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Mechanistic understanding of low methylmercury bioaccessibility from crayfish (*Procambarus clarkii*) muscle tissue

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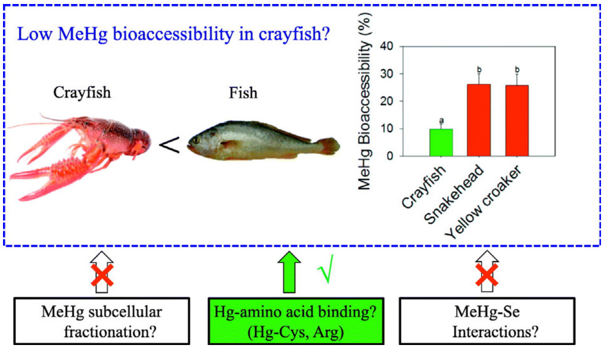
HIGHLIGHTS

- Bioaccessibility of MeHg in crayfish muscle was lower than that in fish muscle.
- MeHg-Se interactions did not account for the low MeHg bioaccessibility.
- MeHg bioaccessibilities in certain sub-cellular fractions were species-specific.
- Higher Cys/Arg levels may explain the lower MeHg bioaccessibility in crayfish.
- Bioaccessibility must be considered when assessing human exposure to crayfish MeHg.

GRAPHICAL ABSTRACT



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ABSTRACT

Recent research indicates that dietary exposure to mercury and other metals from crayfish consumption poses a human health concern, particularly in regions with high crayfish-consuming populations. To better understand consumption risk from methylmercury (MeHg), we quantified MeHg bioaccessibility in edible tail muscle of cooked red swamp crayfish (*Procambarus clarkii*, collected from seven cities in China), versus cooked fillet tissue of two finfish species: yellow croaker (*Larimichthys polyactis*) and snakehead (*Channa argus*). Results indicated that digestive solubilization rate (DSR) of MeHg in crayfish (7.8 ± 3.9% for restaurant-crayfish and 9.8 ± 0.8% for market-crayfish) was lower than the rate in yellow croaker (25.8 ± 2.7%) and snakehead (26.2 ± 4.7%) tissue, suggesting that relatively low MeHg bioaccessibility in crayfish may reduce dietary exposure to humans. Three possible mechanisms for the reduced MeHg DSR in crayfish tissue were examined: MeHg-Se interactions, MeHg subcellular fractionation, and Hg-amino acid binding. Selenium concentrations were comparable among the examined species, and no significant relationship was observed between tissue Se and MeHg DSR. Similarly, observed differences in subcellular fractionation of MeHg could not explain the species-specific MeHg DSR. Therefore, MeHg-Se interactions and MeHg subcellular fractionation do not explain the relatively low MeHg bioaccessibility in crayfish. Significantly higher cysteine and arginine content was found in crayfish than in the finfish. We suspect

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Abbreviations: CD, cellular debris; CRM, certified reference material; DSR, digestive solubilization rates; dw, dry weight; FAO, Food and Agriculture Organization; HDP, heat-denatured protein; HSP, heat-stable protein; MeHg, methylmercury; MLYR, the middle and lower reaches of the Yangtze River; MRG, metal-rich granules; MT, metallothioneins; NBSC, National Bureau of Statistics of the People's Republic of China; ORG, organelles; TAM, the trophically available metal; THg, total mercury; USITC, United States International Trade Commission.

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that the lower MeHg bioaccessibility of crayfish tail muscle may be attributed to the higher cysteine concentrations, and thus, stronger MeHg-protein binding in crayfish. These results support the interpretation that bioaccessibility differences will alter risk interpretations for MeHg, especially when comparing hazard across aquatic food types.

1. Introduction

Consumption of mercury-contaminated aquatic food (i.e., finfish and shellfish) has been found to be a major route of human exposure to methylmercury (MeHg, Cantoral et al., 2017; Environment Canada, 2015; Mahaffey et al., 2009; USEPA, 2011). This is a health concern, because high dietary exposure to mercury (especially MeHg) can result in nervous system toxicity, adverse neurobehavioral and cardiovascular effects, and in extreme cases, fetal Minamata disease (effects of which include microcephaly, blindness, and severe mental and physical developmental retardation, NRC, 2000). Factors influencing human dietary exposure to MeHg in aquatic food include MeHg concentrations in the food (Carrasco et al., 2011; Oken et al., 2012), human consumption rates (Mahaffey et al., 2009; Wang et al., 2013), and MeHg bioaccessibility (He and Wang, 2011; Ouédraogo and Amyot, 2011; Torres-Escribano et al., 2010). Compared to other factors, MeHg bioaccessibility to humans (defined as the fraction of MeHg in food that could be solubilized by the digestive fluid of human beings) has received less attention (Moreda-Piñeiro et al., 2011), perhaps due to difficulty and cost of in vivo bioaccessibility experiments using animals. Various in vitro extraction methods (mimicking metal solubilization by digestive fluids) have been developed for metal bioaccessibility assessment (Oomen et al., 2003; Rodriguez and Basta, 1999; Versantvoort et al., 2005), based on the assumption that metal desorption from food particles in the digestive tracts could be a limiting step for metal assimilation by humans (Oomen et al., 2002; Versantvoort et al., 2005). Especially, in vitro extraction methods have been validated by correlating metal extraction with its accumulation in organisms (Koch et al., 2013; Li et al., 2014a; Li et al., 2014b). Nevertheless, studies of MeHg bioaccessibility in human food are limited; existing studies focus on finfish, aquatic mammals, and caribou (He and Wang, 2011; Moreda-Piñeiro et al., 2011; Wang et al., 2013), with few published examinations of MeHg bioaccessibility from shellfish tissue.

