Effect of cooking methods on bioaccessibility of Zn, Se, Cd, Cu in sea cucumber (*Apostichopus japonicus*)

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Abstract In this study, the total concentration and bioaccessibility of four metals (Zn, Se, Cd, Cu) in sea cucumbers (*Apostichopus japonicus*) before and after cooking were measured. The concentration of Zn, Se, Cd, and Cu were 22.24 ± 0.75 , 0.75 ± 0.06 , 0.32 ± 0.07 , and 1.88 ± 0.09 mg/kg in raw cucumber, respectively. The contents of Zn, Se, and Cu in high-pressure samples were significantly higher than that in the raw sea cucumber ($p < 0.01$). The levels of Cd were all decreased after three thermal treatments. The intake of Zn and Cu increased in sea cucumber cooked by all thermal processes. While the bioaccessibility of Se and Cd decreased after cooking. A significant correlation was observed between the concentration and bioaccessibility of minerals. These data provide useful information for dietary risk assessments of minerals in sea cucumbers.

Keywords Sea cucumber · Bioaccessibility · In vitro · Mineral

Introduction

The sea cucumber (*Apostichopus japonicus*), which belongs to the Echinoderm phylum, is a typical temperate benthic organism. It has a high nutritional value and has long been used for traditional food and functional food in many Asian countries [1, 2]. The major edible part of sea cucumber is the body wall, consisting mainly of collagen, mucopolysaccharides and minerals such as Zn, Se, Fe, and Cu. Due to the high market value, the sea cucumber has become the most important cultured aquatic species in recent years. In 2015, the harvesting of this species reached 20 million tons in China [3]. Sea cucumber could easily self digest after removal from seawater due to its highly active enzyme. So more than 80% of the fresh sea cucumber throughout the world is processed into dehydrated products [4].

With the continuous development of sea cucumber aquaculture, food safety issues have become increasingly prominent [5]. Due to the booming industrialization and urbanization, varieties of pollutants are brought into the coastal waters. Heavy metals are considered as critical contaminants due to their high toxicity, non-degradability, and persistence. In aquatic environments, sediments serve as the largest reservoir for heavy metals [6]. Sea cucumbers which feed on sediments may accumulate heavy metals more easily and thereby represents a risk to consumers. There were less reports about heavy metals accumulated in sea cucumber compared with fish and shellfish. Mohamadizadeh et al. [7] investigated the heavy metals in tissues of two sea cucumbers in the Persian Gulf and found Cu and Zn concentrations were below permissible limits for human consumption while Cd and Pb were above the limits. Five kind of heavy metals (Pb, Cd, Hg, As, and Cr) in sea cucumber from Jinan market, China were analyzed

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and it was found that all of them were below the permissible limits, and among which Cd concentration was the highest [8].

Commonly, the information published about the levels of heavy metals in sea cucumbers report only the total amounts. However, this is not sufficient as it is necessary to know about the bioaccessibility of each element. Bioaccessibility refers to the percentage of the ingested amount of the element that potentially can be absorbed during the digestion process and subsequently transformed into metabolically active species [9, 10].

Several approaches have been developed to assess the bioaccessibility of heavy metals from different food matrixes [11–13]. Considering the cost and ethical concerns of in vivo tests, an in vitro digestion model provides a simple and reliable method to assess metal bioaccessibility [14, 15]. The bioaccessibility of metals depends not only on its binding forms but also on the physicochemical properties of the food and food processing method [16]. Some studies have been carried out to estimate possible changes in bioaccessibility and content after thermal processing in seafood [17–19].

Prior to the work described in this manuscript, little information was available describing the bioaccessibility of heavy metals in sea cucumber. In this paper, Cu, Zn, and Se, which are rich in sea cucumber were selected as beneficial trace elements and Cd as the toxic one [20, 21]. The primary objective of the present study was to assess the content and bioaccessibility of heavy metals (Cu, Zn, Se, and Cd) in sea cucumber before and after thermal processing.

Materials and methods

Samples

Sea cucumber (*Apostichopus japonicus*) was used as the test materials in this study. Samples were purchased from a local fishing area in the North Yantai region of China in May of 2016. Samples were randomly sampled and corresponded to a homogeneous batch of individuals of a single species (similar size, caught on the same day).

