Food and Chemical Toxicology 55 (2013) 627–636

Contents lists available at SciVerse ScienceDirect

Food and Chemical Toxicology

journal homepage: www.elsevier.com/locate/foodchemtox

Bioaccessibility of 12 trace elements in marine molluscs

Mei He [a,b], Wen-Xiong Wang [a,c,*]

[a] State Key Laboratory for Marine Environmental Science, College of Environment and Ecology, Xiamen University, Xiamen 361005, China
[b] State Key Laboratory of Applied Microbiology, Ministry-Guangdong Province Jointly Breeding Base, South China, Guangdong Institute of Microbiology, Guangzhou 510070, China
[c] Division of Life Science, The Hong Kong University of Science and Technology (HKUST), Clearwater Bay, Kowloon, Hong Kong

ARTICLE INFO

Article history:
Received 18 July 2012
Accepted 22 January 2013
Available online 14 February 2013

Keywords:
Bioaccessibility
Metal
Metalloid
In vitro model
Subcellular distribution

ABSTRACT

We conducted a large scale investigation of the bioaccessibility of 12 trace elements from 11 marine mollusc species (scallop, oyster, clam, abalone, snail, and mussel) collected from five locations in Chinese coastal waters. The bioaccessibility of all the 12 trace elements was generally high, with the average values ranging from 42.5% to 90.7%. The highest bioaccessibility was observed for As, Cu, Ni and Se, and the lowest for Fe, Co and Pb. Steaming decreased the bioaccessibility of all 12 trace elements and thus diminished their risks. No correlation was observed between the bioaccessibility and the total concentration of the 12 elements. However, there was a significant correlation between the bioaccessibility of the 12 elements and their subcellular distribution. For most trace elements, a significantly negative relationship was demonstrated between the bioaccessibility and the elemental partitioning in the metal-rich granule fraction or in the cellular debris fraction, and a significantly positive correlation was observed between the bioaccessibility and the elemental partitioning in the heat-stable protein fraction and in the trophically available fraction. Hence, the elemental subcellular distribution, especially the elemental partitioning in the trophically available fraction, might be a good predictor of the bioaccessibility and risks of trace elements in molluscs.

1. Introduction

Molluscs are abundant in variety, and are widely cultivated and consumed in China. Most molluscs such as oyster, abalone, clam, and scallop are delicious and highly nutritious food with high levels of proteins, essential elements, amino acids and unsaturated fatty acids (Arino et al., 2003; National Academy of Sciences/National Research Council, 2005, 2006; Mahaffey et al., 2008). However, they also accumulate a substantial amount of toxic elements, presenting a great risk to human health. Additionally, even the essential elements can be harmful to humans when they reach high concentration (Wang, 2012). Consequently, accurate human health risk assessment of trace elements in molluscs is greatly needed.

In human health risk assessment, the total concentration of contaminants in food is generally used. Nevertheless, total concentration of contaminants may not always reflect the available amount of the contaminants ingested with food. Bioaccessibility indicates the fraction of the contaminants ingested with food which are released from the food matrix into the digestive tracts (mouth, stomach and intestine). It refers to the maximum bioavailability of the contaminants from food (Amiard et al., 2008; Brandon et al., 2006; Intawongse and Dean, 2006; Oomen et al., 2002, 2003; Versantvoort et al., 2005). Therefore, quantifying the bioaccessibility of contaminants may improve the human health risk assessments as compared to the total concentration.

Bioaccessibility study is of great importance to the health risk assessment of trace elements in molluscs. However, there were only a few studies on the bioaccessibility of trace elements in molluscs. Previous studies showed that cooking caused some metals, metalloids and radiocuclides in mussels less bioaccessible to humans (Houlbreque et al., 2011; Metian et al., 2009). Amiard et al. (2008) determined the bioaccessibility of several trace elements in clam, oyster, mussel as well as the effects of cooking and diet habits on the bioaccessibility. Their results showed that bioaccessibility varied with elements, and cooking could diminish the bioaccessibility of trace elements in these molluscs. Another study indicated that the bioaccessibility of As in clam was related to its speciation, with the inorganic As being the most bioaccessible species (Koch et al., 2007).

