Inductively coupled plasma orthogonal acceleration time-of-flight mass spectrometry (ICP-oa-TOF-MS) analysis of heavy metal content in *Indocalamus tesselatus* samples

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**Abstract**

*Indocalamus tesselatus* is one of the most popular packing materials in China. Heavy metals in Chinese *I. tesselatus* samples have received great interest because they are related to health. A simple and fast method for the determination of Pb, Cd, Mn, Ni, Cr, As, Hg, Cu and Zn, by inductively coupled plasma orthogonal acceleration time-of-flight mass spectrometry (ICP-oa-TOF-MS), following microwave closed vessel digestion of samples, was proposed. The method was validated using standard reference material (GBW 07605-Tea). Samples of *I. tesselatus* from five different regions of China were analysed using the proposed method. Heavy metals contents from different regions were found at different levels. Their low contents of heavy metals showed that collection areas were not polluted and all collected *I. tesselatus* samples could be unreservedly used as food packing materials without any health risk.

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**1. Introduction**

*Indocalamus tesselatus* (family Graminales) is grown predominately in South China, including Hubei, Jiangxi, Guangdong, Guangxi and Yunnan province. It is often uncultivated in mountain slope, shady slope and semi-shady slope of cleugh at elevations no more than 1000 above sea level. In China, it is often used as packing materials. It is wrapped with glutinous rice, green bean and pork, named ‘Zongzi’ or ‘traditional Chinese rice-pudding’. As the Dragon Boat Festival comes, Chinese will make traditional Chinese rice-pudding to commemorate ancestors. Chinese rice-pudding is also exported to America, Canada, Australia, Japan and Korea.

*I. tesselatus* is rich with polysaccharides and flavonoid. It is known that polysaccharides have high biological activity. Polysaccharides from *I. tesselatus* were extracted and its activity was studied. The results showed that some of them had high anticancer, antitumor and antioxidative effects (Ding, Li, Chen, Elmahadi, & Xu, 1998). *I. tesselatus* also has high antimicrobial activity, but its antimicrobial mechanism is still not clear. Just because of its antioxidative and antimicrobial activity, *I. tesselatus* can successfully prevent the stuffing deterioration for up to 7 days at room temperature. There is general understanding that packing materials should form an effective barrier between outer environment and the food, and the concentration of all elements in packing materials should be below the safe level for human consumption (Chen et al., 1999; Gomez, Cerutti, Sombra, Silva, & Martinez, 2007; Zhang & Li, 2010). Using *I. tesselatus* as packing materials should ensure that it will not contaminate the food.

Reliable control of a range of packaging materials required analytical methods allowing the determination of traces metals in a large variety of matrices with satisfactory sensitivity and selectivity (Batista, Rodrigues, de Souza, Oliveira Souza, & Barbosa, 2011; Capar, Mindak, & Cheng, 2007; Kim et al., 2008; Llorent-Martínez, Ortega-Barrales, Fernández-de Córdova, Domínguez-Vidal, & Ruiz-Medina, 2011; Skrzydlewska, Balcerzak, & Vanhaecke, 2003). The use of both microwave digestion in closed-vessel for sample preparation and the inductively coupled plasma mass spectrometry (ICP-MS) for detection permitted an accurate determination of multi-element at trace and ultra-trace level in food samples (Millour et al., 2011; Sanchez Lopez, Gil Garcia, Sanchez Morito, & Martinez Vidal, 2003). However, some limitations occurred, including in elemental coverage when detecting rapid transient signals, and distinguishing very small differences between isotope ratios, etc.

The introduction of ICP time-of-flight mass spectrometers to the analytical practice has brought many practical advantages, e. g. enhanced sample throughput and elemental coverage, enhanced resolution, unlimited use of internal standards without performance compromises, improved isotope ratio precision capability owing to the high correlation of the noise sources for all isotopes which occur with simultaneous sampling and measurement of transient
signals (Dick et al., 2008; Husáková, Urbanová, Šrámková et al., 2011).

Since only a small number of laboratories operated with ICP-oa-TOF-MS system, the practical experience including the analysis of food packing materials was still missing. The present study relates to analysis the presence of heavy metals (Pb, Cd, Mn, Ni, Cr, As, Hg, Cu and Zn) in I. tesselatus from five different regions of China, by inductively coupled plasma orthogonal acceleration time-of-flight mass spectrometry (ICP-oa-TOF-MS), following microwave closed vessel digestion of samples.

2. Experimental

2.1. Reagents and chemicals

All solutions were prepared with analytical reagent grade chemicals. The water used in all studies was ultrapure water obtained from a Barnstead Easy pure RF compact ultrapure water system. Nitric acid was obtained from Beijing Chemical Works (Beijing, China). All calibration standard solutions were prepared by appropriate dilutions of 1000 μg/L stock solutions (National analysis centre for iron and steel (Beijing, China)) immediately before use.

