Evaluation of the Effect of Different Cooking Methods on the Heavy Metal Levels in Crayfish Muscle

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Highlights

- The levels of Cu in crayfish abdominal muscle increased significantly after cooking processes.
- A high level of As was determined in commercial Spicy-ready-to-eat crayfish abdominal muscle.
- Household cooking methods are relatively safer than commercial cooking for crayfish abdominal muscle, and children should consume crayfish in moderation.

Abstract: The current study investigated the effects of various cooking styles (boiling, frying, and steaming) and seasoning methods (home cooking and ready-to-eat commodity) on levels of nine heavy metals in the crayfish (*Procambarus clarkii*) muscle. The estimated daily intake (EDI), target hazard quotients (THQ), and target cancer risk (TCR) were used to assess the health risk in the crayfish muscle. The results showed that cooking processes significantly increased the concentration of Cu, which raises a potential risk for children (the THQ values > 1). The levels of toxic heavy metals in the ready-to-eat crayfish muscle were significantly higher than those in household cooking. Especially for As, the THQ values rose to 7.1 and 13.2 for adults and children respectively. Therefore, home cooking is safer than ready-to-eat crayfish, and children should consume crayfish within a limited range. The recommended consumption of the cooked abdominal muscle of crayfish should be 257 and 58 g/day, for children (16 kg) and adults (70 kg), respectively.

Keywords: ***Heavy metals; Crayfish abdominal muscle; Cooking process; Condiments; Risk assessment***

1. Introduction

Crayfish (*Procambarus clarkia)*, which originated from the southern United States and northern Mexico, was introduced into, and cultivated in China in the early 20th century (Zhang et al., 2021). China is currently the world's largest producer and consumer of crayfish (FAO, 2019). According to statistics from China, in 2021, the total yield of crayfish farming reached 2.39 million tons and continues to grow. The major crayfish farming area is located in the middle and lower reaches of the Yangtze River basin.

Crayfish is indeed a great food to promote, because of its health benefits; it is naturally packed with minerals, vitamins, and protein (Adebiyi et al., 2020). Moreover, it is also considered a very popular dish due to its taste and impact on the local food culture. However, the exposure to heavy metals in the aquatic biota through the food chain is a problem that cannot be ignored (Bosch et al., 2016; Varol & Sünbül, 2017), the same is true for crayfish. Crayfish have a special digestive gland, which not only absorbs and stores food, but also handles heavy metals (Antón et al., 2000), that can accumulate, and cause potential harm to the human body. Moreover, toxic metals such as mercury (Hg), cadmium (Cd), chromium (Cr), and lead (Pb) can induce a series of cancers, neurotoxicity, nephrotoxicity, and organ failure even at very low doses (Ikem et al., 2021; Zhou et al., 2021). Furthermore, copper (Cu), zinc (Zn), nickel (Ni), manganese (Mn), and other essential trace elements may be harmful to the human body if consumption is excessive (Wang et al., 2019; Zhou et al., 2021). Therefore, crayfish as one of the most popular aquatic products in China, has recently attracted more and more attention as there is a need to monitor heavy metal pollution.

The cooking process may cause some changes in the physical chemistry properties of food, such as the content and biological acceptability of metals (Rodríguez-Estival et al., 2019). Indeed, the variability of metal levels after different cooking methods in aquatic products is still a controversial issue. Abd-Elghany et al. (2020) found that certain heavy metals in crab and shrimp could dissolve and be released during boiling. Mercury content in scallop viscera also reduced by 59% during boiling (Toyes-Vargas et al., 2016). In contrast, Wiech et al. (2017) reported that boiling resulted in ten-fold higher Cd concentrations of claw meat compared to raw claw meat. Steaming also caused a strong increase in As, Cr, Cu, and Pb of mussels (Barbosa et al., 2018). It seems that sometimes aquatic products can further accumulate or reduce the levels of heavy metals during various cooking processes. At present, most studies of heavy metal residues in crayfish and health assessment focus mainly on raw crayfish (Baki et al., 2018; Tan et al., 2021; Xiong et al., 2020). The human risk assessment related to edible crayfish may be incomplete, therefore, the effect of cooking processes on levels of heavy metals in the crayfish abdominal muscle should be investigated.

The purpose of this study is to evaluate the effects of cooking styles (boiling, frying, and steaming), and seasoning methods (household and ready-to-eat) on the levels of nine heavy metals including arsenic (As), barium (Ba), cadmium (Cd), chromium (Cr), copper (Cu), mercury (Hg), manganese (Mn), nickel (Ni) and lead (Pb) in crayfish abdominal muscle. The human health risk of consuming the crayfish abdominal muscle treated with different cooking methods was investigated using the daily intake (EDI), target risk quotient (THQ), and cancer risk (TCR), to provide instructive advice on food safety.

2. Materials and methods

2.1 Sample collection

Raw samples were purchased in the market around the Huazhong Agricultural University, located in Hubei Province, China. These samples were immediately packed in ice boxes and transported to the laboratory. After cleaning, the crayfish samples were sealed and stored in polyethylene bags, then stored in the refrigerator at -18 ℃ for further experiments. Spicy-ready-to-eat crayfish were obtained from the supermarket around Huazhong Agricultural University.