Freshwater crayfish (Decapoda: Parastacidae and Astacidae) is known to accumulate high levels of metals, including mercury (Kuklina et al., 2014; Peng et al., 2016a; Peng et al., 2016b), possibly due to its omnivory and necrophagy (Alcorlo et al., 2006; Martin-Diaz et al., 2006; Schilderman et al., 1999). Meanwhile, crayfish is an important worldwide aquatic food, and is particularly popular for consumption in China, the southeastern United States, and western Europe (FAO, 2014; FAO, 2016; Holdich, 1993; NBSC, 2013; USITC, 2003). Regionally elevated methylmercury in crayfish is a potential health hazard for high rate consumers of crayfish (Hothem et al., 2007; Peng et al., 2016a; Schuler et al., 2000). Especially, our recent research indicates that crayfish consumption could pose a health risk to high consumption individuals in China (Peng et al., 2016a; Peng et al., 2016b). While crayfish mercury levels as well as the associated health risk have been documented (Faria et al., 2010; Hothem et al., 2007; Peng et al., 2016a; Schuler et al., 2000), there are no published measurements of MeHg bioaccessibility from crayfish. This lack of knowledge impedes accurate prediction of MeHg consumption risk.

Several biochemical mechanisms could influence MeHg bioaccessibility in crayfish. Crayfish exhibit relatively high Se (260–4100 ng/g dry weight [dw], Peng et al., 2016b; Xu et al., 2011) and protein levels (68.4%–70.8%, dw, Chen et al., 2010; Ding et al., 2006). In view of the strong affinity between Se or protein, and Hg in tissues (Khan and Wang, 2009; Lemes and Wang, 2009; Zhong and Wang, 2006), the potential effects of MeHg-Se or MeHg-protein interactions on MeHg bioaccessibility in crayfish warrant examination. Different subcellular fractionation can also cause differences in MeHg bioaccessibility among species (Dang and Wang, 2010), because metal mobility varies among subcellular fractions (He and Wang, 2011; He and Wang, 2013). Finally, differences in amino acid content can affect MeHg bioaccessibility. In particular, cysteine exhibits high binding affinity with MeHg, potentially reducing bioaccessibility (Leaner and Mason, 2002; Lemes and Wang, 2009; Zhong and Wang, 2006; Zhong and Wang, 2008). No previous studies have compared these factors and their influence on MeHg bioaccessibility in crayfish.

The main objectives of this study are to investigate MeHg bioaccessibility in cooked crayfish and examine its controlling factors. We hypothesized that bioaccessibility of MeHg in crayfish could be different compared to that in other aquatic food, considering the potential interactions between MeHg and its binding sites within crayfish tissues. To test the hypothesis, the bioaccessibility of MeHg in crayfish was compared to other aquatic food obtained in China, using a common in vitro extraction method developed by Versantvoort et al. (2005). The underlying mechanisms were then evaluated to explain the lower MeHg bioaccessibility observed in crayfish, compared to other aquatic food. We specifically investigated the possible impact of (1) MeHg subcellular fractionation, (2) MeHg-Se interactions and (3) amino acid content on MeHg bioaccessibility.

2. Materials and methods

2.1. Sample collection and preparation

Two batches of crayfish (*Procambarus clarkii*) samples were obtained, including cooked crayfish collected from restaurants (hereafter referred to as restaurant-crayfish) and raw crayfish collected from markets (hereafter referred to as market-crayfish) (Supporting information: Table S1). Twenty-six individual restaurant-crayfish were collected in seven cities (Table 1) in China in September 2012, as part of a survey of metal contamination in crayfish (Peng et al., 2016a; Peng et al., 2016b). All selected cities are located in the middle and lower reaches of the Yangtze River (MLYR), the main area of crayfish production and consumption in China. One restaurant was randomly selected in each city, except Nanjing (normally regarded as one of the Chinese cities with the highest crayfish consumption), from which three restaurants were chosen. Cooking techniques included frying and stewing. Samples were vacuum-packed, stored on ice and transported to the lab within 1–3 days of collection. After determination of tail length, edible tail muscle was dissected, weighed, rinsed with ultrapure water, freeze-dried, ground into a fine

Table 1
Digestive solubilization rate (DSR) of MeHg in restaurant-crayfish samples (mean ± SD).

Sampling sites	Sample size (n)	Cooking method	DSR (%)
Nanjing-1 (NJ-1)	3	Frying	8.0 ± 3.5
Nanjing-2 (NJ-2)	2	Frying	7.8 ± 4.4
Nanjing-3 (NJ-3)	3	Frying	6.5 ± 2.5
Suqian (SQ)	3	Frying	4.3 ± 0.6
Suzhou (SZ)	3	Frying	5.8 ± 0.4
Anqing (AQ)	3	Stewing	5.7 ± 2.4
Hangzhou (HZ)	3	Frying	10.8 ± 3.3
Anji (AJ)	3	Frying	10.4 ± 2.6
Xinyang (XY)	3	Stewing	11.2 ± 7.8
Total	26	/	7.8 ± 3.9