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Bioaccessibility of Hg, Cd and As in cooked black scabbard fish and edible crab

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ABSTRACT

Regular consumption of seafood has been widely recommended by authorities. Yet, some species accumulate high levels of contaminants like Hg, Cd and As. In addition, the risks associated to the consumption of such seafood may increase if consumers use cooking practices that enhance the concentration of contaminants and their bioaccessibility. In this study, the bioaccessibility of Hg, Cd and As was assessed with *in vitro* human digestion of raw and cooked black scabbard fish (Hg; steamed, fried and grilled) and edible crab (Cd and As; steamed and boiled) tissues. Additionally, the toxicological hazards associated with the consumption of these products were also discussed. Generally, Hg, Cd and As bioaccessibility increased throughout the digestion process. Cadmium and As revealed high bioaccessibility rates in raw and cooked samples (up to 100%), whereas lower bioaccessible fractions of Hg was observed (up to 40%). Furthermore, this study pointed out the importance of food matrix, elemental chemical properties and cooking practices in the bioaccessibility of Hg, Cd and As. The toxicological hazards revealed that edible crab brown meat (Cd) and grilled black scabbard fish (MeHg) consumption in children should be moderated. In contrast, edible crab muscle (Cd) and fried or steamed black scabbard fish (MeHg) should be consumed to minimize exposure. The use of bioaccessible contaminant data strongly reduced the toxicological risks of MeHg, whereas less risk reduction occurred with Cd and inorganic As.

1. Introduction

Seafood is a high nutritional quality food item rich in ω3 polyunsaturated fatty acids, essential amino acids, trace elements and vitamins, and with low cholesterol and saturated fatty acids content (Sioen et al., 2007). Its consumption has been widely encouraged to prevent coronary heart disease, hypertension and cancer (Simopoulos, 1997; Sioen et al., 2007). However, some marine resources can accumulate high levels of contaminants in their edible tissues, such as mercury (Hg), cadmium (Cd), lead (Pb) and arsenic (As), as a result of natural processes (e.g. volcanic activity) or due to anthropogenic actions (Dugo et al., 2006). It is well recognized that these mutagenic, neurotoxic and teratogenic elements can interfere with the human body functions, by damaging the renal, endocrine, gastrointestinal, cardiovascular and nervous systems (Goyer et al., 1995). In order to regulate human exposure to these contaminants, several international agencies established maximum permissible concentrations (MPC's) and tolerable weekly intakes (TWI's) in seafood products (e.g. EC, 2006a, 2008; EFSA, 2011; FAO/WHO, 2011). The current limits are mostly set for raw products and vary according to the element and seafood species, but do not consider several edible tissues (e.g. crustaceans brown meat and livers) much appreciated by some consumers. However, seafood is normally cooked before consumption, and the culinary procedures can affect contaminants' content (e.g. Domingo, 2011). Therefore, regular consumers of seafood with contaminant levels close to tolerable values can be exposed to greater risks due to cooking practices.

Determining the amount of contaminants that are available for absorption in the intestinal epithelium after the digestion process, i.e. the bioaccessibility, is an important tool in health risk assessment (Metian et al., 2008). This tool provides accurate information and guidelines for seafood consumption to authorities, industry and consumers. Presently, well recognized *in vitro* methods that simulate the human digestion process are available, most of them comprising two phases: stomach and small intestinal (e.g. Krul et al., 2000; Kulp et al., 2003; Versantvoort et al., 2005; Van de Wiele et al., 2007; Goicoechea et al., 2011). Yet, so far, only few studies addressed the bioaccessibility of Hg, Cd and As in raw and cooked seafood.

Black scabbard fish (*Aphanopus carbo*) and edible crab (*Cancer pagurus*) are two marine resources of major economic and gastronomic importance in Southern European countries. Black scabbard fish is mostly consumed grilled and fried, whereas edible crab is gen-

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erally boiled before consumption. However, high levels of Hg, Cd and As have been reported for both species, i.e. Hg in black scabbard fish muscle (range: 0.14–2.76 mg kg^{-1} wet weight; Afonso et al., 2008; Costa et al., 2009), Cd in edible crab brown meat and As in edible crab muscle and brown meat (range: 0.03–53.16 mg kg^{-1} wet weight for Cd; 14.26–44.40 mg kg^{-1} wet weight for As; Barrento et al., 2009), often exceeding the limits set by international agencies (EC, 2006a, 2008; US NAS, 2010). Therefore, the excessive consumption of these seafood products might place at risk regular consumers.

In this context, the present work aimed to assess the bioaccessibility of Hg, Cd and As in raw and cooked black scabbard fish (Hg) and edible crabs' brown meat (Cd and As) and muscle (As). Additionally, the toxicological hazards associated with the consumption of these products were also discussed.

2. Material and methods

2.1. Sample preparation and cooking

Black scabbard fish (*A. carbo*; 1141 ± 31 cm total length; 2192 ± 301 g total weight; $n = 5$) and edible crab (*C. pagurus*; 171 ± 8 mm total length; 102 ± 4 mm total width; 991 ± 104 g total weight; $n = 15$) were collected in a Portuguese local market. Specimens were prepared using common household practices (Fig. 1). Briefly, black scabbard fish were eviscerated, beheaded, skin removed and sliced in eight portions that were weighted and randomly allocated to uncooked (control), fried, grilled and steamed treatments (two slices per treatment). Edible crabs were washed and divided in uncooked (control; 5 animals), boiled (5 animals) and steamed (5 animals). The edible tissues (fish muscle, crabs muscle and crabs brown meat, i.e. gonads and hepatopancreas) of raw and cooked specimens were separated, weighted, homogenized with a grinder until complete visual disruption of the tissue (Retasch Grindomix, GM200, Germany; 5000 rpm; polypropylene cup and stainless steel knives) and stored at −80 °C until further analyzes.