2.2 Sample treatment and analysis

2.2.1 Cooking processes of the crayfish abdominal muscle

Sufficient raw frozen crayfish in cold storage were thawed by Micro-flow water and subsequently, 120 individual crayfish were randomly selected. In addition to the raw samples used as references, the remaining samples were cooked using four processing methods: (1) boiling at 100 ℃ was completed for 15 min, (2) frying by immersion in rapeseed oil at 180 °C for 5 min, (3) steaming above vapor at 105 °C for 15 min, (4) reference to traditional Chinese household seasoning and cooking, frying samples for 5 min, followed by boiling with spicy condiments for 15 min. (see Table 1). Similarly, 24 individual ready-to-eat crayfish commodities were randomly selected. Edible abdominal muscle was collected from crayfish with plastic tweezers after cooking and weighed in order to calculate the cooking loss (CL). The samples were put in clean beakers for drying to constant weight at 65°C.

2.2.2 Digestion method of crayfish

The crayfish abdominal muscle samples were dried and ground. Samples (0.5g) were placed in 50 mL digestion vessels with ultrapure HNO_3 (65%, 5 mL) and H_2O_2 (30%, 1 mL) was added. The digestion lasted for 10 h in an autoclave and heated in 4 stages: the first stage was at low temperature (50 °C) for 1 h, the second stage at 70 °C for 1 h, the third stage at 100 °C for 1 h, and the final fourth stage at 120 °C for 7 h. The samples were cooled to room temperature, transferred to volumetric flasks, and then diluted to 25 mL with HNO3 (1%). As, Ba, Cd, Cr, Cu, Hg, Mn, Ni, and Pb were measured using the Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES/ AES: Varian (720-ES)).

2.2.3 Quality assurance

The quality assurance/quality control (QA/QC) system included blanks, parallel samples, duplicate measurements, and certified reference materials that meet the Chinese National standard of GSB 04-1767-2004. The detection limits for As, Cd, Ba, Cr, Hg, Cu, Ni, Mn, and Pb, are 0.0001 mg/L(ppm).

2.3 Measurement of cooking loss of the crayfish abdominal muscle

Cooking loss (CL) is calculated as the percentage of weight loss after cooking relative to the initial weight of the sample. The equation is expressed as follows (Fan et al., 2020; Wan et al., 2019):

$$CL = (M_i - M_j)/M_i \times 100\% \quad (1)$$

Where M_i represents the weight of the crayfish abdominal muscle before cooking and M_j represents the weight of the crayfish abdominal muscle after cooking.

2.4 Health risk assessment

Edible abdominal muscles of crayfish were selected to assess the risk to human health. Estimated daily intake (EDI) and target hazard quotients (THQ) were used to determine the non-carcinogenic health risk assessment. Target cancer risk (TCR) assessment is based on the cancer slope factor (CSF).

2.4.1 Calculation of EDI

EDI aims to evaluate risk by assessing the consumption of contaminated food. EDI is determined by the following formula (Arisekar et al., 2020):

$$EDI = \frac{C_i \times FIR}{a \times BW} \quad (2)$$

Where C_i is the concentration of heavy metals in the crayfish abdominal muscle (mg/kg, dry weight); a is the conversion factor between dry weight (DW) and wet weight (WW); FIR is the ingestion rate of the crayfish abdominal muscle, assuming a daily meal size of the abdominal muscle: children:72 g; adults:168 g (Peng et al., 2022). BW is the average body weight (adult: 70 kg, children: 16 kg).

2.4.2 Calculation of THQ

The THQ proposed by USEPA (2012) is a method to evaluate the health risks caused by the ingestion of chemical pollutants based on the ratio of the amount absorbed by the human body to the reference amount, it is calculated by the following formula (Arisekar et al., 2020):

$$THQ = \frac{EDI \times EF \times ED}{RfD \times AT} \quad (3)$$

EF is the population exposure frequency (365 days/year). ED is the duration of exposure years (considering a lifetime of 6 years for children and 70 years for adults). RfD is the reference oral dose of heavy metals (μg/kg/day; As,0.3; Ba,200; Cd,1; Cr,1500; Cu,40; Hg,0.5; Mn,140; Ni,20; Pb,4). AT is the average exposure time of the non-carcinogenic risk (365 days/year×ED).

To assess the overall health risk posed by more than one metal, the Hazard index (HI) originating from THQ was utilized and is expressed as the sum of the hazard quotients (USEPA, 2011). The HI values through daily consumption of the crayfish abdominal muscle for human beings were evaluated using the following equation:

$$HI = THQ\,(As) + THQ\,(Ba) + THQ\,(Cd) + THQ\,(Cr) + THQ\,(Cu) + THQ\,(Hg) + THQ\,(Mn) + THQ\,(Ni) + THQ\,(Pb) \quad (4)$$

Where HI < 1 is safe, HI > 1 is hazardous.

2.4.3 Calculation of TCR

TCR refers to the probability that a person will develop cancer due to exposure to potential carcinogens in his/her lifetime (USEPA, 1989). TCR is calculated as follows (Yu et al., 2020):

$$TCR = \frac{EDI \times EF \times ED}{AT} \times CSF \times 10^{-3} \quad (5)$$

CSF is the cancer slope factor (mg/kg; As,1.5; Cd,6.3; Cr,0.5; Hg,8.5E-06; Pb,0.0085). AT is the average exposure time of carcinogenic exposure (365 days/year×ED).

2.5 Graph and statistics

Bar charts were performed with GraphPad (Prism 9) and MATLAB (R2018a, MathWorks, Inc., USA) software. Significant differences among means were determined by ANOVA and Duncan multiple range test (SPSS V27, IBM). Pearson correlation was used to analyze the relationship of variables, cluster analysis was used to classify the association of heavy metals, and principal component analysis (PCA) was used to further verify correlation analysis.

