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Cooking makes cadmium contained in Chilean mussels less bioaccessible to humans

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ABSTRACT

In Chile, upwellings occur periodically along the coasts, resuspending metals from the seafloor and reintroducing them to the food web. Chilean blue mussels, *Mytilus chilensis*, accumulate these toxic compounds and show high concentrations of cadmium. An *in vitro* simulated digestion method has been applied to specimens of *M. chilensis* previously contaminated with ^{109}Cd, to measure the bioaccessibility of cadmium for humans. The effects of the cooking process on the cadmium content of this species and on the resulting change in dietary bioaccessibility have also been evaluated. While cooking resulted in an increase in cadmium concentration in mussel flesh, cadmium remaining in the cooked flesh was also significantly less bioaccessible than cadmium occurring in the raw tissue. Estimations made in this study show that the intake of Cd from mussels by the Chilean population does not exceed the toxicological reference values established by the FAO/WHO; consequently, a health risk situation is not indicated.

1. Introduction

Utilisation of marine resources for human consumption has increased rapidly worldwide and consumption of fish and fishery products reached 137 millions tons by 2003 (FAO, 2004). Shellfish have the ability to bioaccumulate metals from their surroundings as they feed by filtering particles from water (e.g., Fisher, Teyssié, Fowler, & Wang, 1996; Poulsen, Riisgard, & Mohlenberg, 1982; Wang, 2009). While metals like cobalt, chromium, iron, manganese or zinc can be essential for humans in trace amounts, other non-essential elements, such as mercury, arsenic, lead and cadmium, can be toxic for humans, even at very low concentrations (Belitz & Grosch, 1999; EC, 2006; Reilly, 2002).

In Chile, upwellings occur periodically along the coasts, resuspending metals from the seafloor and reintroducing them into the food web (Lares, Flores-Muñoz, & Lara-Lara, 2002; Lares, Rivero, & Huerta-Diaz, 2005; Mandal & Suzuki, 2002; Romero et al., 2003; Valdés, Román, Dávila, Ortlieb, & Guiñez, 2006). Shellfish accumulate these toxic compounds that can become highly concentrated in their tissues (Astorga España, Rodríguez, & Diaz Ramero, 2004; De Gregori, Pinochet, Delgado, Gras, & Muñoz, 1994; Ober, González, & Santa Maria, 1987; Pinochet et al., 1995). Recently, Tapia et al. (2010) recorded cadmium concentrations in a group of three mollusc species collected from different locations in the Maule Region (Chile) and revealed levels reaching 4.32 ± 0.12 mg kg^{-1} dry weight for the bivalve *Ameghinomya antiqua*. The Chilean blue mussel, *Mytilus chilensis* (Hupe, 1854), also shows high concentration in cadmium (Bruhn, Campos, Diaz, Cid, & Nobrega, 2002; Hervé-Fernández et al., 2010; Tapia et al., 2010). Cadmium levels equivalent to 4.4 ± 0.3 mg kg^{-1} dry weight have been measured in lyophilised bivalve samples from the Chilean coast (Bruhn et al., 2002), appreciably exceeding the upper limit for Cd of 1.0 mg kg^{-1}, as currently defined by the EU and Norway (Muñoz et al., 2005). Cadmium is one of the most toxic metals to man. Low Cd exposure may give rise to skeletal damage, as evidenced by low bone mineral density (Nordberg et al., 2002; Staessen et al., 1999). *M. chilensis* is one of the most important bivalves commercially exploited along the Chilean coast. Its aquaculture production reached 100,000 tons in 2008 (Fernández-Reiriz, Navarro, Contreras, & Labarta, 2008). Its exports have also significantly increased in the last 2 years, focused towards North America, Japan, Latin America and the European Community. Unfortunately, due to the presence of cadmium in its tissues, Chilean mussels have suffered several occurrences of rejection by European countries since 1989 (Figueroa, 2008), representing direct monetary losses for Chilean companies.