2.2. Indocalamus tesselatus samples

5 I. tesselatus samples were collected randomly from Guangdong (sample region 1), Hubei (sample region 2), Jiangxi (sample region 3), Guangxi (sample region 4) and Yunnan province (sample region 5), China. Samples were dried under vacuum and grinded. The best amount of sample was fixed in 0.2 g to fix the minimum amount of acids required for treatment of that sample mass and to decide the final volume of sample solutions.

2.3. Sample preparation

Samples were digested using a MARSS microwave digestion system (CEM, American), equipped with HP-1500 PTFE (polytetrafluoroethylene) vessels. Sample digestion procedure was applied according to the method of Millour et al. (2011) with slight modifications. Before use, PTFE vessels were decontaminated in a bath of 10% nitric acid (65%, v/v), then rinsed with ultra pure water and dried in a 40°C oven.

0.2 g of I. tesselatus sample was weighed precisely in a PTFE digestion vessel and wet-oxidised with 8 mL nitric acid (65%, v/v) and 2 mL hydrogen peroxide (30%, w/w) in the microwave digestion system. The vessels were closed and samples were heated at the same time in the microwave oven. 1200 W maximum power was used for the digestion of samples and reference materials. The operating program for the sample microwave digestion system was carried out according to these conditions: 1200 W (13 min of ramp), 1200 W for 20 min and 20 min for cooling to room temperature. One randomly selected vessel was filled with reagents only and taken through the entire procedure as a blank. After cooling to room temperature, digests were transferred into vessels and Rh was added as internal standard at a 2 μg/L concentration. Then, the digested samples were diluted with ultra pure water to 50 mL in vessels for further analysis by ICP-oa-TOF-MS.

2.4. Instrumentation and analysis

The Optimass 9500 ICP-oa-TOF-MS instrument (GBC Scientific Equipment Pty. Ltd., Australia) was used throughout this study. The equipment was fitted with a nebuliser and a spray chamber for conventional nebulisation (Husáková, Urbanová, Audrlícká-Vavrušová et al., 2011). After preliminary experiments, the final instrumental condition selected for ICP-oa-TOF-MS operation was given in Table 1. The operational performance of the instrument was optimised daily with a multi-elemental solution.

2.5. Calibration

A multi-element standard working solution with the elements to be analysed at a concentration of 1000 μg/L was prepared by stock solutions. All solutions were 1% (v/v) in nitric acid. For calibration, multi-element solutions were prepared with the following concentrations: 0, 0.5, 4, 6 and 20 μg/L of Cd, Ni, As and Hg multi-element solution; 0, 5, 40, 60, 200 μg/L of Cu, Zn and Cr multi-element solution; 0, 25, 200, 300, 1000 μg/L of Mn solution and 0, 1, 8, 12, 40 μg/L of Pb solution.

2.6. Statistical analysis

The data was analysed using one-way analysis of variance (ANOVA) to examine statistical significance of differences in the mean concentration of heavy metals in samples. Results were considered significant at p < 0.05. Statistical analyses were conducted using SPSS version 15 statistical analysis packages.

3. Results and discussion

3.1. Operational condition for microwave digestion

The main object of microwave digestion was to accomplish complete dissolution of samples in the shortest time and with the minimum amounts of acids. Nitric acid is a strong oxidising agent and is widely used for liberating trace elements from biological and botanical matrices because it behaves ideally under microwave conditions (Wang et al., 2006). Since it is known that hydrogen peroxide has clarifying effect, the mixture of nitric acid and hydrogen peroxide had already been tested as digestion re-

<table>
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<tr>
<th>Sample introduction and plasma</th>
<th>Optimass 9500</th>
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<tr>
<td>ICP-oa-TOF-MS</td>
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<td>Generator set power</td>
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<tr>
<td>Plasma gas flow rate</td>
<td>10.0 L min⁻¹</td>
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<tr>
<td>Auxiliary gas flow rate</td>
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<td>Nebulizer gas flow rate</td>
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<td>Z1</td>
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<td>Y mean</td>
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<tr>
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<td>Z lens mean</td>
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<tr>
<td>Z lens deflection</td>
<td>0 V</td>
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<td>Push-out plate</td>
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<td>Blanker</td>
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<td>Reflection</td>
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agents by other authors (Sanz-Segundo, HernaÁndež-Artiga, Hidalgo-Hidalgo de Cisneros, Bellido-Milla, & Naranjo-Rodriguez, 1999). Employing 8 mL nitric acid (65%, v/v) and 2 mL hydrogen peroxide (30%, w/w) led to a total and simultaneous dissolution of samples in a short time. If a small amount of hydrogen peroxide was used, it would allow greater sample masses to be digested adequately. However, too much hydrogen peroxide used would slow down the digestion speed and a charred residue was obtained (Eilola & Perämäki, 2001).

