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Mineral Composition and Germanium Contents in Some *Phellinus* Mushrooms in the Northeast of Thailand

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Abstract: Ash and mineral (Na_2O, K_2O, MgO, CaO, Al_2O_3, SiO_2, TiO_2, MnO, Fe_2O_3 and P_2O_5) content of 5 *Phellinus* mushrooms from the Northeast part of Thailand has been measured using X-Ray Fluorescence (XRF) spectrometry. A method for determination of trace levels of germanium by Graphite Furnace Atomic Absorption Spectrometry (GFAAS) with chemical matrix modifier and Inductively Coupled Plasma Mass Spectrometry (ICP-MS) was compared. The preparation of sample solutions for determination of trace levels of germanium by wet method and dry ashing method was studied. It was found that none of the cations interfered GFAAS by using palladium strontium and nickel as new matrix modifier and the linear correlation range was 0.0040-1.00 mg L^{-1} and the detection limit was 0.0041 mg L^{-1}. The GF-AAS was applied to determine of trace levels of germanium in part per million (ppm) level in some *Phellinus* mushrooms with a recovery range of 75-95% which the results agree with the ICP-MS (81-111%). The ICP-MS was applied to determine of germanium in part per billion (ppb) levels in some *Phellinus* mushrooms with containing 0.32-1.56 ppm. The wet method for preparation sample solutions was not successful while dry ashing method was successful.

Key words: *Phellinus* mushrooms, germanium, aluminum oxide, graphite furnace atomic absorption spectrometry, palladium-strontium-nickel

INTRODUCTION

Mushrooms are considered as alternative food source to provide adequate nutrition to world's increasing population. The consumption of mushroom will prevent increasing of serum cholesterol (Konuk *et al*., 2006). Especially, *Phellinus* mushroom has been used as a traditional medicine in oriental countries for the treatment of stomachaches, inflammation, arthritis of the knee, gastroenteric disorders, tumors and lymphatic disorders (Samchai *et al*., 2009).

Germanium-containing dietary supplements were interested in remedies for certain diseases. Organo-germanium compounds especially carboxyethylgermanium sesquioxide or Ge-132 was considered to promote health and cure diseases. Organo-germanium compounds are described as antioxidants and inhibiting the progress of cancer and AIDS and destroying cancer cells (Krystek and Ritsema, 2004; McMahon *et al*., 2006). Small amounts of organo-germanium were found in some plant-based foods such as garlic, ginseng,

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comfrey, aloe and mushrooms. Therefore, germanium is an important element, the determination of germanium in botanical samples is necessary. Several methods for determination of germanium in trace levels have been developed such as atomic absorption spectrometry (Yoshiki *et al*., 1980), hydride generation atomic absorption spectrometry (Zaijun *et al*., 2007), inductively coupled plasma mass spectrometry (Krystek and Ritsema, 2004; Li *et al*., 1998; Shinohara *et al*., 1999), spectrophotometry and graphite furnace atomic absorption spectrometry (McMahon *et al*., 2006; Studnicki, 1980; Dittrich *et al*., 1985; Ueda and Kitadani, 1989; Haug and Chonghua, 1990; Tao and Fang, 1993; Matsusaki *et al*., 1994; Xiao-Quan and Bei, 1995; Dong-Qun *et al*., 1995; Ni and He, 1995; Ni and Zang, 1995; Zhang and Ni, 1996; Zhang *et al*., 1997; Peng *et al*., 1999; Yang and Zhang, 2002; López-Garcia *et al*., 2005; Meeravali *et al*., 2007; Mizuno *et al*., 1988).

For the determination of germanium in trace levels, graphite furnace atomic absorption spectrometry (GFAAS) or electrothermal atomic absorption spectrometry is a widely used method due to its simplicity, low cost and decreasing from interferences, especially when palladium- strontium is used as chemical modifier. The GFAAS can be applied to food, botanical samples (Zhang *et al*., 1997; Yang and Zhang, 2002) and real food samples (Zaijun *et al*., 2007). Many interferences such as Na^+, K^+, Ca^{2+}, Mg^{2+}, Cu^{2+}, Co^{2+},PO_4^{3-}, Cl^- and SO_4^{2-} disturb the signal in determination of germanium by GFAAS using tube wall technique (Yang and Zhang, 2002). Many researchers try to overcome these interferences by using matrix modifiers such as Ni, Ba (Dittrich *et al*., 1985), Pd, Pd-Mg (Haug and Chonghua, 1990), Al-Co (Matsusaki *et al*., 1994) and Pd-Sr (Zhang and Ni, 1996), but it can not overcome the sulfate ion in the case of amount sulfates. The GFAAS can be used to determine Ge in more than 4.00 mg kg^{-1}(ppm), whereas ICP-MS can be used in ng g^{-1}(ppb), but ICP-MS has a high cost. Germanium in mushroom has in wide range 0.022-2000 mg kg^{-1} (Mizuno *et al*., 1988), therefore the method was suitably selected.

