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Simultaneous analysis of 18 mineral elements in *Cyclocarya paliurus* polysaccharide by ICP-AES

Jian-Hua Xie[a,1], Ming-Yue Shen[a,b,1], Shao-Ping Nie[a], Xin Liu[a], Jun-Yi Yin[a], Dan-Fei Huang[a], Hui Zhang[a], Ming-Yong Xie[a,*]

[a] *State Key Laboratory of Food Science and Technology, Nanchang University, Nanchang 330047, China*
[b] *Center of Analysis and Testing, Nanchang University, Nanchang 330047, China*

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ABSTRACT

The contents of 18 kinds of mineral elements in *Cyclocarya paliurus* polysaccharide samples were determined by ICP-AES. The limits of detection (LOD) of the method for 18 elements were in the range of 0.01–3.80 mg/kg. The average recoveries obtained by the standard addition method were found between 94.34% and 105.69% (RSD, 1.01–4.23%). The results showed that *C. paliurus* polysaccharides were abundant in major and trace elements which are healthy for human body. The contents of Ca, Al, Mg, K, Fe, Mn and P were very high, ranging from 274.5 ± 10.3 to 5980.0 ± 102.7 mg/kg, while the contents of Zn, Na, Se, Cr, Pb, Cu and As ranged from 0.9 ± 0.1 to 37.1 ± 4.2 mg/kg. Finally, the levels of Ni, Cd, V and Co were not detected in the samples. ICP-AES is a simple, precise and efficient method for the determination of many mineral elements in polysaccharide samples simultaneously.

1. Introduction

Cyclocarya paliurus (Batal.) Iljinskaja (*C. paliurus*), commonly known as "sweet tea tree", is a well-known edible and medicinal plant, grown on cloudy and foggy highlands in Southern China. Traditionally, it is used in China both as drug formulations in traditional Chinese medicine and as an ingredient in functional foods or dietary supplements (Xie, Li, Nie, Wang, & Lee, 2006). Significant attention has recently been drawn to the use of *C. paliurus* for developing functional food, as it has been reported to have many biological activities, such as antihypertensive activity (Kurihara, Asami, Shibata, Fukami, & Tanaka, 2003), antioxidant (Xie, Xie, Nie, et al., 2010; Xie et al., 2012), and antidiabetics (Xie, Li, Nie, Wang, & Lee, 2006). These beneficial effects have been partly attributed to its variety of chemical components, including protein, polysaccharides, triterpenoids, flavonoids, steroids, saponins, phenolic compounds, etc. (Dong et al., 2008; Fang et al., 2011; Li et al., 2011; Xie, Xie, Shen, et al., 2010). In recent years, many polysaccharides isolated from natural sources have been proved to possess excellent bio-activities, which have attracted much attention in the field of biochemistry and pharmacology (Liu, Wang, & Ding, 2013; Sun and Kennedy, 2010; Zhang, Cui, Cheung, & Wang, 2006; Zhang et al., 2012). In the leaves of *C. paliurus* the polysaccharide has also been recognized as a main active component (Xie, Xie, Shen, et al., 2010). Previous studies have shown that polysaccharides extracted from the leaves of *C. paliurus* exhibited a significant free radical scavenging activity (Xie, Xie, Nie, et al., 2010), antimicrobial activities (Xie et al., 2012), and strong inhibitive effect towards breast cancer (Xie et al., 2013).

Meanwhile, most of the minerals contribute significantly to normal growth and play a pivotal role in biochemical functions and essential enzyme systems, even at threshold levels (Bhat, Kiran, Arun, & Karim, 2010). Trace elements also have curative or preventive roles in combating diseases. Some common elements such as K, Na and P are essential for human health and the content of these elements is important for nutritional purposes (Jia, Liu, & Li, 2011). Some trace elements, such as Fe, Cu, Zn and Mn, are known to play important roles in biological systems (Choudhury and Garg, 2007). It is also known that elements like Cr, Co and Ni are very important and essential for humans in a specific concentration, however in higher concentration can be toxic to humans or in lower level may cause disorders of human body and functions (Yang, Yan, Cao, & Zhang, 2012). In contrast, some heavy metals, such as Pb and Cd, are toxic even in trace amounts. Therefore, determination of composition and concentration of trace elements in foods and related products is essential for understanding their nutritional

* Corresponding author. Tel.: +86 791 83969009; fax: +86 791 83969009.
E-mail addresses: jhxie@ncu.edu.cn (J.-H. Xie), myxie@ncu.edu.cn, xmync@163.com (M.-Y. Xie).
[1] These authors contributed equally to this work.

