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Original Research Article

Determination of heavy metals in chinese prickly ash from different production areas using inductively coupled plasma-mass spectrometry

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Abstract

Purpose: *To determine the heavy metal content of Chinese prickly ash (CPA) produced in various areas of China.*
Methods: *CPA samples collected from different production areas in China were subjected to microwave digestion, and the contents of copper (Cu), nickel (Ni), chromium (Cr), lead (Pb), cadmium (Cd), arsenic (As), mercury (Hg), tin (Sn), and antimony (Sb) were determined by inductively coupled plasma-mass spectrometry (ICP-MS).*
Results: *Heavy metal levels in the CPA samples followed the order: Cu (3.29-24.17 mg/kg) > Cr (0.04-7.33 mg/kg) > Ni (0.88-6.86 mg/kg) > Pb (0.00-3.84 mg/kg) > As (0.0011-1.08 mg/kg) > Cd (0.029-0.211 mg/kg) > Sb (0.03-0.21 mg/kg) > Sn (0.00-0.15 mg/kg) > Hg (0.000-0.032 mg/kg). Metal-to-metal correlation studies showed that there were significant correlations between Cu-Cr (p = -4.02), Cu-Ni (p = 0.561), Cu-As (p = 0.554) and Ni-As (p = 0.428) at the 0.01 level. Also, some metal-to-metal correlations were observed in Pb-Cr (p = 0.351), Pb-Cu (p = -0.310), Sb-Cd (p = 0.322), Sb-Hg (p = 0.311) and Cd-Sn (p = 0.309) at the 0.05 level. The highest concentrations of Pb and As in CPA exceeded the maximum permissible limits in China. Based on current safety standards, the concentrations of heavy metals in these CPA samples mean they are safe for human consumption.*
Conclusions: *The status of heavy metal concentrations of CPA should be further investigated in Sichuan, Shaanxi, Shanxi and Jiangsu. In addition, ICP-MS is a reliable and rapid technique for the determination of the heavy metals in CPA.*

Keywords: *Chinese prickly ash, Heavy metals, Inductively-coupled plasma-mass spectrometry, Food safety*

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INTRODUCTION

Currently, food pollution by heavy metals has become a threat to human health because of environmental contamination, industrial waste, and fertilizers used in agriculture, as well as various anthropogenic pollutants [1]. In addition, toxic heavy metals in plants could be influenced by the geochemical constituents of soil, pollution of water and air, and the selective accumulation of some elements in plants themselves [2].

Although some metals, such as Cu, Ni, Cr, Mn, Mo, Zn, Fe, Co, and Al, are essential nutrients for plant growth, they can also present phytotoxic effects in human beings at concentrations greater than threshold concentrations [3]. It has been reported that several diseases, such as disorders of the nervous, digestive, and hematopoietic systems and even organ deformities, could be caused by heavy metal poisoning, including Pb, Cd, As, Hg, Sn, and Sb poisoning [4,5]. Therefore, with increasing industrialization and environmental pollution, it is necessary to monitor the content of some toxic heavy metal elements in various foods.

Chinese prickly ash (CPA), including *Zanthoxylum schinifolium* Sieb. Et Zucc. (ZS) and *Z. bungeanum* Maxim (ZB) (Rutaceae), is a famous condiment widely used in China, and it is mainly distributed in Sichuan, Gansu, and Shaanxi [6]. Although CPA has aroused considerable attention for its chemical constituents, quality control, and possible uses, it is disappointing that systemic research regarding the heavy metal profile of this well-known food is still lacking [7,8]. Therefore, our present study was designed to investigate the contents of some toxic heavy metal elements, including Cu, Ni, Cr, Pb, Cd, As, Hg, Sn, and Sb, in CPA from different CPA-producing areas using inductively coupled plasma mass spectrometry (ICP-MS).