Digestion of mushroom samples is an important consideration for determination of germanium. In most cases, when using wet method (Zaijun *et al*., 2007), the germanium was loosed and using dry ashing method (Mizuno *et al*., 1988) by hydrochloric acid with GFASS was loosed too.

Currently *Phellinus* mushrooms is interested from several researchers (Kim *et al*., 2004; Song *et al*., 2003, 2008; Li *et al*., 2008). This work aims to evaluate the chemical composition of 5 *Phellinus* mushrooms in the Northeast of Thailand: *Phellinus conchatus* (Pers.) Quél., *Phellinus rimosus* (Berk.) Pilát, *Phellinus igniarius* (L.) Quél., *Phellinus gilvus* (Schwein.) Pat. and *Phellinus nigrolimitatus* (Romell) Bourdot and Galzin and to compare method between GFAAS and ICP-MS for determination of germanium in some *Phellinus* mushrooms and preparation of sample solutions between wet method and dry ashing method.

MATERIALS AND METHODS

Instrumentation

Wavelength Dispersive X-Ray Fluorescence Spectrometry (XRF)

A PANalytical XRF spectrometer Model Axios Advanced with an end-window Rh tube was used for determination of chemical composition. Labor-Schoeps Automatic fusion unit Model AAG-2 was used fused ash *Phellinus* mushroom.

Graphite Furnace Atomic Absorption Spectrometry (GFAAS)

Absorbance was achieved and monitored using a Varian Spectr AA Model 880Z and a coated graphite partition tube with wall atomization and platform atomization. The source of radiation used was a germanium hollow cathode lam.

The new chemical modifier (palladium, strontium and nickel solution) and other modifier 5 and 10 μL sample solutions were injected onto the graphite platform before each atomization cycle.

Inductively Coupled Plasma-Mass Spectrometry (ICP-MS)

A Perkin-Elmer ELAN 5000 ICP-MS spectrometer equipped with an HGA-600MS electrothermal vaporizer was used for determination of germanium.

Reagents

The working solutions of germanium were prepared by serial dilution of a stock solution containing 1000 mg L^{-1} of germanium.

Palladium solution (2000 mg L^{-1}) was prepared by dissolving palladium (II) nitrate dihydrate 0.2504 g in deionized water and adjusted in 50 mL with deionized water.

Palladium-Strontium solution was prepared by dissolving strontium nitrate 0.1208 g in 50 mL 2000 mg L^{-1} palladium solution.

Aluminum-Cobalt solution was prepared by dissolving cobalt nitrate hexahydrate 0.0728 g in 50 mL 2 mol L^{-1} aluminum solution.

Nickel solution (5000 mg L^{-1}) was prepared by dissolving nickel (II) nitrate hexahydrate 0.2504 g in deionized water and adjusted in 50 mL with deionized water.

Five microliter of modifier solution and 10 μL of sample solution were used. 10 μL of interfere solution was used and solution of anion (sodium salt) and cation (nitrate salt) were prepared by dissolving the salts in deionized water.

Sample Preparation

Phellinus mushroom samples were cleaned from dirt and soil with brush, chopped up with a plastic knife, finely ground with agate motor and dried at 65-70°C for 24 h.

Dry Ashing

The dry ashing method was modified from standard method (Curdová *et al.*, 1998). The dried *Phellinus* mushroom samples were ashing in 4 steps : 200°C for 1 h, 300°C for 1 h, 400 °C for 1 hour and 450°C for 1 h and cooled down to room temperature.

For XRF

The ashing *Phellinus* mushroom sample, about 1.0 g each, was added with 66 % $Li_2B_4O_7$: 34 % $Li_2B_2O_4$ flux (ratio 1 : 5) in platinum crucible (95 % Pt/5 % Au) and 0.05-0.10 g LiBr. Then, the mixed sample was fused at 1200°C for 5-10 min and the reference material was prepared from certified reference rocks in the same way.

For GFAAS

For the wet method, the dried mushroom, about 3 g, was spiked with the standard germanium 10 mg L^{-1} 1 mL and added with 10 mL concentrated HNO_3 and 1 mL concentrated H_2SO_4 in flask equipped with reflux overnight. Then the sample solution was heated about 80°C until the solution was completely digested. The solution was transferred in 50 mL volumetric flask and diluted to mask with deionized water. This solution was used to compare the preparation of sample solution.

For ICP-MS and GFAAS

For dry ashing method, the ashing *Phellinus* mushroom sample, about 0.2-0.4 g each, was put in 50 mL beaker and 10 mL of 5000 mg L^{-1} of Ni solution was added. For the

determination of the percent recovery, the standard germanium 10 mg L^{-1} 1 mL was spiked in ashing mushroom. After 1 mL 1% HNO_3 was added to dissolve, the solution was transferred in 50 mL volumetric flask and diluted to mask with 1% HNO_3.

RESULTS AND DISCUSSION

This study used dry ashing method which gave the more concentrated solution and less-time consuming than the wet method. For example, dry ashing take 6-8 h and the ash can be dissolved in 5-10 min while wet method need overnight soaking of sample with nitric acid and sulfuric acid mixture and then needed refluxing for about 7 h. For mushroom with low ash content e.g., 4% ash, the use of 3 g ash in 50 mL is equivalent to digest 75 g of mushroom for wet method. The optimum condition of GFAAS furnace analysis conditions was showed in Table 1.

X-Ray Fluorescence Spectrometry (XRF)

Table 2 and 3 show the chemical composition of 5 *Phellinus* mushrooms in the Northeast of Thailand, it was found that the highest levels of SiO_2, TiO_2, K_2O, P_2O_5 and ash were observed in *Phellinus conchatus* (Pers.) Quél. as 11.343, 0.037, 0.289, 0.083 and 17.45%, respectively. In *Phellinus igniarius* (L.) Quél. showed highest level of MgO, CaO and MnO as 0.328, 0.960 and 0.046%, respectively,. The highest level of Al_2O_3 and Fe_2O_3 were 0.376 and 0.174%, respectively, in *Phellinus gilvus* (Schwein.) Pat. The highest Na_2O level was observed in *Phellinus rimosus* (Berk.) Pilát as 0.034%. The ash contents of each mushroom species varied as well as ash composition. The origin of these minerals are still under investigation such as silica, which may be from the defense system of biological species (32). If this is so, the medicinal properties of the mushroom may relate to silica content. Aluminium, which is not an essential in living system (33), worth maintaining. Our interest lies in the relationship of these mineral contents with germanium. Our hypothesis is germanium content may enter mushroom via the some mechanism as aluminium content due to similarity in atomic size as well as periodic table diagonal relationship. These results suggest that *Phellinus* mushrooms are very good mineral source (Table 2, 3). That is, besides being poor in lipid and very rich in protein, ash and fiber, the mushrooms examined could supply minerals.

Pyrolysis and Atomization Temperature for Germanium in the Pd Modifier

The main purpose of a modifier in GFAAS is to decrease the lost of germanium and to reduce the interference by the chemical matrix. The pyrolysis and atomization temperature

Table 1: Shows the GFAAS furnace analysis conditions

Step	Temperature (°C)	Ramp time (sec)	Hold time (sec)	Inner gas (mL min^{-1})
Drying	120	5.0	30.0	300
Pyrolysis (ashing)	900	5.0	22.0	300
Atomization	2600	0.8	4.0	0
Tube clean	2600	0.0	2.0	300

Table 2: The percent of the chemical composition in 5 *Phellinus* mushrooms from the Northeast of Thailand

Phellinus mushroom	Na_2O	K_2O	MgO	CaO	Al_2O_3	TiO_2	MnO	Fe_2O_3
Phellinus conchatus (Pers.) Quél.	0.001	0.289	0.265	0.702	0.227	0.037	0.005	0.105
Phellinus rimosus (Berk.) Pilát	0.034	0.120	0.243	0.600	0.306	0.015	0.027	0.111
Phellinus igniarius (L.) Quél.	0.002	0.092	0.328	0.960	-	0.002	0.046	0.010
Phellinus gilvus (Schwein) Pat.	0.016	0.071	0.253	0.397	0.376	0.020	0.004	0.174
Phellinus nigrolimitatus (Romell) Bourdot and Galzin	ND	0.139	0.176	0.788	0.201	0.025	0.005	0.082

ND: Not done

Table 3: The chemical composition in ash 5 *Phellinus* mushrooms from the Northeast of Thailand

Phellinus mushroom	% Ash	SiO_2	% P_2O_5
Phellinus conchatus (Pers.) Quél.	17.45	11.343	0.083
Phellinus rimosus (Berk.) Pilát	5.08	1.346	0.046
Phellinus igniarius (L.) Quél.	16.53	0.263	0.043
Phellinus gilvus (Schwein.) Pat.	5.09	2.193	0.036
Phellinus nigrolimitatus (Romell) Bourdot and Galzin	12.08	7.577	0.070

Table 4: Comparison of peak area on difference modifiers from sulfate interference

	Peak area		
Interferences (ppm)	Al+Co +Ni	Pd+Sr +Ni	Pd+Sr
SO_4^{2-}, 0	0.2819	0.2308	0.2147
SO_4^{2-}, 0.1	0.2676	0.2340	0.2075
SO_4^{2-}, 1.0	0.2617	0.2462	0.2034
SO_4^{2-}, 10.0	0.1699	0.2121	0.1694
SO_4^{2-}, 20.0	-	0.1845	0.1381
SO_4^{2-}, 40.0	-	0.1571	0.0810

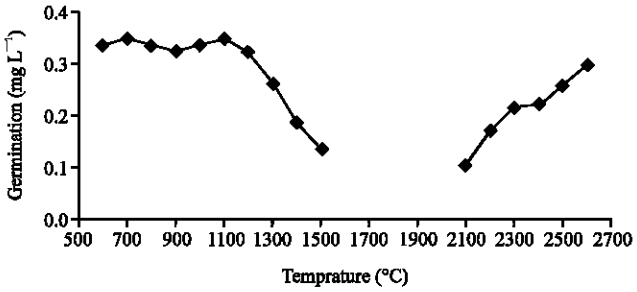

Fig. 1: The pyrolysis and atomization temperature curves of 0.2 mg L^{-1} germanium using 100 mg L^{-1} Pd modifier in platform mode; the left the studied pyrolysis and the right the studied atomization

curves of 0.2 mg L^{-1} germanium using 100 mg L^{-1} Pd modifier in platform mode are shown in Fig. 1 (When the pyrolysis was studied, the atomization was kept at 2600°C. In the case of the studied atomization, the pyrolysis was kept at 900°C). It was found that the pyrolysis temperature range is 700-1100°C and the maximum atomization is raised to 2600°C. Then, the pyrolysis temperature and atomization temperature was selected as 900 and 2600°C, respectively, for this research.

Comparison Between No Modifier and Modifier

The no modifier and difference modifiers using the pyrolysis temperature 900°C and atomization temperature 2600°C of 0.2 mg L^{-1} germanium in platform mode are shown in Table 4 and Fig. 2a-e.

The results indicate that peak area was improved into isothermal temperature for all modifiers, but 0.02 mol L^{-1} Al+0.01 mol L^{-1} Co and 2000 mg L^{-1} Pd + 1000 mg L^{-1} Sr modifiers had the high peak area and were improved successfully. Since Ge could form compound with the two set modifiers to give stable Ge-compound in pyrolysis step and was released slowly Ge atom in atomization step [11, 15, 21 and 22].

