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Effects of Cooking and Subcellular Distribution on the Bioaccessibility of Trace Elements in Two Marine Fish Species

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In current human health risk assessment, the maximum acceptable concentrations of contaminants in food are mostly based on the total concentrations. However, the total concentration of contaminants may not always reflect the available amount. Bioaccessibility determination is thus required to improve the risk assessment of contaminants. This study used an in vitro digestion model to assess the bioaccessibility of several trace elements (As, Cd, Cu, Fe, Se, and Zn) in the muscles of two farmed marine fish species (seabass *Lateolabrax japonicus* and red seabream *Pagrosomus major*) of different body sizes. The total concentrations and subcellular distributions of these trace elements in fish muscles were also determined. Bioaccessibility of these trace elements was generally high (>45%), and the lowest bioaccessibility was observed for Fe. Cooking processes, including boiling, steaming, frying, and grilling, generally decreased the bioaccessibility of these trace elements, especially for Cu and Zn. The influences of frying and grilling were greater than those of boiling and steaming. The relationship of bioaccessibility and total concentration varied with the elements. A positive correlation was found for As and Cu and a negative correlation for Fe, whereas no correlation was found for Cd, Se, and Zn. A significant positive relationship was demonstrated between the bioaccessibility and the elemental partitioning in the heat stable protein fraction and in the trophically available fraction, and a negative correlation was observed between the bioaccessibility and the elemental partitioning in metal-rich granule fraction. Subcellular distribution may thus affect the bioaccessibility of metals and should be considered in the risk assessment for seafood safety.

KEYWORDS: Bioaccessibility; metal; metalloid; bioavailability; in vitro model; subcellular distribution

INTRODUCTION

Fish species are important seafoods with high nutritional value. They are a good source of minerals, unsaturated lipids, phospholipids, vitamins, essential elements, and proteins of high biological value and, thus, have become ideal components of a healthy and balanced diet (*1*, *2*). With the improvement of living standards, the demand for fish has been increasing steadily. In addition to their key nutrients, fish products are also the accumulators of substantial concentrations of toxic metals (e.g., Cd, Pb) and metalloids (e.g., As) and may pose a threat to human health (*3*, *4*). The current human health risk assessments in fish are traditionally based on the total concentration of contaminants and fish consumption. However, total concentration of ingested contaminants may not always reflect the real bioavailable fraction to the consumer. A better insight into the oral exposure of contaminants from a consumer product is required for a more accurate health risk assessment.

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Bioaccessibility describes the fraction of a contaminant ingested with food that is released from its matrix into the digestive juice chime and has the potential to be absorbed by the intestines during digestion, whereas bioavailability is the proportion of a contaminant ingested with food that is absorbed by the intestine with the subsequent potential to reach the systemic circulation and exert toxic effects (*5*−*10*). During food digestion, the release of a contaminant from ingested food is a precondition for uptake and assimilation. The released contaminant can be partially or totally absorbed by the intestines, entering the systemic circulation at last. As a result, bioaccessibility refers to its maximum bioavailability. Thus, when the risk of contaminants ingested with food is assessed, there is a pressing demand to determine the bioaccessibility and evaluate the bioavailability to increase the accuracy of risk evaluation and improve human health risk assessment.

In vitro digestion methods are useful tools to evaluate bioaccessibility and were originally developed to estimate the bioavailability of iron and other minerals (*11*−*13*). Several in vitro approaches and models have been developed to mimic the effects of the human digestion process (*7*). Compared to the in vivo methods, the in vitro method is simple, rapid, and low in cost, conditions are easy to control, and it has a better reproducibility and thus can provide a cost-effective approximation of the in vivo tests (*1*, *14*, *15*).

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Table 1. Total Concentrations in the Two Fish Muscles and the Recovery of Elements Using Oyster Powder as a Standard ($n = 5$)[a]

	seabass (μg/g)		red seabream (μg/g)			
element	small size	large size	small size	medium size	large size	standard recovery (%)
As	3.17 ± 0.48 a	1.75 ± 0.36 b	1.04 ± 0.13 a	2.85 ± 0.21 c	1.72 ± 0.10 b	99.8
Cd	0.05 ± 0.01 a	0.07 ± 0.01 a	0.17 ± 0.11 ab	1.60 ± 0.60 c	0.06 ± 0.03 a	95.9
Cu	0.58 ± 0.11 a	0.42 ± 0.03 b	0.44 ± 0.11 a	0.76 ± 0.13 b	0.45 ± 0.11 a	95.0
Fe	8.17 ± 1.14 a	10.2 ± 1.42 a	12.9 ± 1.89 a	11.7 ± 1.89 a	14.7 ± 5.26 a	95.1
Se	1.35 ± 0.07 a	0.59 ± 0.14 b	0.61 ± 0.11 a	1.14 ± 0.10 c	0.84 ± 0.07 b	101.2
Zn	15.1 ± 1.59 a	8.64 ± 1.86 b	32.8 ± 14.0 a	25.1 ± 2.71 ab	12.1 ± 1.42 c	90.0

[a] For each metal in each fish species, the different letters indicate that the two size treatments were significantly different.

Bioaccessibility studies of contaminants were mainly concerned with soils and foods (fish, shellfish, seaweed, meat, grains, and vegetables). The contaminants considered include metals and organic pollutants as well as nontoxic organics. Previous studies have shown that oral bioavailability varies with the food resource and food processing or preparation (*6*, *16*). Soil properties, contaminant concentration, and aging also have important effects on the bioaccessibility (*17*−*19*). Food may be ingested raw or after various treatments (cooking techniques such as boiling, steaming, frying, or grilling). Heating processes generally change the bioaccessibility (*1*,*5*,*11*,*14*,*20*−*23*), but there is a lack of information concerning the influences of different cooking treatments on the bioaccessibility.

The subcellular distribution of trace elements is an important tool for interpreting the internal mechanisms of toxicity, tolerance, trophic transfer, and detoxification during accumulations in aquatic organisms (*24*−*34*). Only a few studies, however, have addressed the subcellular distribution of trace elements in fish [e.g., freshwater yellow perch (*26*−*28*)], especially marine fish. In the yellow perch, Cd and Cu were found to be dominantly associated with the heat-stable proteins (*26*−*28*). Different subcellular fractions may have different bioaccessibilities, but this remains essentially unknown at present.

In this study, we evaluated the bioaccessibilities of several trace elements in two marine fish species (the seabass *Lateolabrax japonicus* and the red seabream *Pagrosomus major*) that are widely cultured and consumed in Fujian Province, China. There have been substantial concerns regarding the safety of consuming farmed fish as a result of contaminant accumulation. Previously, there were a few studies on the bioaccessibility of trace elements in fish (swordfish, sardine, tuna, and cooked tuna) (*1*, *16*, *35*−*42*). The present study focused on three objectives: (1) to evaluate the bioaccessibility of several trace elements in the two commercial fish of different body sizes; (2) to determine the influence of different cooking processes on the bioaccessibility of these trace elements; and (3) to explore the relationship of bioaccessibility with total concentrations and subcellular distributions of these trace metals in the fish.

MATERIALS AND METHODS

Fish Sampling and Chemicals. Seabass (*L. japonicus*) and red seabream (*P. major*) were sampled from a fish farm located in Xiamen, Fujian, China. For each species, different body sizes of fish were collected. Two sizes of seabass (large, 52.3 ± 2.2 cm body length, 1.72 ± 0.22 kg body weight; and small, 41.7 ± 1.4 cm body length, 0.78 ± 0.07 kg body weight) and three sizes of red seabream (large, 46.8 ± 0.5 cm body length, 1.93 ± 0.07 kg body weight; medium, 31.1 ± 0.5 cm body length, 0.72 ± 0.03 kg body weight; and small, 26.8 ± 0.9 cm body length, 0.40 ± 0.03 kg body weight) were obtained from the sampling site. The number of replicates was $n = 5$ for all samples. After sampling, all of the fish muscle samples were frozen and stored at −70 °C.

All chemicals for the digestive fluids were purchased from Sigma-Aldrich, except for urea (Sangon, China), glucuronic acid (Sangon, China), uric acid (Sangon, China), and BSA (Sangon, China). The standard reference material for oyster tissue (1566a) was obtained from the National Institute of Standards and Technology (Gaithersburg, MD). Purified water was obtained with a Milli-Q water-purifying system. All other reagents were of ultrapure or analytical grade.

Trace Element Bioaccessibility Determination. Bioaccessibility measurements of trace elements were performed using an in vitro digestion method. This in vitro digestion method was based on the procedures described by Versantvoort et al. (*6*) (the composition of the artificial juices is shown in their Table 1). Overall, it mimics human digestion, which consists of three steps simulating the digestive processes in the mouth, stomach, and small intestines. First, 6 mL of artificial saliva was added to a minced fish muscle sample (4.5 g) and incubated for 5 min. Then, 12 mL of artificial gastric juice was added and incubated for 2 h. Finally, a mixture of 12 mL of artificial duodenal juice, 6 mL of artificial bile, and 2 mL of HCO_3^- was added and incubated for a further 2 h. All three simulating processes were incubated at 37 °C. The supernatants and pellets were separated by centrifugation at 2800*g* for 5 min and then acid-digested with 65% HNO_3 using the heat block at 80 °C for 1 h and then at 110 °C until a clear solution was obtained. The element levels in the acid solutions were determined by inductively coupled plasma−atomic emission spectroscopy (ICP-AES, Thermo model IRIS Intrepid II XSP) after dilution with deionized water. Bioaccessibility was expressed as the percentage of trace elements recovered in the supernatant.

Because fish are eaten both raw or after various treatments, bioaccessibility measurements were carried out not only on the raw fish muscle samples but also on boiled, steamed, fried, and grilled samples (*16*). For boiling, the muscle samples were added to 1 L of boiling distilled water for 5 min in a stainless steel pot (25.5 cm in diameter). When steaming, the fish muscles were cooked for 10 min in a rice cooker. In the case of frying, the fish muscle was fried in a Teflon pan (32 cm in diameter) in the presence of 25 mL of cooking quality sunflower oil for 5 min. For the grilled fish muscle, oil was not added and the cooking duration was 20 min. For each sample, the number of replicates was $n = 5$.

Trace Element Concentration and Subcellular Distribution Determination. The total element concentrations of raw seabass and red seabream muscles were first determined. The fish muscles were first dried and then acid digested with 65% HNO_3 using the heating block until a clear solution was obtained. After acid digestion, element concentrations in the muscle tissues were determined by ICP-AES. The number of replicates was $n = 5$ for all samples. In addition, the element subcellular distribution of raw muscles was measured using the methods described by Wallace et al. (*30*−*32*). Five different fractions were obtained altogether, including the cellular debris, metal-rich granules (MRG), organelles, heat-denaturable protein (HDP), and heat-stable protein (HSP) fractions. These five fractions were similarly dried first and then acid-digested; afterward, the element concentrations in the five fractions were determined by ICP-AES. The number of replicates was $n = 5$ for all samples.

Statistics. SPSS 10.0 was used for the statistical analysis. A one-way ANOVA analysis and Tukey test were applied to determine statistical differences. A significance level of $p < 0.05$ was adopted for all comparisons. Results were expressed as mean ± standard deviation ($n = 5$).

RESULTS

Total Element Concentration and Subcellular Distribution in Fish Muscles. The total element concentrations in seabass and red seabream muscles are shown in **Table 1**. In this study, the recovery of the standard oyster reference materials for all measured trace

elements ranged from 90 to 101%. In general, the total concentrations of trace elements in the two fish muscles were element-, species-, and size-dependent. Except for the highest As and Se concentrations in the small-size seabass, the highest concentrations of the other four elements were all detected in red seabream. Thus, the red seabream was a stronger accumulator of trace elements than the seabass.