2.2. In vitro human digestion model

The experimental setup of the *in vitro* model used was adapted from Hur et al. (2009). Uncooked and cooked black scabbard fish muscle and edible crab muscle and brown meat were *in vitro* digested with four digestive juices: saliva, gastric, duodenal and bile (composition shown in Table 1). The components used in simulated juices were the following: KCl (Merck, 99.5%), KSCN (Sigma, P2713), NaH_2PO_4 (Merck, 99.5%), Na_2SO_4 (Merck, 90%), NaCl (Merck, 99.5%), $NaHCO_3$ (Merck, 99.5%), NH_4Cl (Riedel-de Haën, 99.5%), HCl (Merck, 37%), urea (Sigma, U5128), glucose (Sigma, G5400), glucuronic acid (Sigma, G5269), uric acid (Sigma, U2625), albumin from bovine serum (Sigma, A7906), α-amylase (Sigma, 86250), mucin (Sigma, M2378), pepsin (Sigma, P7125), lipase (Sigma, L3126), pancreatin (Sigma, P8096) and bile extract (Sigma, B8631). The methodology is schematized in Fig. 2. Briefly, consisted in digesting 5 g of fish or crab tissue using 50 mL centrifuge tubes (Sarstedt, 114 × 28 m, PP) in a rotating water bath environment (60 rpm; Memmert W1314, Germany) at 37 °C throughout each digestive phase: saliva (5 ml; 5 min), stomach (gastric juice; 12 ml; 2 h) and small intestine (duodenal juice: 12 ml, 5 min; bile juice: 6 ml, 2 h). The reaction was stopped at the end of each digestion phase by diminishing the temperature to 4 °C, and tubes were centrifuged at 10,000*g* during 10 min at 4 °C (Sigma 3k30, Germany), in order to separate the digestion fluids (aqueous phase) from the particulate residue. The digestive procedure was performed in triplicate, i.e. three tubes were used per digestive phase: (a) mouth (saliva only); (b) stomach (saliva and gastric juices); and (c) small intestine (saliva, gastric, duodenal and bile juices). Therefore, nine tubes were used per sample. The digestive fluids and residue at the end of the digestive procedure were stored at −80 °C for quantification of Hg, Cd and As. The bioaccessibility fraction was calculated as the concentration of a particular contaminant (Hg, Cd and As) solubilized in a digestive phase (mouth, stomach and small intestine) to the concentration of the same contaminant in the sample before digestion.

2.3. Quantification of Hg, Cd and As

The quantification of Hg, Cd and As was performed in the seafood sample before digestion, in the three fluid extracts and in the sample residues.

Mercury levels (Hg) were determined by atomic absorption spectrometry according to United States Environmental Protection Agency test method 7473 (US EPA, 2007), using a Hg analyzer (Leco, AMA 254, St. Joseph, MI, USA) that uses the Hg vapor generation technique. The procedure was based on decomposition of at least 10 mg of solid sample or 200 µl of liquid sample ($n = 3$) by combustion, preconcentration of Hg by amalgamation with gold and Hg was detected at 254 nm by atomic absorption spectrometry. Concentrations were calculated from a calibration curve with diluted Hg standard solutions of 1 g L^{-1} of $Hg(NO_3)_2$ (dissolved in 2 M HNO_3; Merck).

Samples for Cd and As assessment were primarily digested in triplicate in a microwave oven (CEM, Mars5, USA). The procedure for solid samples (1 g) was the following: 5:1 v/v mixture of nitric acid (65%, Merck) and hydrogen peroxide (30%, Merck), 15 min at 120 °C, 1200 w and 120 PSI. In contrast, the procedure for digestive liquid extracts (1 mL) was: 5:1:15 v/v mixture of nitric acid (65%, Merck), hydrogen peroxide (30%, Merck) and Milli Q water, 10 min at 200 °C, 300 w and 350 PSI.