EXPERIMENTAL

Materials and reagents

Forty-two CPA samples were collected from the primary of CPA-producing areas in China and were identified by Professor Min Li (College of Pharmacy, Chengdu University of TCM). *Z. schinifolium* Sieb. Et Zucc. (ZS) samples were collected from Yunnan, Chongqing, and Sichuan, and *Z. bungeanum* Maxim (ZB) samples were collected from Gansu, Shaanxi, and Sichuan etc. Concentrated nitric acid and 30 % hydrogen peroxide were purchased from Kermel Chemical Reagent Co, Ltd. (Tianjin, China). Concentrated hydrochloric acid and hydrofluoric acid were purchased from Xilong Chemical Co, Ltd. (Chengdu, China). A multi-elemental mixture of metal standards was purchased from the National Testing Center of Nonferrous Metals and Electronic Materials analysis (Beijing, China). All other reagents used in our present study were of chromatographically pure grade.

Sample preparation

The collected samples were crushed over a sieve III filter, then placed in a dryer and dried before use. The testing samples (0.3 ± 0.0001 g) were precisely weighed and placed in a microwave digestion tank. Next, 8 mL of nitric acid was added and placed in an airtight container at room temperature for 30 min. Microwave digestion was subsequently performed. The digestion process was as follows: the temperature was increased from room temperature to 120 °C and held for 12 min, and then the temperature was increased to 165 °C in 6 min and held for 10 min. The temperature was then increased to 180 °C in 5 min and held for 25 min. Finally, the samples were concentrated and washed for ICP-MS determination.

Instruments and optimum conditions

The ICP-MS spectrometer combined with a quadrupole analyzer and collision chamber (Agilent Technologies, USA) were used to perform the heavy metal determination. In this study, the optimal operating conditions for ICP-MS analysis are shown in Table 1.

Table 1: Optimal ICP-MS operating conditions for analysis of studied samples

Instrument parameter	Condition
RF power	1200 W
RF frequency	21.69 MHz
RF Matching	1.38 V
Carrier gas (inner)	0.82 L/min
Makeup Gas	0.14 L/min
Plasma gas	Ar X 50S 5.0
Plasma gas flow (Ar)	13 L/min
Nebulizer pump	0.1 rps
Sample untake	0.5 mL/min
Spray chamber temperature	3 °C
Resolution m/z	254 amu
Background	< 5cps (9amu)
Short-term stability	< 3%
Long-term stability	< 4%
Isotopes measured	^{52}Cr, ^{60}Ni, ^{63}Cu, ^{75}AS, ^{111}Cd, ^{118}Sn, ^{121}Sb, ^{202}Hg, ^{208}Pb

Method validation

Mixed internal standard solutions with a concentration of 1,000 μg/mL, including ^{45}Sc, ^{72}Ge, ^{115}In, and ^{209}Bi, were used to correct the changes in the sample uptake rate and plasma conditions for the ICP-MS measurements. The analytical characteristic of the proposed method was obtained for the nine elements studied under the optimized conditions. Table 2 presents the linear ranges used for calibration and the coefficients of determination (R) used to assess the linearity (R > 0.999). The concentrations of Pb and As in CPA samples exceeded the maximum permissible limits in China. The limits of detection (LOD) and quantification (LOQ) for each metal were determined as follows: nine independent analyses of a blank solution spiked with the metal at a lower level of concentration of the analytical curve were performed. LOD and LOQ were calculated from the standard deviation (σ) of these determinations (LOD = 3.σ and LOQ = 10.σ).

Statistical analysis

Statistical calculations and analysis were performed using SPSS 19.0 (SPSS Inc., USA). Difference in heavy metal concentrations among different CPA samples was detected using Pearson correlation analysis was used for data analyzing. The level of significance was taken as $p < 0.05$ and $p < 0.01$.

RESULTS

To obtain the reliability of the results, recoveries were calculated by spiking with aliquots of metal standards and then analyzing them as usual, according to the original concentrations of the spiked samples [9]. Acceptable recoveries of 95–100 % were obtained for externally added Cu, Ni, Cr, Pb, Cd, As, Hg, Sn, and Sb, respectively. We included at least one laboratory duplicate for each CPA sample, and the result for each batches was analyzed in three times. Moreover, Table 2 presented a good linear ranges used for calibration and the coefficients of determination.