3.Results and discussion

3.1 Cooking loss and moisture content of the crayfish abdominal muscle under different cooking processes.

As illustrated in Table1, the moisture content (MC) and weight of the crayfish abdominal muscle showed significant differences before and after cooking ($P < 0.05$). Due to the protein denaturation and muscle fiber contraction (Fan et al., 2020); the average MC in the cooked crayfish abdominal muscle decreased, ranging from 7.89% to 17.98% compared with raw samples. And the MC is considered the most important driver of cooking loss (CL), the CL of the cooked crayfish abdominal muscle decreased from 25.13% to 38.77%. The MC in spicy seasoned samples was much lower than in unseasoned samples ($p<0.05$), which might be attributed to the increase in osmotic pressure caused by condiments in processed crayfish liquid, leading to more water loss in spicy seasoned samples. Referring to the calculation method of Rahman et al. (2012), the conversion factor between dry weight and wet weight in samples was calculated using MC. For example, the MC in fish is 79%, and the conversion factor is calculated as 4.8. The conversion factors of the crayfish abdominal muscle under different cooking processes are presented in Table 1.

3.2 Effects of different cooking styles on the heavy metal levels detected in crayfish

The results of heavy metal levels in the crayfish abdominal muscle were determined under different cooking styles (boiling, frying, and steaming) shown in Table 2. The mean values of Cu, Cd, and Ni of cooked samples increased significantly compared to the raw samples. And obvious differences were also observed among the three cooking methods ($p<0.05$). The mean levels of Cu (41.30 ± 6.07mg/kg) and Cd (0.06 ± 0.03 mg/kg) in fried samples increased by 40.9% and 50.0% compared to raw samples, respectively. The mean concentration of Ni (2.21 ± 0.40 mg/kg) in steamed samples was roughly 4 times higher than in raw samples. This is consistent with previous studies, Bao et al. (2021) found that Cu levels in crab meat (WW) increased by 20 - 30% compared to the raw samples after being treated with all cooking methods (steaming, frying, and boiling). Similarly, the average Cu concentration (39 ± 18 mg/kg, WW) in brown crab meat increased by 160% after steaming (Maulvault et al., 2012). Barbosa et al. (2018) reported that steaming increased the concentration of Cu by about 36% (DW) in mussels. Similarly, Cd concentrations (0.30 ± 0.29 mg/kg, WW) in boiled crab claw meat increased tenfold compared to raw claw meat (Wiech et al., 2017). The levels of Ni (0.62 ± 0.13 mg/kg, WW) and Cu (20 ± 5 mg/kg, WW) in shrimp increased 110.5% and 29.4% respectively after Pan-frying (Kalogeropoulos et al., 2012). The phenomena may be explained by changes in CL (Table 1), lipid volatilization, carbohydrate, and protein degradation during the cooking process (Schmidt et al., 2017), which could have reduced the actual dry weight of the sample and increased heavy metal levels per unit weight of the samples. Another explanation for this trend is that heavy metals from the hepatopancreas may have contaminated the abdominal muscle of crayfish during cooking processes, resulting in higher levels of heavy metals in the cooked crayfish abdominal muscle. On the contrary, the boiling method obviously decreased the levels of Cr ($p<0.05$) in the crayfish abdominal muscle compared to raw samples in this study. Barbosa et al. (2018) also reported that Cr concentration decreased (by 28%) in cooked mussels. In addition, the boiling process has been shown to exhibit a significant reduction of up to 99.8% for Cr in boiled shrimp (Ulaganathan et al., 2022). These results indicate that the changes in heavy metal levels of aquatic products are influenced largely by subjects and the cooking method. For example, cooking can reduce mercury by 59% in scallop viscera (Toyes-Vargas et al., 2016), but significantly increased ($p <0.01$) the Hg levels in the Norway lobster (Perugini et al.,

2016) and fish (Costa et al., 2016).

3.3 Effects of different seasoning and cooking methods on metal levels in the crayfish abdominal muscle

The concentration of heavy metals in the crayfish abdominal muscle treated with different seasoning methods (home and commercial styles) was investigated and illustrated in Table 2. The mean levels of toxic heavy metals As (2.56 ± 0.46 mg/kg), Hg (0.22 ± 0.06 mg/kg), and Pb (0.34 ± 0.09 mg/kg) in RE were significantly higher than those of HC ($p<0.01$). Especially the concentration of As in RE for some reason exceeded the maximum limit value of national standards (GB2762-2017). On one hand, the original heavy metal accumulation in crayfish may be different. In the study of Peng et al. (2022), the distribution of As concentration in crayfish obtained from the Hubei province was greatly dissimilar (0.67-4.05mg/kg, DW). On the other hand, the commercial seasoning method seems to have a greater chance to toxic heavy metals contamination than homemade. Similar results were reported by Peng et al. (2016), who found that As contributed to most of the risk (hazard index) of metals in 210 commercial seasoning crayfish samples collected from the Yangtze River Basin area. In Malaysia, most processed species (canned fish and salted seafood products) showed significantly higher As levels than the local permitted limit (1.0 mg/kg) (Jeevanaraj, Foat, et al., 2020).

