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Spectrometric method for determination of inorganic contaminants (arsenic, cadmium, lead and mercury) in Smooth weakfish fish

T.S. Silva [a, *], C. Conte [b], J.O. Santos [b], E.S. Simas [b], S.C. Freitas [b], R.L.S. Raices [a], S.L. Quitério [a]

[a] Instituto Federal de Educação, Ciência e Tecnologia do Rio de Janeiro (IFRJ), Mestrado Profissional em Ciência e Tecnologia de Alimentos (PGCTA), Rua Senador Furtado, 171/175, Maracanã, 20270-021 Rio de Janeiro, Brazil

[b] Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA), Centro Nacional de Pesquisa de Tecnologia Agroindustrial de Alimentos (CTAA), Av. das Américas, 29.501, 23020-470, Rio de Janeiro, RJ, Brazil

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ABSTRACT

This study aimed to investigate methodologies to quantify the elements arsenic, cadmium, lead and mercury in fish and verify the occurrence of these metals in Smooth weakfish (*Cynoscion leiarchus*), often consumed in Rio de Janeiro. Samples were freeze-dried and arsenic, cadmium and lead were quantified by Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES). The methods were validated according to the validation procedures of the Brazilian National Institute of Metrology, Quality and Technology (Inmetro) and the European Community. The results met the acceptance criteria for validation, and the methods were suitable for detecting the elements in the following ranges: As and Hg: 1000 ng g^{-1}, Pb: 300 ng g^{-1}, and Cd:100 ng g^{-1}. The limits of detections found for As, Cd, Pb and Hg were 2.02 ng g^{-1}, 0,11 ng g^{-1} and 1,9 ng g^{-1}, 0,03 ng g^{-1} respectively. Then samples of smooth weakfish fish was evaluated and the fish species presented levels ranging from 5.17 ng g^{-1} to 14.05 ng g^{-1}; 119.23 ng g^{-1} to 272.74 ng g^{-1}, and 6.95 ng g^{-1} to 10.83 ng g^{-1} for the elements Hg, As, and Cd, respectively. In contrast, the Pb levels were below the limits of quantification.

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1. Introduction

Consumption of fish and seafood has increased in recent years as they have been associated with reduced risk of developing cardiovascular disease, some cancers, rheumatoid arthritis and other inflammatory diseases (Lund, 2013; Rahman, Molla, Saha, & Rahman, 2012). This remarkable demand leads to a concern in food security and the need to establish procedures and measures to minimize the risks of intake of contaminated food, due the presence of pollutants in the environment. Some examples of major water pollutants include biodegradable organic compounds, refractory organics, pathogens, excess nutrients and metals (Bandowe et al., 2014). Although metals are in the aquatic environment in very low concentrations of the order of part per million or per billion, concentration may increase significantly due to industrial, agricultural, mining, transport, and incineration activities, biomass burning, improper disposal of waste and effluents and atmospheric deposition (Djedjibegovic, Larssen, Skrbo, Marjanovi, ć, & Sober, 2012).

In the aquatic environment, toxic metals can harm the diversity of species and ecosystems due to their toxicity, persistence and cumulative behavior, which can cause health problems (Bandowe et al., 2014; Kehrig et al., 2009). Biomagnification and bioaccumulation can occur through several levels of a food chain, thus larger animals tend to accumulate higher contaminant levels when compared to smaller organisms (Kehrig et al., 2009).

Therefore, concern about this issue has increased worldwide, especially in developing countries, since the determination of the concentrations of these contaminants allow evaluating the potential risks to which the population is exposed (Morgano, Rabonato, Milani, Miyagusku, & Quintaes, 2014; Rahman et al., 2012; Syversen & Kaur, 2012). Several studies have focused on the accumulation of trace metals in fish and seafood (Qin, Jiang, Bai, Tang, & Mou, 2015; 2014; Psoma, Pasias, Rousis, Barkonikos, & Thomaidis, 2014; Leung et al., 2014; Medeiros et al., 2012).

Various regulatory agencies have set limits for contaminants in foods, called Maximum Residue Limit (MRL). According to the European Commission, MRL values for Hg, As, Cd, and Pb in catching fish for the Fishery Products Export Certification are: 1000, 1000, 100, and 300 ng g^{-1} (wet weight), respectively (Commission of the

* Corresponding author.
E-mail address: tania.silva@embrapa.br (T.S. Silva).

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European Communities, 1881/2006).

Several analytical methods can be used to measure metals in fish and seafood, including Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) (Fernández et al., 2015; Morgano et al., 2014), inductively coupled plasma mass spectrometry (ICP-MS) (Qin et al., 2015; Djedjibegovic et al., 2012), flame (FAAS) and graphite furnace atomic absorption spectrometry (GFAAS), after matrix destruction with concentrated acids.

Atomic spectroscopy is a very efficient tool for the determination of trace elements. Its principle is based on the measure of the energy absorbed or emitted intensity of an atom to be subjected to very high energies, capable of converting the elements into gaseous atoms in a process called atomization. This process can be obtained by flame, graphite furnace or inductively coupled plasma (Harris, 2009). In optical emission spectroscopy, atoms are excited by an external energy as heat or electricity and power is supplied by a plasma. This external energy external power promotes the outer electrons of the orbital ground state to the excited orbital and after a short time, the excited atoms relax to the ground state, providing their energy as visible or ultraviolet photons (Skoog et al., 2006). However, before quantification, a treatment step (digestion) of samples must be performed to ensure the quality of results. The digestion is performed to solubilize the analyte of interest and to destroy the organic matter. The microwave oven digestion technique has been chosen since it has advantages such as speed, efficiency, lower consumption of reagents, lower risk of sample contamination, low volatilization losses, and good accuracy and reproducibility (Cindrić et al., 2012; Nardi et al., 2009).

