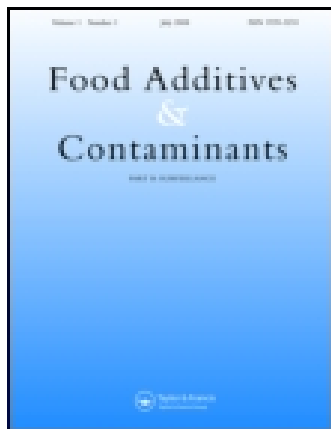


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A survey on the heavy metal contents in Chinese traditional egg products and their potential health risk assessment

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Pb, Zn and Cu were determined in 35 Preserved Egg (PE) samples, 25 Salted Egg (SE) samples and 40 Egg Preserved in Rice Wine (EPRW) samples collected from Jiangxi province by ICP-MS. The corresponding health risk for consumers was assessed by the target hazard quotients (THQ) and hazard index (HI). Average Pb, Zn and Cu content in all samples was 0.125 mg/kg, 10.939 mg/kg and 2.094 mg/kg, respectively. Average Pb content in PE was significantly higher than in SE and EPRW. THQ and HI values were less than 1, indicating that intake of heavy metals from PE, SE and EPRW will not pose a significant hazard risk to humans. However, more attention should be paid to control the ingestion by PE, which is the main source of Pb, Zn and Cu for consumers among these three egg products.

Keywords: egg products; food safety; heavy metals; risk assessment; ICP-MS

Introduction

Egg products are highly nutritive and cheap food for humans and have become an essential part of daily diet in the world. Egg production of China was up to 28.114 million tons in 2011 (National Bureau of Statistics of China 2012) and the majority of egg products were consumed by Chinese. It was reported that egg intakes for adults and children were 39.7 and 19.1 g/d, respectively (Zheng et al. 2007). Besides fresh eggs, Preserved Egg (PE), Salted Egg (SE) and Egg Preserved in Rice Wine (EPRW) are traditional egg products in China. They are the favourite food for Chinese due to their unique taste. PE, also known as pidan or century egg, is a special food of egg preserved in a mixture of salt, tealeaves and alkaline substances (quicklime and ash) for several weeks to several months. SE, also known as xiandan, is egg soaked in brine or packed in salted charcoal for one to two months. EPRW is egg packed in jars and covered by distillers' grains and salt for several months.

Food safety has been a major public concern worldwide in recent decades because food consumption is a major pathway of toxic elements to human beings (Liu et al. 2013). Eggs have been demonstrated to be capable of accumulating heavy metals from diets and surrounding environment (Burger et al. 2009), which may pose a health threat to human, especial for children. Heavy metals, including lead (Pb), zinc (Zn), copper (Cu) and cadmium (Cd), have been detected in egg-associated foodstuffs. Cu and Zn contents in fresh eggs were in the range from 0.61 to 0.67 mg/kg, and 10.96 to 12.33 mg/kg, respectively (Shang & Wang 1997).

Mean concentrations of Cd, Pb and Cu were reported to be 0.021–0.049 mg/kg, 0.06–0.48 mg/kg and 2.29–3.26 mg/kg, respectively (Abdulkhalik et al. 2012). Demirulus (2013) and Giannenas et al. (2009) reported that eggs were rich in Zn and Cu and contained low Cd content. In the report of Zheng et al. (2007), eggs were calculated to contribute 1.13%–4.51% and 1.20%–3.67% of heavy metals for adults and children in Huludao city, respectively. During the processing progress of PE by traditional and modern ways, Pb or Zn may exceed the limits for humans. Furthermore, information concerning the content of heavy metals in SE and EPRW are still limited. Therefore, it is important to measure heavy metals contents in PE, SE and EPRW and assess their potential health risk to human beings.