Until now, studies on heavy metals in Chilean food have been mainly focused on vegetables (Muñoz et al., 2002; Queirolo et al., 2000). In the study of Muñoz et al. (2005), cadmium content in Chilean fish and shellfish (277 ng kg^{-1} w/w) appears much greater than reported for the same food group in other total diet studies (Urieta, Jalon, & Eguileor, 1996; Ysart et al., 1999). According to

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these studies and by comparison with European legislation regarding the contents measured in Chilean foods, fish and shellfish are the only groups that could be problematic.

The objective of the present work is to assess the health risks associated with the presence of Cd in mussels consumed by the Chilean population. We therefore applied an innovative approach, the *in vitro* simulated digestion method, on specimens of *M. chilensis* previously contaminated with ^{109}Cd (following seawater exposure or feeding with contaminated algae) to measure the bioaccessibility of cadmium for human consumers. We also evaluated the effects of the cooking process (raw vs. cooked mussels) on the cadmium content in mussel soft tissues and on the resulting change in dietary bioaccessibility.

In parallel, the combination of cadmium concentrations determined in a group of mussels and information on their consumption rates allowed us to calculate a daily exposure for humans. To estimate health risk, this calculated dietary exposure has been compared with the Provisional Tolerable Weekly Intake (PTWI) recommended by the Joint FAO/WHO Expert Committee for Food Additives (JECFA).

2. Material and methods

2.1. Collection and acclimation of mussels

The Chilean mussels *M. chilensis* were collected at Leimo Beach in Calbuco, Chile (45°45′53″S; 73°07′58″W). At this location, mussels are hung on cords at between 3 and 5 m depth. Forty mussels of uniform size (5–6 cm length) were selected and washed to remove encrusting organisms. They were then transported to experimental facilities in Monaco where they were acclimated to laboratory conditions (12 °C, 30‰ salinity). These environmental parameters were similar to those encountered at the Chilean sampling site. To reach a salinity of 30‰, Mediterranean seawater (with a salinity of 38‰) was mixed with dechlorinated freshwater using precise flow meters. During this acclimation period, mussels were fed every 2 days with algal cells from a batch culture (*Isochrysis galbana*; 5×10^3 cells ml^{-1}).

2.2. Cadmium concentration in M. chilensis

The "background" cadmium concentration of *M. chilensis* was measured in the tissue of nine specimens. Samples were freeze-dried and then ground in an agate mortar to obtain a fine homogeneous powder. About 300 mg of dried sample material were solubilised in 5 ml nitric acid (Suprapur, Merck) using a microwave oven (Mars X, CEM); then Cd concentrations were determined by Zeeman graphite furnace AAS (SpectraAA10, Varian, Australia). Analytical quality control included analysis of procedural blank and certified reference material SRM 2976 (Mussel, NIST).

2.3. Radiotracer exposure

2.3.1. Contamination via seawater

Mussels were placed in a 700-l covered aquarium filled with Mediterranean seawater, under the same environmental conditions as described above. They were experimentally exposed to ^{109}Cd via seawater. The seawater was spiked with low nominal activities of ^{109}Cd to get a concentration of 0.3 Bq ml^{-1}. In terms of stable metal concentration, this addition corresponds to 1.5×10^{-3} ng Cd l^{-1}, which is five orders of magnitude lower than the background concentration of Cd in the seawater bordering the Chilean coast (0.002 mg l^{-1}) (Alarcón, 2003) and 5000 times lower than the Cd level in the Mediterranean Sea (8 ng l^{-1}, Copin-Montegut, Courau, & Nicolas, 1986). The seawater was renewed and spiked every 2 days to maintain the ^{109}Cd activity (Hervé-Fernández et al., 2010). During the whole experiment, mussels were fed each time the spike was renewed, with the algal solution of *I. galbana*, at a cell concentration of 5×10^3 cells ml^{-1}. When the activity accumulated began to approach a steady state after 43 days (Hervé-Fernández et al., 2010), 20 mussels were blotted dry on absorbent paper to eliminate any adhering radioactive medium, and then weighed before being gamma-counted. They were then frozen at −20 °C to be later exposed to the *in vitro* digestion process (see below).