ICP-MS results for CPA samples are listed in Table 3. The basic statistical data such as the number of samples, mean values, minimum and maximum values can be seen. Analysis of results indicate that, the highest concentration of Cr was found in Sichuan (7.33 mg/kg), and that of Ni was found in Shandong (6.86 mg/kg). Moreover, we obtained a high content of As in Sichuan (1.08 mg/kg). In the case of CPA samples, Cu is the most abundant metal in all CPA samples, the range of Cu concentration was 3.29 (in Sichuan) and 24.17 mg/kg. while for As 0.0011-1.08 mg/kg (in Sichuan and Gansu, respectively), and Sb 0.03-0.21 mg/kg (for Gansu, Sichuan). Cd levels ranged from 0.029-0.21 mg/kg (Gansu, Chongqing). Sn was the lowest concentration all analyzed heavy metals (most production areas were not detected), follow by Hg were not detected only in Sichuan and Shanxi. Furthermore, the highest concentration of Pb level was 3.84 mg/kg in Shaanxi, and the lowest was not detected in Gansu.

Correlation coefficients between metal concentrations were presented in Table 4. Statistical analysis by SPSS showed metal-to-metal correlations for all samples between Cu-Cr (p = -4.02), Cu-Ni (P = 0.561), Cu-As (p = 0.554) and Ni-As (p = 0.428) at the 0.01 level. Also, some metal-to-metal correlations were observed in Pb-Cr (p = 0.351), Pb-Cu (p = -0.310), Sb-Cd (p = 0.322), Sb-Hg (p = 0.311) and Cd-Sn (p = 0.309) at the 0.05 level. Moreover, others metal-to-metal correlations for CPA samples were not significant, and each ion appeared independent.

DISCUSSION

The aim of this study was to determine the concentrations of heavy metals in CPA. We obtained results indicate that the concentration of heavy metals contamination differs significantly depending on the production area. Furthermore, the result showed that the concentrations of elements may also differ even within the same production area and the same variety, depending on climate and soil conditions where the CPA were grown.

Based our investigations, the Chinese food standards only regulated the concentrations of As, Pb, Cd and Hg in CPA, which sets a limit for dried samples (0.3 mg/kg for As, 0.50 mg/kg for Cd, 0.03 mg/kg for Hg, and 1.86 mg/kg for Pb). However, for other heavy metals we have not found any requirements.

Table 2: Linear range, regression correlation coefficient (R), LOD, LOQ

Element	Linear range (mg/kg)	Regression	R^2	LOD (mg/kg)	LOQ (mg/kg)
Cr	0-100	y=0.00027x-1.74815	0.99915	0.3258	1.0755
Ni	0-100	y=0.00022x-0.48819	0.99997	0.3452	1.1392
Cu	0-100	y=0.00011x-2.19595	0.99996	0.6794	2.2421
As	0-100	y=0.00048x-0.78168	0.99996	0.00011	0.00037
Cd	0-100	y=0.00025x+0.22823	0.99998	0.0184	0.0599
Sn	0-100	y=0.00007x-0.36808	0.99999	0.0132	0.0433
Sb	0-100	y=0.00008x+0.10945	0.99998	0.028	0.0932
Hg	0-100	y=0.00028x+0.11307	0.99985	0.0081	0.0267
Pb	0-100	y=0.00003x-1.14500	0.99999	0.0935	0.3009

Table 3: Concentrations of heavy metals in different production areas of CPA (mg/kg)