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Table 1
Operating conditions of the ICP-AES.

Parameter	Value
Radio frequency incident power	1300 W (Optimized)
Flow rate of auxiliary argon	0.2 L/min
Flow rate of nebulizer argon	0.9 L/min (Optimized)
Flow rate of plasma gas	15 L/min
Flow rate of sample uptake	1.5 mL/min
Viewing mode	Axial
Spray chamber type	Cyclonic
Sample propulsion	Peristaltic pump, three channel
Torch type	Fassel type
Detector	Segmented-array charge-coupled (SCD)

importance (Ajasa, Bello, Ibrahim, Ogunwande, & Olawore, 2004). Some polysaccharides have been commercially developed into important therapeutic drugs and functional foods (Ajith and Janardhanan, 2007). Xie, Nie, Fu, and Wang (2006) reported that metal elements in tea polysaccharide were closely related to the biological actions of polysaccharide. To our knowledge, there are little researches regarding the content of mineral elements in polysaccharides. Thus, further research of the inorganic matters especially the trace elements and heavy metals in polysaccharides is being considered more and more important.

Many techniques have been utilized for the elemental analysis of a range of matrices, including atomic fluorescent spectroscopy (Chen, 2003), capillary zone electrophoresis or wide extended flame atomic absorption spectrometry (F-AAS) (Lavilla, Vilas, & Bendicho, 2008), multi-element inductively coupled plasma-optical emission spectrometry (ICP-OES) (Rezic and Steffan, 2007), inductively coupled plasma atomic emission spectroscopy (ICP-AES) (Gong, Luo, Gong, Gao, & Xie, 2012; Zachariadis and Sahanidou, 2009), and inductively coupled plasma-mass spectrometry (ICP-MS) (Cubadda, Raggi, & Coni, 2006). Compared with other instrumental techniques, ICP-AES has many advantages, such as low cost, rapid analysis, wide linear range, low detection limit, high sensibility and accuracy, having the characteristics of good selectivity and simultaneous determination of multi-elements, etc. (Garavaglia, Rebagliati, Roberti, & Batistoni, 2002). In this study, 18 kinds of major and trace elements (Ca, Al, Mg, K, Fe, Na, Zn, Cr, Mn, Se, Cu, P, Ni, As, Cd, V, Co and Pb) in *C. paliurus* polysaccharide samples were determined simultaneously by ICP-AES.

2. Materials and methods

2.1. Reagents

All reagents were of analytical reagent grade unless otherwise stated. HNO_3 and H_2O_2 were of suprapure quality (Merck, Darmstadt, Germany). All the plastic and glassware were cleaned by soaking in dilute HNO_3 (1:9) and were rinsed with ultra pure water prior to use. The element standard solutions used for calibration were prepared by diluting stock solutions of 1000 μg/mL of each element (Sigma). Aqueous solutions were prepared with ultra pure water from a Milli-Q water purification system (Millipore, Bedford, MA, USA).

2.2. Instrumentation

The contents of the elements were determined by an inductively coupled plasma atomic absorption spectrometer (Perkin Elmer Optima model, 5300D) with axial viewing of the emitted radiation. The main instrumental specifications and conditions are summarized in Table 1. A peristaltic pump was used to introduce the solutions into the ICP-AES at a flow rate of 1.5 mL/min. The dissolved samples were delivered through the pump using Tygon PVC tubing. Operating parameters for the instrument included forward power 1300 W, coolant gas flow rate 15.0 L/min, auxiliary gas flow rate 0.2 L/min and nebulizer gas flow rate 0.9 L/min.

