Determination of total mercury in nuts at ultratrace level

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HIGHLIGHTS

- Direct analysis of Hg in nuts has been improved by a previous fat removal.
- Comparison of cold vapour atomic fluorescence and direct analysis of Hg in nuts.
- Mercury content in tree nuts was determined.

GRAPHICAL ABSTRACT
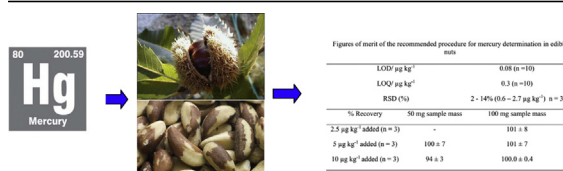
ABSTRACT

Total mercury, at $\mu g\,kg^{-1}$ level, was determined in different types of nuts (cashew nut, Brazil nuts, almond, pistachio, peanut, walnut) using a direct mercury analyser after previous sample defatting and by cold vapour atomic fluorescence spectrometry. There is not enough sensitivity in the second approach to determine Hg in previously digested samples due to the strong matrix effect. Mercury levels in 25 edible nut samples from Brazil and Spain were found in the range from 0.6 to 2.7 $\mu g\,kg^{-1}$ by using the pyrolysis of sample after the extraction of the nut fat. The accuracy of the proposed method was confirmed by analysing certified reference materials of Coal Fly Ash-NIST SRM 1633b, Fucus-IAEA 140 and three unpolished Rice Flour NIES-10. The observed results were in good agreement with the certified values. The recoveries of different amounts of mercury added to nut samples ranged from 94 to 101%. RSD values corresponding to three measurements varied between 2.0 and 14% and the limit of detection and quantification of the method were 0.08 and 0.3 $\mu g\,kg^{-1}$, respectively.

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1. Introduction

Foods are the main source of toxic elements intake for humans and have detrimental effects on various metabolic processes if they are present in relatively high concentrations [1]. In recent years, the concentration of toxic elements in foods has received great attention because of the potential risk to human health [2–4] of inadequate foodstuffs.

Mercury is a toxic element, included among the most hazardous heavy metals found in all foodstuffs, known to bioaccumulate and biomagnify in the food chain [5]. It gives rise to various toxic effects to human health, even exposure in low doses, acting on different parts of the body including skin, digestive system and especially the central nervous system [6].

Tree nuts (cashew nut, Brazil nuts, almond, pistachio, peanut, walnut) are commonly consumed products around the world. Nutritionally, they are a good source of essential minerals, carbohydrates, proteins, unsaturated fat, dietary fibre and vitamins, although they present high calorie content [7]. Scientific evidence has demonstrated that the ingestion of tree nuts contributes to positive health impacts in the prevention of cancer;

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also, helps to reduce the risk of type 2 diabetes and coronary heart disease [8]. These studies raise the possibility that their consumption will be recommended as an important component in the diet [9]. However, populations that consume tree nuts can be exposed to high levels of mercury when nuts are contaminated or produced from polluted areas.

The Joint FAO/WHO Expert Committee on Food Additives (JECFA), which also evaluates chemical contaminants in food supplies, has established provisional tolerable weekly intake (PTWIs) for total mercury at $4 \mu g \, kg^{-1}$ of body weight, or $34 \mu g$ per day for an individual weighing around 60 kg [10]. The GTZ German Agency for Technical Cooperation produced a guide that establishes the limit of Hg in cashew nut as $30 \mu g \, kg^{-1}$ [11].

Brazil is a great producer of nuts and the principal one are Brazil nuts (*Bertholletia excelsa*) from the Amazon region, that have an important nutritional, social and economic value for the nation. Annual production of about 45,000 tons generates a turnover of 33 million dollars [12]. Another commonly consumed tree nut is the cashew nut (*Anacardium accidentale* L.) typically produced in the north and northwest regions of Brazil, which contributes with 200 million dollars per year to the Brazilian economy [13].

In the last few years, a number of studies have focused on the contents of trace elements in various tree nuts [14–17]. These studies, however, do not include the determination of mercury, and to our knowledge, only three of these published in the last four years have included experimental data on Hg in nuts [18–20].

Kannamkumarath et al. developed a methodology using inductively coupled plasma mass spectrometry (ICP-MS) that employs different molecular weight fractions and various types of extractants in order to determine different elements. The concentrations of Hg in Brazil nuts samples reported were $8.2 \mu g \, g^{-1}$ with shell and $0.4 \mu g \, g^{-1}$ without shell [18]. However, Rodushkin et al. evaluated inorganic constituents of thirteen types of raw nut and seeds employing microwave assisted digestion and Sector field ICP-MS detection. The concentration of Hg ranged from $0.12 \mu g \, kg^{-1}$ for Brazil nuts to $3.6 \mu g \, kg^{-1}$ for pine nuts [19]. Welna et al. investigated the concentration of trace elements in Brazil nuts in two fractions (lipid and non-lipid) using hydride generation inductively coupled plasma optical emission spectroscopy (CV-ICP-OES) to determine Hg. The concentrations found were in all the case below the limit of detection ($2 \mu g \, kg^{-1}$) [20]. In spite of the relatively low concentrations found for Hg in nuts, Madejón et al. reported on the accumulation capacity of some potentially toxic elements in plants [21]. Although very low concentrations can be found in some areas, environmental pollution can cause accumulation of heavy metals and produce changes in the concentration of toxic elements as a function of the production area. Thus, the monitoring of mercury levels in tree nuts could be important to offer guarantees on the safety of these products and to assure their safe exportation.