Molluscs are often eaten upon domestic cookeries. Cooking and some diet habits (e.g., chewing, co-ingestion with vinegar, freezing before consumed) affect the elemental bioaccessibilities of some foods, as demonstrated in previous studies (Amiard et al., 2008; Houlbreque et al., 2011; Metian et al., 2009). However, the current health risk assessment generally considers trace elements in the raw molluscs. The effects of cooking and other factors such as

* Corresponding author at: Division of Life Science, HKUST, Clearwater Bay, Kowloon, Hong Kong.
E-mail address: wwang@ust.hk (W.-X. Wang).

0278-6915/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.fct.2013.01.046

the diet habits on the bioaccessibility of trace elements in molluscs also need to be considered. In addition, subcellular distribution of metals could provide valuable information about metal toxicity and trophic transfer. Five subcellular fractions including metal-rich granules (MRGs), organelles, cellular debris, heat-stable protein (HSP) and heat-denatured protein (HDP) are generally identified in the subcellular partitioning studies. Different subcellular fractions may have different ecotoxicological significances, for example the combination of MRG and HSP can be considered as the biologically detoxified metal pool, and the combination of organelles, HSP and HDP can be considered as the trophically available metals (TAMs, Wallace and Luoma, 2003). Accordingly, the distribution of metals in the subcellular pools of organisms may affect the metal toxicity and bioaccessibility (Wallace et al., 1998, 2003; Wallace and Luoma, 2003; Wang and Rainbow, 2006). In our previous studies on marine fish, we found that the bioaccessibility of seven trace elements (As, Cd, Cu, Fe, Se, Zn and MeHg) in several marine fish was significantly correlated with the elemental subcellular distribution (He et al., 2010; He and Wang, 2011). However, such relationship, if any, between bioaccessibility and subcellular distribution of trace elements in marine molluscs is still unclear.

In this study, the bioaccessibility of 12 trace elements (Ag, As, Cd, Cr, Co, Cu, Fe, Mn, Ni, Pb, Se, and Zn) in 11 mollusc species collected from five locations (Dalian, Shenzhen, Haikou, Wenzhou and Xiamen) in Chinese coastal waters was determined to improve the accuracy of health risk assessment. Important factors affecting bioaccessibility of trace elements were studied with an aim to decrease the bioaccessibility of elements and then diminish their risks to humans. Our main objectives were: (1) to determine the elemental bioaccessibility in different mollusc species collected from different locations in Chinese coastal waters; (2) to examine the effects of steaming on the bioaccessibility of trace elements in molluscs; and (3) to explore the relationship of the bioaccessibility of trace elements in molluscs with their total concentration and subcellular distribution.

Table 1
The size and species of collected molluscs and the corresponding sampling locations.

Molluscs species	Shell length (cm)	Notes
Dalian		
Ruditapes philippinarum	2.87 ± 0.10	Clam, wild, from Heishijiao
Mytilus galloprovincialis	5.40 ± 0.31	Mussel, wild, from Heishijiao
Crassostrea gigas	9.77 ± 0.65	Oyster, wild, from Dalian Wan
Haliotis discus hannai	7.67 ± 0.21	Abalone, wild, from Longwangtang
Patinopecten yessoensis	9.50 ± 0.32	Scallop, wild, from Zhangzi Island
Shenzhen		
Ruditapes philippinarum	4.02 ± 0.22	Clam, wild, from Daya Bay
Perna viridis	4.79 ± 0.38	Mussel, wild, from Daya Bay
Crassostrea angulata	9.20 ± 1.12	Oyster, wild, from Daya Bay
Haliotis diversicolor	4.95 ± 0.31	Abalone, farmed, from Daya Bay
Chlamys nobilis	4.02 ± 0.15	Scallop, farmed, from Daya Bay
Haikou		
Ruditapes philippinarum	2.57 ± 0.09	Clam, farmed, from a local market
Babylonia areolata	2.63 ± 0.17	Snail, farmed, from a local market
Crassostrea angulata	11.6 ± 0.70	Oyster, farmed, from a local market
Haliotis diversicolor	4.22 ± 0.11	Abalone, farmed, from a local market
Chlamys nobilis	4.90 ± 0.09	Scallop, farmed, from a local market
Wenzhou		
Meretrix meretrix	3.40 ± 0.27	Clam, farmed, from a local farm
Mytilus galloprovincialis	5.43 ± 0.23	Mussel, farmed, from a local market
Crassostrea angulata	4.94 ± 0.30	Oyster, farmed, from a local market
Haliotis discus hannai	3.88 ± 0.32	Abalone, farmed, from a local farm
Babylonia areolata	3.26 ± 0.32	Snail, farmed, from a local farm
Xiamen		
Ruditapes philippinarum	3.15 ± 0.13	Clam, farmed, from a local market
Perna viridis	6.40 ± 0.33	Mussel, farmed, from a local market
Babylonia areolata	5.24 ± 0.26	Snail, farmed, from a local market
Haliotis discus hannai	5.23 ± 0.16	Abalone, farmed, from a local market
Chlamys nobilis	6.50 ± 0.33	Scallop, farmed, from a local market