The potential health risk caused by heavy metals can be classified into carcinogenic and non-carcinogenic effects. As a neurotoxic element, long-term exposure of Pb would result in serious health problems (Oliver 1997). Cu and Zn are essential elements; however, they will also pose non-carcinogenic effects to human health if their exposure doses exceed the thresholds (or tolerance reference does) for humans (US EPA 2013). The non-carcinogenic effects of heavy metals can be estimated by Target Hazard Quotients (THQ) and the Hazard Index (HI), an integrated risk index by comparing the ingestion amount of a pollutant with a standard reference dose, proposed by US EPA (Storelli 2008). THQ has been successfully used to qualitatively evaluate the possible non-carcinogenic effects of heavy metals for humans through food consumption (Han et al. 1998; Huang et al. 2008; Khan et al. 2013; Li et al. 2013). The HI index was set to assess the overall potential risk

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resulting from multiple toxic elements (US EPA 1986; Zhuang et al. 2013). A lot of studies on the health risk of heavy metals via food consumption have been focused on vegetables (Wang et al. 2005; Khan et al. 2008; Gebrekidan et al. 2013; Ji et al. 2013; Saha & Zaman 2013), rice (Fu et al. 2008; Zhuang et al. 2009), fish (Yi et al. 2011; Copat et al. 2012; Saha & Zaman 2013), Puerh tea (Cao, Qiao, et al. 2010), herbal flowers (Zhu et al. 2013) and fruits (Radwan & Salama 2006; Saha & Zaman 2013). Few studies have been conducted to compare the health risk of heavy metals in eggs (Zheng et al. 2007) and egg-based foods (Cabrera-Vique et al. 2011) with other foodstuffs, where it was found that eggs contributed importantly to the dietary intake of heavy metals.

However, the information concerning the heavy metal contents of Chinese traditional egg products (PE, SE and EPRW) and the evaluation of their health risk are still scarce. Therefore, the objectives of the present study were to measure the concentration of Zn, Cu and Pb in PE, SE and EPRW and assess their health risk for adults and children based on THQ and HI values. In this study, only Pb, Cu and Zn were measured, since only these are compulsory due to the Hygiene standard for egg products of China (SAC 2003a) and Hygiene standard for preserved eggs of China (SAC 1988).

Materials and methods

Sampling

A total of 100 egg samples were collected from markets in June 2013 in Jiangxi province, China. These included 35 PE, 25 SE and 40 EPRW samples. Several eggs from the same sample were pooled and homogenised with a clean plastic mixer and stored at 4°C prior to analysis.

Sample analysis

All samples (2–3 g for each one, fresh weight) were digested according to China's national standard methods for the determination of Pb, Cu and Zn in foodstuffs (SAC 2010, 2003b, 2003c) and the elemental content was measured by inductively coupled plasma mass spectroscopy (ICP-MS) using an ELAN 9000 instrument (Perkin-Elmer-Sciex, Waltham, MA, USA). For the purpose of detection accuracy, the stock in solution was used as an

internal standard during the analysis procedures. The most abundant and less interfered isotopes (^{63}Cu , ^{66}Zn , ^{208}Pb and ^{115}In) were chosen to determine the concentration of studied elements. The limits of detection (LOD) were defined as three times the standard deviation of 11 replicates of standard blanks as proposed by Li et al. (2013). The LOD values of Pb, Cu and Zn were 0.004, 0.50 and 0.20 $\mu\text{g}/\text{kg}$, respectively and their recovery ratios ranged from 98% to 102% throughout the analysis procedures. In this study, the correlation coefficients (R^2) of Pb, Cu and Zn were 0.9998, 0.9997 and 0.9995, respectively. The operation parameters were set as proposed by Zhang et al. (2006). All chemicals used in this study were of guaranteed grade. All experiments were conducted in duplicate and the mean values were used for further calculation.