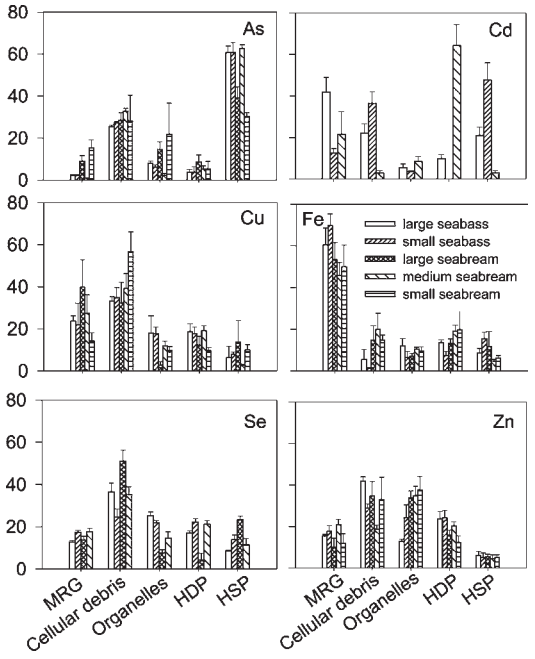

Figure 1. Element subcellular distribution in the muscles of seabass and red seabream of different sizes. Values are mean ± standard deviation (n = 5). MRG, metal-rich granule; HDP, heat-denaturable protein; HSP, heat-stable protein.

Figure 1 indicates the element subcellular distributions in the muscles of seabass and red seabream. It was clear that the subcellular distributions were element-specific, whereas the distributions of As, Cu, Fe, Se, and Zn were similar between the seabass and red seabream. HSP, MRG, and cellular debris fractions were the largest pool for As, Fe, and Se, respectively. For Cu, cellular debris and MRG fraction were the dominant pools. For Zn, cellular debris and organelles fraction were the main pools. For Cd, MRG, HSP, and HDP were the largest pool in large-size seabass, small-size seabass, and medium-size red seabream, respectively. No Cd was found in the HDP fraction of small-size seabass or in any of the five fractions of large-size and

Table 2. Regression Coefficients for the Relationships between the Total Element Concentration and Element Subcellular Distribution in both Seabass and Red Seabream Muscles[a]

	As	Cd	Cu	Fe	Se	Zn
MRG	0.469**↓	0.005	0.161	0.092	0.003	0.189*↑
cellular debris	0.006	0.220	0.002	0.079	0.612**↓	0.077
organelles	0.190	0.051	0.183	0.160	0.034	0.030
HDP	0.073	0.239	0.091	0.010	0.377	0.024
HSP	0.570**↑	0.203	0.010	0.206*↓	0.002	0.065
BDM (MRG + HSP)	0.422*↑	0.194	0.401*↑	0.001	0.006	0.082

[a] *, significant at $p < 0.05$; **, significant at $p < 0.01$; ↓, negative correlation; ↑, positive correlation.

Table 3. Element Bioaccessibility (Expressed as Mean ± SD, Percent) of Seabass and Red Seabream Muscles (n = 5)[a]

fish	As	Cd	Cu	Fe	Se	Zn
seabass						
small size						
raw	90.8 ± 0.9 a	93.2 ± 2.9 a	84.2 ± 0.5 a	51.3 ± 3.2 a	60.8 ± 7.1 ab	72.3 ± 2.0 a
boil	84.3 ± 0.9 b	81.9 ± 3.6 b	65.3 ± 1.8 bc	48.5 ± 1.8 a	62.2 ± 6.6 a	52.2 ± 0.9 b
steam	86.2 ± 0.9 b	86.5 ± 1.9 ab	65.7 ± 2.7 bc	43.0 ± 2.2 ab	54.0 ± 3.3 abc	46.3 ± 1.4 bc
fry	83.9 ± 0.6 b	85.2 ± 0.9 ab	71.2 ± 4.1 b	53.7 ± 4.4 a	38.7 ± 4.9 bc	40.1 ± 2.1 cd
grill	84.4 ± 0.6 b	80.3 ± 2.1 b	59.0 ± 3.4 c	27.7 ± 5.5 b	34.2 ± 3.9 c	38.7 ± 1.5 d
large size						
raw	81.2 ± 3.5 a	84.8 ± 1.9 a	81.4 ± 0.7 a	58.0 ± 1.2 a	61.0 ± 3.5 a	70.4 ± 1.7 a
boil	75.1 ± 1.4 a	72.4 ± 2.9 b	63.3 ± 2.1 bc	49.8 ± 0.8 ab	56.3 ± 3.2 a	46.1 ± 1.5 b
steam	77.0 ± 1.6 a	82.7 ± 1.6 ab	64.3 ± 1.3 b	47.7 ± 2.0 bc	56.0 ± 1.6 a	36.8 ± 1.6 c
fry	76.8 ± 1.8 a	36.2 ± 4.1 c	54.2 ± 3.3 c	50.8 ± 2.2 ab	29.0 ± 3.7 b	40.4 ± 2.0 bc
grill	73.3 ± 4.0 a	76.4 ± 3.4 ab	63.7 ± 3.1 bc	39.8 ± 3.0 c	37.2 ± 2.5 b	40.3 ± 1.5 bc
red seabream						
small size						
raw	74.0 ± 2.5 a	77.1 ± 4.9 a	82.7 ± 0.6 a	45.4 ± 4.5 ab	58.1 ± 7.0 ab	73.1 ± 1.0 a
boil	71.7 ± 4.1 a	64.4 ± 5.6 a	71.0 ± 0.7 b	50.8 ± 1.2 a	63.6 ± 3.3 a	51.5 ± 0.8 b
steam	75.2 ± 2.4 a	79.3 ± 3.8 a	69.5 ± 1.4 b	47.0 ± 2.4 a	66.2 ± 3.0 a	50.7 ± 0.8 b
fry	70.6 ± 4.6 a	78.1 ± 3.6 a	65.8 ± 1.5 bc	47.9 ± 3.7 a	27.8 ± 4.5 c	31.7 ± 3.1 d
grill	72.8 ± 2.5 a	65.6 ± 5.1 a	60.1 ± 2.1 c	28.5 ± 6.2 b	39.6 ± 2.6 bc	38.9 ± 1.1 c
medium size						
raw	87.9 ± 0.2 a	89.9 ± 1.9 a	85.4 ± 0.3 a	52.0 ± 3.2 a	60.9 ± 7.3 a	70.2 ± 0.9 a
boil	83.5 ± 1.7 ab	78.2 ± 4.5 bc	71.2 ± 0.2 b	50.9 ± 2.6 a	63.0 ± 4.8 a	49.4 ± 1.0 b
steam	86.5 ± 0.7 ab	88.0 ± 1.3 ab	67.5 ± 1.2 bc	51.4 ± 1.4 a	63.8 ± 2.6 a	43.4 ± 0.6 c
fry	82.8 ± 1.2 b	76.3 ± 1.9 c	60.9 ± 3.1 c	52.7 ± 3.9 a	27.4 ± 0.9 b	31.3 ± 2.3 d
grill	84.9 ± 1.0 ab	79.0 ± 2.0 bc	61.2 ± 1.0 c	24.6 ± 8.5 b	33.4 ± 1.5 b	31.9 ± 1.1 d
large size						
raw	79.9 ± 0.7 a	73.7 ± 2.8 a	84.2 ± 1.4 a	51.8 ± 2.0 a	48.0 ± 3.0 bc	66.6 ± 1.4 a
boil	72.9 ± 1.5 b	63.9 ± 4.5 ab	66.9 ± 1.6 b	49.2 ± 2.6 a	59.8 ± 2.5 ab	40.3 ± 1.2 b
steam	7.3 ± 2.3 ab	80.4 ± 0.7 a	63.2 ± 2.1 b	52.5 ± 2.3 a	61.6 ± 1.2 a	36.9 ± 1.6 b
fry	4.3 ± 2.1 ab	52.2 ± 11.0 b	63.9 ± 1.0 b	53.2 ± 3.3 a	32.4 ± 3.9 d	35.2 ± 2.1 b
grill	3.7 ± 0.9 ab	68.1 ± 2.0 ab	58.2 ± 3.3 b	34.7 ± 4.2 b	38.5 ± 3.0 cd	28.1 ± 1.4 c