### 3.2. Selection of isotopes

The development of a method for the determination of heavy metals (Pb, Cd, Mn, Ni, Cr, As, Hg, Cu and Zn) entailed careful selection of isotopes for monitoring their concentrations in samples. When the element of interest had two or more isotope masses, several specific isotopes were considered. Interference correction formulas were included in the software of the ICP-oa-TOF-MS instrument (Larcher, Nicolini, & Pangrazzi, 2003).

### 3.3. Calibration, validation studies and detection limit

Calibration graphs were obtained by analysis of multi-element standard working solution. Equations and correlation coefficient for calibration curves were given in Table 2.

The method was validated using standard reference material (GBW 07605-Tea). The measured values were compared with the certified values following a procedure described by Linsinger (2005). To evaluate method performance, the value of $\Delta_m$ was compared with the value of $U_\lambda$. If the value of $U_\lambda$ was larger than the value of $\Delta_m$, the measured value was not significantly different ($p > 0.05$) from the certified value.

The value of $\Delta_m$ was calculated by the following formula:

$$\Delta_m = |C_m - C_{CRM}|$$

where $\Delta_m$—absolute difference between mean measured value and certified value; $C_m$—mean measured value; $C_{CRM}$—certified value.

The value of $U_\lambda$ was calculated as follow:

$$U_\lambda = 2 \sqrt{ u^2 + u^2_{CRM}}$$

where $U_\lambda$—expanded uncertainty of difference between result and certified value; $u_m$—uncertainty of the measurement result; $u_{CRM}$—uncertainty of the certified value.

As can be seen in Table 3, the measured values of all heavy metals were not significantly different ($p > 0.05$) from the certified values and they were in good agreement with the certified values. This indicated that validity of the proposed method for analysis of *I. tesselatus* samples.

The ICP-oa-TOF-MS proposed method detection limit, was evaluated as three times the standard deviation (SD) of the average of the procedure blank solution (10 replicates) and slope of the standard calibration solution: DL = 3SD/slope. Detection limit of heavy metals was 0.011 $\mu$g/g of Pb, 0.047 $\mu$g/g of Cd, 0.016 $\mu$g/g of Mn, 0.019 $\mu$g/g of Ni, 0.036 $\mu$g/g of Cr, 0.139 $\mu$g/g of As, 0.017 $\mu$g/g of Hg, 0.013 $\mu$g/g of Cu, and 0.034 $\mu$g/g of Zn.

### 3.4. Determination of heavy metals in samples

Heavy metal contents in *I. tesselatus* samples were measured by ICP-oa-TOF-MS following microwave digestion. Five different *I. tesselatus* samples were collected from different regions of China. The results of the elemental analysis were summarised in Table 4.
Heavy metal contents from different region were found at different levels. Pb, Cd, Mn, Ni, Cr, As, Hg, Cu and Zn were detectable in all samples, and their contents ranged from 0.810 to 4.49, 0.128 to 0.268, 8.44 to 305, 2.28 to 5.77, 0.081 to 0.549, 0.945 to 1.84, 0.025 to 0.140, 3.20 to 549, 10.7 to 15.8 µg/g, respectively. Some factors, including the environmental features of each region (water, temperature, pH and salinity), may affect metal accumulation (Rodenas de la Rocha, Sanchez-Muniz, Gomez-Juaristi, & Larrea Marín, 2009).

At present, there are still no maximum levels for heavy metals in *I. tesselatus*. Because *I. tesselatus* were used as food packing material, it would be cooked in water for several hours after packed. So, *I. tesselatus* could be applied to standard for tea established by Chinese national standard. Heavy metals values in the packing material under normal conditions, should not affect human health.

Different regions have different chemical compositions. Majority of samples was processed by immersing into copper sulphate solution, to make it returning green. Although Cu element is no longer contamination element of food by Chinese national standard in 2010, massive addition of Cu is still harmful to health.

The results (Table 4) indicated that major values for heavy metals between different regions were significant differences (p < 0.05). This may be because that *I. tesselatus* samples from different regions have different chemical compositions. Majority of samples were collected from mountain slope, shady slope and semi-shady slope of cleugh, and were not processed before used for packing materials. Samples were uncultivated in rural area, heavy metal accumulation was little.

4. Conclusion

The proposed ICP-oa-TOF-MS method allows the fast determination of the content of Pb, Cd, Mn, Ni, Cr, As, Hg, Cu and Zn in Chinese *I. tesselatus* samples, following microwave digestion. The method was validated using standard reference material (GBW 07605-Tea). The study showed that the doses of heavy metals associated with *I. tesselatus* samples which could be used as food packing material under normal conditions, should not affect human health.

References