Production area	N	Analyzed Metals (mg/kg)									
		Statistics	**Cr**	**Ni**	**Cu**	**As**	**Sb**	**Cd**	**Sn**	**Hg**	**Pb**
Gansu	13	Mean	1.84	1.85	6.59	0.05	0.075	0.039		0.016	
		Min.	0.08	1.29	4.06	0.0011	0.03	0.029	ND	0.011	ND
		Max.	3.21	3.57	8.19	0.12	0.15	0.06	0.02	0.023	1.25
		SD	0.02	0.05	0.06	0.0168	0.006	0.003		0.0002	
Sichuan	10	mean	2.14	2.71	5.83	0.18	0.09	0.10			0.86
		Min.	0.14	1.59	3.29	0.004	0.01	0.03	ND	ND	0.2
		Max.	7.33	5.88	13.2	1.08	0.21	0.18	0.15	0.032	3.12
		SD	0.02	0.05	0.05	0.02	0.02	0.02			0.05
Shaanxi	6	Mean	1.52	1.93	8.77	0.105	0.063	0.07	0.055	0.022	1.63
		Min.	0.04	1.12	4.69	0.02	0.02	0.05	0.03	0.02	0.01
		Max.	2.89	2.96	13.5	0.27	0.09	0.10	0.08	0.024	3.84
		SD	0.02	0.04	0.05	0.014	0.013	0.011	0.002	0.0005	0.03
Chongqing	2	Mean	1.055	3.54	5.36	0.07	0.20	0.16		0.0225	0.94
		Min.	0.73	2.45	5.33	0.002	0.19	0.11	ND	0.021	0.43
		Max.	1.38	4.63	5.39	0.14	0.20	0.211	ND	0.022	1.44
		SD	0.025	0.02	0.06	0.016	0.035	0.03		0.0003	0.065
Jiangsu	2	Mean	2.17	2.96	10.8	0.081	0.07	0.065		0.021	1.07
		Min.	0.16	1.66	4.88	0.064	0.06	0.06	ND	0.020	0.02
		Max.	4.17	4.25	16.8	0.099	0.08	0.07	0.06	0.023	2.12
		SD	0.02	0.10	0.07	0.06	0.025	0.007		0.0009	0.045
Shanxi	4	Mean	0.995	1.68	11.10	0.09	0.078	0.065			0.95
		Min.	0.14	0.88	4.25	0.07	0.07	0.05	ND	ND	0.01
		Max.	1.94	3.88	14.5	0.11	0.08	0.08	0.04	0.041	2.12
		SD	0.02	0.04	0.10	0.04	0.003	0.006			0.03
Shandong	2	Mean	0.95	4.28	14.4	0.068	0.06	0.055		0.0215	1.27
		Min.	0.14	1.67	4.65	0.063	0.06	0.05	ND	0.021	1.14
		Max.	1.76	6.86	24.17	0.072	0.06	0.06	ND	0.022	1.4
		SD	0.01	0.09	0.15	0.003	0.05	0.001		0.0008	0.035
Guizhou	2	Mean	1.02	2.95	13.40	0.31	0.06	0.055		0.0235	0.43
		Min.	0.35	1.60	6.75	0.063	0.05	0.05	ND	0.023	0.02
		Max.	1.69	4.29	19.96	0.084	0.07	0.06	0.04	0.024	0.83
		SD	0.01	0.04	0.05	0.074	0.03	0.02		0.009	0.03
Yunnan	1	Mean	4.32	1.84	4.70	0.05	0.06	0.10		0.025	1.13
		Min.	4.32	1.84	4.70	0.05	0.06	0.10	ND	0.025	1.13
		Max.	4.32	1.84	4.70	0.05	0.06	0.10	ND	0.025	1.13
		SD									
All samples		Mean	1.76	2.38	7.95	0.897	0.088	0.063			
		Min.	0.04	0.88	3.29	0.0011	0.03	0.029	ND	ND	ND
		Max	7.33	6.86	24.17	1.08	0.21	0.211	0.15	0.032	3.84
		SD	0.02	0.05	0.074	0.03	0.018	0.022			

Note: *ND = not detected*

Table 4: Correlation coefficients between metal concentrations

	Cr	Ni	Cu	As	Sb	Cd	Sn	Hg	Pb
Cr	1.000	-0.153	-4.200**	-0.126	-0.071	-0.104	-0.126	0.062	0.351*
Ni		1.000	0.561**	0.428**	0.185	0.175	0.096	0.186	-0.029
Cu			1.000	0.554**	-0.302	0.004	0.117	-0.028	-0.310*
As				1.000	-0.113	0.037	0.144	0.119	-0.070
Sb					1.000	0.322*	-0.202	0.311*	0.063
Cd						1.000	0.309*	-0.084	0.099
Sn							1.000	-0.260	-0.245
Hg								1.000	0.261
Pb									1.000