2.3.2. Contamination via food

The alga *I. galbana* was grown in seawater enriched with f/2 medium (with no Zn, Si or EDTA) and used to feed the mussels. A given volume of this cell culture was filtered through a 1-μm Nucleopore filter to get an initial cell density of around 1×10^5 cells ml^{-1} in the 20-l incubation medium. This algal solution was spiked with microlitre quantities of the radiotracer ^{109}Cd in acidic solution, to reach an activity of 5 kBq l^{-1} in the incubation medium. The algae were grown in this incubation medium until they reached a cell density of 3.4×10^6 cells ml^{-1}. A given quantity of ^{109}Cd-labelled algae was then filtered through a 1-μm Nucleopore filter to give an algal cell density of 10^4 ml^{-1} in the experiment medium. Three replicates of 10 ml of the ^{109}Cd-labelled algae solution were collected and analysed to determine the initial ^{109}Cd activity in the algae cells and their density. A final activity of 74 μBq cell^{-1} was measured in the algal solution given to the mussels during the experiment. This filtrate was then resuspended in a 20-l aquarium maintained under the same conditions as described above.

The remaining mussels were placed in the 20-l aquarium for 3 h. After 3 h, the ^{109}Cd activity of the mussels (expressed in Bq g^{-1}) was measured as described above, before they started to produce faecal pellets.

2.4. In vitro digestion

All mussels were thawed before starting the *in vitro* digestion procedure. In Chile, consumers eat the mussels either raw, after adding lemon juice, or cooked. Thus cadmium bioaccessibility was determined from soft tissues of *M. chilensis* cooked (boiled in tap water) or raw with lemon juice ($n = 10$ for each treatment).

For each contamination pathway (via seawater or via food), some mussels ($n = 10$) were kept raw and seasoned with 5 ml of lemon juice, whereas the remaining samples ($n = 10$) were placed individually in 50-ml glass beakers, covered and heated for 10 min at 150 °C on a hotplate. ^{109}Cd activity was determined in the mixture of lemon juice and liquid (for the raw mussels) and in the cooking juice (for the cooked mussels).

Soft tissues were submitted whole to *in vitro* digestion. This *in vitro* digestion procedure consists in mimicking human digestion, according to the procedure described by Versantvoort, Oomen, Van de Kamp, Rompelberg, and Sips (2005). Briefly, it consists of a three-step procedure simulating the digestive processes in the mouth, stomach and small intestine. The soft tissue was first exposed to artificial saliva at pH 6.8 for 5 min, then artificial gastric juice at pH 1.3 was added for 2 h, and thirdly a mixture of artificial duodenal juice, bile and $NaHCO_3$ at pH 8.1–8.2 was added for a further 2 h. The incubation temperature was 37 °C. After centrifugation at 2800*g* for 5 min, the supernatants and pellets were separated. Pellets were collected using HCl (2 N). ^{109}Cd activities were determined in pellets and supernatants (see below). Extractability (and hence bioaccessibility) was calculated as the percentage of ^{109}Cd activity recovered in the supernatant. The number of replicates was 10 for all treatments.

The γ emissions of ^{109}Cd were determined at 88.03 keV in whole mussel, soft parts, supernatants, pellets and seawater, using a well-type NaI detector. The activities of samples were corrected for background, radioactive decay, counting efficiency and compared with standard of appropriate geometry. Counting times were adjusted to give relative propagated errors <5% at the 1 SD level, i.e., 20 min for the whole mussels and seawater, 1 h for soft parts, supernatants, pellets.

2.5. Data analyses

All statistical analyses were performed with Statistica 5.0. All data were analysed for normality with a Lilliefors test ($p > 0.2$) and homoscedasticity with a Levene's test ($p > 0.05$); to check differences among treatments (cooked or raw) and/or contamination pathways (dissolved or feeding), a factorial ANOVA and *a posteriori* test of Tukey were performed when statistically significant differences were observed ($\alpha = 0.05$).

2.6. Estimation of chemical doses

The standard US Environmental Protection Agency (US EPA, 1992) method was used to estimate the risk for human consumption due to the ingestion of Chilean mussels. The daily intake doses were determined using the equation below:

$$D = (C \times I_i)/W$$

where, D is the estimated dose ($\mu g\ kg^{-1}\ day^{-1}$) for cadmium at ingestion rate I. C is the concentration of cadmium in the mussels ($\mu g\ kg^{-1}$). I_i is the ingestion rates in the Chilean population ($kg^{-1}\ day^{-1}$). W is the assumed human body weight.