2.3. Sample preparation

The leaves of *C. paliurus*, grown in Xiushui County, Jiangxi Province, China. A voucher specimen was deposited at the State Key Laboratory of Food Science and Technology, Nanchang University, China. The leaves were air dried and ground into fine powder in a mill, then stored in the sealed bags for the analysis of mineral elements.

Extraction of polysaccharides was conducted according to the method of Xie, Shen, Nie, Li, and Xie (2011) with some modifications. Briefly, the dried leaf powder (5 kg) was firstly extracted with 10 L of 80% ethanol for 24 h to remove interference components such as monosaccharide, disaccharide, oligosaccharide and polyphenols in the samples. Then the pretreated samples were extracted in 80 °C hot water. The aqueous extract was concentrated to 20% of the original volume under reduced pressure in a rotary evaporator, and proteins were removed with Sevag method. After removal of the Sevag reagent, the solution was dialyzed (MW cut-off 14 kDa) for 36 h in tap water and 12 h in ultra pure water before concentration. The extract was precipitated with three times volume of 95% ethanol at 4 °C for overnight and the precipitate was centrifuged at 8400 × *g* for 15 min. The precipitate was dissolved in ultra pure water, collected, frozen and freeze-dried, then the polysaccharide was obtained.

2.4. Analytical procedures

The analysis of mineral elements was performed according to the method of Saracoglu, Saygi, Uluozlu, Tuzen, and Soylak (2007) with some modifications. Briefly, approximately 0.5 g of *C. paliurus* polysaccharide was digested by dry ashing in porcelain containers by adding 10 mL of concentrated HNO_3 (10% solution, w/v). The mixture was first maintained over a hot plate until dryness and then in muffle furnace at 450–500 °C for 16 h. The ashed sample was then treated with 1 mL of concentrated HNO_3 for ash whitening and this mixture was ashed again for 6 h. Then, the residue was dissolved in 5 mL of 10% (v/v) HNO_3 and filtered through a filter paper. The sample was diluted to 25 mL with ultra pure water. Blank solutions were prepared in the same way as the *C. paliurus* polysaccharide samples. The accuracy of the method was checked by recovery assays in the analytical samples by adding analyte concentrations similar to the sample value and always in the linear calibration range.

2.5. Calibration procedure

For the quantitative analysis of the samples, calibration curves were established on six different concentrations. Standard solutions were prepared in 10% (v/v) HNO_3 (the same percentage of acid present in the samples) by diluting a multi-element standard solution containing all the elements. A blank was carried out in the same way. The calibration ranges were selected according to the expected concentrations of the elements of interest.

2.6. Statistical analysis

Data were expressed as means ± standard deviations (S.D.) and analyzed by the SPSS statistical software (SPSS Inc., Chicago, IL, USA), and the results were taken from at least three independent experiments performed in triplicate.

Table 2
Analysis wavelength and the limit of detection ($n = 6$).

Element	Wavelength (nm)	Limit of detection (LOD, mg/kg)
Na	589.592	1.45
K	766.490	3.00
Mg	285.213	0.80
Ca	317.933	1.50
Fe	238.204	0.25
Cr	267.716	0.10
Pb	220.353	0.12
Zn	206.200	0.10
Mn	257.610	0.05
Se	196.026	0.45
Al	396.153	0.55
Cu	327.393	0.25
P	213.617	3.80
Ni	231.604	0.75
Cd	228.802	0.01
As	188.979	2.20
V	313.107	0.01
Co	228.616	0.05

Table 3
Calibrations of the elements by ICP-AES.