*Correlation is significant 95% CI; **Correlation is significant at 99% CI

Our studies have demonstrated that the content of heavy metals do not exceed the maximum permissible limits in CPA samples according to Chinese food standards, except for Pb and As. Meanwhile, a summary on maximum values for heavy metals set by countries in different regions of the world has been recently published by the WHO in 2007 [10], which proposes a limit of 10 mg/kg for Pb and 0.3 mg/kg for Cd in dried herbs. In this research, the highest concentrations of Pb, Cd were 3.84 mg/kg, 0.211 mg/kg in the CPA samples from Shaanxi, Chongqing, respectively, and do not exceed the standard level of 10 mg/kg, 0.3 mg/kg. However, the highest level of Pb was over the upper limits imposed (1.86 mg/kg) for Chinese food standards in Sichuan (3.12 mg/kg), Shaanxi (3.84 mg/kg), Shanxi (2.12 mg/kg) and Jiangsu (2.12 mg/kg). This may be caused by various reasons, such as air, soil pollution, etc. Also, Pb has no beneficial role in human metabolism and can cause some health disorders. Thus, it must be considered seriously. In addition, the result of analysis showed that the content of As (1.08 mg/kg) in Sichuan exceeded the maximum permissible limits in China food standards. For this reason, may be caused by soil enrichment and pesticide pollution.

According to the World Health Organization (WHO) and Food and Agriculture Organization (FAO), regarding health risks, the intake of Pb compounds will cause acute poisoning in humans, and metallic Pb is possibly carcinogenic [11]. The provisional tolerable weekly intake (PTWI) for Pb, set as a limit for metal intake based on body weight for an average adult (60 kg body weight) is 1.5 mg. The researchers reported that Pb intake through food and drinking water was about 0.3 mg (Europe) and 0.61 mg (China) in every week [12,13]. Then, the per capita annual consumption of CPA was calculated is 0.5 kg. Based on the Chinese food standards, when the concentration of Pb reached the limit requirement (1.86 mg/kg), the consumption of Pb is about 0.017 mg, only accounting for 1.192 % in PTWI. The present study showed that the per capita annual consumption of CPA was similar to the values observed in Shaanxi, China [13]. Thus, based on current safety standards, the concentrations of heavy metals in these CPA samples mean they are safe for human consumption.

In recent years, environmental pollution constantly aggravates the constant improvement in the economic level and ecological environment destruction. Consequently, food safety problems, including heavy metal pollution, microorganisms causing foodborne diseases, pesticide residues, organic pollutants, and chemical pollution, have been reported frequently at home and abroad, and have become serious threats to human life [14]. Additionally, more and more investigations have demonstrated that heavy metals are the key factor in food pollution due to their harmful effects on human health at low concentrations.

Heavy metals that enter the body through food, interfere with normal physiological function, and harm human body health are known as toxic heavy metals. These heavy metal elements, including Hg, Cd, Cr, Pb, As, and Sn, which are grouped into medium toxicity (Cu, Sn, etc.) and strong toxicity elements (e.g., Hg, As, Cd, Pb, and Cr) depend on the harm of heavy metal elements on human [15]. The contamination and enrichment of heavy metal elements may occur during production, processing, storage and transportation of food. Recently, heavy metal pollution has become the focus of much international attention, primarily because of the voluminous discharge into the environment from industrial activities. Heavy metals are toxic to ecosystems as well as humans, so their bioaccumulation in the food chain causes serious disorders [16-18]. The elements (Pb, Hg, Gr, As, and Cd) have significant poisonous activities against humans and cannot be degraded in the body. In addition, these heavy metals could present violent toxicities combined with other toxins in the human body. Commonly, Pb, As, and Hg are considered highly toxic elements that can cause serious harm to people’s health and are involved in brain cell damage and hypothermia, leading to dementia, brain death, neurological disorders, and even cancer [19,20].

CONCLUSION

The findings of this study indicate that Cu, Ni, Cr, Pb, Cd, As, Hg, Sn, and Sb are the characteristic heavy metal elements found in prickly ash, this is worth-noting in the safety monitoring of this famous Chinese famous condiment. The concentrations of Pb and As in CPA exceeded the maximum permissible limits in Sichuan, Shaanxi, Shanxi and Jiangsu. Thus, the status of heavy metal concentrations of CPA should be further investigated in Sichuan, Shaanxi, Shanxi and Jiangsu. In addition, ICP-MS is a reliable and rapid technique for the determination of the heavy metals in CPA. Based on the obtained data and current safety standards, CPA in this study are safe for human consumption.

DECLARATIONS

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Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

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