3. Results

A mean value of 0.95 ± 0.20 mg Cd kg^{-1} was measured for the nine specimens of *M. chilensis* analysed.

^{109}Cd activities were 10 times higher in the soft parts of mussels contaminated via seawater (310.3 ± 19.3 Bq g^{-1}) than in those contaminated via food (30.2 ± 6.1 Bq g^{-1}).

For both contamination modes, cooking increased the Cd concentration in the mussel flesh; cooked mussels contained 153.8 ± 49.2 and 48.3 ± 8.5 Bq g^{-1} while the raw mussels contained only 72.4 ± 6.5 and 29.8 ± 8.2 Bq g^{-1}, when contaminated via dissolved or via food pathways, respectively (Fig. 1). ^{109}Cd activities were also higher in the cooked juice (5.3 ± 0.7 and 3.3 ± 0.8 Bq g^{-1} for mussels previously contaminated, respectively, via dissolved or via food ($p > 0.9$) than in the liquid and lemon juice accompanying raw mussels (0.7 ± 0.2 and 0.4 ± 0.2 Bq g^{-1} for mussels previously contaminated respectively via water or via food ($p < 0.1$); Fig. 1).

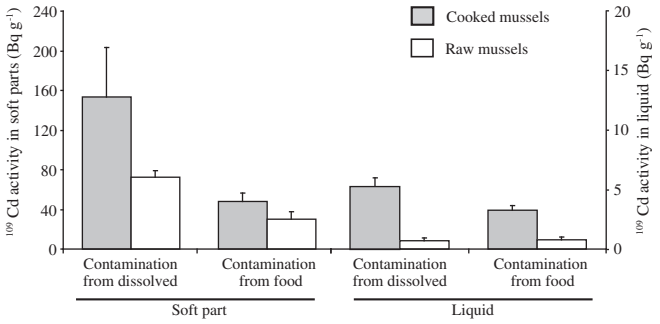

Fig. 1. ^{109}Cd activities (Bq g^{-1}) (mean ± SD, $n = 10$) in the soft parts and liquids in the mussels *Mytilus chilensis* either cooked or kept raw and seasoned with lemon juice, called respectively "cooked" and "raw" mussels. These mussels previously accumulated ^{109}Cd either via seawater (from dissolved) or via the ingestion of radiolabelled algae (from food).

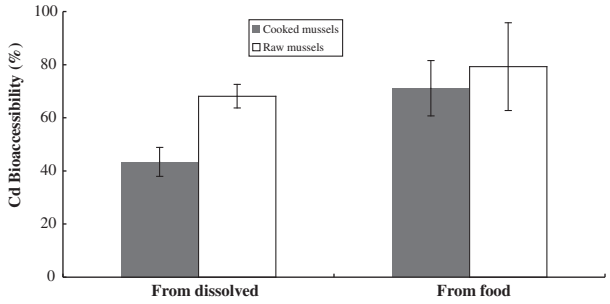

Fig. 2. ^{109}Cd Bioaccessibility (%; mean ± SD, $n = 10$) from mussel soft parts (cooked or raw) to human consumers determined using *in vitro* digestion method. Mussels previously accumulated ^{109}Cd either via seawater (from dissolved) or via the ingestion of radiolabelled algae (from food).

The *in situ* digestion method showed that the bioaccessible fraction of ^{109}Cd in the mussels *M. chilensis* varied from 42.4 ± 5.5% (in cooked mussels after contamination via water) to 79.3 ± 16.6% (in raw mussels after feeding with ^{109}Cd labelled algae; Fig. 2). When dissolved ^{109}Cd was accumulated, the bioaccessible fraction was higher in raw mussels (68.1 ± 4.4%) than in cooked ones (42.4 ± 5.5%; $p < 0.001$) while there was no difference between the bioaccessible fractions in raw and cooked mussels, when they were previously contaminated via the ingestion of radiolabelled algae ($p > 0.7$; Fig. 2). While, the cooking procedure increased the Cd concentration in the mussel soft parts and liquids, cadmium remaining in the cooked flesh was less bioaccessible than when present in the raw tissues.

The daily intake dose of Cd was estimated and compared with the PTWI. The annual consumption of molluscs in Chile is 2 kg per person per year (FAO, 2003), which gives a daily ingestion rate (I_i) of 5.479 g of molluscs per person, per day. In the present experiment, a mean value of 0.95 ± 0.20 mg Cd kg^{-1} has been measured for nine specimens of *M. chilensis* analysed and an average body weight of 69 kg (to conform with the body weight used by Muñoz et al. (2005) has been considered. The intake dose of cadmium for mussel consumption is thus estimated to be 0.077 ± 0.016 $\mu g\ kg^{-1}\ day^{-1}$.

4. Discussion

The *in vitro* digestion method developed by Versantvoort et al. (2005) and used in the present study allowed us to evaluate the bioaccessibility of cadmium from Chilean mussels by humans, as it mimics quite closely the conditions occurring along the human digestive tract, i.e., constant temperature, a succession of enzymatic activities and pH, corresponding to each digestive step occurring from the mouth to the intestine. Moreover, few studies have examined how cooking affects the amount of contaminants in edible mussel tissue. The results of our study show clearly that the cadmium bioaccessibility fraction was higher in the raw than in the cooked mussels (by a factor of up to 2).

First, it is clear that the cooking treatment had a direct effect on the cadmium contained in the matrix of the cooked mussels. The analysis of ^{109}Cd content in raw and cooked mussels demonstrated that the cooking process resulted in a concentration of the cadmium by a factor of two in the soft parts, due to both the loss of moisture as well as the release of cadmium into the cooking juice. Indeed, cooking juice had enhanced cadmium levels by up to one order of magnitude, compared to liquid plus lemon juice, accompanying raw mussels. In *M. chilensis*, around one third of the total cadmium was stored in the gonads (Hervé-Fernández et al.,

2010). During the cooking process, the expulsion of gametes might be responsible for the higher cadmium concentration observed in the cooking juice. In contrast, raw mussels sprinkled only with lemon juice (presenting a low pH of 2.59) are not able to release the cadmium absorbed in their tissue. Our results confirmed the previous analyses of radiotracer content made in raw and cooked specimens of *Mytilus galloprovincialis*, which had demonstrated that the cooking process resulted in a concentration by 20–70% of most of the elements studied (including cadmium, Metian et al., 2009). In this study, as in our investigation, cooking juice also displayed metal concentrations higher by up to one order of magnitude compared to the inter-valve fluid of raw mussels. However, depending on the study, the effects of cooking on metal content in seafood are different and they may vary according to the element considered and its chemical speciation. Whereas Atta, El-Sebaie, Noaman, and Kassab (1997) demonstrated that the cooking process did not concentrate elements like Cd, Cu, Pb and Zn in the fish *Tilapia nilotica*, Devesa et al. (2001) reported both reductions and increases in total arsenic contents after the cooking process in fish and bivalve, due either to solubilisation/volatilisation or concentration of the metalloid. The element concentrations in tissues after cooking appear also to depend on the type of cooking (Ersoy, Yanar, Kucukgulmez, & Celik, 2006; Perelló, Martí-Cid, Llobet, & Domingo, 2008) and the considered species, as, for example, ^{134}Cs is not concentrated in cooked mussel tissue (Metian et al., 2009) whereas it is in fish tissues (Burger et al., 2004).