Modifiers for the Reduction of Interferences

The loss of germanium as volatile GeO and GeS during the pyrolysis and atomization steps results in a loss of analytical signal in the determination of germanium. To reduce this

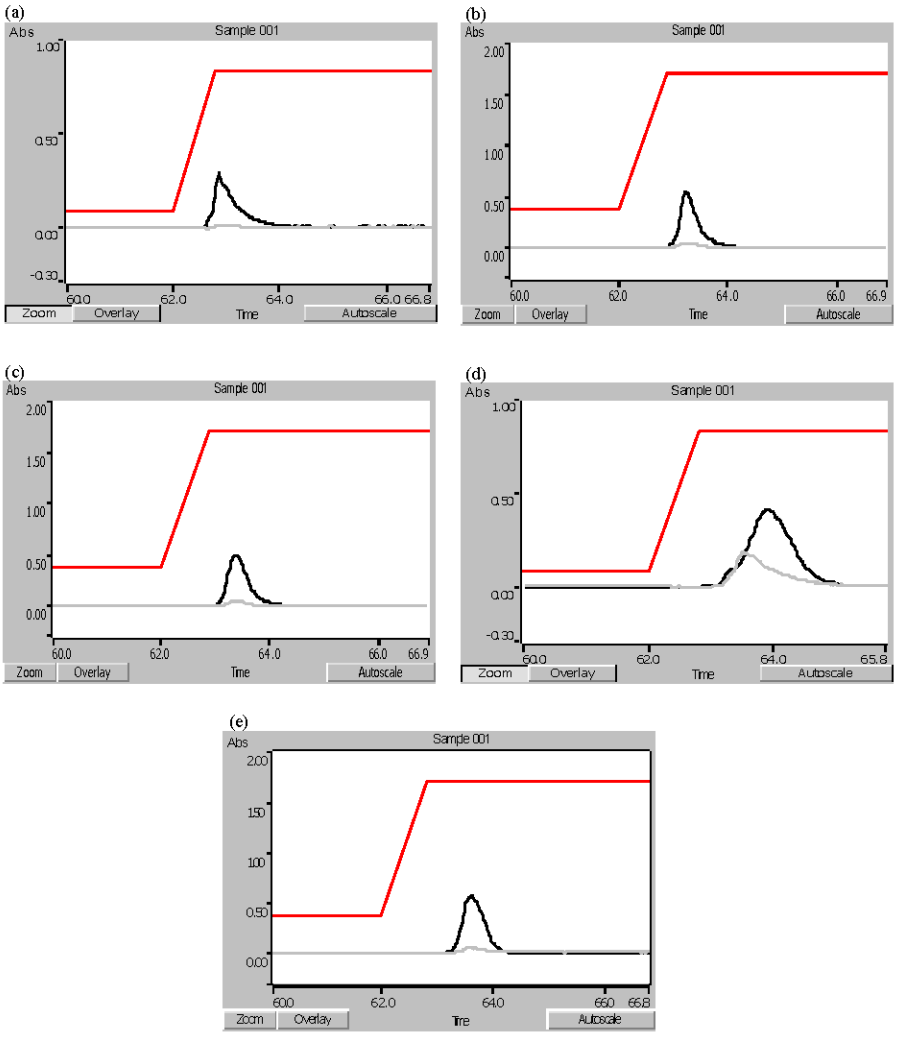

Fig. 2: The comparison of peak area between no modifier and modifiers. (a) No modifier, (b) 100 mg L^{-1} P d modifier, (c) 500 mg L^{-1} Pd modifier, (d) 0.02 mol L^{-1} Al + 0.01 mol L^{-1} Co and (e) 2000 mg L^{-1} Pd+1000 mg L^{-1} Sr modifier

effect Aluminum-cobalt and Palladium-strontium were used to increase the analytical signal. Dittrich *et al.* (1985) has been reported the Ni $(NO_3)_2$ modifier increased the thermal stability of germanium by form as $NiGeO_3$ in pyrolysis step. Therefore, the effect of interference such as Na^+, K^+, Mg^{2+}, Ca^{2+}, Cl^-, PO_4^{3-} and SO_4^{2-} etc to analytical signal in determination of germanium was studied by addition of 1000 mg L^{-1} Ni in prepared sample solution with Aluminum-cobalt and Palladium-strontium as modifiers.

