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

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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

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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.