3.4 The correlations and cluster analysis of nine heavy metals in different crayfish abdominal muscle samples

The results of hierarchical cluster analysis based on nine heavy metals autocorrelations coefficient of the crayfish abdominal muscle are shown in Fig. 1. Nine heavy metals were separated into three clusters: 1) Cu, Ni, and Mn; 2) Cd, Cr, and Ba; 3) As, Hg and Pb. Correlations between heavy metals can reveal the information about their source and their association. A significant positive correlation was observed between Cu and Ni, implying that they may have originated from similar sources. The reason may be that crayfish abdominal muscle absorbed these free metals transferred from the cooking ware or the other additives during cooking. Even for high-quality corrosion-resistant alloy cooking wares, low levels of metals are also released from the stainless-steel surface into the solvent (Hedberg & Odnevall Wallinder, 2015). Disyawongs & Mukprasert (2005) also showed a trend of Pb transference in prawns cooked in ceramic containers. Mn, at relatively high concentrations, occurs naturally and is not associated with human activities (Xiong et al., 2020).

The positive correlations among Ba, and Cd imply that the condiments may be one of the sources of these heavy metals. For instance, peppers have great potential for absorbing trace metals (Khan et al., 2020). Some literatures have reported the accumulation of Cd in peppers (Huang et al., 2015; Ugulu et al., 2021). Significant correlations were observed between As, Hg, and Pb, and differences in toxic metal levels of RE and HC, implying that these metals may be derived from extraneous contaminants during the commercial seasoning process.

3.5 The PCA analysis of heavy metal levels in the crayfish abdominal muscle treated with different cooking processes

Principal component analysis (PCA) was used to trace more relationships between heavy metal levels in the crayfish abdominal muscle and different cooking processes. Three principal components explained 84.79% of the total variability. The first principal component (PC1) accounted for 41.78%, the second PC2 31.21%, and the third PC3 11.80% of the total variance. The 3D plot of PCA in Fig. 2. shows three groups formed very clearly in the three-dimensional space, one group is the raw samples, the samples treated with three preliminary cooking methods including boiling, frying, and steaming clustered together, two kinds of seasoning cooking styles including home cooking and commercial cooking form the third group. This phenomenon verified the results of the relevant analysis, implying the close association of heavy metals during cooking processes. Likewise, strong correlations of heavy metals have also been observed in processing of fruit products (Abbasi et al., 2020), which might be due to similarities in sources of contamination during food processes.

4. Health risk assessment

4.1 EDI

Based on the conversion factor, EDIs of nine heavy metals obtained from consumption of the crayfish abdominal muscle treated with different cooking and seasoning styles in children (16 kg) and adults (70 kg) were calculated (Table 3). The EDI values of heavy metals both in children and adults were almost all below the PTDI limits set by the joint FAO/WHO Expert Committee on Food Additives (JECFA,1999), except the EDIs of As and Hg in RE for children, 3.98 $\mu g^{-1} kg^{-1} d^{-1}$ and 0.34 $\mu g^{-1} kg^{-1} d^{-1}$ respectively. These results indicate that children in Wuhan consume ready-to-eat crayfish commodities that may cause certain health risks.

4.2 THQ

THQ is an estimation of the risk level (non-carcinogenic) due to pollutant exposure to heavy metals. Estimated THQ values for each heavy metal through consumption of the crayfish abdominal muscle treated with different cooking processes are presented in Fig. 3.

All THQ values of nine heavy metals in raw samples were less than 1. But the THQ value of Cu in the processed crayfish abdominal muscle exceeded 1 for children, the THQs of Cu are 1.21, 1.22, and 1.14 for three cooking styles (boiling, frying steaming) respectively. And 1.29, 1.24 for two seasoning methods (home cooking and commercial cooking). The cooked crayfish abdominal muscle represents a good source of Cu for consumers, as a necessary trace element. However, continuous, and excessive intake of the cooked crayfish abdominal muscle may pose a potential risk for children's health, since a balance in the dietary intake of Cu is crucial for health (Tan & Mitra, 2021). Elevated serum Cu levels increase the risk of obesity in children and adolescents (Ge et al., 2020). Maternal Cu status during pregnancy is also related to the neuropsychological development of infants (Amorós et al., 2019).

Furthermore, the THQ value of As in RE is up to 7.1 and 13.2 for children and adults respectively, implying that As in RE could cause certain health risks. As is a neurotoxic element, therefore, exposure to high concentrations of As causes severe central nervous system impairment in infants (Mochizuki, 2019). International Agency for Research on Cancer (IARC) has categorized As a class-I human carcinogen (IARC 2004). It has been confirmed to be associated with cancers in humans, such as lung, blood, and breast cancers (Taheri et al., 2016). But the analysis of levels of As cannot fully reflect the toxicity information, as the harm of As often depends on its chemical species that exist in aquatic products. Organic arsenic basically has no impact on humans, while inorganic arsenic (iAs) is toxic and can induce the occurrence of a variety of cancers and diseases. In this study, to roughly calculate the THQ value of iAs from RE, the proportion of 3% iAs in TAs (total arsenic) was assumed (Núñez et al., 2018; Qin et al., 2021). The values of iAs for adults and children are 0.21 and 0.40 respectively, roughly estimating the THQ of iAs to be less than 1.

The HI values of the hazard quotient of the crayfish abdominal muscle treated with different cooking processes are displayed in Fig. 4, which shows that the HI values of the processed crayfish abdominal muscle are significantly higher than raw samples. The average HI values of different cooking methods followed the order of Commercial seasoning cooking (RE) > Home seasoning cooking (HC)> Boiling > Frying > Steaming > Raw. The HI values for children were all greater than 1, indicating that the health risks raised by the consumption of crayfish for children cannot be ignored. It seems that aquatic products are subjected to further metal accumulation during processing (Jeevanaraj, Ahmad Foat, et al., 2020).