In this study, Aluminum-Cobalt plus Ni and Palladium-Strontium plus Ni mixed modifier was investigated to reduce interference on the peak area of germanium 0.2 mg L^{-1} and the results are shown in Fig. 3. The evaluation of optimum condition for Aluminum-Cobalt plus

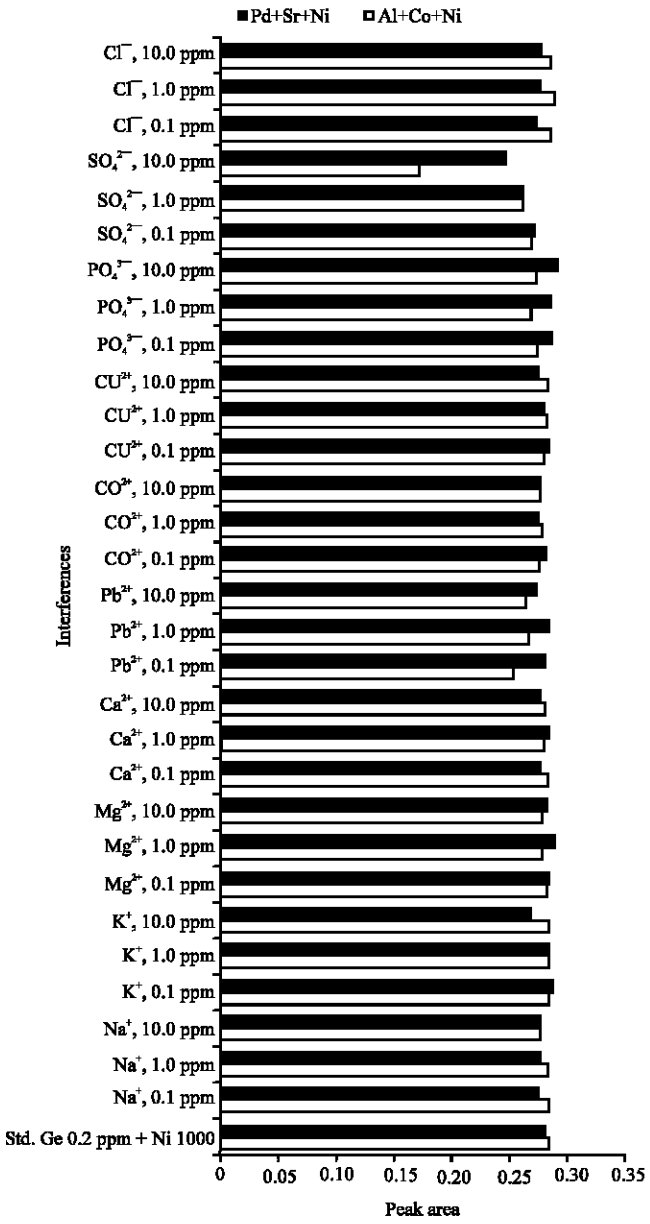

Fig. 3: Comparison of mixed modifier on germanium 0.2 mg L^{-1} in difference interferences

Ni and Palladium-Strontium plus Ni mixed modifier showed to reduce sulfate ions on the peak area of germanium 0.2 mg L^{-1} and the results was shown in Table 5.

The results indicate that Aluminum-cobalt plus Ni mixed modifier can eliminate these ion except sulfate ion. Since, the reduction of sulfate ion is very important in determination of germanium by GFAAS. Whereas, Palladium-Strontium plus Ni mixed modifier can reduce interference from these ions. Then Palladium-Strontium plus Ni mixed modifier is good modifier with respect to Aluminum-cobalt plus Ni mixed modifier. Because Ni and Pd stabilize

Table 5: Comparison of peak area on difference modifiers from sulfate interference

	Peak area		
Interferences	Al+Co +Ni	Pd+Sr +Ni	Pd+Sr
SO_4^{2-}, 0 ppm	0.2819	0.2308	0.2147
SO_4^{2-}, 0.1 ppm	0.2676	0.2340	0.2075
SO_4^{2-}, 1.0 ppm	0.2617	0.2462	0.2034
SO_4^{2-}, 10.0 ppm	0.1699	0.2121	0.1694
SO_4^{2-}, 20.0 ppm	-	0.1845	0.1381
SO_4^{2-}, 40.0 ppm	-	0.1571	0.0810

Table 6: Recovery of germanium from various *Phellinus* mushrooms with Palladium-Strontium plus Ni as mixed modifier

	Recovery (%)			
	GF-AAS		ICP-MS	
Phellinus mushroom	$\bar{X}$	SD (n = 3)	$\bar{X}$	SD (n = 3)
Phellinus conchatus (Pers.) Quél.	91	4.6	102	1.3
Phellinus rimosus (Berk.) Pilát	91	1.3	95	1.0
Phellinus igniarius (L.) Quél.	95	2.3	111	1.5
Phellinus gilvus (Schwein.) Pat.	75	1.8	81	2.3
Phellinus nigrolimitatus (Romell) Bourdot and Galzin	86	3.5	92	1.4