Statistical analysis

The descriptive statistics (mean, standard deviation (SD) and range) were summarised and calculated using Microsoft Excel 2010. The average concentration of each heavy metal was used for further calculations. Student's *t*-test was employed for statistical analysis of heavy metal concentrations in three types of egg products and performed by Origin 9.0. A *p*-value <0.05 was considered to indicate a significant difference across the compared groups.

Results and discussion

Heavy metals in egg products

The analytical results are given in Table 1. Concentrations of Pb, Zn and Cu in all analysed egg products samples varied largely, indicating remarkable effects of egg origin (Nisianakis et al. 2009) and processing procedures on metal concentrations. Generally, Zn concentration was significantly larger than Pb ($p < 0.01$) and Cu ($p < 0.01$). Moreover, Zn and Cu content were two and one orders of magnitude higher than that of Pb, respectively, which confirmed the fact that eggs are rich in both Zn and Cu. Pb content in PE was significantly high compared with SE and EPRW ($p = 0.02$ and $p = 0.03$, respectively). The relatively high concentration of Pb in PE could be attributed to the accumulation of Pb by PE from its several essential ingredients (salt and alkaline substances) during the processing procedures (Hou 1981).

Table 1. Heavy metal concentrations (mg/kg) in investigated egg products.

Foodstuffs	Number of samples	Pb		Zn		Cu	
		Range	Mean \pm SD	Range	Mean \pm SD	Range	Mean \pm SD
PE	35	0.006–1.361	0.212 \pm 0.317	4.780–31.144	10.436 \pm 4.388	0.617–7.903	2.684 \pm 1.979
SE	25	0.004–0.183	0.053 \pm 0.048	6.312–23.244	11.221 \pm 4.071	0.709–5.439	1.758 \pm 1.138
EPRW	40	0.007–0.578	0.094 \pm 0.115	6.567–20.231	11.204 \pm 3.064	0.408–6.012	1.788 \pm 1.688
Total	100	0.004–1.361	0.125 \pm 0.212	4.780–31.144	10.939 \pm 3.803	0.408–7.903	2.094 \pm 1.724

Table 2. Maximum limits (mg/kg) for the investigated metals in egg products.

Foodstuff	Contaminant		
	Pb	Zn	Cu
PE	2.0 ^a /0.5 ^b	50 ^a /30 ^c	10 ^c
SE	0.2 ^a /0.2 ^b	50 ^a	NA ^d
EPRW	1.0 ^a /0.2 ^b	50 ^a	NA ^d

Note: ^aMaximum limit of contaminants in egg products (SAC 2003a).

^bMaximum limit of contaminants in foodstuffs (SAC 2012).

^cMaximum limit of contaminants in preserved eggs (SAC 2002).

^dNo data available.

As shown in Table 2, national standard maximum limits of Pb, Zn and Cu in egg products are in effect in China (SAC 2003a, 2002) to assure safe egg consumption. Pb concentration in all samples was below the limits as set in GB 2749–2003 (SAC 2003a). However, Pb content in three PE samples and six EPRW samples exceeded the Pb maximum limit of GB 2762–2012 (SAC 2012), with rates being 8.57% and 15%, respectively. Furthermore, Zn concentration in all samples was below the limits in both GB 2749–2003 (SAC 2003a) and NY 5143–2002 (SAC 2002), except for one PE sample whose Zn content (31.144 mg/kg) exceeded the maximum limit of contaminants in preserved eggs (SAC 2002). In terms of Cu, there are still no maximum limits for SE and EPRW in the current national standard of China (SAC 2003a). However, Cu content in all analysed samples was within the Cu limits as set in NY 5143–2002 (SAC 2002) and their mean concentrations in each type of sample were far below this limit. In general, studied SE and EPRW samples can be safely ingested by consumers, while people better control their daily intake of PE.

The metal content in this study was comparable with previous investigations. Average Cu and Zn content in eggs were reported to be 1.608 mg/kg and 13.93 mg/kg, respectively (Zheng et al. 2007) and their concentration ranges in fresh eggs were 0.61 to 0.67 mg/kg and 10.96 to 12.33 mg/kg, respectively (Shang & Wang 1997), both of which were close to the results of this study. For Pb, the average contents in SE and EPRW are close to the

value (0.052 mg/kg) (Zheng et al. 2007). While, the average value in PE is fourfold higher than that of Zheng et al. (2007), suggesting that Pb is abundant in PE. In addition, both Pb and Cu concentrations of this study are similar to the results reported by Abdulkhalik et al. (2012).

Estimated daily intake of heavy metals by consuming egg products

The estimated daily intake of heavy metals is a fundamental parameter for health risk assessments. It can be calculated with

$$EDI = \frac{C \times E_F \times E_D \times F_{IR}}{W_{AB} \times T_A \times 1000} \quad (1)$$

where E_F is the exposure frequency (365 days/year); E_D is the exposure duration (70 years); F_{IR} is the fresh food ingestion rate (g/person/day); C is the metal concentration ($\mu\text{g/g}$); W_{AB} is the average body weight (60 kg for adults and 32.7 kg for children (Fu et al. 2013) and T_A is the average exposure time for non-carcinogens. According to the report of Zheng et al. (2007), it is assumed that each of PE, SE and EPRW accounted for 10% of the daily intake of egg for both adults and children (so in the present study $F_{IR} = 3.97$ and 1.91 g/day for adults and children, respectively).

Health risks for adults and children are considered separately because of the discrepancy in between them (Wang et al. 2005) due their difference in body weight and daily intake of foodstuffs. Estimated Daily Intake (EDI) values of Pb, Zn and Cu for adults and children via the consumption of PE, SE and EPRW were presented in Table 3. Both for adults and children the trend of EDI for heavy metals in PE, SE and EPRW was in the order $Zn > Cu > Pb$ and intake of each metal was higher for adults than that for children. The trend in this study is the same as found for rice and garden vegetables (Cao, Chen, et al. 2010), Puerh tea (Cao, Qiao, et al. 2010), rice (Zhuang et al. 2009) and other foodstuffs including eggs (Zheng et al. 2007). It means

Table 3. Estimated daily intake (EDI) (mg/kg bw/d) of heavy metals for adults and children due to egg products consumption.

Population	Foodstuffs	EDI of heavy metals		
		Pb	Zn	Cu
Adults	PE	1.406×10^{-5}	6.905×10^{-4}	1.776×10^{-4}
	SE	3.515×10^{-6}	7.425×10^{-4}	1.163×10^{-4}
	EPRW	6.207×10^{-6}	7.413×10^{-4}	1.183×10^{-4}
Children	PE	1.111×10^{-5}	5.457×10^{-4}	1.404×10^{-4}
	SE	2.777×10^{-6}	5.868×10^{-4}	9.194×10^{-5}
	EPRW	4.906×10^{-6}	5.859×10^{-4}	9.352×10^{-5}

that the contribution of various foodstuffs to Zn, Cu and Pb follows the same order for both adults and children. It is observed that EDI values of Pb are several times smaller than that for shellfish (Li et al. 2013), fish (Saha & Zaman 2013), rice and vegetables (Zhuang et al. 2009), but fairly comparable with those for fruits (Saha & Zaman 2013) and Puerh tea (Cao, Qiao, et al. 2010). Our findings imply that PE, SE and EPRW contribute less importantly to daily heavy metal intake than main foodstuffs (e.g. rice, vegetables and fish).

As shown in Figures 1 and 2, PE contributed most to the daily intake of Pb for adults and children in all 3 egg products, followed by EPRW and SE. It indicates that the main source of Pb to the diet is PE. The largest contributions to the daily intake of Cu for adults and children were also from PE, followed by SE and EPRW. In addition, the contributions of PE, SE and EPRW were roughly equivalent for Zn intake, both for adults and children. Overall, PE is the most important source of Pb, Zn and Cu for both adults and children among these three Chinese traditional egg products.

Potential health risk of heavy metals

Health risk of individual heavy metals

The potential non-carcinogenic effects of individual heavy metals can be expressed as THQ (US EPA 1986) and be calculated as

$$\text{THQ} = \frac{\text{EDI}}{\text{RfD}} \quad (2)$$

where RfD is the oral reference dose. The RfD values of Zn and Cu are set to be 0.3 and 0.04 mg/kg/day (US EPA 2013), while Pb is set at 3.57 $\mu\text{g}/\text{kg}/\text{day}$ according to provisional tolerable daily intake (PTDI) suggested by

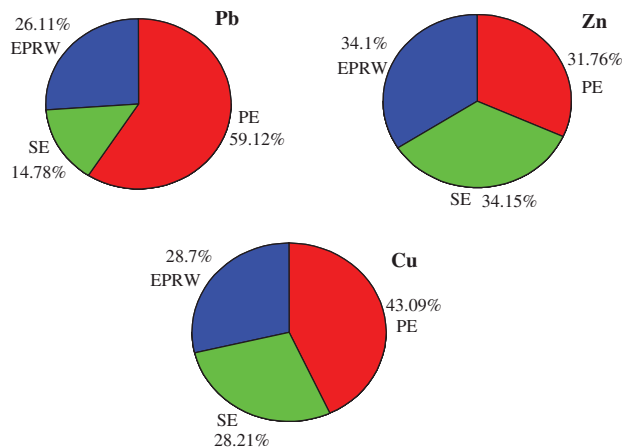


Figure 2. The contribution of individual egg products to the total daily intake of heavy metals for children.

FAO/WHO (JECFA 2003) in this study, since the RfD for Pb in US EPA (2013) is not available.

The THQ of individual heavy metals through consumption of PE, SE and EPRW for adults and children were derived and listed in Table 4. It was observed that there was no THQ value over 1 via the consumption of PE, SE and EPRW, indicating that people would not experience significant health risks (Zheng et al. 2007; Zhu et al. 2013) if they only ingested individual heavy metals from PE, SE and EPRW. Similar results have been reported by earlier studies (Wang et al. 2005; Zheng et al. 2007; Saha & Zaman 2013). Additionally, eggs also had no noticeable human health risk for egg consumers based on a THQ study (Abduljaleel & Shuhaimi-Othman 2011). It is notable that THQ of a single metal for adults is higher than that of children, which coincidences with a previous report (Zheng et al. 2007). THQ values of Pb were the lowest, which should be related to its low content. THQ of

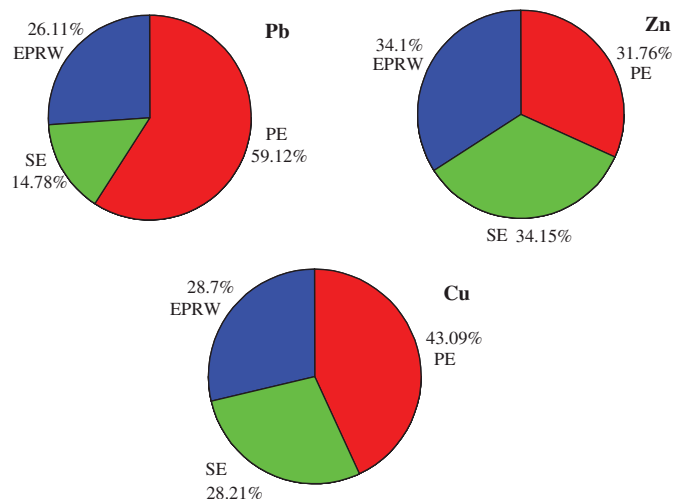


Figure 1. The contribution of individual egg products to the total daily intake of heavy metals for adults.

Table 4. THQ, TTHQ and HI of heavy metals for adults and children due to egg products consumption.

Population	Foodstuffs	THQ			TTHQ	HI
		Pb	Zn	Cu		
Adults	PE	0.00394	0.00230	0.00444	0.0107	0.0242
	SE	0.000983	0.00247	0.00291	0.00636	
	EPRW	0.00173	0.00247	0.00296	0.00716	
	TDHQ	0.006653	0.00724	0.01031		
Children	PE	0.00311	0.00182	0.00351	0.00844	0.0191
	SE	0.000778	0.00196	0.00230	0.00504	
	EPRW	0.00137	0.00195	0.00234	0.00566	
	TDHQ	0.005258	0.00573	0.00815		

Pb in the consumption of eggs and other foodstuffs are also found to be the minimal, compared with that of Cu and Zn (Wang et al. 2005; Zheng et al. 2007; Huang et al. 2008), while THQ values of Cu were the biggest, indicating that more attention should be paid to the health risk of Cu due to egg consumption.

Combined health risk of heavy metals

Additive and/or interactive effects may result from exposure of two or more contaminants. Total THQ (TTHQ) has been reported to assess the overall non-carcinogenic effects of multiple heavy metals in individual foodstuff (Zheng et al. 2007; Saha & Zaman 2013). TTHQ can be calculated as

$$\text{TTHQ}(\text{individual foodstuff}) = \sum_{i=1}^n \text{THQ}_i \quad (3)$$

where THQ_i is the THQ value of toxicant i .

The health index, HI, was used to estimate the total no-carcinogenic health risk caused by multiple heavy metals (US EPA 1986). HI for a specific receptor/pathway combination (e.g. diet) was calculated as

$$\text{HI} = \sum_{i=1}^n \text{TTHQ}_i \quad (4)$$

where TTHQ_i is the TTHQ value of foodstuff i .

The total diet THQ (TDHQ) of each metal in PE, SE and EPRW for adults and children was generally less than 1 (Table 4), suggesting that adult and children consumers of PE, SE and EPRW would not confront adverse health risks by ingestion of individual metal Pb, Cu and Zn (Zheng et al. 2007). In addition, TTHQ and HI values for adults and children were less than 1 (Table 4), also indicating that there would be no significant potential health risk for human. Compared with other kind of foodstuffs, egg consumption posed the lowest health risk for humans (Zheng et al. 2007). However, the TTHQ values in the present

study are lower than those in eggs as reported by Zheng et al. (2007), which can be explained by the fact that PE, SE and EPRW only account for a relative small part of the total amount of egg consumption. Even though TTHQ values of PE for adults and children are less than one, it will be of great importance for consumers to control the dietary intake of PE because increased PE consumption will result in a higher health risk.

Throughout this study, HI values for adults were higher than those of children, meaning that adults experience a higher level of risk than children. The relative contributions of PE, SE and EPRW to HIs were 44.18%, 26.26% and 29.56% for adults and 44.10%, 26.33% and 29.57% for children, respectively. The relative contributions of Pb, Zn and Cu to HI were 27.49%, 29.91% and 42.60% for adults and 27.47%, 29.94% and 42.59% for children, respectively. The results of this study show that in PE, Cu is the major component contributing to the potential health risk, followed by Zn and Pb.

Conclusion

Pb, Zn and Cu concentrations in three Chinese traditional egg products ranged from 0.0045 to 1.361 mg/kg, 4.780 to 31.144 mg/kg and 0.408–7.903 mg/kg, respectively. The average content of Pb in PE was significantly higher than that of SE and EPRW. According to limits of national standards of China, SE and EPRW are safe egg products for human consumption, while PE is of concern. PE is the main source of Pb, Zn and Cu for consumers. THQ values of each studied metal in PE, SE and EPRW and the HI values for adults and children were smaller than 1. The obtained results demonstrated that consumption of PE, SE and EPRW will not result in significant health risks for human consumption, when too high consumption of PE is avoided.

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