4.3 TCR

All TCR values of As, Cd, Cr, Hg, and Pb for adults and children under different cooking processes in Fig.5. were far below the unacceptable threshold (10^{-4}), and the As in RE had maximum TCR values, 5.97×10^{6} and 3.18×10^{6} for adults and children respectively. This demonstrates that the crayfish abdominal muscle could pose a carcinogenic risk for consumers. Likewise, cooking processes also have a lower effect on the cancer risk of heavy metals in the crab (Bao et al., 2021). It, therefore, seems that cooking processes have less effect on the carcinogenic risk of aquatic products.

4.4 Estimation of the consumption threshold of crayfish

Based on the threshold of THQ (THQ $\leqslant$ 1), Equation (2) was used to estimate the annual consumption threshold of the cooked crayfish abdominal muscle for children and adults, and the corresponding annual consumption of cooked crayfish was based on abdominal muscle/crayfish weight of a proportional calculation of 13.5% (Tan et al., 2021). Under home cooking methods, a rough combination of THQ threshold and Cu levels in fried samples indicates that the maximum daily intake of crayfish abdominal muscle for adults (70kg) and children (16kg) is 257g and 58g respectively, and the maximum annual intake is 694kg and 156kg respectively, higher than the consumption thresholds calculated by Tan et al. (2021) (139kg and 488kg for children (20kg) and adults (70kg) crayfish, respectively). Under commercial cooking methods, the maximum annual intake for consumers suggests, based on the health risk of As, that the maximum annual intake of crayfish for adults and children is 64kg and 14kg respectively.

5. Conclusion

A significant correlation between heavy metal levels in the crayfish abdominal muscle and cooking processes was observed. The levels of Cu in the cooked crayfish abdominal muscle increased significantly compared to raw samples. For some unknown reason, the commercially seasoned crayfish abdominal muscle has a very high level of the As, the THQ values were up to 7.1 and 13.2 for children and adults respectively. All cooking processes can thus, increase the risk of heavy metals in the crayfish abdominal muscle,

Overall, household cooking is much safer than commercial cooking. The impact of cooking methods should be considered, to avoid overestimating or underestimating the health risk assessment of consumers. For crayfish and related products, more strict quality detection and food consumption limit may be adopted for customers, especially for children.

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Conflict of Interest

The authors have no conflict of interest.

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Table 1. The basic parameters of the crayfish abdominal muscle (average weight (AW), cooking loss (CL), moisture content (MC)) and conversion factor for wet weight to dry weight under different cooking methods.

Cooking processes	AW (g)	MC (%)	CL (%)	Conversion factor	Condiments
Raw	3.74±0.17^{a}	79.76±0.50^{a}	/	4.9	
Boiled	2.80±0.09^{b}	74.03±0.78^{b}	25.13%		Colza oil
Fried	2.38±0.19^{c}	73.47±0.79^{b}	36.36%	3.8	
Steamed	2.46±0.17^{c}	73.58±0.41^{b}	34.22%		
HC	2.29±0.24^{c}	65.42±0.83^{c}	38.77%	2.9	Spicy condiments [1]
RE	1.95±0.36^{d}	64.50±0.52^{c}	/		

All values are means ± standard deviations of eight values. Different small letters in the same column indicate significant differences between means ($p < 0.05$).

[1] Spicy condiments include *Zingiber officinale, Zanthoxylum genu, red pepper,* Beer, Colza oil, and broad bean paste.

Table2. Levels of nine heavy metals (mg/kg, dry weight) in the crayfish abdominal muscle during different cooking processes. Results are reported as mean ± standard deviation (SD) on dry weight.

mg/kg	Statistics	Cooking styles				Seasoning styles		GB	CAC
		Raw	Boiled	Fried	Steamed	HC	RE		
As	Mean ± SD	ND	ND	ND	ND	0.10±0.06b	2.56±0.46a	2.45	
	CV					0.63	0.19		
Ba	Mean ± SD	4.44±1.38c	3.82±1.31c	2.99±0.79c	3.12±0.95c	23.38±4.05a	7.15±1.87b		
	CV	0.33	0.37	0.28	0.32	0.19	0.28		
Cd	Mean ± SD	0.04±0.05c	0.06±0.04c	0.06±0.03c	0.04±0.03c	0.28±0.05a	0.17±0.06b	2.45	9.8
	CV	1.46	0.74	0.60	0.79	0.17	0.36		
Cr	Mean ± SD	1.80±0.66ab	0.77±0.39d	1.38±0.35bc	1.10±0.16cd	2.13±0.50a	1.04±0.15cd	9.8	
	CV	0.39	0.54	0.27	0.15	0.25	0.15		
Cu	Mean ± SD	29.32±3.69b	41.00±3.86a	41.30±6.07a	38.83±4.92a	33.47±2.62b	32.07±1.80b		
	CV	0.13	0.10	0.16	0.14	0.12	0.06		
Hg	Mean ± SD	ND	ND	ND	ND	0.11±0.09b	0.22±0.06a	2.45	
	CV					0.90	0.29		
Mn	Mean ± SD	21.21±4.54cd	27.13±4.91ab	18.09±4.25cd	24.33±7.12bc	30.96±3.80a	15.62±2.78d		
	CV	0.23	0.19	0.25	0.31	0.13	0.19		
Ni	Mean ± SD	0.58±0.38c	1.63±0.41b	2.00±0.65ab	2.21±0.40a	1.60±0.34b	0.73±0.25c		
	CV	0.70	0.27	0.35	0.19	0.21	0.36		
Pb	Mean ± SD	ND	ND	ND	ND	0.14±0.04b	0.34±0.09a	2.45	1.44
	CV					0.32	0.29		

CV: Coefficient of Variation

ND means below detection level.