[a] For each size of each fish species, the different letters indicate that the cooking methods were significantly different.

Table 4. Regression Coefficients for the Relationships of Element Bioaccessibility with Total Element Concentration and Element Subcellular Distribution in both Seabass and Red Seabream Muscles[a]

	As	Cd	Cu	Fe	Se	Zn
total concentration	0.673***↑	0.007	0.292**↑	0.199*↓	0	0.013
MRG	0.732***↓	0.360*↓	0.060	0.000	0.130	0.002
cellular debris	0.020	0.381*↓	0.116	0.081	0.022	0.001
organelles	0.092	0	0.037	0.002	0.114	0.014
HDP	0.126	0.198	0.166	0.013	0.134	0.062
HSP	0.509**↑	0.206	0.203	0.166	0.210	0.002

[a] *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ↓, negative correlation; ↑, positive correlation.

small-size red seabream. Se was undetectable in all five fractions of small-size red seabream.

Table 2 analyzes the relationship between the total element concentration and subcellular distribution in seabass and red seabream muscles. Wallace et al. (*30*) defined the biologically detoxified metals (BDM) as the summation of MRG and HSP. For As, there was a significant negative correlation between its total concentration and the fraction in MRG ($p < 0.01$) and a significant positive correlation between its total concentration and the HSP ($p < 0.01$) or BDM fraction ($p < 0.05$). No significant correlation was found between total Cd concentration and Cd in any of the subcellular fractions. For Cu and Zn, there was a significant positive correlation of total concentration with the fraction in BDM ($p < 0.05$) and MRG ($p < 0.05$). For Fe and Se, a significant negative correlation of total concentration with the element fraction in HSP ($p < 0.05$) and cellular debris ($p < 0.01$) was also found. In addition, the total element concentration was not correlated with the fraction in organelles and HDP for any of the studied elements.

Element Bioaccessibility of Fish Muscles. Bioaccessibility was measured on the basis of the extraction. For all of the raw muscle samples, the bioaccessibilities of all the studied elements were high, ranging from 45 to 93%. The highest and lowest values were recorded for Cd in the small-size seabass muscle and for Fe in the small-size red seabream muscle (**Table 3**). Within the same element, no notable difference of element bioaccessibility was observed between the two fish species. The bioaccessibility was element-dependent. For seabass and medium-size red seabream, Cd was the highest bioaccessible element. For small- and large-size red seabream, Cu was the highest. However, Fe was the least bioaccessible element in the two fish species except for the large-size red seabream. Overall, the bioaccessibility of both fish species followed the pattern Fe < Se < Zn < As ≈ Cu ≈ Cd.

The influences of cooking methods on element bioaccessibility are presented in **Table 3**. Heating processes, including boiling, steaming, and frying as well as grilling, generally decreased the bioaccessibility of all studied elements in the two fish muscles, especially Cu and Zn. For these two metals, frying and grilling had a greater influence on the bioaccessibility than boiling and steaming. The decrease in bioaccessibility of Cu ranged from 13.0 to 27.2% and from 17.7 to 26.0% for frying and grilling, respectively, whereas it ranged from 11.7 to 18.9% and from 13.2 to 21.0% for boiling and steaming. Similarly, as a result of frying and grilling, the decrease in bioaccessibility of Zn ranged from 30.0 to 41.4% and from 30.1 to 38.5%, respectively, and decreased 20.1−26.3 and 22.4−33.6% after boiling and steaming, respectively.

Relationship between Element Bioaccessibility and Total Tissue Concentration and Subcellular Distribution. We also performed the correlation analysis between the bioaccessibility and total tissue concentration and subcellular distribution in the two fish species (**Table 4**). There were a few cases when significant correlations between bioaccessibility and total tissue concentration were found, for example, positive correlations for As ($p < 0.001$) and Cu ($p < 0.01$) and a negative correlation for Fe ($p < 0.05$). Furthermore, bioaccessibility also correlated significantly with subcellular distribution in a few cases, for example, As in HSP (positive, $p < 0.01$), As in MRG (negative, $p < 0.001$), and Cd in MRG and cellular debris (negative, $p < 0.05$). For the other four elements (Cu, Fe, Se, and Zn), no significant correlation between bioaccessibility and subcellular fraction was observed.

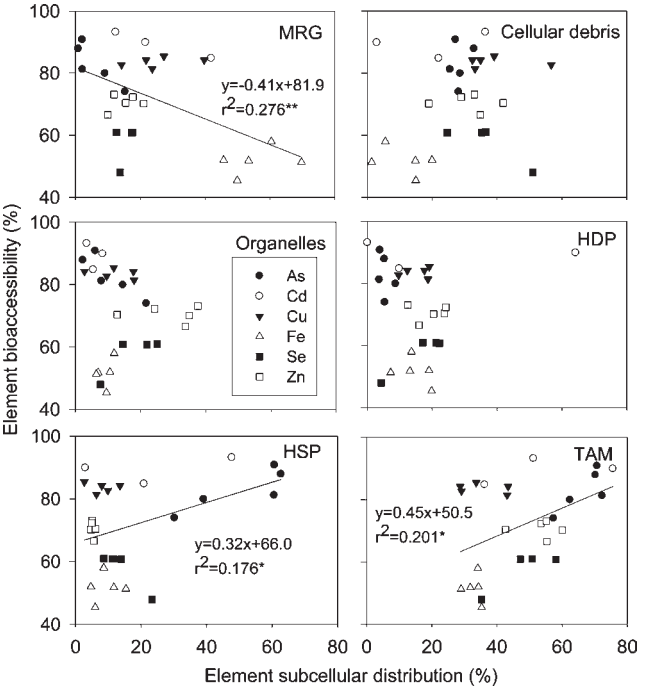

Figure 2. Relationship between element bioaccessibility and subcellular distribution in the muscle of seabass and red seabream. MRG, metal-rich granule; HDP, heat-denaturable protein; HSP, heat-stable protein; TAM, organelles + HDP + HSP. *, $p < 0.05$; **, $p < 0.01$.

Correlation analysis was also conducted for all of the trace elements and the two fish species combined between the bioaccessibility and subcellular fraction (**Figure 2**). The proposed trophically available metal (TAM) component proposed by Wallace and Luoma (*32*) is defined as the sum of organelles, HDP and HSP. Significant positive correlation was found for HSP ($p < 0.05$) and TAM ($p < 0.05$), and significant negative correlation was found for MRG ($p < 0.01$). No correlation was obvious for the other subcellular fractions (cellular debris, organelles, and HDP).

DISCUSSION

For the raw muscles of seabass and red seabream, the bioaccessibility of all studied elements varied between 45 and 93%. Thus, all six elements were highly bioaccessible on the basis of our in vitro extraction measurements. Bioaccessibility varied little between the two fish species, but was highly variable depending on the element studied (Fe < Se < Zn < As ≈ Cu ≈ Cd). In previous studies, the bioaccessibility measured using the in vitro digestion approach for raw food products was 38−87% (edible seaweed) for As (*20*) and was 20−84, 26−97, and 34−82% for Cd, Cu, and Zn, respectively, for shellfish products (*5*, *43*).

Previously, several studies have addressed the bioaccessibility of trace elements in fish, but most of these studies were on mercury bioaccessibility. In fish, the bioaccessibility of Se and

Hg varied from 50 to 83% and from 9 to 17%, respectively (*1*). Our results of Se bioaccessibility in the two marine cultured fish were consistent with these earlier studies. Many factors can affect the bioaccessibility of trace elements, including the chemical species. For example, methylmercury (MeHg) is the more hazardous form of Hg, but Cabanero et al. (*36*) indicated that MeHg had a much lower bioaccessibility than the inorganic Hg in fish. For different As speciation in seafood, the bioaccessibility was 67.5−100% for arsenobetaine, 30% for dimethylarsinic acid, 45% for tetramethylarsonium ion, and >50% for trimethylarsine oxide (*40*). Laird et al. (*39*) demonstrated that gastrointestinal microbes may affect Hg bioaccessibility of 16 country foods including fish. Co-consumption of foods containing phytochemicals (green tea extract, soy protein, wheat bran, and psyllium) with fish may potentially reduce mercury bioaccessibility compared to eating fish alone (*42*). Selenium is recognized to decrease mercury toxicity and affect mercury bioaccessibility when both elements are simultaneously administrated (*1*, *35*, *36*, *44*).

In most cases, seafood products are processed prior to consumption. These thermal treatment processes might change the total concentrations or alter the bioaccessibility of some trace elements. For example, the bioaccessibility of Cd, Cu, and Zn in shellfish decreased upon cooking (*5*). In some seaweeds, after cooking, the As bioaccessibility either did not change in *Hizikia fusiforme* or increased in *Porphyra* sp. (*14*, *20*). In addition, in another two species of fish, cooking induced no change in the bioaccessibility of As (*40*). Se bioaccessibility in cooked tuna was slightly higher than in uncooked tuna (*1*). Except for Se and As, there was no previous study on the influence of cooking processes on bioaccessibility of trace elements in fish; thus, the results obtained from this study were compared only with other seafood products. In this study, cooking processes, including boiling, steaming, and frying as well as grilling, generally decreased the bioaccessibility of six studied elements in the two fish muscles, especially for Cu and Zn. Decrease in bioaccessibilities after cooking was also found in shellfish (*5*). Cooking processes accelerated the protein degradation and enhanced the loss of water and other soluble constituents and may subsequently affect the bioaccessibility (*5*, *11*, *14*). On the one hand, cooking induces denaturalization of the proteins of meat fibers, such that the tissues shrink and then become harder and more compact. Cooking may also cause the formation of disulfide-bonded proteins, which render the proteins less digestible, as demonstrated in the study of cooking of sorghum (*11*, *45*).