2. Materials and methods

2.1. Molluscs sampling and reagents

Eleven marine mollusc species, including clams, scallops, abalones, snails, mussels, and oysters, were collected from five sampling locations (Dalian, Wenzhou, Xiamen, Shenzhen, Haikou) in Chinese coastal waters. These five locations were the coastal cities from the North to South in China. All the collected mollusc species were consumed by the Chinese people, and the habitats (farmed or wild) of these molluscs were chosen randomly. The sizes and species of the collected molluscs and the corresponding sampling locations are shown in Table 1. Five replicates were collected for each mollusc species from each sampling site. The edible tissues of these molluscs (i.e., the soft tissues of clams, mussels and oysters, the foot muscle of abalones and snails, and the adductor muscle and digestive gland of scallops) were dissected and then stored at −80 °C.

Urea, uric acid, glucuronic acid, and bovine serum albumin (BSA) used for the *in vitro* digestion were purchased from Sangon, China. Other chemicals for the *in vitro* digestion (α-amylase, mucin, glucose, glucoseamine hydrochloride, pepsin, pancreatin, lipase, bile, KCl, KSCN, NaCl, $CaCl_2$) were all purchased from Sigma–Aldrich. Oyster tissue 1566a (National Institute of Standard and Technology, Gaithersburg, MD, USA) was used as the standard reference material. All the other reagents were of analytical grade.

2.2. Total element concentration and subcellular distribution

Total concentrations of 12 elements (Ag, As, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, Se and Zn) in both raw and steamed mollusc tissues from the five sampling locations were determined. For the steamed mollusc tissues, they were first steamed for 10 min and then measured for their elemental total concentrations. Mollusc tissues were first digested in 9 ml 65% HNO_3 until a clear solution was obtained, and then the element concentrations based on the wet weight were measured by Inductively Coupled Plasma Mass Spectrometry (ICP-MS, Agilent 7700x). Internal standards (Internal Standard Mix Part# 5183–4681, Agilent) were used to correct the sensitivity drift and matrix effect. External standards were used to calibrate the instruments, and a control standard was repeatedly measured after every 30 samples. Additionally, raw mollusc tissues were subjected to subcellular fractionation, and five fractions including the metal-rich granules (MRGs), organelles, cellular debris, heat-stable protein (HSP), and heat-denaturable protein (HDP) were obtained, according to the method described by Wallace et al. (1998, 2003). The obtained five fractions were similarly digested and the element contents in the different fractions were then measured by ICP-MS. Oyster tissue 1566a was used as the standard reference material and was digested concurrently with the mollusc samples.

2.3. Element bioaccessibility and the effects of cooking

Element bioaccessibility was measured using an *in vitro* digestion model described by Versantvoort et al. (2005). The *in vitro* digestion model simulated the three digestion processes in the mouth, stomach and intestine of humans. Firstly, the mollusc tissues (five replicates for each mollusc species in each sampling site) were minced and homogenated and then incubated for 5 min with 6 ml artificial saliva. Secondly, they were incubated for 2 h in 12 mL artificial gastric juice. Finally, they were incubated for 2 h with a mixture of 12 mL artificial duodenal juice, 6 mL artificial bile and 2 mL 1 M HCO_3^-. The incubations were all carried out in a 55 rpm shaker at 37 °C. After incubation, the supernatants and pellets were obtained by a 5 min centrifugation with a speed 2800*g*. The supernatants were digested in 2 ml 65% HNO_3 until a clear solution was obtained, and the element contents in the supernatants were measured by ICP-MS. The percentages of the trace elements in the supernatants to the mollusc tissues were calculated as the element bioaccessibility.

Molluscs were usually consumed after processing by some common cooking methods. In this study, we also examined the effects of steaming on the elemental bioaccessibility in molluscs. Mollusc tissues were first steamed for 10 min (100 °C) in a normal domestical rice cooker and then the elemental bioaccessibility in 4.5 g steamed mollusc tissues were determined by the *in vitro* model with each sample of five replicates, as described above.