GB is the Chinese Maximum level for contaminants in crustacean aquatic products (mg/kg, dry weight) in GB2762-2017.

CAC is the Maximum level for the contaminant in aquatic products (mg/kg, dry weight) in CXS 193-1995 proposed by WHO and FAO.

Different small letters in the same column indicate significant differences between means ($p < 0.05$).

Table 3. Estimated daily intake (EDI) of heavy metals under different cooking processes (μg-1kg−1day−1). EDI value > PTDI is reported in boldface.

Cooking processes		As	Ba	Cd	Cr	Cu	Hg	Mn	Ni	Pb
Raw	Adult	0	2.18	0.02	0.88	14.36	0	10.39	0.29	0
	Child		4.08	0.03	1.66	26.93		19.48	0.54	
Boiled	Adult	0	2.41	0.04	0.48	25.89	0	17.13	1.03	0
	Child		4.52	0.07	0.91	48.55		32.13	1.93	
Fried	Adult	0	1.89	0.04	0.87	26.09	0	11.42	1.26	0
	Child		3.54	0.07	1.64	48.91		21.42	2.37	
Steamed	Adult	0	1.97	0.03	0.69	24.53	0	15.37	1.40	0
	Child		3.69	0.05	1.30	45.99		28.82	2.62	
SH	Adult	0.008	19.35	0.24	1.77	27.70	0.09	25.63	1.33	0.11
	Child	0.016	36.28	0.44	3.31	51.93	0.17	48.05	2.49	0.21
SC	Adult	2.12	5.92	0.14	0.86	26.54	0.18	12.93	0.60	0.28
	Child	3.98	11.10	0.26	1.62	49.76	**0.34**	24.24	1.13	0.52
PTDI		2.14	-	1	3	500	0.23	140	4.26	3.57

PTDI, provisional tolerable daily intake.

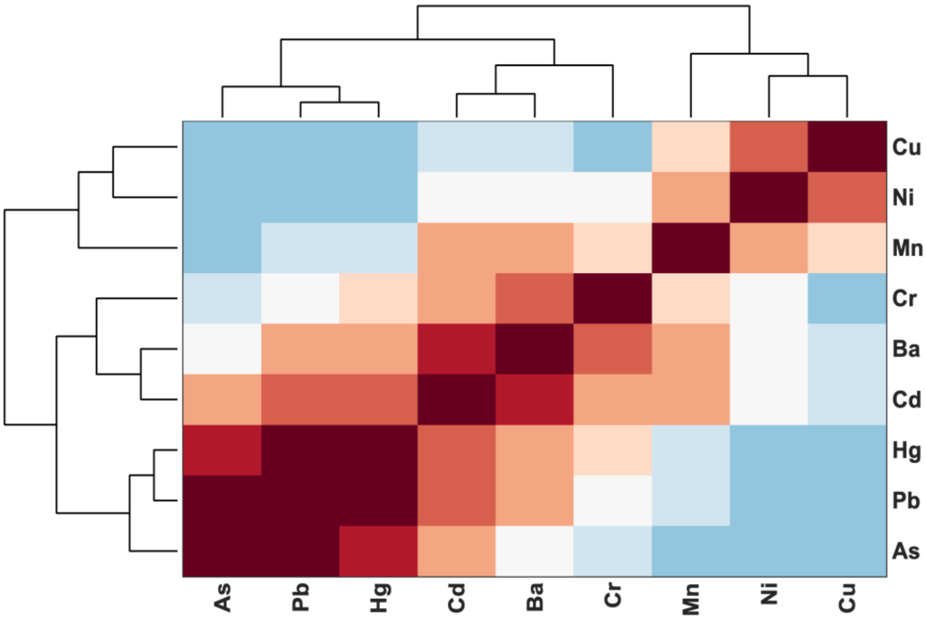

Fig. 1. Cluster analysis of the correlation coefficient matrix-based variables. Different colors represent different correlations. Red represents positive correlations, blue represents negative correlations, the darker red color, represents stronger correlations.

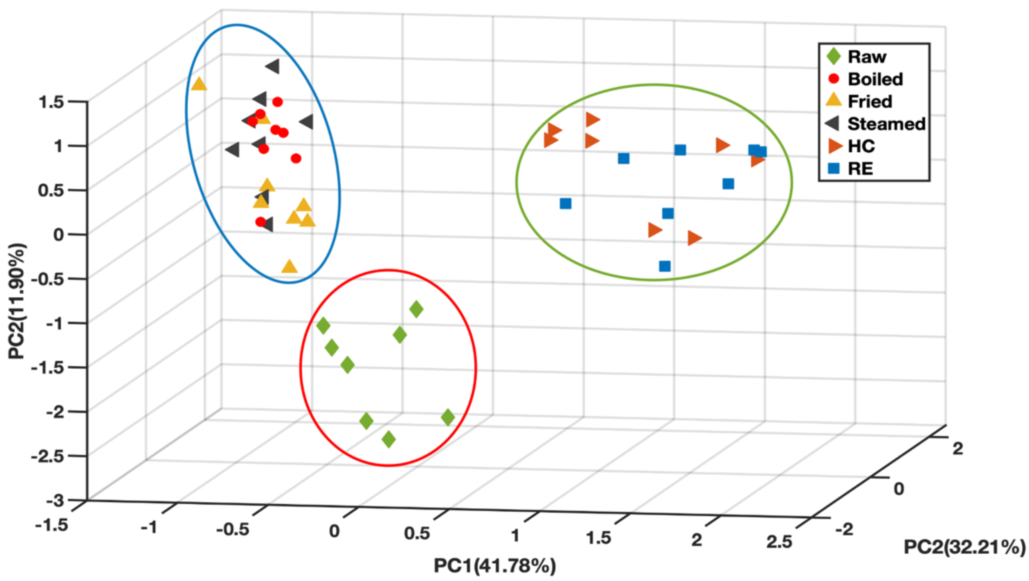

Fig. 2. The principal components under different cooking processes are based on the 1st, 2nd, and 3rd eigenvectors.