Element	Regression equation	Correlation coefficient (r)
Na	$Y = 13{,}670x + 3060.1$	0.99999
K	$Y = 3336x + 163.5$	0.99989
Mg	$Y = 119{,}600x + 156{,}402.6$	0.99885
Ca	$Y = 2787x + 5423.2$	0.99879
Fe	$Y = 22{,}570x + 24{,}672.7$	0.99924
Cr	$Y = 16{,}810x + 12{,}029.7$	0.99967
Pb	$Y = 1980x$	0.99983
Zn	$Y = 6491x + 209.1$	0.99997
Mn	$Y = 1{,}157{,}200x + 257{,}363.1$	0.99827
Se	$Y = 733.3x - 6.0$	0.99988
Al	$Y = 63{,}530x + 695.3$	0.99996
Cu	$Y = 104{,}600x + 63.7$	1.00000
P	$Y = 668x + 2248.4$	0.99991
Ni	$Y = 9131x - 2.6$	0.99998
Cd	$Y = 29{,}240x - 41.7$	1.00000
As	$Y = 471.7x + 1.8$	0.99997
V	$Y = 11{,}857x + 223.1$	0.99999
Co	$Y = 18{,}150x - 14.7$	0.99994

3. Results and discussion

3.1. Optimization of ICP parameters

The effect of the plasma incident power was examined in the range of 1100–1500 W. It was observed that the highest sensitivity was obtained at 1300 W incident radio frequency power for all analytes, thus 1300 W was selected in this study. Another critical factor is the flow rate of nebulizer gas, which was reported to significantly affect the solution transportation into the ICP and also the atomization performance (Zachariadis and Sahanidou, 2009). Accordingly, the nebulizer gas flow rate was studied in the range of 0.7–1.1 L/min, with the best performance at 0.9 L/min. Therefore, a flow rate of 0.9 L/min was used in further analysis. When the pumping rate was greater than 1.5 mL/min, the signal intensity did not increase, and the mass drained from the chamber increased. As a result, the sample uptake flow rate of 1.5 mL/min was selected for further study.

3.2. Selection of optimum elemental analysis wavelength and detection limit

The interference-free determination was investigated for each element by recording the spectra of the sample solution and potentially interfering elements near the analytical line. The analysis wavelengths for detection with little spectral interference and high precision were selected. A compromise between the most sensitive spectral line of each analyte and the lower background was used to select the optimum spectral lines for this study. The elemental analysis wavelengths for ICP-AES instrument are presented in Table 2.

3.3. Limits of detection

The limit of detection (LOD, mg/kg) was calculated as the lowest concentration level that is statistically different from a blank (LOD = 3 S.D./m; m is the slope of the addition graph, S.D. is the within-run standard deviation of single blank determination), corresponding to a 99% confidence level (Yang et al., 2012). Under the above-described optimum conditions, the LOD values for the most sensitive spectral lines of each analyte are given in Table 2. The results showed that the LOD values of the 18 mineral elements by ICP-AES method ranged from 0.01 to 3.80 mg/kg.

3.4. Calibration studies

To determine the linearity of the response versus concentration for the elements, a series of standard solutions (0.5, 2.0, 5.0, 10.0, 50.0 and 100.0 μg/mL) for ICP-AES were analyzed. The calibration curves and regression analysis on calibration curves are presented in Table 3. A good linear relationship between the corresponding sensitivities and the concentrations of the elements was achieved. For all the elements analyzed, the correlative coefficients (r) of the calibration curves were at least 0.9983.

3.5. Accuracy and precision of the method

In order to evaluate the accuracy and precision of the proposed method, the standard adding method was adopted to determine the recovery rate of each element, and the precision of the method was determined by repeatability and reproducible studies of method and expressed as relative standard deviation (RSD). The average recovery rate and RSD of each element were determined for each experiment. The replicate experiments were performed under the chosen conditions. The average recoveries and relative standard deviations (RSD) from these experiments are given in Table 4. The average recovery rates ranged from 94.34% to 105.69% and the RSD values of each element in millet ranged from 1.01% to 4.23% (less than 5%), for all elements in millet (Table 4). These results suggest that the method used is of good precision, accurate and can be used to determine each element.

3.6. Application in experimental samples

The contents of 18 kinds of mineral elements in *C. paliurus* polysaccharide are summarized in Table 5. The results indicated that the contents contents of Ca, Al, Mg, K, Fe, Mn and P were very high (>260 mg/kg), averaging 5980.0 ± 102.7 mg/kg, 885.3 ± 25.6 mg/kg, 1126.6 ± 42.2 mg/kg, 1810.5 ± 30.5 mg/kg, 633.0 ± 21.8 mg/kg, 274.5 ± 10.3 mg/kg and 2359.1 ± 69.7 mg/kg, respectively, while the contents of Zn, Na, Se, Cr, Pb, Cu and As were ranging from 0.9 ± 0.1 to 137.1 ± 4.2 mg/kg, averaging 31.2 ± 0.3 mg/kg, 37.1 ± 4.2 mg/kg, 17.7 ± 0.2 mg/kg, 1.7 ± 0.1 mg/kg, 12.7 ± 0.4 mg/kg, 7.9 ± 0.1 mg/kg and 0.9 ± 0.1 mg/kg, respectively. The elements Ni, Cd, V and Co were not detected in the samples (Table 5).

The abundances of Ca, K and Mg in *C. paliurus* polysaccharide are similar to that in *C. paliurus* leaves samples (Xie, Li, Nie, Wang, & Lee, 2006). Xie, Li, Nie, Wang, and Lee (2006) determined the

Table 4
Analysis of mineral elements in polysaccharide sample, recovery and precision of determination method ($n = 3$).

Element	Content[a] (μg/mL)	RSD (%)	Added value (μg/mL)	Found value[a] (μg/mL)	Recovery (%)
Na	2.74	2.54	5	7.78	100.52
K	36.21	3.17	30	66.19	99.97
Mg	22.53	4.23	30	55.52	105.69
Ca	119.60	2.59	100	219.01	99.73
Fe	12.66	3.14	5	17.79	100.68
Cr	0.03	1.34	0.5	0.50	94.34
Pb	0.25	1.01	0.5	0.74	98.67
Zn	0.62	1.45	0.5	1.16	103.57
Mn	5.49	2.14	5	10.69	101.91
Se	17.71	1.48	5	22.51	99.12
Al	5.31	2.88	5	10.21	99.03
Cu	0.16	1.11	0.5	0.67	101.52
P	47.18	2.87	30	77.10	99.89
Ni	n.d.[b]	3.55	0.5	0.50	100.00
Cd	n.d.[b]	1.29	0.5	0.52	104.00
As	0.02	2.53	0.5	0.51	98.08
V	n.d.[b]	1.07	0.5	0.49	98.40
Co	n.d.[b]	2.12	0.5	0.49	98.40

[a] Data presented are the average of three determinations.
[b] n.d., not detected in the sample.

most abundant mineral elements in *C. paliurus* leaves samples such as K (22,000 ± 800 mg/kg), Ca (15,700 ± 200 mg/kg), and Mg (3760 ± 160 mg/kg). Mn (718 ± 88 mg/kg), Zn (66.8 ± 2.9 mg/kg), Fe (254 ± 5 mg/kg) and Cu (40.6 ± 0.5 mg/kg) were also detected in *C. paliurus* leaves. The level of Cr in *C. paliurus* polysaccharide was also similar to that of *C. paliurus* leaves. However, the contents of Fe and Se in *C. paliurus* polysaccharide were higher than that of *C. paliurus* leaves, and the contents of Mn, Cu and Zn in *C. paliurus* polysaccharide were lower than that of *C. paliurus* leaves. Mineral elements play important roles in health and disease states of humans and domestic animals. Usually deficiency in mineral elements can lead to undesirable pathological conditions in human, which may be prevented or reversed by adequate supplementation (Fraga, 2005). Xie, Li, Nie, Wang, and Lee (2006) showed that the therapeutic properties of *C. paliurus* were related to the enriched concentrations of several trace elements in their leaves, among them the notable ones included Mn, Zn, Mg, Cu and Ni. The results showed that *C. paliurus* polysaccharide samples were also rich in some of the essential minerals including Na, K, Ca, Fe, Mg, Cu, Mn, Se and Zn, which are known to be beneficial for human health. Ca is a major component of bone, assists in tooth development, helps regulate endo- and exoenzymes, and plays a significant role in regulating blood pressure (Brody, 1994). Using plant-based Ca as a nutritional supplement might be advantageous for consumers who are allergic to animal-based food sources or who practice strict vegetarianism. Zn is necessary for the function of over 300 different enzymes and plays a vital role in an enormous number of biological processes (Aberoumand and Deokule, 2009). K is multifunctional nutrient that form an essential part of many important enzymes. Fe is found both as a structural component of some enzymes and as an activator of others. Adequate Fe in a diet is very critical for decreasing the incidence of anaemia (Lynch and Baynes, 1996). Cu is important in the process of biological transfer of electrons, and is vital for the synthesis of red blood cells and the maintenance of structure and function of nervous system. Se is an essential element for nutrition of a capital importance in the human biology. This element is a cofactor of a large number of selenium-dependent enzymes such as an antioxidant enzyme, which is involved in cellular protection from severe oxidation by free radicals (Wang, Zhao, Wang, Yao, & Zhang, 2012).

Table 5
Analysis of mineral element in polysaccharide sample.

Element	Content[a] (mg/kg)	RSD (%)
Na	137.1 ± 4.2	1.79
K	1810.5 ± 30.5	1.95
Mg	1126.6 ± 42.2	3.89
Ca	5980.0 ± 102.7	1.72
Fe	633.0 ± 21.8	3.44
Cr	1.7 ± 0.1	5.88
Pb	12.7 ± 0.4	3.15
Zn	31.2 ± 0.3	0.96
Mn	274.5 ± 10.3	3.75
Se	17.7 ± 0.2	1.12
Al	885.3± 25.6	2.89
Cu	7.9 ± 0.1	1.27
P	2359.1 ± 69.7	2.95
Ni	n.d.[b]	–
Cd	n.d.[b]	–
As	0.9 ± 0.1	3.19
V	n.d.[b]	–
Co	n.d.[b]	–

[a] Values were expressed as means ± S.D. ($n = 3$).
[b] n.d., not detected in the sample.

Xie, Nie, Fu, and Wang (2006) have determined the content of Zn, Ca, Fe, Cu, or Se in tea polysacchairde using ICP-MS. In tea polysacchairde, the contents of these five metals were 25.72, 2354, 77.47, 6.432, and 0.001 mg/kg, respectively. In this study, the contents of these five elements in *C. paliurus* polysaccharide samples were 31.2 ± 0.3, 5980.0 ± 102.7, 633.0 ± 21.8, 7.9 ± 0.1 and 17.7 ± 0.2 mg/kg, respectively. However, the content of Mg in *C. paliurus* polysaccharide (1126.6 ± 42.2 mg/kg) was lower than the value of 3448 mg/kg in tea polysacchairde (Xie, Nie, Fu, & Wang, 2006). Therefore, we can conclude that *C. paliurus* polysaccharide has high nutritional values from the viewpoint of elements such as Zn, Ca, Fe, Cu and Se. To our knowledge, this is the first study of determining the levels of the trace elements and heavy metals in *C. paliurus* polysaccharide using ICP-AES method. Results of the present study have provided vital information on the mineral composition and heavy metal contents in *C. paliurus* polysaccharide samples, which may be explored as a food supplement or used in the development of new food products.

4. Conclusions

This study has for the first time reported the determination of 18 mineral elements in *C. paliurus* polysaccharide by ICP-AES method. The method is highly reliable, has a short analysis time and a LOD of 3.8 mg/kg. The average recoveries obtained by the

standard addition method ranged from 94.34% to 105.69% with the RSD values in the range of 1.01–4.23%. The variation in the results obtained from the analysis of polysaccharide samples showed that the method was well suited for the determination of mineral elements. From a human nutritional point of view, *C. paliurus* polysaccharide was rich in mineral elements such as Ca, Al, Mg, K, Fe, Mn and P. For toxic elements, the contents of As and Cr were very low, and even below the detection limit in some samples. In addition, the levels of Ni, Cd, V and Co were not detected in the samples. These results may provide the scientific data for further study of the relationship between the content of elements and its medical therapy. The results may also provide references for the quality evaluation of *C. paliurus* polysaccharide.

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