Cooking had a more significant influence on the bioaccessibility of Cu and Zn than on the other trace elements, which may be attributed to their storage patterns. Both Cu and Zn were stored mainly in metallothionein and insoluble ligands in the form of less easily degradable complexes and less digestible proteins (*5*). Therefore, there was a marked decrease in the bioaccessibility after cooking. Frying and grilling caused a more rapid decrease in bioaccessibility of both Cu and Zn than boiling and steaming. One of the possible explanations was the greater degree of water and weight loss and more acute heating conditions, which caused the tightly coiled peptide chains to unfold and form large and insoluble aggregates.

It has been recognized that the subcellular partitioning of metals is a useful tool to interpret the internal processing in aquatic organisms during metal accumulation, including toxicity, tolerance, trophic transfer, and detoxification (*24*−*34*). There are very few reports of the subcellular distributions of metals in fish collected from natural environments. Earlier studies in freshwater yellow perch (liver) showed that Cd and Cu were associated with HSP dominantly, and Zn distribution followed the order of organelles > HDP > cellular debris > HSP (*26*−*29*). Our measurements of Zn distribution in the two marine-cultured fish were consistent with these earlier studies, but we found that the main subcellular fractions to bind with Cu were the cellular debris and MRG. The Cd subcellular distribution was fish- and size-dependent. Organisms are able to detoxify accumulated metals, and in most cases, metal concentrations increased in the detoxified subcellular fractions with increasing whole tissue concentrations (*46*). Metal detoxification and storage can take place by binding to HSP or sequestration into MRG; the summation of both pools was termed as BDM (*30*). An increasing BDM compartmentalization with increasing total metal concentration indicated a higher degree of detoxification by the organisms. In this study, we found a significant positive correlation between the BDM and the total concentrations of As and Cu for both fish species, but no correlation was documented for Cd, Fe, Se, and Zn, presumably because the total concentration ranges in the fish muscles were rather narrow.

Significant positive correlation was also observed between the bioaccessibility and the total concentrations for As and Cu, whereas no relationship was found for Cd, Se, and Zn, and a negative relationship was found for Fe. Such different relationships for different elements may be due to different element storage forms and different subcellular distributions in the fish muscles. Again, the insignificant correlation for Cd, Se, and Zn was probably caused by the rather narrow range of total element concentrations.

In the present study, using an in vitro digestion model to mimic human digestion, we for the first time demonstrated that there was a significant relationship between the bioaccessibility and elemental distribution in TAM when all six elements were considered together. Earlier, Wallace and Luoma (*32*) showed a significant relationship between TAM in prey and dietary assimilation by predators (from oligochaete *Limnodrilus hoffmeisteri* to grass shrimp *Palaemonetes pugio*; from bivalve *Potamocorbula amurensis* to grass shrimp *Palaemon macrodactylus*). They suggested that TAM represented the bioavailable metals from the diet and may be used as a tool to predict trophic transfer. Such a hypothesis has been tested in a few marine predators [gastropod and fish (*29*, *34*)]. Our present study demonstrated that the different subcellular fractions of trace elements in the fish tissues had different bioaccessibilities in the human gut environments. The negative correlation between bioaccessibility and element partitioning in MRG fraction strongly suggested that the MRG fraction was less bioaccessible. In contrast, an element bound with the HSP fraction may be more bioaccessible. Such differences in the bioaccessibilities may have been due to the different abilities of the digestive enzymes to digest different chemical components.

In conclusion, the bioaccessibilities of the six studied elements from seabass and red seabream were generally high. Given such high bioaccessibilities, it is still reasonable to use the total concentrations to evaluate food safety of these elements in the two farmed marine fish species. BDM was the dominant subcellular detoxified compartmentalization for both fish species, and As concentrations increased in BDM with increasing total element concentrations. For all of the studied trace elements in both fish species, there was a significant negative correlation between element bioaccessibility and element partitioning in MRG and a positive correlation of element bioaccessibility with element partitioning in HSP and TAM. Consequently, risk assessment for seafood safety may also need to consider the subcellular distribution of trace elements. Cooking processes (boiling, steaming, frying, and grilling) generally decreased the bioaccessibilities of these trace elements, especially frying and grilling. The results of cooking processes on the bioaccessibilities

of trace elements suggest that fish may be eaten raw, boiled, or steamed to improve nutritional values for essential elements and may be eaten fried and grilled to diminish the toxicity and health risk from toxic elements.

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LITERATURE CITED

(1) Cabanero, A. I.; Madrid, Y.; Camara, C. Selenium and mercury bioaccessibility in fish samples: an in vitro digestion method. *Anal. Chim. Acta* **2004**, *526*, 51–61.

(2) Domingo, J. L.; Bocio, A.; Marti-Cid, R.; Llobet, J. M. Benefits and risks of fish consumption Part II. RIBEPEIX, a computer program to optimize the balance between the intake of omega-3 fatty acids and chemical contaminants. *Toxicology* **2007**, *230*, 227–233.

(3) Falco, G.; Llobet, J. M.; Bocio, A.; Domingo, J. L. Daily intake of arsenic, cadmium, mercury, and lead by consumption of edible marine species. *J. Agric. Food Chem.* **2006**, *54*, 6106–6112.

(4) Marti-Cid, R.; Llobet, J. M.; Castell, V.; Domingo, J. L. Dietary intake of arsenic, cadmium, mercury, and lead by the population of Catalonia, Spain. *Biol. Trace Elements Res.* **2008**, *125*, 120–132.

