eberhardt, K. R.; Beary, E. S.; Fassett, J. D., *Metrologia*, **1997**, 34, 87.
12. BIPM, IEC, IFCC, ILAC, ISO, IUPAC, IUPAP and OIML, *Evaluation of measurement data — Guide to the expression of uncertainty in measurement*, JCGM 100:2008, **2008**.
13. ISO, *Statistical methods for use in proficiency testing by interlaboratory comparison*, ISO 13528:2015, Geneva, Switzerland, **2015**.
14. Cohen, E. R.; Cvitas, T.; Frey, J. G.; Holmstrom, B.; Kuchitsu, K.; Marquardt, R.; Mills, I.; Pavese, F.; Quack, M.; Stohner, J.; Strauss, H. L.; Takami, M.; Thor, A. J., *Quantities, units and symbols in physical chemistry, IUPAC Green Book, 3rd ed*, IUPAC and RSC Publishing, Cambridge, UK, **2008**.