GF-ASS: Graphite furnace atomic abomic absoption, ICP-MS: Inductively coupled plasma mass spectroscopy

Table 7: Ash *Phellinus* mushroom Analysis by ICP-MS

	Concentration of Ge (ng g^{-1})	
Ash *Phellinus* mushroom	$\bar{X}$	SD (n = 3)
Phellinus conchatus (Pers.) Quél.	0.65	0.01
Phellinus rimosus (Berk.) Pilát	1.56	0.02
Phellinus igniarius (L.) Quél.	0.32	0.02
Phellinus gilvus (Schwein.) Pat.	1.70	0.10
Phellinus nigrolimitatus (Romell) Bourdot and Galzin	0.64	0.06

the Ge thermally and chemically in pyrolysis step owing to the formation of stable $NiGeO_3$ [11] and Pd-Ge [20], respectively. Strontium combined with sulphate ions to form $SrSO_4$ [21], but Aluminum combined with chloride ions and caused the spectral interference in Ge signal at 265.15 nm [15].

Analytical Merit

The absolute detection limit (3σ) of germanium based on the variability of the reagent blank which was carried out in the same way as the ashing method was found to be 0.0041 mg L^{-1}. The linearity of the method was interested and the linear range was determined to be 0.0040- 0.5 mg L^{-1}, with an R^2 value of 0.9999.

Sample Analysis

The proposed method was applied to the determination of germanium in *Phellinus* mushrooms. For the wet method, the added germanium, for study recoveries, in the acid could not detect. For dry ashing method, the recoveries of spiked germanium in *Phellinus* mushrooms are in range of 75-95 % which agree with ICP-MS (81-111%) in Table 6.

When GF-AAS was applied to determination of germanium in real *Phellinus* mushrooms, the signal germanium was lower than detection limit (0.0041 mg L^{-1}). The ICP-MS was used to determine germanium in real *Phellinus* mushrooms, it was shown in Table 7.

Comparison between ppm Ge and oxide of Al, Fe, Mg and Ca in ash *Phellinus* mushrooms were shown in Table 8.

Table 8: Comparison between ppm Ge by ICP-MS and oxide of Al, Fe, Mg and Ca by XRF in Ash of *Phellinus* mushroom

Phellinus mushroom	Ge (ppm)	Al_2O_3 (%)	Fe_2O_3 (%)	MgO (%)	CaO (%)
Phellinus conchatus (Pers.) Quél.	0.65	1.59	0.74	1.86	4.92
Phellinus rimosus (Berk.) Pilát	1.56	9.17	3.32	7.28	17.99
Phellinus igniarius (L.) Quél.	0.32	ND	0.38	13.13	38.40
Phellinus gilvus (Schwein.) Pat.	1.70	9.40	4.34	6.32	9.94
Phellinus nigrolimitatus (Romell) Bourdot and Galzin	0.64	2.01	0.82	1.76	7.88

The results show that ppm Ge was related to the oxide of Al and Fe especially ppm Ge and % Al_2O_3. Since Ge and Al was diagonal in periodic table and the properties was correspond, then amount of Al_2O_3 may be a useful indicator for amount of Ge in *Phellinus* mushroom. It has been reported of Ge content in food and fruits (Zaijung *et al*., 2001) but it has never been reported the amount of Ge in *Phellinus* mushroom before. This is the first reported of Ge and mineral content in *Phellinus* mushroom which could be supported the used this mushroom as medicinal mushroom.

CONCLUSION

Palladium and Strontium plus Nickel as mixed modifier can be used in determination of germanium by GF-AAS to reduce the matrix interference especially the serious interference of sulfate ion. The recoveries obtained by spiked germanium were found to be 75-95% for GF-AAS and 81-111% for ICP-MS. The GF-AAS was suitable for determination of germanium more than 4.0 ppm, whereas ICP-MS can use to determine Ge in ppb level. The preparation of sample solution for determination of germanium was suitable as the dry ashing method. The ppm Ge by ICP-MS and % Al_2O_3 by XRF in ash *Phellinus* mushrooms was closely relationship.

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