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Reduction of mercury bioaccessibility using dietary strategies

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ABSTRACT

Food is the main source of mercury for most people. To promote its toxic effect, ingested mercury must be solubilised during digestion and absorbed. The present work aimed to seek dietary components that reduce the quantity of soluble (bioaccessible) mercury after digestion.

The effect of 28 compounds on solubility of inorganic mercury and methylmercury in aqueous solution after *in vitro* gastrointestinal digestion was evaluated. Compounds that reduced mercury solubility were assayed in seafood subjected to gastrointestinal digestion. Lignin (95% CI: 77–88%), tannic acid (95% CI: 61–75%), pectin (95% CI: 48–65%), hydroxypropylmethylcellulose (95% CI: 40–59%), methylcellulose (95% CI: 44–53%) and carboxymethylcellulose (95% CI: 34–51%) produced the highest reductions in mercury bioaccessibility from food. Apart from tannic acid, lignin, pectin and methylcellulose, which reduced Fe bioaccessibility, they did not affect bioaccessibility of essential cations. This information may be useful for defining dietary strategies to reduce mercury bioavailability, although *in vivo* studies are required to confirm their suitability.

1. Introduction

Inorganic divalent mercury [Hg(II)] and methylmercury (CH_3Hg) are the major forms of mercury (Hg) in the diet. Seafood products, especially large predators, are the main dietary sources of CH_3Hg, although they also contain considerable concentrations of Hg(II) (EFSA, 2012). Vegetable products such as mushrooms make an important contribution to intake of Hg(II) (Kalač & Svoboda, 2000).

The toxic effects of Hg are well known, consisting mainly of neurological, immune, haematological and renal alterations (NTP, 1993; NRC, 2000), and are dependent on the dose, chemical form and exposure route. Continuous exposure to Hg takes place mainly as a result of exposure in the workplace, but population groups exposed to Hg through the diet have also been identified, such as subsistence fishing populations (Oliveira et al., 2010) and frequent consumers of certain seafood products (EFSA, 2012). In fact, various organisations have issued recommendations to limit the consumption of some seafood products in susceptible populations (pregnant women, breastfeeding women, and children).

The fact that many food matrices with high concentrations of Hg are important sources of nutrients and antioxidants, and that in some cases they form the basis of the local diet, makes it difficult to reduce their consumption. Therefore various approaches have been adopted in order to reduce the concentrations of Hg in food before it is marketed. Washing seafood products with cysteine, NaCl and EDTA has been assayed (Hajeb & Jinap, 2012). Some of these approaches have been successful in laboratory studies, but they have not been extrapolated to the industry, possibly because of technical difficulties and/or because of organoleptic, structural or nutritional changes in the food product.

Another possible form of intervention involves acting after intake of the food product. During gastrointestinal digestion, food undergoes a series of physical and chemical processes that enable solubilisation of components of the food. Moreover, the Hg released from the matrix can bind to other food components present in the lumen, forming complexes with characteristics different from those of the metal that was present in the food. This conditions its arrival in the systemic circulation.

It has been stated that the bioavailability of Hg (the quantity absorbed after ingestion, which reached the systemic circulation) depends on the chemical form in which the metal is present. In fact, *in vivo* studies have reported that Hg(II) is less extensively absorbed than CH_3Hg (Sasser, Jarboe, Walter, & Kelman, 1978). It has also been shown that this bioavailability is influenced by the presence of certain food components. Janle et al. (2015) showed that the Hg present in swordfish is absorbed more efficiently in rats when it is administered together with green tea extract. The same effect is observed in humans fed with 2 daily fish meals and 6 cups of tea for

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3 consecutive days (Canuel et al., 2006). However, the presence of insoluble fibre reduces absorption of CH_3Hg *in vivo* (Rowland, Mallett, Flynn, & Hargreaves, 1986). Thus there are components in the diet that influence the quantity of Hg that reaches the systemic circulation and that accumulates in target organs. This makes it possible to evaluate the possible use of food components as dietary strategies to reduce the quantity of this metal that reaches the bloodstream.

The aim of this study was to seek dietary components that could reduce the bioaccessibility of Hg from food, in order to decrease the amount of Hg available for intestinal absorption.

2. Materials and methods

2.1. Food samples

Swordfish (*Xiphias gladius*) and yellowfin tuna (*Thunnus albacares*) samples were purchased at various stores in the city of Valencia. Fillets were cooked in a frying pan without additional ingredients and then homogenised and stored at 4 °C until the analysis (maximum 2 days).

2.2. Study of the influence of dietary components

Twenty-eight dietary components (Table 1) were selected, at the concentrations that are customary in food. The effect of each of them on the solubility of Hg(II) and CH_3Hg in aqueous solution (both 1 mg/L) prepared from commercial standards of $Hg(NO_3)_2$ (Merck, Spain) and CH_3HgCl (Alfa Aesar, Spain), respectively, was evaluated. In the first approach, the solutions of Hg were placed in contact (30 min, shaking) with the compounds and then the Hg was analysed in an aliquot of the supernatant (Section 2.4). In the second approach, after the period of contact an *in vitro* digestion was applied (Section 2.3). Only the compounds that reduced the solubility of the aqueous standards were subsequently assayed in the *in vitro* digestion of the food matrices (Section 2.1). The results obtained in the presence of the dietary components were compared with those obtained without them.

2.3. In vitro gastrointestinal digestion

The enzymes and bile salts for the *in vitro* digestion were purchased from Sigma–Aldrich (Spain): porcine pepsin (enzyme activity 944 U/mg protein), porcine pancreatin (activity equivalent to 4 × US Pharmacopeia specifications/mg pancreatin) and bile extract (glycine, taurine conjugates and other bile salts).

The *in vitro* digestion method described by Jadan, Clemente, Devesa, and Vélez (2016) was used. Cooked food sample (10 g) or standard Hg solution (1 mg/L final concentration) with or without the dietary component was weighed and 85 g of deionised water was added. Then the pH was adjusted to 2.0 with 6 mol/L HCl. A solution of pepsin prepared in 0.1 mol/L HCl was added in order to obtain 0.02 g of pepsin/100 g solution. The mixture was made up to a weight of 100 g with deionised water and incubated at 37 °C for 2 h with constant shaking (120 rpm).

The digest was then subjected to the intestinal step. The pH was increased to 6.5 by means of 1 mol/L $NaHCO_3$. A solution of pancreatin and bile extract prepared in 0.1 mol/L $NaHCO_3$ was added to provide 0.005 g of pancreatin/100 g solution and 0.03 g of bile extract/100 g solution. The mixture was incubated at 37 °C for 2 h with constant shaking (120 rpm).

After the digestion, the samples were transferred to tubes and centrifuged (10,000 rpm, 4 °C, 30 min). The total concentrations of Hg were quantified in the soluble fraction obtained (bioaccessible fraction), and the bioaccessibility was determined by means of the following equation:

$$\text{Bioaccessibility} = [A/B] \times 100$$

where A is the concentration of Hg in the bioaccessible fraction

Table 1
Dietary compounds used in the study (concentrations expressed in g per 100 mL).

Dietary compound	Concentration (aqueous Hg solution)[a]	Concentration (food matrix)[a]	Brand
N-acetylcysteine (NAC)	0.40	n.a	Sigma
$CaCl_2$ anhydrous/Phytic acid dipotassium	0.55/0.11	n.a	Panreac/Sigma
Carboxymethylcellulose (CMC)	0.20	0.20; 0.40	AKUCELL
λ carrageenan	0.2	n.a	Cargill
Catechin hydrate	0.04	n.a	Fluka
Chitosan medium molecular weight	0.48	0.5; 1.0	Aldrich
Citric acid 1-hydrate	0.04	n.a	Panreac
L-Cysteine	0.5	n.a	Merck
Gelatin from porcine skin	0.2	n.a	Sigma
Guar gum	1.0	n.a	Fluka
Gum arabic from acacia tree	1.0	1.0; 3.0	Fluka
Hydroxypropyl cellulose (HPC)	0.4	0.5; 2.0	Aldrich
Hydroxypropyl methylcellulose (HPMC)	1.6	1.6; 3.2	Dow Chemical Company
Iron(III) sulfate hydrate [Fe(III)]	0.048	n.a	Scharlau
(±)-α-Lipoic acid	0.02	0.02; 0.1	Sigma
Lignin, alkali	1.4	0.5; 1.5	Aldrich
Malic acid	0.8	na	Sigma–Aldrich
Methylcellulose (MC)	1.0	1.0; 3.0	Dow Chemical Company
Oxalic acid dihydrate	0.12	n.a	Merck
Pectin from apple	1.0	1.0; 2.0	Sigma
Phytic acid dipotassium salt	0.5	n.a	Sigma–Aldrich
Saponin	2.0	1.0; 3.0	Santa Cruz Biotech.
Seleno-D-L-methionine (SeMet)	0.1×10^{-3}	n.a	Sigma
Sodium alginate	0.2	n.a	EPSA
Sodium selenate [Se(VI)]	0.1×10^{-3}	n.a	Merck
Tannic acid	3.2	0.5; 1.0	Merck
Xanthan gum from *Xanthomonas campestris*	1.0	n.a	Sigma
Xylan from oat spelts	1.4	1.5; 3.0	Sigma

[a] Numbers indicate the low and high concentrations of dietary compound employed in the food assays; n.a: not assayed.