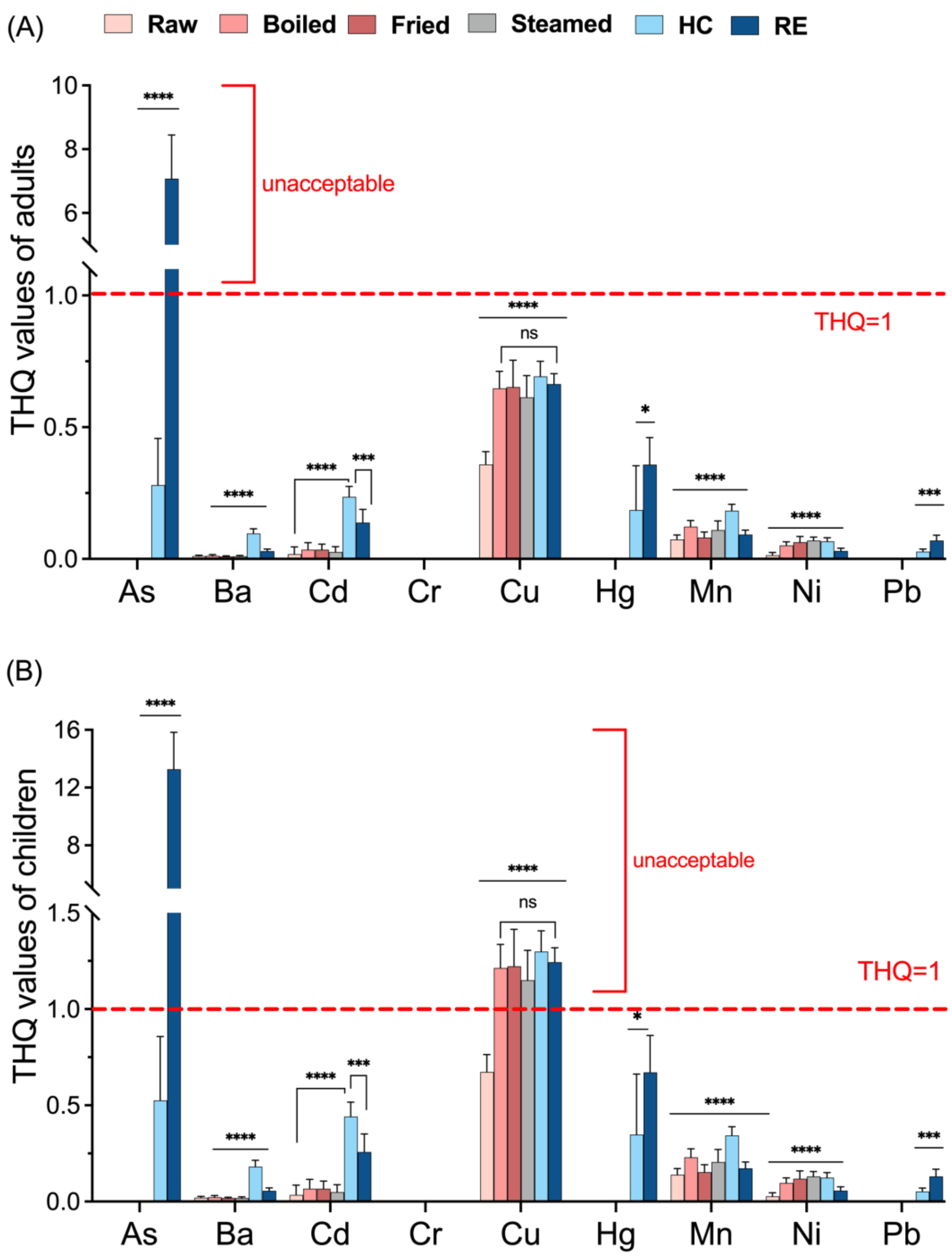

Fig. 3. The THQ values of nine heavy metals in the crayfish abdominal muscle under different cooking processes (Raw, Boiled, Fried, Steamed, Spicy crayfish abdominal muscle cooked using the household method (HC) and the ready-to-eat spicy crayfish abdominal muscle (RE): (A) adult (B) child. Values represent the means of THQ values.

Significant correlation levels： *:P < 0.05,**: P < 0.01,***: P < 0.001,****: P < 0.001, ns: P>0.05.