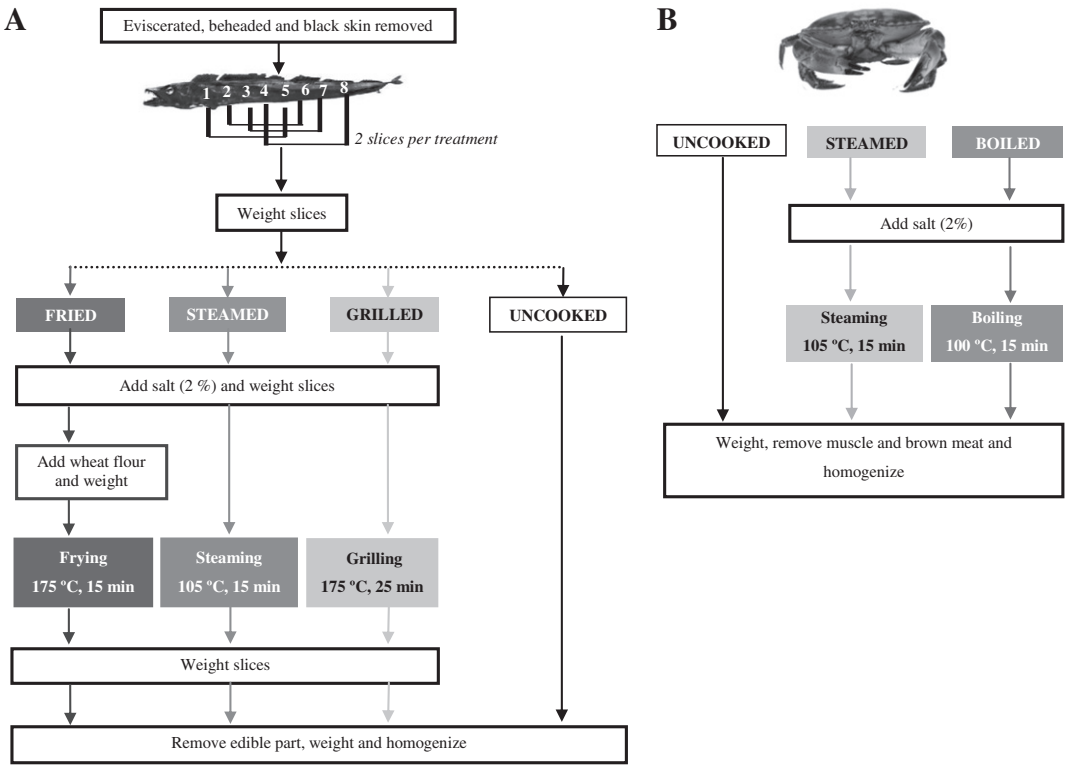

Fig. 1. Preparation of uncooked and cooked black scabbard fish (A) and edible crab (B).

Table 1
Composition of digestive juices used per 100 ml of ultrapure water (adapted from Hur et al., 2009).

Components	Digestive juices			
	Saliva (pH 6.8 ± 0.2)	Gastric (pH 1.30 ± 0.02)	Duodenal (pH 8.1 ± 0.2)	Bile (pH 8.2 ± 0.2)
Inorganic	−90 mg KCl −20 mg KSCN −89 mg NaH_2PO_4 −58 mg Na_2SO_4 −30 mg NaCl −170 mg $NaHCO_3$	−275 mg NaCl −27 mg NaH_2PO_4 −82 mg KCl −31 mg NH_4Cl −HCl 1 M (adjust pH)	−700 mg NaCl −339 mg $NaHCO_3$ −8 mg KH_2PO_4 −56 mg KCl −HCl 1 M (adjust pH)	−526 mg NaCl −579 mg $NaHCO_3$ −38 mg KCl −HCl 1 M (adjust pH)
Organic	−20 mg Urea	−65 mg Glucose −2 mg Glucuronic acid −8.5 mg urea −33 mg Glucosamine hydrochloride −100 mg BSA	−100 mg Urea −100 mg BSA	−25 mg Urea −180 mg BSA
Bioactive	−29 mg α-Amylase −1.5 mg Uric acid −2.5 mg Mucin	−250 mg Pepsin	−900 mg Pancreatin −150 mg lipase	−3 g Bile salt extract

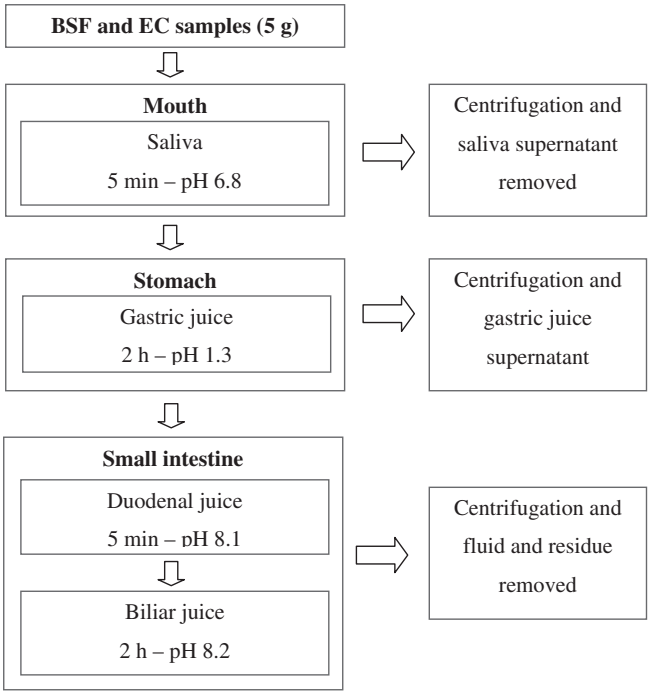

Fig. 2. Flow diagram of the *in vitro* digestive procedure; *Abbreviations:* BSF, black scabbard fish; EC, edible crab.

The Cd content was then analyzed on a graphite furnace atomic absorption spectrometer (Varian, Spectr AA 220Z, Australia) according to the EN 14084:2003 (CEN, 2003), calibrated with a Cd standard solution of 1 g L^{-1} of $Cd(NO_3)_2$ (dissolved in 0.5 M HNO_3, Merck). In contrast, As content was analyzed by a quadrupole inductively-coupled plasma mass spectrometry unit (ICP-MS; Thermo Elemental, X-series 2, UK; see conditions in Table 2) according to the EN 15763:2009 (CEN, 2009). The As calibration curve was performed and checked with two independent certified As standard solutions containing 1 g L^{-1} of H_3AsO_4 (dissolved in 0.5 M HNO_3; Merck). ICP-MS tuning was performed on a daily basis with a diluted 10 mg L^{-1} multi-element solution (Analytika, UNICAM, Portugal). Yttrium (1000 mg L^{-1}; Merck) was chosen as internal standard to correct for instrumental drift.

Certified biological material (Lobster hepatopancreas; Tort-2; National Research Council, Canada) was used to check the methods' accuracy for Hg, Cd and As quantification (see Table 3).

All material used was cleaned before use with a warm solution of 20% of nitric acid (HNO_3, Merck) to avoid external contaminations.

2.4. Exposure calculations

The toxicological risks associated with the intake of MeHg, Cd and inorganic As from consumption of raw and cooked black scabbard fish and edible crab before *in vitro* digestion and its bioaccessible fraction were quantified. Since the toxic form of Hg (MeHg) was not determined, it was assumed that 86% Hg detected was MeHg, according to findings of Afonso et al. (2008). Similarly, for As it was assumed that 0.05% and 0.23% of all As was in the inorganic form (most toxic) in edible crab muscle and brown meat, respectively, according to findings of Sloth et al. (2005).

The average contaminants levels found in the samples were evaluated by using the guideline values recommended by international organizations, i.e. the provisional tolerable weekly intake (PTWI) set by FAO/WHO (2011) for MeHg (1.6 μg/kg bw/week) and inorganic As (15 μg/kg bw/week inorganic As withdrawn in 2011) and the tolerable weekly intake (TWI) set by the European Food Safety Authority for Cd (2.5 μg/kg bw/week; EFSA, 2011).

The toxicological risks were assessed for adults and children (7–12 years old), using adults and children average body weights in Portugal (69 and 31.5 kg, respectively; Padez et al., 2004; EC, 2006b). Three consumption scenarios were considered: one portion per day, one portion per week and one portion per month (black scabbard fish portion: 200 g for adults and 150 g for children; edible crab brown meat portion: 50 g for adults and children; edible crab muscle portion: 75 g for adults and children).

Table 2
Instrument operating conditions for As determination by ICP-MS.

Instrument	Thermo XSeries2
Autosampler	Cetac ASX-520
Nebulizer	1 ml min^{-1} Concentric glass nebulizer
Spray chamber	Air cooled quartz spray chamber
Torch	Quartz 1.5 mm injector
Cones	Skimmer Ni Sampler Ni
Forward power (W)	1400
Cool gas (Ar; L min^{-1})	13
Auxiliary gas (Ar; L min^{-1})	0.82
Nebulizer gas (Ar; L min^{-1})	0.80–0.85
Pole Bias (V)	−0.5
Hexapole Bias (V)	−3
Correction equations	75As = 75 M −3.13220 * 77ArCl77ArCl = 77 M −0.826 * 82Se

Table 3
Methodology accuracy for Hg, Cd and As with certified reference material (Tort-2 – National Research Council, Canada; mg kg^{-1} dry weight; $n = 4$) and the detection limits (± standard deviation; μg kg^{-1} DW).

Contaminant	Technique	Detection limit	Certified biological material	Certified value	Present work
Mercury (Hg)	AAS	0.5	Lobster hepatopancreas (TORT-2)	0.27 ± 0.06	0.28 ± 0.00
Cadmium (Cd)	GFAAS	0.04	Lobster hepatopancreas (TORT-2)	26.7 ± 0.6	27.0 ± 0.0
Arsenic (As)	ICP-MS	0.3	Lobster hepatopancreas (TORT-2)	21.6 ± 1.8	22.4 ± 2.4

Abbreviations: AAS, atomic absorption spectrometry; GFAAS, graphite furnace atomic absorption spectrometry; ICP-MS, inductively coupled plasma mass spectroscopy.

2.5. Statistical analysis

One-way analysis of variance (ANOVA) followed by Tukey's test were used to detect significant differences in bioaccessibility of Hg, Cd and As between the culinary treatments. Data was log-transformed whenever the assumptions of normality (Kolmogorov–Smirnov's test) and homogeneity of variances (Levene's *F*-test) were not corroborated. Non-parametric analysis of variance (Kruskall–Wallis) and multiple comparisons test were applied whenever transformed data could not meet these requirements. Statistical significance was established at $p < 0.05$ using the software STATISTICA 8.0© (Statsoft, Tulsa, USA).

3. Results and discussion

3.1. Mercury bioaccessibility

Total Hg content detected in *A. carbo* samples varied according to culinary treatment (Table 4). The Hg variability found between specimens is likely due to physiological and environmental factors, such as food, maturation stage, age and size (Nussey et al., 2000; Canli and Atli, 2002; Costa et al., 2009). Overall, the highest Hg content before *in vitro* digestion was found in cooked black scabbard fish muscle, probably as a result of water loss during the culinary procedure, though only significantly in grilled samples. Similar findings were obtained in previous studies with other fish species (e.g. Perelló et al., 2008). Grilled *A. carbo* showed 80% of samples above the MPC set for uncooked black scabbard fish (1 mg kg^{-1}; EC, 2006a, 2008), whereas steamed, fried and uncooked fish had respectively 40%, 32% and 8% of samples above this limit.

Regardless of the culinary procedure, throughout the *in vitro* digestion, the bioaccessible Hg fraction in black scabbard fish muscle was lower in the mouth step and higher at the end of the digestion process (small intestine) (Table 4). Food is partly broken down in the mouth by chewing and chemical action of salivary enzymes that break down starches into smaller molecules, whereas the remaining digestion processes of proteins and lipids occur in the stomach and small intestine, with the release of specific enzymes (like pepsine and pancreatine), pH alterations and peristaltic movements (Hur et al., 2009). Overall, non statistical differences in bioaccessible Hg was observed between uncooked (45%) and grilled (42%) or steamed (33%) samples in small intestine, whereas statistically lower Hg bioaccessibility occurred in fried (24%) samples compared to uncooked samples (Fig. 3A). Such differences can be due to structural changes during the cooking treatments that might have affected the solubility and release of Hg, or induced Hg complexation with other components. Shim et al. (2009) evidenced that the presence of insoluble fibers, such as those from wheat, decrease Hg bioaccessibility, since these compounds can bond and diminish Hg solubility. This fact might explain the low Hg bioaccessibility in fried black scabbard fish, as wheat flour was added during the culinary preparation. In contrast, previous authors reported lower Hg, As, Cd, Cu, Fe, Se, and Zn bioaccessibility in grilled fish muscle than in uncooked samples (He et al., 2010; Torres-Escribano et al., 2011a). Lower Hg bioaccessibility in cooked fish could be attributed to the loss of native proteins and structural alterations during the heat exposure, thus limiting the activity of gastrointestinal enzymes that degrade protein structures bonded with Hg (Torres-Escribano et al., 2011a).

Hg bioaccessibility obtained in the gastrointestinal step was higher than those obtained in previous studies for uncooked black scabbard fish (10.1% and 16.5% in the gastric and gastrointestinal steps, respectively) (Cabañero et al., 2004, 2007). These differences are likely related to variations in the digestion model used, such as enzyme concentrations and activity, type of recipients used, volume of digestive juice, amount of sample and water content. Torres-Escribano et al. (2011b) reported different bioaccessibility of As, Cd, Pb and Hg for the same certified reference material using a static and a dynamic multicompartment *in vitro* digestion method, and suggested that the specific form in which the elements occur in the samples might explain the bioaccessibility values. In fact, Liard et al. (2009) reported that Hg bioaccessibility is affected by the dynamic Hg transfer between its bioaccessible (i.e. <10 kDa) and non-bioaccessible (>10 kDa) fractions. Gastro intestinal microorganisms may also influence the speciation of dietary Hg in the intestinal lumen via the conversion of MeHg to Hg^{II}, though varying with the type of food consumed (Liard et al., 2009). Additionally, *in vitro* digestors have difficulty to reproduce the whole gastro intestinal process, such as: sequential secretion of enzymes in physiological concentrations, appropriate pH for the enzymes and addition of relevant cofactors such as bile salts and coenzymes, removal of the digested products, appropriate mixing at each stage of the digestion, physiological transit time for each step of the digestion, physical mechanisms of absorption in the small intestine, and absence of brush-border enzymes (Liard et al., 2009).

3.2. Cadmium bioaccessibility

Cadmium content in edible crabs' brown meat was significantly lower in uncooked crabs compared with cooked, whereas no significant differences were detected between boiled and steamed crabs (Table 4). Currently, Cd MPC for crustaceans is only set for muscle (0.5 mg kg^{-1}; EC, 2006a, 2008) but not for brown meat. In this study, Cd levels in this tissue largely exceeded the MPC in all cooked and uncooked samples.

Bioaccessible Cd in brown meat crab was always lower in the mouth step and higher in the small intestine, as it was observed with Hg (Table 4). No statistical differences were detected between cooked and uncooked samples in saliva and small intestine, whereas in the stomach significantly higher bioaccessibility was found in steamed brown meat compared to uncooked samples (Table 4; Fig. 3B). A high Cd fraction was bioaccessible by the end of the digestive process (small intestine), reaching 95.3% in uncooked, 83.9% in steamed and 83.7% in boiled samples (Fig. 3B). In fact, 5.8–13.1% and 45.9–72.0% of the total Cd content was already accessible in the mouth and stomach, respectively. The nutritional and textural characteristics of brown meat might explain such high values, as this tissue is very soft, liquidized and fatty, thus enabling higher sample area in contact with the digestion juices and homogeneous emulsions. Amiard et al. (2008) evidenced the high bioaccessibility of Cd in various seafood products, such as the green mussel (*Perna viridis*; 36%), scallop (*Chlamys nobilis*; 20%), clam (*Marcia hiantina*; 48%), oyster (*Saccostrae cucullata*; 68%) and neogastropod (*Buccinum undatum*; 72%). However, the results obtained

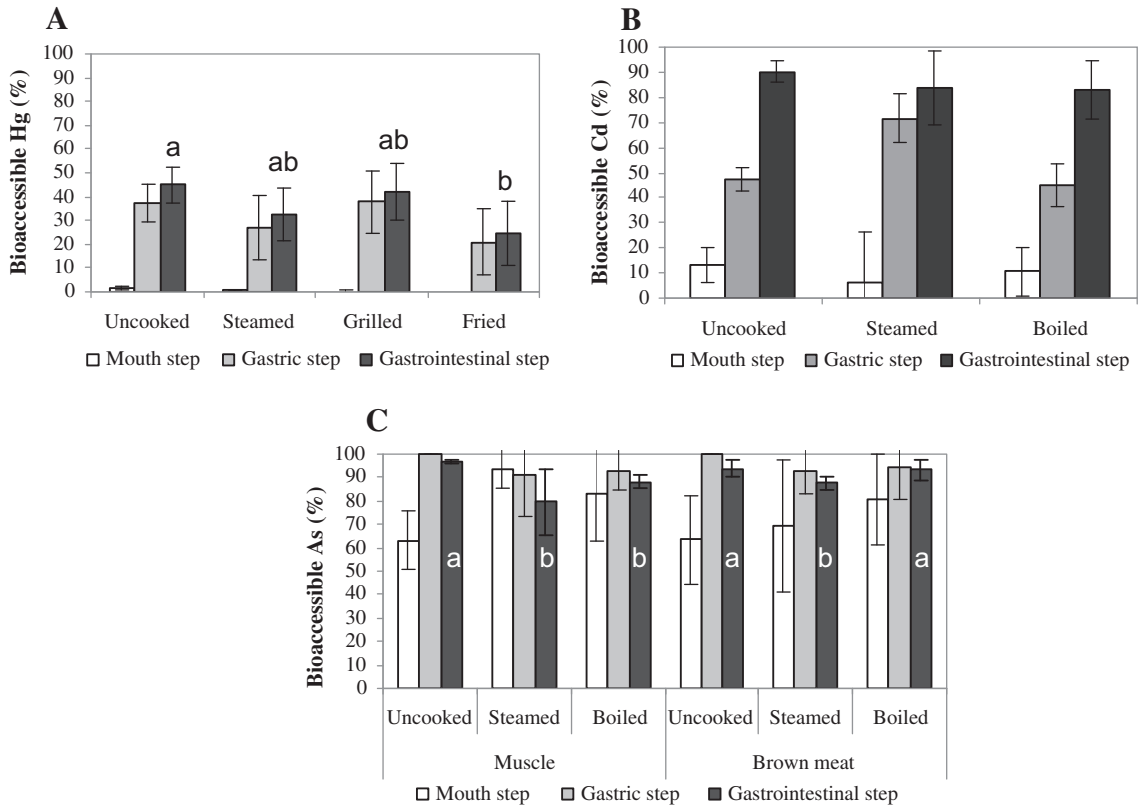

Fig. 3. Bioaccessibility of Hg in black scabbard fish (A), Cd in edible crab brown meat (B) and As in edible crab muscle and brown meat (C). In the columns of the small intestine different letters denote significant differences ($p < 0.05$), whereas the absence of letters indicate no significant differences.

Table 4
Moisture, lipid, Hg, Cd and As content of cooked and uncooked seafood samples before *in vitro* digestion, as well as bioacessible and non-bioacessible Hg, Cd and As during *in vitro* human digestion.

Aphanopus carbo (Muscle; $n = 5$)	Hg before digestion ($\mu g\ kg^{-1}$)	Bioaccessible Hg			Hg in residue ($\mu g\ kg^{-1}$)
		Mouth step ($\mu g\ kg^{-1}$)	Gastric step ($\mu g\ kg^{-1}$)	Gastrointestinal step ($\mu g\ kg^{-1}$)	
Uncooked	861 ± 115^{b}	13 ± 6^{a}	331 ± 75^{ab}	398 ± 90^{ab}	483 ± 73^{b}
Steamed	988 ± 92^{ab}	5.7 ± 1.3^{b}	274 ± 140^{ab}	328 ± 125^{ab}	665 ± 98^{a}
Grilled	1263 ± 216^{a}	4.7 ± 1.0^{b}	486 ± 249^{a}	553 ± 243^{a}	710 ± 88^{a}
Fried	957 ± 105^{ab}	1.9 ± 0.8^{c}	192 ± 128^{b}	225 ± 121^{b}	742 ± 206^{a}
Cancer pagurus (Brown meat; $n = 5$)	Cd before digestion ($\mu g\ kg^{-1}$)	Bioaccessible Cd			Cd in residue ($\mu g\ kg^{-1}$)
		Mouth step ($\mu g\ kg^{-1}$)	Gastric step ($\mu g\ kg^{-1}$)	Gastrointestinal step ($\mu g\ kg^{-1}$)	
Uncooked	5.6 ± 0.8^{b}	0.70 ± 0.35	2.8 ± 1.2^{b}	$5.3\ 0 \pm 0.8$	0.23 ± 0.08^{b}
Steamed	8.0 ± 3.4^{a}	0.49 ± 0.54	5.5 ± 1.7^{a}	6.4 ± 1.9	1.9 ± 1.3^{a}
Boiled	8.5 ± 1.7^{a}	0.84 ± 0.25	4.0 ± 1.8^{ab}	7.2 ± 2.4	1.2 ± 0.9^{a}
Cancer pagurus ($n = 10$)	As before digestion ($\mu g\ kg^{-1}$)	Bioaccessible As			As in residue ($\mu g\ kg^{-1}$)
		Mouth step ($\mu g\ kg^{-1}$)	Gastric step ($\mu g\ kg^{-1}$)	Gastrointestinal step ($\mu g\ kg^{-1}$)	
Muscle					
Uncooked	18 ± 5	12 ± 5^{b}	18 ± 5	17 ± 5	0.61 ± 0.23^{c}
Steamed	24 ± 4	23 ± 5^{a}	22 ± 7	19 ± 2	6.3 ± 4.0^{a}
Boiled	16 ± 5	14 ± 7^{ab}	14 ± 4	14 ± 4	2.3 ± 1.1^{b}
Brown meat					
Uncooked	17 ± 6	10 ± 3	17 ± 6^{a}	16 ± 6	0.90 ± 0.49
Steamed	13 ± 3	8.7 ± 3.3	12 ± 3^{ab}	11 ± 3	1.5 ± 0.2
Boiled	11 ± 3	8.8 ± 3.3	9.4 ± 1.7^{b}	9.5 ± 2.2	1.2 ± 0.4

In each column different superscript letters denote significant differences ($p < 0.05$), whereas values without superscript letters indicate no significant differences.

by these authors were lower than those observed in the present study, probably due to the specific characteristics of the food matrix.

Interestingly, Cd bioaccessibility percentage was lower in cooked brown meat compared to uncooked samples (Fig. 3B), likely due to the solid texture exhibited by steamed and boiled brown

Table 5
Estimated intake of MeHg, Cd and inorganic As through the consumption of black scabbard fish and edible crab, and comparison with the established tolerable intake values (PTWI or TWI).

Contaminant	Uncooked		Boiled		Steamed		Grilled		Fried	
	Total (%)	Bioaccessible (%)	Total (%)	Bioaccessible (%)	Total (%)	Bioaccessible (%)	Total (%)	Bioaccessible (%)	Total (%)	Bioaccessible (%)
MeHg in black scabbard fish muscle[a]										
Children intake (7–12 years old; 150 g portion)	*D*: 1794	*D*: 829	–	–	*D*: 2058	*D*: 683	*D*: 2631	*D*: 1152	*D*: 1994	*D*: 469
	W: 256	*W*: 119			*W*: 294	*W*: 97.6	*W*: 376	*W*: 165	*W*: 285	*W*: 67.0
	M: 59.8	*M*: 27.6			*M*: 68.6	*M*: 22.8	*M*: 87.7	*M*: 67.0	*M*: 66.5	*M*: 15.6
Adult intake (200 g portion)	*D*: 1092	*D*: 505	–	–	*D*: 1253	*D*: 416	*D*: 1602	*D*: 701	*D*: 1214	*D*: 285
	W: 156	*W*: 72.1			*W*: 179	*W*: 59.4	*W*: 229	*W*: 100	*W*: 173	*W*: 40.8
	M: 36.4	*M*: 16.8			*M*: 41.8	*M*: 13.9	*M*: 53.4	*M*: 23.4	*M*: 40.5	*M*: 9.5
Cd in edible crabs' brown meat										
Children intake (7–12 years old; 50 g portion)	*D*: 2489	*D*: 2356	*D*: 3778	*D*: 3200	*D*: 3556	*D*: 2844	–	–	–	–
	W: 356	*W*: 337	*W*: 540	*W*: 457	*W*: 508	*W*: 406				
	M: 83.0	*M*: 78.5	*M*: 126	*M*: 107	*M*: 119	*M*: 94.8				
Adult intake (50 g portion)	*D*: 1136	*D*: 1075	*D*: 1725	*D*: 1461	*D*: 1623	*D*: 1299	–	–	–	–
	W: 162	W: 154	*W*: 246	*W*: 209	*W*: 232	*W*: 186				
	M: 37.9	*M*: 35.8	*M*: 57.5	*M*: 48.7	*M*: 54.1	*M*: 43.3				
Inorganic As in edible crabs' brown meat[b]										
Children intake (7–12 years old; 50 g portion)	*D*: 2.9	*D*:*D*: 2.7	*D*: 1.9	*D*: 1.6	*D*: 2.2	*D*: 1.9	–	–	–	–
	W: 0.41	*W*: 0.39	*W*: 0.27	*W*: 0.23	*W*: 0.32	*W*: 0.27				
	M: 0.10	*M*: 0.09	*M*: 0.06	*M*: 0.05	*M*: 0.07	*M*: 0.06				
Adult intake (50 g portion)	*D*: 1.3	*D*: 1.2	*D*: 0.86	*D*: 0.74	*D*: 1.0	*D*: 0.86	–	–	–	–
	W: 0.19	*W*: 0.18	*W*: 0.12	*W*: 0.11	*W*: 0.14	*W*: 0.12				
	M: 0.04	*M*: 0.04	*M*: 0.03	*M*: 0.02	*M*: 0.03	*M*: 0.03				
Inorganic As in edible crab muscle[b]										
Children intake (7–12 years old; 75 g portion)	*D*: 1.0	*D*: 0.94	*D*: 0.89	*D*: 0.78	*D*: 1.3	*D*: 1.1	–	–	–	–
	W: 0.14	*W*: 0.13	*W*: 0.13	*W*: 0.11	*W*: 0.19	*W*: 0.15				
	M: 0.03	*M*:0.03	*M*: 0.03	*M*: 0.03	*M*: 0.04	*M*: 0.04				
Adult intake (75 g portion)	*D*: 0.46	*D*: 0.43	*D*: 0.41	*D*: 0.36	*D*: 0.61	*D*: 0.48	–	–	–	–
	W: 0.07	*W*: 0.06	*W*: 0.06	*W*: 0.05	*W*: 0.09	*W*: 0.07				
	M: 0.02	*M*: 0.01	*M*: 0.01	*M*: 0.01	*M*: 0.02	*M*: 0.02				

Data was calculated using adults and children mean body weights in Portugal (bw; 69 and 31.5 kg, respectively; Padez et al., 2004; EC, 2006b). *Abbreviations: D*, estimated daily intake; *W*, estimated weekly intake; *M*, estimated monthly intake; *x*, number of times that exceed intake values.

[a] Assuming that 86% Hg is present as MeHg (according to Afonso et al., 2008).

[b] Assuming that 0.05% and 0.23% of all As is in the inorganic form in edible crab muscle and brown meat, respectively (according to Sloth et al., 2005).

meat, thus being less accessible to digestive enzymes. Lower Cd bioaccessibility in cooked samples compared to uncooked has also been previously reported for fried, grilled, steamed and boiled seabass and red sea bream muscle (He et al., 2010).

3.3. Arsenic bioaccessibility

Generally, no significant differences in the As content of samples before the *in vitro* digestion were found between cooked and uncooked crab muscle and brown meat samples (Table 4). Previous studies of Perelló et al. (2008) reported higher As content in grilled, fried and roasted fish (sardine, hake and tuna) compared to uncooked samples, thus indicating that the cooking procedure might affect As content, So far, no MPC has been set in EU for total As in seafood, whereas the United States Food and Drug Administration set an Action Level limit of 76 mg kg^{-1} for As in crustaceans (US NAS, 2010). All samples did not exceed this limit for As.

The As bioaccessible fraction of crab muscle and brown meat attained the highest level in the stomach and remained constant or slightly decreased in the small intestine (Table 4). High As fractions were bioaccessible in crab muscle throughout the digestive process, reaching 63.2–93.7% in the mouth, 91.1–100.0% in the stomach and 79.6–96.6% in the small intestine (Fig. 3C). Similar high As bioaccessibility was obtained in crab brown meat, with 63.3–80.6% in the mouth, 92.6–100.0% in the stomach, and 87.7–93.7% in the small intestine (Fig. 3C). Similar findings have been reported in previous studies of As bioaccessibility with clams and seaweed (Kock et al., 2007). The low pH during the gastric step has been pointed out as the major prerequisite for As solubilization and consequent availability in the gastrointestinal step (Oomen et al., 2003).

Overall, As bioaccessibility in the gastrointestinal step was statistically lower in steamed (muscle and brown meat) and boiled (muscle only) samples (Fig. 3C). This is in accordance with the findings of He et al. (2010) in boiled samples of small seabass (*Lateolabrax japonicus*) and large seabream (*Pagrosomus major*). Such decrease in As bioaccessibility in boiled samples can be due to the As release to the boiling water and/or proteins' denaturation that shrink the muscle and turn it more compact and less accessible to digestive enzymes, similarly to the findings of Kulp et al. (2003) with heterocyclic amines in cooked meat. In fact, the culinary procedure and food matrix seems to play an important role in As bioaccessibility, as Laparra et al. (2007) reported an increase in microwave cooked Greenland halibut (*Reinhardtius hippoglossoides*) compared to uncooked samples, whereas this culinary practice did not affect As bioaccessibility in sole (*Solea solea*).

3.4. Toxicological hazards

The weekly average consumption of cooked black scabbard fish muscle by adults and children exceeded the PTWI set for MeHg in all cooked and uncooked samples (1.3–3.2 times; Table 5). However, taking into account the Hg bioaccessible fraction, an evident decrease in the risk of exceeding the PTWI was observed, with values only exceeded in uncooked and grilled fish for children. Therefore, black scabbard fish might be consumed by adults (once per week) and children (once per month) with few health hazards, and consumers should preferably use steaming and frying as culinary procedures for this species, as far as Hg is concerned.

The risks of exceeding the Cd TWI in edible crab brown meat were high in adults and children either in before *in vitro* digestion samples (1.6–5.4 times) and in its bioaccessible fraction (1.5–4.6 times) (Table 5). Nevertheless, the consumption of brown meat once per month never exceeded the TWI in adults, whereas for children the consumption of brown meat once a month still exceeded the TWI in cooked crabs, particularly in boiled specimens. The high Cd values detected in edible crab brown meat suggest that consumers should moderately consume this product due to the potential heath-associated risks and that more accurate MPC's should be established for the presence of contaminants in different edible tissues, such as brown and white meat for Cd.

The risks of exceeding the PTWI of inorganic As in adults and children with the daily ingestion of edible crab brown meat and muscle were extremely reduced either in the sample (<2.9%) and in the bioaccessible fraction (<2.7%) (Table 5). Therefore, results indicate that As intake does not represent a health concern through consumption of edible crab muscle and brown meat.

4. Conclusion

This work evaluated the bioaccessibility of Hg, Cd and As in cooked black scabbard fish and edible crab and the toxicological risks for children and adults assuming three different consumption scenarios. Several conclusions can be made:

- The bioaccessibility of Hg, Cd and As is largely influenced by several factors, such as the type of matrix, cooking preparation, simulated digestion model and the chemical properties of the contaminant.
- The incorporation of bioaccessible Hg, Cd and As in the toxicological assessment reduces the risks compared to the whole contaminant levels found in samples before digestion.
- The toxicological hazards associated to the consumption of edible crab brown meat (Cd) and grilled black scabbard fish (MeHg) revealed that the consumption in children should be moderated.
- Edible crab muscle and fried or steamed black scabbard fish should be consumed to minimize exposure to Cd and MeHg, respectively.
- It is extremely important to refine and harmonize/validate *in vitro* digestion methods for different samples to better simulate *in vivo* situations. Recently, Wragg et al. (2011) harmonized an *in vitro* digestion model for soil samples through inter-laboratorial trials.
- The use of *in vitro* digestion models to study bioaccessibility of contaminants in seafood provides relevant information for risk assessment analysis, thus enabling improvements to the current legislation.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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