Reagents and standards

Standard stock solutions containing 1 mg/mL of each element were prepared by the National Research Center for Geoanalysis (Beijing, China). Porcine pepsin (1:3000), pancreatin-5.0, Ox bile, dihydrate and porcine mucin were purchased from Sigma-Aldrich (St. Louis, MO, USA). The other chemicals were obtained from Sangon Biotech Co. Ltd. (Shanghai, China).

To avoid contamination, all glassware and storage bottles were kept in 10% nitric acid for at least 24 h, rinsed three times with ultrapure water, and kept dry prior to use.

Thermal processing procedure

The most commonly used thermal treatments by consumers for processing sea cucumber were employed. After washing with distilled water to remove foreign matter and dirt, samples were dissected to remove the digestive tracts and divided into four groups. The first group was uncooked and considered as raw. The other three groups were cooked by the following methods: boiling, high-pressure, dried-rehydration. For the boiling sample, around 100 g sea cucumber was placed in 1500 mL of boiling Milli-Q water for 10 min. The high-pressure sea cucumber samples were performed in a pressure cooker of 80 kPa for 20 min. The processes of dried-rehydration sea cucumber samples were cumbersome. According to the traditional manufacturing and eating process, the gutted fresh sea cucumber samples were boiled in water for 30 min, fully packed with dry salt for 24 h, then dehydrated by oven at 40 °C for 12 h. After completing the dry salting process, the sea cucumber samples were soaked in Milli-Q water for 48 h at 4 °C, boiled in water for 30 min, then soaked in Milli-Q water for another 48 h at 4 °C. The raw and thermally processed samples were homogenized separately in a blender (Joyoung, JYL-G12E, China) for 2 min. Each sample was divided into two portions: one for minerals analysis by ICP-MS and the other for in vitro digestion.

In-vitro digestion

The operating conditions of the in vitro model were designed using the protocol described by Oomen et al. [22]. Sea cucumber sample (2.5 ± 0.01 g, three subsamples of each) was combined with 9 mL of saliva and the mixture was shaken for 5 min at 55 rpm and 37 °C. Subsequently, 14 mL of gastric juice was added, and the mixture was returned to the shaker for 2 h. Finally, 27 mL of duodenal juice and 9 mL of bile were added simultaneously, and the mixture was shaken for another 2 h. The mixture was centrifuged at 8000 g for 10 min; the supernatant was filtered over a 0.45 µm filter and frozen at -20 °C for further ICP-MS analysis.

Determination of minerals by ICP-MS

The minerals concentration of all samples was determined by ICP-MS [Thermo Jarrel-Ash Quadriion (POEMS), Thermo Fisher Scientific, Waltham, MA, USA]. The raw and thermal treatment samples were dried, weighed, and analyzed after digestion of 0.2 g samples with 10 mL

HNO₃ by microwave acid digestions. A procedural blank was used to determine any possible contamination during the experiment. The detailed measurement condition of ICP-MS was described by GB 5009.14-2017. The limit of detection (LOD) values were calculated on the basis of three SD criteria, with SD being the standard deviation of eleven measurements of a reaction blank. Comparison of concentrations among groups was conducted using one-way ANOVA.

Results and discussion

Mineral contents in raw and thermal processed samples by ICP-MS

The mineral contents of raw and cooked sea cucumber are presented in Table 1. The Zn content of raw sample was found to be 22.24 mg/kg. In the cooked samples, the levels of Zn found were, in increasing order, boiling < dried-rehydration < high-pressured; the statistical analysis showed that these differences were significant ($p < 0.01$). The Se content of dried-rehydration and high-pressured samples were significantly higher than that of the raw sea cucumber ($p < 0.01$). After three thermal treatments, the levels of Cd were all decreased and the significantly lowest amount of Cd content (0.11 ± 0.02 mg/kg) was found in dried-rehydration sample. The significantly highest amount of Cu content (2.89 ± 0.16 mg/kg) was found in high-pressured sample, and the lowest (1.13 ± 0.07 mg/kg) was in boiled sea cucumber ($p < 0.01$). The minerals content in raw samples were similar to the results reported by Liu [23], which were Zn content ranging from 21.8 to 30.36 mg/kg, Se content ranging from 0.73 to 1.01 mg/kg, Cu content ranging from 0.87 to 2.7 mg/kg, Cd content ranging from 0.08 to 0.75 mg/kg.