(5) Amiard, J. C.; Amiard-Triquet, C.; Charbonnier, L.; Mesnil, A.; Rainbow, P. S.; Wang, W.-X. Bioaccessibility of essential and non-essential metals in commercial shellfish from western Europe and Asia. *Food Chem. Toxicol.* **2008**, *46*, 2010–2022.

(6) Versantvoort, C. H. M.; Oomen, A. G.; Van de Kamp, E.; Rompelberg, C. J. M.; Sips, A. J. A. M. Applicability of an in vitro digestion model in assessing the bioaccessibility of mycotoxins from food. *Food Chem. Toxicol.* **2005**, *43*, 31–40.

(7) Oomen, A. G.; Hack, A.; Minekus, M.; Zeijdner, E.; Cornelis, C.; Schoeters, G.; Verstraete, W.; Van de Wiele, T.; Wragg, J.; Rompelberg, C. J. M.; Sips, A. J. A. M.; Van Wijnen, J. H. Comparison of five in vitro digestion models to study the bioaccessibility of soil contaminants. *Environ. Sci. Technol.* **2002**, *36*, 3326–3334.

(8) Brandon, E. F. A.; Oomen, A. G.; Rompelberg, C. J. M.; Versantvoort, C. H. M.; van Engelen, J. G. M.; Sips, A. J. A. M. Consumer product in vitro digestion model: bioaccessibility of contaminants and its application in risk assessment. *Regul. Toxicol. Pharmacol.* **2006**, *44*, 161–171.

(9) Oomen, A. G.; Rompelberg, C. J. M.; Bruil, M. A.; Dobbe, C. J. G.; Pereboom, D. P. K. H.; Sips, A. J. A. M. Development of an in vitro digestion model for estimating the bioaccessibility of soil contaminants. *Arch. Environ. Contam. Toxicol.* **2003**, *44*, 281–287.

(10) Intawongse, M.; Dean, J. R. In-vitro testing for assessing oral bioaccessibility of trace metals in soil and food samples. *Trends Anal. Chem.* **2006**, *25*, 876–886.

(11) Kulp, K. S.; Fortson, S. L.; Knize, M. G.; Felton, J. S. An in vitro model system to predict the bioaccessibility of heterocyclic amines from a cooked meat matrix. *Food Chem. Toxicol.* **2003**, *41*, 1701–1710.

(12) Fairweather-Tait, S.; Hurrell, R. F. Bioavailability of minerals and trace elements. *Nutr. Res. Rev.* **1996**, *9*, 295–324.

(13) Miller, D. D.; Schricker, B. R.; Rasmussen, R. R.; Van Campen, D. An in vitro method for estimation of iron availability from meals. *Am. J. Clin. Nutr.* **1981**, *34*, 2248–2256.

(14) Laparra, J. M.; Vélez, D.; Montoro, R.; Barberá, R.; Farré, R. Estimation of arsenic bioaccessibility in edible seaweed by an in vitro digestion method. *J. Agric. Food Chem.* **2003**, *51*, 6080–6085.

(15) Laparra, J. M.; Glahn, R. P.; Miller, D. D. Bioaccessibility of phenols in common Beans (*Phaseolus vulgaris* L.) and iron (Fe) availability to Caco-2 cells. *J. Agric. Food Chem.* **2008**, *56*, 10999–11005.

(16) Perello, G.; Marti-Cid, R.; Llobet, J. M.; Domingo, J. L. Effects of various cooking processes on the concentrations of arsenic, cadmium, mercury, and lead in foods. *J. Agric. Food Chem.* **2008**, *56*, 11262–11269.

(17) Stewart, M. A.; Jardine, P. M.; Barnett, M. O.; Mehlhorn, T. L.; Hyder, L. K.; McKay, L. D. Influence of soil geochemical and physical properties on the sorption and bioaccessibility of chromium(III). *J. Environ. Qual.* **2003**, *32*, 129–137.

(18) Stewart, M. A.; Jardine, P. M.; Brandt, C. C.; Barnett, M. O.; Fendorf, S. E.; McKay, L. D.; Mehlhorn, T. L.; Paul, K. Effects of contaminant concentration, aging, and soil properties on the bioaccessibility of Cr(III) and Cr(VI) in soil. *Soil Sediment Contam.* **2003**, *12*, 1–21.

(19) Yang, J. K.; Barnett, M. O.; Jardine, P. M.; Brooks, S. C. Factors controlling the bioaccessibility of arsenic(V) and lead(II) in soil. *Soil Sediment Contam.* **2003**, *12*, 165–179.

(20) Almela, C.; Laparra, J. M.; Velez, D.; Barbera, R.; Farre, R.; Montoro, R. Arsenosugars in raw and cooked edible seaweed: Ccharacterization and bioaccessibility. *J. Agric. Food Chem.* **2005**, *53*, 7344–7351.

(21) Veda, S.; Kamath, A.; Platel, K.; Begum, K.; Srinivasan, K. Determination of bioaccessibility of β-carotene in vegetables by *in vitro* methods. *Mol. Nutr. Food Res.* **2006**, *50*, 1047–1052.

(22) Mulokozi, G.; Hedren, E.; Svanberg, U. In vitro accessibility and intake of β-carotene from cooked green leafy vegetables and their estimated contribution to vitamin A requirements. *Plant Foods Hum. Nutr.* **2004**, *59*, 1–9.

(23) Hemalatha, S.; Platel, K.; Srinivasan, K. Influence of heat processing on the bioaccessibility of zinc and iron from cereals and pulses consumed in India. *J. Trace Elem. Med. Biol.* **2007**, *21*, 1–7.

(24) Campbell, P. G. C.; Giguere, A.; Bonneris, E.; Hare, L. Cadmium-handling strategies in two chronically exposed indigenous freshwater organisms − the yellow perch (*Perca flavescens*) and the floater mollusc (*Pyganodon grandis*). *Aquat. Toxicol.* **2005**, *72*, 83–97.