Several techniques are currently available to determine mercury in food matrices and plants [22–24]. Among the analytical methodologies applied for the quantitative determination of low concentrations of mercury, the most commonly used methods involve atomic spectrometry, particularly cold vapour atomic fluorescence spectrometry (CV-AFS) [25] due to its high sensitivity and selectivity and relatively low instrumental cost. This method, however, involves a previous digestion of the sample and the introduction of acid solutions for the generation of Hg vapour. Because of this, the direct volatilization of Hg through sample ashed in an oxygen flow followed by the pre-concentration of Hg in a gold trap could provide a direct high sensitive alternative for Hg determination of trace levels in complex samples, thus avoiding problems found in multistep methods [26]. The aforementioned methodology has been applied to the direct determination of Hg in fish [27] and soil [28] and the concentration levels found in these samples were at mg kg^{-1}.

We have not found any study regarding Hg determination in tree nuts, probably due to the low concentrations expected. Therefore, the aim of the present work has been the quantification of total mercury in tree nut samples using the pyrolysis of samples and Hg determination by atomic absorption, with the results found compared with those obtained by CV-AFS.

2. Materials and methods

2.1. Reagent and standards

Ultra-pure water with a resistivity of 18.2 MΩ cm Milli Q from Millipore (Bedford-USA) was employed. The following chemicals were used for mineralization: 69% (w/w) HNO$_3$, 35% (w/w) H$_2$O$_2$, and 30% (w/w) HCl, all of them from Scharlau (Barcelona, Spain). The reduction solution was prepared with stannous chloride at 2% (w/v), dissolving SnCl$_2$·2H$_2$O from Scharlau in 10% (v/v) HCl which remained for 30 min in a stream of nitrogen with constant stirring to eliminate any mercury traces. A stock solution of KBr and KBrO$_3$, both from Scharlau was prepared daily containing 0.1 mol L^{-1} KBr and 0.017 mol L^{-1} KBrO$_3$. The use of this solution is important because it allows a complete conversion of bromate to Br$_2$. A stock solution of 12% (w/v) hydroxylamine hydrochloride from Scharlau was used in order to complete the digestion and to remove the excess of oxidant and the nitrous oxides generated during the digestion, which have an unfavourable impact on the reduction of mercury with stannous chloride. The mercury analyser was calibrated using aqueous Hg (II) with different concentrations and two calibration sets prepared by dilution from 1000 mg L^{-1} Merck standard solution (Darmstadt, Germany). The concentration of mercury for the first interval ranged from 0 to 20 ng Hg; while the second curve ranged from 20 to 1000 ng Hg. The calibration range automatically switched over, depending on the signal obtained for the unknown samples, allowing accurate measurements at lower mercury concentrations without shortening the overall mercury working range.

2.2. Instrumentation and apparatus

An ultrasonic water bath of 350 mL with 50 W power and 50 Hz frequency from Selecta (Barcelona, Spain) was employed for sample sonication. For sample mineralization, a microwave system Ethos SEL from Milestone (Sorisole, Italy) equipped with an thermocouple for automatic temperature control, an automatic gas detector and ten high pressure closed digestion vessels made of high purity TFM (a thermally resistant form of Teflon) with 100 mL inner volume was used. The system was operated at a maximum exit power of 1000 W. After digestion, the measurements were carried out with an atomic fluorescence spectrometer (AFS) model Millennium Merlin instrument PSA 10.025 (Orpington, UK) in the range of 0.025 to 0.2 ng mL^{-1} Hg(II). The flow rates were adjusted at 9 mL min^{-1} for the carrier and sample and to 4.5 mL min^{-1} for the reducing agent. The mercury vapour generated on-line was separated from the liquid phase in a gas–liquid separator, swept from it with argon (250 mL min^{-1}) and dried in a Perma Pure dryer membrane with argon (2.5 L min^{-1}).

A direct mercury analyser Milestone DMA-80 was used for direct determination of Hg in untreated and fat-free samples by atomic absorption after sample ashing and Hg pre-concentration in a gold trap. The instrument was equipped with an auto sampler with a capacity of forty sample boats, an interference filter of 254 nm with 9 nm width and a silicon detector which measured the absorbance signal of Hg at 253.6 nm. The maximum mass of solid sample that can be analysed in the instrument is 500 mg for solids and 500 µL for liquids. The oxygen was used as carrier gas. The operating conditions for DMA-80 were performed according to