Recently, many researchers have determined the concentration of metals in sea cucumber [7, 24]. However, most of these studies focused on the levels of minerals based on an unprocessed form. Information which compares the effect of the processing on minerals in sea cucumber is scarce. The concentration of minerals in thermal processed sea cucumber had a significant

difference compared with the raw sample. Various thermal treatments have different effects on the concentration of minerals in sea cucumber. The contents of Zn, Se, and Cu in high-pressured samples were significantly higher than that in the raw sea cucumber ($p < 0.01$). We suspect that the high-pressured process is better to break the structure of sea cucumber than other processes, thus during the processing, it is easy for composition changes to. The dry weight content of the sample was decreased due to the loss of volatiles and, to a lesser extent, the other gross sample constituents (lipids, carbohydrates, and proteins) during the processing. This resulted in an increasing content of minerals per unit weight. On the other hand, boiling treatments caused varying degrees of reduction in the concentration of four minerals. The results may be related to the solubilization of metal in the water. Raw sea cucumbers do not stay fresh for a long time due to their high water content and high-activity enzymes. Therefore, a considerable amount are sold as dried sea cucumbers, which are usually rehydrated before being consumed. After dried-rehydration, the changes were different. There was an increase in Zn and Se, and a decrease in Se and Cu compared with the raw sample.

The effects of thermal processing methods on the minerals concentration in the seafood have intensively focused on the common heavy metals [25, 26]. Compared with the metals mentioned in the literature, there were different results regarding the relationship between the mineral content of raw and thermal processed seafood [19]. These differences could be ascribed to the mineral chemistry, processing technique or processing conditions [16]. For example, Marimuthu et al. [27] studied the content of Mg, Zn, Ca, and Cu in various cooked fish meat. Mg and Zn content decreased in fish cooked by almost all methods, Cu concentration increased while the content of Ca decreased in fried fish samples. Diaconescu et al. [17] studied the concentration of Cr, Ni, Cd, and Pb in various cooked fish meat. There were no significant differences in Pb concentrations between the raw, grilled, fried, microwaved, and baked fish, but Cr concentrations in grilled and microwave-cooked fish decreased significantly. García-Sartal et al. [18] studied the effects of cooking on the concentration of As, Co, Cr, Cu, Fe, Ni, Se, and Zn in four edible seaweeds

Table 1 Mineral composition of raw and thermal processing of *A. Japonicus* ($n = 3$, mg/kg dry wt)

	Zn	Se	Cd	Cu
Raw	22.24 $\pm$ 0.75 ^b	0.75 $\pm$ 0.06 ^b	0.32 $\pm$ 0.07 ^a	1.88 $\pm$ 0.09 ^b
Boiling	17.18 $\pm$ 1.35 ^c	0.74 $\pm$ 0.09 ^b	0.19 $\pm$ 0.01 ^{ab}	1.13 $\pm$ 0.07 ^c
High-pressure	36.9 $\pm$ 0.47 ^a	1.12 $\pm$ 0.06 ^a	0.19 $\pm$ 0.02 ^{ab}	2.89 $\pm$ 0.16 ^a
Dried-rehydration	25.13 $\pm$ 0.74 ^b	1.4 $\pm$ 0.11 ^a	0.11 $\pm$ 0.02 ^b	1.54 $\pm$ 0.08 ^b

Values are shown as mean $\pm$ standard deviation of triplicates. Values with different superscripts in the same row are significantly different ($p < 0.01$)

and found that the metals were released into the cooking water during the heat treatment with the exception of Ni and Zn in Kombu, Cr, Fe, and Co in Wakame, and Zn in Nori, for which almost 100% was retained in the seaweed.

It was argued that the resulting increased minerals level was due to the loss of volatiles and other soluble compounds during thermal processing, whereas decreases were the result of mineral losses because of solubilization or volatilization.

Minerals bioaccessibility of raw and thermal processed samples

The bioaccessibility percentage in raw and thermal processed sea cucumber are shown in Fig. 1. The bioaccessibility of Zn, Se, Cd, and Cu before and after processing were 76.56–105.34, 93.06–104.23, 14.1–97.27, and 46.4–105.34%, respectively. Among them, bioaccessibility of three examples exceeded 100%. Previous studies demonstrated that the percent bioaccessibility of Cd, Cu, and other trace elements occasionally exceed 100% [15, 28]. Bioaccessibility values in excess of 100% were occasionally observed due to heterogeneity of samples [15]. Se bioaccessibility were high in all samples, so it meant bioaccessibility would generally had little effect on Se exposure estimates from sea cucumber. Cd bioaccessibility in dried-rehydration sea cucumber was the lowest, indicating that this process could modulate the absorption of Cd.

Fig. 1 Mean percent in-vitro bioaccessibility of Cu, Zn, Se and Cd in raw and thermal processed sea cucumber. The digestion protocol was performed as described in in-vitro digestion section

Differences in mineral bioaccessibility between studies are difficult to directly compare due to the different in vitro extraction protocol and the kinds of seafood [15]. He et al. [29] studied Cd, Cu, and Se in two fish species of different body sizes and the bioaccessibility were 73.7–93.2, 81.4–85.4, and 48–61%, respectively. Laird and Chan [15] investigated Cd, Se, and Cu in fish, shellfish, and seaweed, the bioaccessibility ranged from 18 to 107, 50 to 98, and 59 to 117%, respectively. Gao and Wang [30] analyzed the toxic metals in oysters and the oral bioaccessibility of Zn ranged from 75.1 to 90.0%. It could be seen that the Cu bioaccessibility values in raw sea cucumber were lower than previous reports, while the Cd, Zn, and Se bioaccessibility were consistent to the reports.

The intake of Zn and Cu increased in sea cucumber cooked by all thermal processes. While the bioaccessibility of Se and Cd decreased after cooking. The results showed that thermal treatments have an effect on the bioaccessibility of metals. Furthermore, the variation trend was different in minerals. In summary, the thermal processing procedure affects the major nutrient composition and consequently, the metal bioaccessibility. In general, after thermal processing, the texture of sea cucumber was soft and easily digestible compared with the raw sample. This textural characteristic may allow leaching of the minerals into the digestive juices quite easily. While the increase of the bioaccessibility of Zn and Cu in sea cucumbers after thermal processing may be the result of the two effects: (a) the decrease in weight resulting from loss of water, volatiles, and the other instability sample constituents [31], and (b) thermal processing causing denaturation of the proteins in sea cucumbers, so that the tissues shrink and become harder and more compact.

The bioaccessibility of minerals depends not only on its binding forms, but also on the physicochemical properties of the food, the food processing method and the manner by which the mineral has entered the food [16]. Almela et al. [32] found that baking *Porphyra* sp. increased As bioaccessibility, while Wang et al. [16] observed microwave-cooked oysters decreased Cd bioaccessibility. In addition, Koch et al. [33] found that the bioaccessibility of As in clams was related to its speciation.

Intercorrelations of contents and bioaccessibility

No correlation was observed between the percent bioaccessibility of Cd and Se (Table 2). In contrast, significant and positive correlations were observed between the total concentration of Zn and Se ($r = 0.93$), Zn and Cd ($r = 0.75$), Se and Cd ($r = 0.94$), Cd and Cu ($r = 0.31$). Additionally, weak but significantly negative correlations were observed between Cu and Se. The correlation between the total concentration of mineral and its

Table 2 Pearson correlation coefficients for the concentration and percent bioaccessibility of Zn, Se, Cd, and Cu

	Total concentration (mg/kg)				Percent bioaccessibility			
	Zn	Se	Cd	Cu	Zn	Se	Cd	Cu
Total concentration (mg/kg)								
Zn	1	0.93**	0.75**	− 0.39**	− 0.66**			
Se		1	0.94**	− 0.02**		− 0.99**		
Cd			1	0.31**			0.97**	
Cu				1				− 0.26**
Percent bioaccessibility								
Zn					1	0.94**	− 0.99**	− 0.75**
Se						1	− 0.90	− 0.93**
Cd							1	0.68**
Cu								1

** $p < 0.01$

respective percent bioaccessibility tended to be significantly negative except Cd which presented a strong significant positive correlation. These results demonstrated that the intake of minerals through sea cucumbers were related not only to the cooking methods but also to the interaction of minerals.

A few earlier studies have been conducted on the correlation between the concentration and bioaccessibility of minerals in seafood. In contrast to our results, He and Wang [34] observed no correlation between Se bioaccessibility and concentration in marine molluscs, while Laird and Chan [15] found that the correlation was significant but positive in fish and shellfish. The bioaccessibility of Cd and Cu was negatively correlated with their total concentration in oyster ($p < 0.05$) [30].