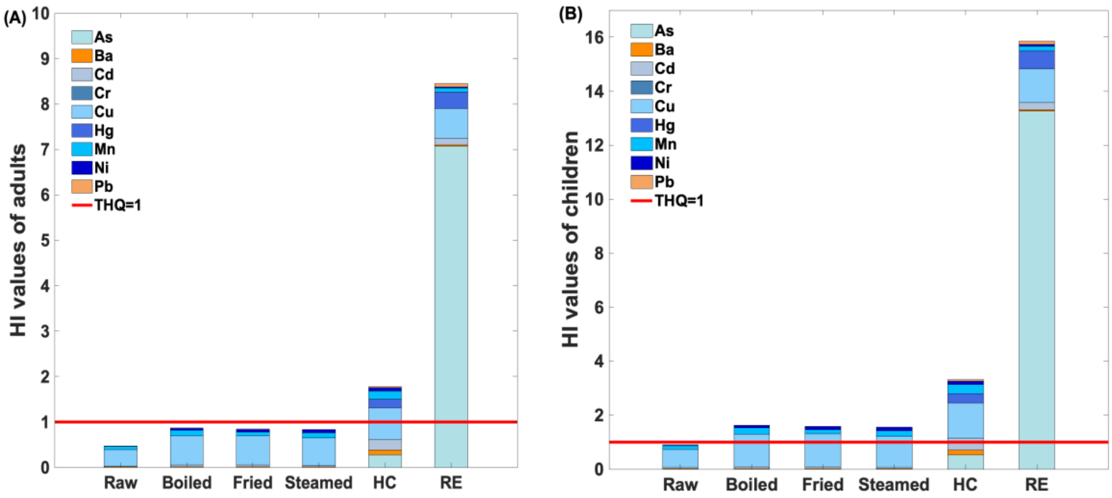

Fig. 4. The HI values of nine heavy metals in the crayfish abdominal muscle under different cooking methods and seasoning styles (Raw, Boiled, Fried, Steamed, spicy crayfish abdominal muscle cooked using the household method (HC) and the ready-to-eat spicy crayfish abdominal muscle (RE): (A) adult (B) child.

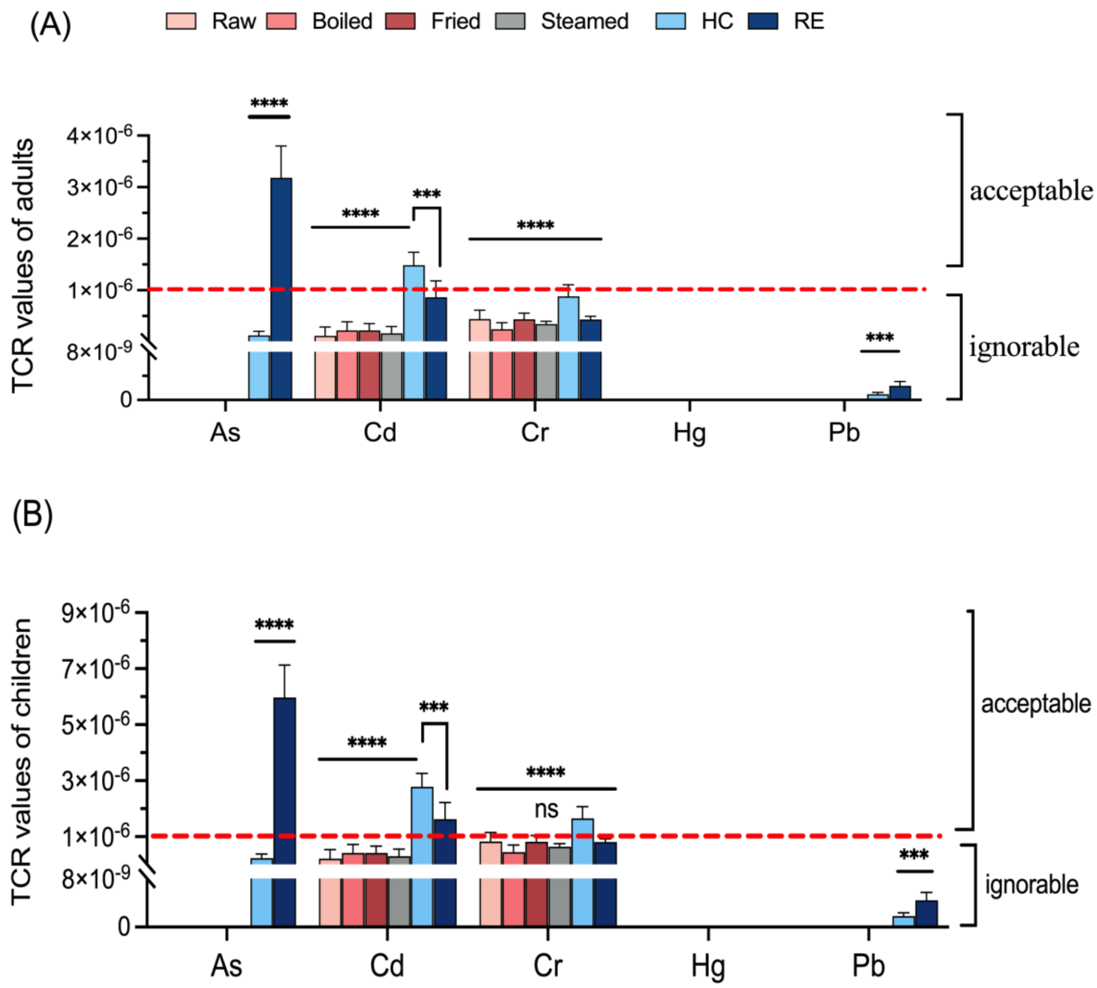

Fig. 5. The TCR values of five heavy metals in the crayfish abdominal muscle under different cooking processes (Raw, Boiled, Fried, Steamed, Spicy crayfish abdominal muscle cooked using the household method (HC) and the ready-to-eat spicy crayfish abdominal muscle (RE): (A) adult (B) child. Values represent the means of TCR values.

Significant correlation levels: *:P < 0.05,**: P < 0.01,***: P < 0.001,****: