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Benefits and risks associated with consumption of raw, cooked, and canned tuna (*Thunnus* spp.) based on the bioaccessibility of selenium and methylmercury

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ABSTRACT

The Se, Hg, and methylmercury (MeHg) levels in raw, cooked (boiled and grilled), and canned tuna (*Thunnus* spp.) were determined before and after an *in vitro* digestion, thereby enabling the calculation of the respective bioaccessibility percentages. A risk–benefit evaluation of raw and canned tuna on the basis of the Se and MeHg data was performed. Selenium bioaccessibility was high in tuna, though slightly lower in canned than in raw products. Mercury levels were high in raw and cooked tuna. Hg bioaccessibility percentages were low (39–48%) in the cooked tuna and even lower (< 20%) in canned tuna. For the bioaccessible fraction, all molar Se:MeHg ratios were higher than one (between 10 and 74). A probabilistic assessment of MeHg risk *vs* Se benefit showed that while a weekly meal of canned tuna presents very low risk, raw, boiled, and grilled tuna consumption should not exceed a monthly meal, at least, for pregnant and nursing women.

1. Introduction

Fish accumulate significant concentrations of Hg in their tissues and are considered as the main source of this toxic metal to humans. Indeed, fish are considered to be the largest Hg source for man (with the exception of occupational exposure). Particularly, methylmercury (MeHg) is a great concern, given its significant neurotoxic nature (Grandjean et al., 2010). On the other hand, fish are an important source of vitamins, fatty acids (EFSA, 2012a), and minerals (*e.g.* calcium, Ca, iron, Fe, zinc, Zn, iodine, I, copper, Cu, and selenium, Se) (Dahl et al., 2006).

Based on the assessment of results from various epidemiological studies involving fish-eating populations and developmental neurotoxicity, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) established a provisional tolerable weekly intake (PTWI) for MeHg of 1.6 μg/kg body weight (b.w.) (FAO/WHO, 2010). Recently, EFSA CONTAM Panel set a tolerable weekly intake (TWI) for MeHg of 1.3 μg/kg b.w. expressed as Hg (EFSA, 2012b). Selenium is a natural antagonist for MeHg that may counteract the effects of high exposures to this contaminant (Ralston and Raymond, 2010). The Se recommended daily allowance (RDA) for individuals aged between 14 and 52 years (excluding the states of pregnancy and lactation) has been set at 0.055 mg (IOM, 2000).

Fish can be consumed raw or subjected to several culinary treatments or even different industrial processes, like canning. It has been reported that physical, chemical, and sensory changes occur during fish confection (weight loss, modifications of water-holding capacity and texture due to protein denaturation and fat and water losses, colour, and aroma development) (Alipour et al., 2010; Kong et al., 2007). Similar important changes have been reported for canned products (Aubourg, 2001).

There is scarce information about the effects of culinary treatment on bioaccessibility of Hg, MeHg, and Se. Bioaccessibility can be seen as an indicator for the maximal oral bioavailability of any given food constituent, that is, the fraction of that constituent

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reaching systemic circulation (Cardoso et al., 2015a). Besides, depending on numerous factors such as type and processing of food, the studied food constituent may be more or less bioavailable (Afonso et al., 2015; Cardoso et al., 2015a; Van Het Hof et al., 2000; Wienk et al., 1999). Recent experimental work has been carried out with the aim of finding a suitable *in vitro* digestion model able to simulate in a realistic way the human digestive system, thereby making possible a reliable determination of the bioaccessible fraction of any specific constituent (Cardoso et al., 2015a). Some novel studies (Afonso et al., 2015; Ouédraogo and Amyot, 2011; Yu et al., 2011) have produced valuable bioaccessibility data.

Among the most consumed fish species, the highest levels of MeHg are found in tuna, which is mostly caught from the wild (EFSA, 2005). This makes tuna a case study for the potential MeHg risks and the possible balancing effects of Se. Therefore, this study's goal is to determine the levels of Hg, MeHg, and Se in frequently consumed tuna products (raw, boiled, grilled, and canned) both before and after *in vitro* digestion (bioaccessible fraction) and to assess the relationship between risks and benefits through a probabilistic methodology.

2. Materials and methods

2.1. Samples and cooking methods

With the purpose of learning about the effect of culinary process and bioacessibility, twenty five cans of tuna (*Thunnus* spp.) in olive oil and twenty five in water (*Thunnus* spp.) were acquired from different Portuguese grocery stores. Five pools were made of each type of canned tuna, oil and water, corresponding to 5 different brands, each containing 5 cans from the same batch.

Five samples of fresh tuna fish (*Thunnus* spp.), each containing three steaks of the same fish (average weight of each steak was 300 g), were purchased from five different Portuguese grocery stores. For each traditional cooking treatment, five individual steaks were used (one for each sample). In the boiling process (1:2 fish:water ratio, water containing 2 g of salt per 100 g of fish), steaks were cooked for about 8 min. The grilling process was carried out in a domestic griller (Flama Sketch 230 V, 50 Hz, 2000 W) operated at about 180 °C. Each side of the salted steaks (1.5 g/100 g) was grilled for about 12 min.