In the present study, although cooking resulted in an increase in cadmium concentration in whole mussel flesh, it also appears that cadmium remaining in the cooked flesh was significantly less bioaccessible than cadmium occurring in the raw tissue. In the same way, Amiard et al. (2008) and Metian et al. (2009) showed that bioaccessibility was significantly lowered for Cd after cooking eight species of molluscs from France, United Kingdom and Hong Kong, including clams, mussels, oysters, scallops and gastropods. Moreover in our study, the bioaccessible fraction is higher in cooked mussels previously contaminated via the ingestion of radiolabelled algae than in cooked mussels contaminated via dissolved cadmium. During the digestion process, previously ingested phytoplankton cells may have been destroyed and the additional cadmium could have been more easily released by the mussels and so become more bioaccessible to humans. So even if cadmium is mainly accumulated via the dissolved pathway (Hervé-Fernández et al., 2010), the contamination via feeding is not negligible, since this way of contamination might increase by a factor of two the bioaccessibility of metals. Therefore, exported mussels should be previously starved or fed with uncontaminated algae in order to lower the elevated bioaccessibility of this trace metal.

The enforcement of current Cd standards in developed countries has affected developing countries like Chile, mainly through rejection of their export products. In fact, many developed countries have prohibited the access to their markets of foreign products containing more cadmium than the levels accepted by their regulations (Figueroa, 2008). In the present experiment, a mean value of 0.95 ± 0.20 mg Cd kg^{-1} has been measured for nine specimens of *M. chilensis* analysed, with four samples in this batch containing Cd levels above the accepted limit of 1.0 mg kg^{-1} as defined by the European Union (Muñoz et al., 2005). Chilean mussels also contained six times more Cd than Spanish mussels (0.14 mg kg^{-1}; Falcó, Llobet, Boccio, & Domingo, 2006).

In the present study, the intake dose of cadmium for mussel consumption is estimated to be 0.077 ± 0.016 μg kg^{-1} day^{-1}, equivalent to 0.54 μg kg^{-1} body weight or 5.31 μg day^{-1}. The FAO/WHO (1993) recommends a PTWI for Cd of 7 μg kg^{-1} body weight. Here, the estimated daily cadmium intake by Chilean mussels (0.54 μg kg^{-1} body weight) is well below this recommended PTWI and consequently a health risk is not indicated to exist. To achieve the recommended PTWI, the approximate amount of *M. chilensis* to be consumed would be in the order of 479 g, which is a factor of 16 higher than the average mollusc consumption in Chile of 30 g per week (FAO, 2007). If we consider an average human body weight of 69 kg, this cadmium intake (equivalent to 5.31 μg day^{-1}) is higher than the Cd intake through fish and seafood found in Greece (0.7–1.1 μg day^{-1}; Karavoltsos, Sakellari, & Scoullos, 2003) and Spain (1.86–3.33 μg day^{-1}; Llobet, Falco, Casas, Teixido, & Domingo, 2003) (Table 1). This value obtained for *M. chilensis* is also consistent with the estimation made by Muñoz et al. (2005) of the Chilean dietary intake of Cd from fish and shellfish (equivalent to 9.2 μg day^{-1}; Table 1).

Table 1
Contribution from seafood (expressed in μg d^{-1}) to dietary intake of Cd, according to different countries. An average body weight of 69 kg has been considered.

Food source	Contribution	Country	Source
Fish and seafood	0.7–1.1	Greece	Karavoltsos et al. (2003)
Fish and seafood	1.9–3.3	Spain	Llobet et al. (2003)
Fish and shellfish	9.2	Chile	Muñoz et al. (2005)
M. chilensis	5.3	Chile	Present study

5. Conclusion

In this study we found that the intake of Cd from mussels by the Chilean population does not exceed the toxicological reference values as established by the FAO/WHO. Furthermore, if we consider that the complete bioaccessibility for cadmium absorbed in cooked *M. chilensis* is only 42% of the total cadmium content, cadmium intake is even more reduced and we can thus conclude that a health risk situation does not seem to exist. This information should also be taken into account before rejecting shellfish products such as mentioned by Figueroa (2008).

Moreover, as in the present work it has been observed that cooking resulted in an increase in cadmium concentration in whole mussel flesh, it is suggested that risk assessors, who do not take cooking method into account, but use contaminant data from raw seafood, may be overestimating safe consumption levels. It has also been reported that cadmium bioaccessibility is also related to the ingestion of other nutrients such as calcium, zinc and iron (Reeves & Chaneyb, 2008). These additional factors should be also be considered by state agencies that are responsible for setting consumption levels for high risk populations.

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