(25) Cheung, M. S.; Fok, E. M. W.; Ng, T. Y. T.; Yen, Y. F.; Wang, W.-X. Subcellular cadmium distribution, accumulation, and toxicity in a predatory gastropod, *Thais clavigera*, fed different prey. *Environ. Toxicol. Chem.* **2006**, *25*, 174–181.

(26) Giguere, A.; Campbell, P. G. C.; Hare, L.; Couture, P. Sub-cellular partitioning of cadmium, copper, nickel and zinc in indigenous yellow perch (*Perca flavescens*) sampled along a polymetallic gradient. *Aquat. Toxicol.* **2006**, *77*, 178–189.

(27) Kraemer, L. D.; Campbell, P. G. C.; Hare, L. Dynamics of Cd, Cu and Zn accumulation in organs and sub-cellular fractions in field transplanted juvenile yellow perch (*Perca flavescens*). *Environ. Pollut.* **2005**, *138*, 324–337.

(28) Kraemer, L. D.; Campbell, P. G. C.; Hare, L. Seasonal variations in hepatic Cd and Cu concentrations and in the sub-cellular distribution of these metals in juvenile yellow perch (*Perca flavescens*). *Environ. Pollut.* **2006**, *142*, 313–325.

(29) Zhang, L.; Wang, W.-X. Significance of subcellular metal distribution in prey in influencing the trophic transfer of metals in a marine fish. *Limnol. Oceanogr.* **2006**, *51*, 2008–2017.

(30) Wallace, W. G.; Lee, B. G.; Luoma, S. N. Subcellular compartmentalization of Cd and Zn in two bivalves. I. Significance of metal-sensitive fractions (MSF) and biologically detoxified metal (BDM). *Mar. Ecol.: Prog. Ser.* **2003**, *249*, 183–197.

(31) Wallace, W. G.; Lopez, G. R.; Levinton, J. S. Cadmium resistance in an oligochaete and its effect on cadmium trophic transfer to an omnivorous shrimp. *Mar. Ecol.: Prog. Ser.* **1998**, *172*, 225–237.

(32) Wallace, W. G.; Luoma, S. N. Subcellular compartmentalization of Cd and Zn in two bivalves. II. Significance of trophically available metal (TAM). *Mar. Ecol.: Prog. Ser.* **2003**, *257*, 125–137.

(33) Wang, W.-X.; Rainbow, P. S. Subcellular partitioning and the prediction of cadmium toxicity to aquatic organisms. *Environ. Chem.* **2006**, *3*, 395–399.

(34) Cheung, M.-S.; Wang, W.-X. Influence of subcellular metal compartmentalization in different prey on the transfer of metals to a predatory gastropod. *Mar. Ecol.: Prog. Ser.* **2005**, *286*, 155–166.

(35) Cabanero, A. I.; Madrid, Y.; Camara, C. Selenium long-term administration and its effect on mercury toxicity. *J. Agric. Food Chem.* **2006**, *54*, 4461–4468.

(36) Cabanero, A. I.; Madrid, Y.; Camara, C. Mercury−selenium species ratio in representative fish samples and their bioaccessibility by an in vitro digestion method. *Biol. Trace Elem. Res.* **2007**, *119*, 195–211.

(37) Camara, F.; Amaro, M. A.; Barbera, R.; Clemente, G. Bioaccessibility of minerals in school meals: comparison between dialysis and solubility methods. *Food Chem*. **2005**, *92*, 481–489.
(38) Camara, F.; Amaro, M. A.; Barbera, R.; Lagarda, M. J. Speciation of bioaccessible (heme, ferrous and ferric) iron from school menus. *Eur*. *Food Res*. *Technol*. **2005**, *221*, 768–773.
(39) Laird, B. D.; Shade, C.; Gantner, N.; Chan, H. M.; Siciliano, S. D. Bioaccessibility of mercury from traditional northern country foods measured using an in vitro gastrointestinal model is independent of mercury concentration. *Sci*. *Total Environ*. **2009**, *407*, 6003–6008.
(40) Laparra, J. M.; Velez, D.; Barbera, R.; Montoro, R.; Farre, R. Bioaccessibility and transport by Caco-2 cells of organoarsenical species present in seafood. *J*. *Agric*. *Food Chem*. **2007**, *55*, 5892–5897.
(41) Pedrero, Z.; Madrid, Y.; Camara, C. Selenium species bioaccessibility in enriched radish (*Raphanus sativus*): a potential dietary source of selenium. *J*. *Agric*. *Food Chem*. **2006**, *54*, 2412–2417.
(42) Shim, S. M.; Ferruzzi, M. G.; Kim, Y. C.; Janle, E. M.; Santerre, C. R. Impact of phytochemical-rich foods on bioaccessibility of mercury from fish. *Food Chem*. **2009**, *112*, 46–50.
(43) Bragigand, V.; Berthet, B.; Amiard, J. C.; Rainbow, P. S. Estimates of trace metal bioavailability to humans ingesting contaminated oysters. *Food Chem*. *Toxicol*. **2004**, *42*, 1893–1902.
(44) Peterson, S. A.; Ralston, N. V. C.; Peck, D. V.; Sickle, J. V.; Robertson, J. D.; Spate, V. L.; Morris, J. S. How might selenium moderate the toxic effects of mercury in stream fish of the western U. S.? *Environ*. *Sci*. *Technol*. **2009**, *43*, 3919–3925.
(45) Duodu, K. G.; Nunes, A.; Delgadillo, I.; Parker, M. L.; Mills, E. N. C.; Belton, P. S.; Taylor, J. R. N. Effect of grain structure and cooking on sorghum and maize in vitro protein digestibility. *J*. *Cereal Sci*. **2002**, *35*, 161–174.
(46) Voets, J.; Redeker, E. S.; Blust, R.; Bervoets, L. Differences in metal sequestration between zebra mussels from clean and polluted field locations. *Aquat*. *Toxicol*. **2009**, *93*, 53–60.

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