Methylmercury is the most important form due to its toxicity. The toxic effects of mercury in the body is related to the chemical form in which it appears. The methylmercury is the most toxic form of living beings, so it is very important to have validated methods for determination of this compound, which can analyzed by speciation Batista et al. (2011). The methods validated in this study are able to quantify the total mercury present in fish. It is worth emphasizing that the determination of mercury levels does not require a preliminary step of sample preparation. The *Environmental Protection Agency* (EPA, 2007) has suggested the direct Analyzer method (EPA Method 7473), which has been widely used to determine mercury in fish and other seafood (Panichev & Panicheva, 2014; Ruiz-De-Cezano et al., 2014).

The objective of this study was to develop and validate spectroscopic methods for determination of inorganic contaminants (arsenic, cadmium, lead, and mercury) in fish, meeting the requirements set out in Brazilian law (INMETRO) and in the European Community. Mercury was determined by the direct analyzer method, while the elements As, Cd and Pb were quantified by inductively coupled plasma optical emission spectrometry (ICP-OES, Perkin Elmer, Model DV Optma 2100, Waltham, MA, USA). Hg was quantified by direct mercury analyzer, (Teledyne Leeman, model Hydra C, Hudson, NH, USA). After validation, the method was used to quantify As, Cd, Hg, and Pb in Smooth weakfish (*Cynoscion leiarchus*), a fish species widely consumed in Rio de Janeiro.

2. Material and methods

2.1. Reagents and analytical solutions

All glassware has previously been immersed in a nitric acid solution (1:1) for 12 h and in ultrapure water for 2 h. Then, washing with ultrapure water were performed, and the material was dried in an oven at 60 °C. The volumetric glassware was dried at room temperature.

Ultrapure water (18.2 MΩ cm^{-1} at 25 °C) was obtained through a water purifier (PURELAB Option-Q – ELGA, Woodridge, IL, USA). Concentrated nitric acid (65% w. w^{-1}) and hydrogen peroxide (30% v. v^{-1}) were of analytical grade (Merck, Darmstadt). The purity of argon was 99.999%.

Standard Hg solutions were prepared by dilution of a standard stock dilution of 1000 mg.L^{-1} (SCP Science, Quebec, Canada). The Cd and Pb standard solutions were prepared from the appropriate dilution of the stock solution at 1000 mg.L^{-1} (Fluka). All the solutions were from ISO Guide 34.

2.2. Samples

For sample validation, fish containing lower Cd, Pb, and Hg levels than the concentration range studied were used. Thus, canned tuna available in the domestic market was used at this step.

Ten Smooth weakfish (*Cynoscion leiarchus*) samples were purchased in retail stores of Pedra de Guaratiba, region of Rio de Janeiro, in 2013. These fish are widely consumed by the local population and are found in Sepetiba Bay, which was contaminated for many years with trace metals such as zinc, mercury, cadmium, and lead from industries located in the surrounding area (Rocha, Cunha, Geraldes, Pereira, & Almeida, 2010).

2.2.1. Sample preparation

The method evaluation was carried out using canned tuna preserved in water as matrix. The matrix was drained for 10 min and then processed in a Wiley mill (knife mill) (Restch Mod GRINDOMIX, Haan, Germany) at 8000 rpm for 45 s. Finally, it was subjected to freeze-drying for 30 h (Edwards Pirani 501, pressure: 0.1 bar; temperature range - 40° C to +30 °C). The samples were stored in plastic bags, sealed under vacuum and kept refrigerated for 15 days at 4 °C for later analysis. This matrix was spiked and used in the validation process.

For the application of the validated method, the smooth weakfish samples were prepared. After fish scales, heads and viscera have been removed, the samples were subjected to the same procedures performed for canned tuna. It is noteworthy that, before and after freeze-drying, samples were weighed on analytical balance balance (Mettler Toledo AL210, Columbus, OH, USA) for moisture determination and analytical results are expressed in terms of wet weight.

For the analysis of Cd and Pb by ICP-OES, 0.5000 g of freeze-dried sample was weighed in Teflon vessels and 6 mL concentrated HNO_3 and 2 mL 30% H_2O_2 were added. Samples were digested in a microwave oven Cem (Mars 5 Middle Slade, Unided Kingdom), 1500 W, for 40 min. The digested material was transferred to a 25 mL volumetric flask and the volume was completed with ultrapure water. All the samples were analyzed in triplicate.

2.3. Determination of mercury by direct analyzer

In analytical balance (Mettler Toledo AL210, Columbus, OH, USA) approximately 0.015 g sample was placed in pre-weighed nickel boats for 15 min at 800 °C. Mercury was quantified in direct Hg analyzer (Teledyne Leeman, model Hydra C, Hudson, NH, USA) under the following conditions: Power 1300 W; Oxygen Flow rate (99.999% purity) from 13 to 15 L min^{-1}; decomposition and catalyst temperatures of 850 °C and 600 °C, respectively; integration time of 180 s, and wavelength (λ) of 254 nm. Data were analyzed by the software Envoy (Teledyne Leeman, model Hydra C, Hudson, NH, USA). The calibration curve was made from a diluted stock solution to 100 ng g^{-1}. Aliquots were taken from this solution. These aliquots were weighed to calculate the amount of mercury and a curve was constructed in ng of mercury per g of total solution. Before calibration, are made several background readings (ultrapure water). These readings are made until microabsorbance values observed

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