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References

- Haider MS, Sultana R, Jamil K, Lakht-e-Zehra, Tarar OM, Shirin K, Afzal W. A study on proximate composition, amino acid profile, fatty acid profile and some mineral contents in two species of sea cucumber. *J. Anim. Plant Sci.* 25(1):168–175 (2015).
- Widianingsih, Zaenuri M, Anggoro S, Panca H, Kusumaningrum S. Nutritional value of sea cucumber [*Paracaudina australis* (Semper, 1868)]. *Aquatic Procedia.* 7:271–276 (2016).
- MOAC. Ministry of Agriculture, China. China fisheries yearbook, 2015. Beijing, China: China Agriculture Publisher (2016).
- Moon JH, Yoon WB. Size dependence of the salting process for dry salted sea cucumber (*Stichopus japonicus*). *J. Food Eng.* 170:170–178 (2016).
- Zhang XF, Liu Y, Li Y, Zhao XD. Identification of the geographical origins of sea cucumber (*Apostichopus japonicus*) in northern China by using stable isotope ratios and fatty acid profiles. *Food Chem.* 218:269–276 (2017).
- Li HM, Kang XM, Li XM, Li Q, Song JM, Jiao NZ, Zhang YY. Heavy metals in surface sediments along the Weihai coast, China: Distribution, sources and contamination assessment. *Mar. Pollut. Bull.* 115:551–558 (2017).
- Mohammadizadeh M, Bastami KD, Ehsanpour M, Afkhami M, Mohammadizadeh F, Esmaeilzadeh M. Heavy metal accumulation in tissues of two sea cucumbers, *Holothuria leucospilota* and *Holothuria scabra* in the northern part of Qeshm Island, Persian Gulf. *Mar. Pollut. Bull.* 103:354–359 (2016).
- Yin LL. Nutritional assessment and safety evaluation of the sea cucumber and abalone in Jinan city. MS thesis, Shandong University, Jinan, Shandong Province, China (2013).
- Lu YT, Yang DY, Song XN, Wang S, Song M, Hang T. Bioaccessibility and health risk assessment of mercury in cinnabar containing traditional Chinese medicines. *J. Trace Elem. Med. Bio.* 44:17–25 (2017).
- Machado I, Cesio MV, Pistón M. In vitro bioaccessibility study of As, Cd, Cu, Fe, Ni, Pb and Zn from raw edible artichoke heads (*Cynara cardunculus* L. subsp. *Cardunculus*). *Microchem. J.* 133:663–668 (2017).
- Silva JGS, Rebellato AP, Greiner R, Pallone JAL. Bioaccessibility of calcium, iron and magnesium in residues of citrus and characterization of macronutrients. *Food Res. Int.* 97:162–169 (2017).
- Leufroy A, Noël L, Beauchemin D, Guérin T. Use of a continuous leaching method to assess the oral bioaccessibility of trace elements in seafood. *Food Chem.* 135:623–633 (2012).
- Torres-Escribano S, Denis S, Blanquet-Diot S, Calatayud M, Barrios L, Vélez D, Alric M, Montoro R. Comparison of a static and a dynamic in vitro model to estimate the bioaccessibility of As, Cd, Pb and Hg from food reference materials Fucus sp. (IAEA-140/TM) and Lobster hepatopancreas (TORT-2). *Sci. Total Environ.* 409:604–611(2011).
- Li HB, Cui XY, Li K, Li J, Juhasz AL, Ma LQ. Assessment of in vitro lead bioaccessibility in house dust and its relationship to in vivo lead relative bioavailability. *Environ. Sci. Technol.* 48:8548–8555 (2014).
- Laird BD, Chan HM. Bioaccessibility of metals in fish, shellfish, wild game, and seaweed harvested in British Columbia, Canada. *Food Chem. Toxicol.* 58:381–387 (2013).
- Wang C, Duan HY, Teng JW. Assessment of microwave cooking on the bioaccessibility of Cadmium from various food matrices using an in vitro digestion model. *Biol. Trace Elem. Res.* 160:276–284 (2014).
- Diaconescu C, Urdes L, Diaconescu S, Popa D. Effects of cooking methods on the heavy metal concentrations in the fish