The homogenized canned tuna (after draining) as well as the raw and cooked steaks were separated into two sub-samples: one was frozen stored at −80 °C and the other frozen at −20 °C and then freeze dried (for 48 h at −45 °C and low pressure) and afterwards stored at −80 °C until further analysis.

2.2. In vitro digestion model

The bioaccessibility of nutrients (Se) and contaminants (Hg and MeHg) in raw and cooked tuna samples were studied by using an *in vitro* method. This method includes three steps, simulating the digestive processes in the mouth, stomach, and small intestine. The composition of digestive juices (saliva, gastric, duodenal, and bile) was the same described by Versantvoort et al. (2005) with some modifications performed by Afonso et al. (2015). The chemicals KCl, NaH_2PO_4, Na_2SO_4, NaCl, $NaHCO_3$, HCl, $CaCl_2 \cdot 2H_2O$, KH_2PO_4, and $MgCl_2$ used for preparation of the digestive fluids, were obtained from Merck (Darmstadt, Germany). NH_4Cl was obtained from Fluka (Buchs, Switzerland) and all other chemicals were obtained from Sigma (St. Louis, MO, USA). α-Amylase, pepsin, pancreatin, trypsin, α-chymotrypsin, lipase and bile salts were also purchased from Sigma (St. Louis, MO, USA). About 1.5 g muscle of homogenized fish was weighed in order to simulate a consumption of approximately 160 g of fish based on the energetic value (Afonso et al., 2015). Raw and cooked fish was homogenised with 4 ml of artificial saliva at a pH 6.8 ± 0.2 for 5 min, then 8 ml of artificial gastric juice (pH 1.3 ± 0.02 at 37 ± 2 °C) was added, and afterwards the pH was adjusted to a final pH 2.0 ± 0.1. This mixture was agitated during 2 h in a head-over-heels movement (37 rpm at 37 ± 2 °C). Finally, a mixture of 8 ml of artificial duodenal juice (pH 8.1 ± 0.2 at 37 ± 2 °C), 4 ml of bile (pH 8.2 ± 0.2 at 37 ± 2 °C), and 1.33 ml of HCO_3^- solution (1 M) was added simultaneously. The final pH of the mixture was set at pH 6.5 ± 0.5 and then agitated for a further 2 h period in a head-over-heels movement (37 rpm at 37 ± 2 °C). The obtained solution was centrifuged at about 2750*g* during 5 min in order to separate the non-digested from the bioaccessible fraction.

2.2.1. Calculation of bioaccessible and non-digested Se and Hg

The percentage (%) of Se and Hg in the bioaccessible and in the non-digested fraction was estimated as follows:

% Se or Hg bioaccessible = [Se or Hg] bioaccessible × 100/[S]
and
% Se or Hg non-digested = [Se or Hg] non-digested × 100/[S]
Being:
[Se or Hg] = concentration of Se or Hg.
[S] = [Se or Hg] in the bioaccessible fraction + [Se or Hg] in the non-digested fraction.

The recovery (%) of Se and Hg was calculated as the ratio: $S \times 100/(\text{Se or Hg})_i$ where S is the amount of Se or Hg in the bioaccessible fraction + the amount of Se or Hg in the non-digested fraction and $(\text{Se or Hg})_i$ is the amount of Se or Hg in the fish sample (raw, cooked or canned) before the digestion. The mean recovery for Hg was 101 ± 5%, 98 ± 5%, 95 ± 3%, 95 ± 7%, and 104 ± 9%, respectively for samples of raw, boiled, and grilled tuna as well as canned tuna in olive oil and water. In the case of Se the recovery was 110 ± 5%; 98 ± 27%; 106 ± 10%, 115 ± 8%, and 109 ± 8%, respectively for samples of raw, boiled, and grilled tuna as well as canned tuna in olive oil and water.

2.2.2. Calculation of bioaccessible protein and MeHg

The percentage (%) of protein or MeHg in the bioaccessible fraction was estimated as follows:

% Protein or MeHg bioaccessible = [Protein or MeHg] bioaccessible × 100/[Protein or MeHg] in the fish sample (raw, cooked or canned) before the digestion.

In order to evaluate the efficiency of this digestion method the protein content was determined in raw and cooked samples as well as in the bioaccessible fraction.

2.3. Analyses

2.3.1. Crude protein

The protein level in raw and cooked samples and bioaccessible fractions was determined using the FP-528 DSP LECO nitrogen analyzer (LECO, St. Joseph, USA) calibrated with EDTA according to the Dumas method (Saint-Denis and Goupy, 2004). The limit of detection (LOD) was 0.84 mg N.

2.3.2. Se, Hg, and MeHg

For Se determination, approximately 0.25 g of each sample (fish and non-digested fraction) was weighed into PTFE vessels and 8 ml of a mixture of nitric acid (65%, Merck), previously purified with a sub-boiling distillation system (Milestone, SubPUR), hydrogen peroxide (30%, suprapur, Merck), and ultra pure water (ratio 4:1:3) was added. Samples were digested in duplicate using