- meat originating from different areas of Danube River. *J. Anim. Sci.* 55:265–267(2012).
18. Garcia-sartal C, Barciela-alonso MC, Moreda-piñeiro A. Study of cooking on the bioavailability of As, Co, Cr, Cu, Fe, Ni, Se and Zn from edible seaweed. *Microchem. J.* 108:92–99 (2013).
 19. Beyza E. Effects of cooking methods on the heavy metal concentrations of the African Catfish (*Clarias Gariepinus*). *J. Food Biochem.* 35:351–356 (2011).
 20. Protano C, Zinnà L, Giampaoli S, Spica VR, Chiavarini S, Vitali M. Heavy metal pollution and potential ecological risks in rivers: a case study from southern Italy. *B. Environ. Contam. Tox.* 92:75–80 (2014).
 21. Li YB, Duan ZW, Liu GL, Kalla P, Scheidt D, Cai Y. Evaluation of the possible sources and controlling factors of toxic metals/metalloids in the Florida Everglades and their potential risk of exposure. *Environ. Sci. Technol.* 49:9714–9723 (2015).
 22. Oomen AG, Rempelberg CJ, Bruil MA, Dobbe CJ, Pereboom DP, Sips AJ. Development of an in vitro digestion model for estimating the bioaccessibility of soil contaminants. *Environ. Contam. Toxicol.* 44:281–287 (2003).
 23. Liu XF. Study of regional differences in nutrient compositions and bioactivities of phospholipids in sea cucumber (*Apostichopus japonicus*). PhD thesis, Ocean University of China, Qingdao, Shandong Province, China (2014).
 24. Noël L, Testu C, Chafey C, Velge P, Guérin T. Contamination levels for lead, cadmium and mercury in marine gastropods, echinoderms and tunicates. *Food Control.* 22:433–437 (2011).
 25. Kalogeropoulos N, Karavoltos S, Sakellari A, Avramidou S, Dassenakis M, Scoullou M. Heavy metals in raw, fried and grilled Mediterranean finfish and shellfish. *Food Chem. Toxicol.* 50:3702–3708(2012).
 26. Ersoy B, Yanar Y, Küçükgülmez A, Çelik M. Effects of four cooking methods on the heavy metal concentrations of sea bass fillets (*Dicentrarchus labrax* Linne, 1785). *Food Chem.* 99:748–751(2006).
 27. Marimuthu K, Geraldine AD, Kathiresan S, Xavier R, Arockiaraj J, Sreeramanan S. Effect of Three different cooking methods on proximate and mineral composition of Asian sea bass (*Lates calcarifer*, Bloch). *J. Aquat. Food Prod. Tech.* 23:468–474 (2014).
 28. Moreda-Piñeiro J, Alonso-Rodríguez E, Romaris-Hortas V, Moreda-Piñeiro A, López-Mahia P, Muniategui-Lorenzo S, Prada-Rodríguez D, Bermejo-Barrera P. Assessment of the bioavailability of toxic and non-toxic arsenic species in seafood samples. *Food Chem.* 130:552–560 (2012).
 29. He M, Ke CH, Wang WX. Effects of cooking and subcellular distribution on the bioaccessibility of trace elements in two marine fish species. *J. Agric. Food Chem.* 58:3517–3523 (2010).
 30. Gao S, Wang WX. Oral bioaccessibility of toxic metals in contaminated oysters and relationships with metal internal sequestration. *Ecotoxicol Environ. Saf.* 110:261–268 (2014).
 31. Duodu KG, Nunes A, Delgadillo I, Parker ML, Mills ENC, Belton PS, Taylor JRN. Effect of grain structure and cooking on sorghum and maize in vitro protein digestibility. *J. Cereal Sci.* 35:161–174(2002).
 32. Almela C, Laparra JM, Vélez D, Barberá R, Farré R, Montoro R. Arsenosugars in raw and cooked edible seaweed: characterization and bioaccessibility. *J. Agric. Food Chem.* 53:7344–7351(2005).
 33. Koch I, Mepherson K, Smith P, Easton L, Doe KG, Reimer KJ. Arsenic bioaccessibility and speciation in clams and seaweed from a contaminated marine environment. *Mar. Pollut. Bull.* 54:586–594(2007).
 34. He M, Wang WX. Bioaccessibility of 12 trace elements in marine molluscs. *Food Chem. Toxicol.* 55:627–636 (2013).