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Total content and *in vitro* bioaccessibility of tellurium in Brazil nuts

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ABSTRACT

Alongside the Brazil nut's role as an excellent source of vitamins, oil, fatty acids, lipids and nutrients, it is also recognized as a rich source of selenium. The pathway along which selenium and sulfur are metabolized in plants is theorized to be the same as that used for tellurium. Total tellurium content and its bioaccessibility are then evaluated by ICP-MS. Interferences and sample preparation are evaluated for the accurate determination of tellurium, and the accuracy determined through analysis of the certified reference material 1643e. A concentration of 4.02 ± 0.391 ng g^{-1} is obtained as an average concentration through external and internal calibrations. Through this reliable result, tellurium bioaccessibility in Brazil nuts is obtained via an *in vitro* validated unified bioaccessibility method. Values of 32% and 30% of total tellurium are available in the gastric and gastrointestinal fractions, respectively.

1. Introduction

Brazil nut, Amazon nut, tocari and tururi [1] are several of the names used for the tree and seeds currently known to Brazilians as *castanha-do-pará* (Pará nut). Since the arrival of Europeans to South America, travelers, missionaries and naturalists have reported these nuts. Appreciated for its flavor, the Brazil nut is currently largely utilized in both the culinary and the cosmetic sectors, and its oil is used to manufacture shampoos, conditioners and soaps [1]. Notably, Brazil's average Brazil nut crop production in the last year was 30 M tons [2]. Additionally, the literature reports a number of benefits from the consumption of Brazil nuts, such as improvement of heart health, weight loss, boosting of immune system, and facilitated nutrient uptake, among others [3].

In terms of nutritive value, the Brazil nut is rich in vitamins A, B1, B2, C and E, as well as minerals such as Ca, Cu, K, Mg, P, and Se [4]. Additionally, Brazil nut oil contains lipid levels of *ca*. 60% to 70%, and the levels of saturated and unsaturated fatty acids are higher than those in other oilseeds [5].

Regarding minerals, it is well-known that the Brazil nut contains relatively large amounts of Se (from 5.8 to 170 µg g^{-1}) [6–9] such that eating only one nut per day is sufficient to fulfill the Se needs of an adult (55 µg Se daily) [3]. Due to this high concentration, plants thus carry out the biosynthesis of Se-amino acids as a form of detoxification. This phenomenon explains the high concentration of amino acids, such as methionine and cysteine (and arginine and leucine, in lower quantities), found in Brazil nuts compared to similar nuts [10].

As reported by Cowgill [11], plants that accumulate Se, such as Indian mustard (*Brassica juncea*), broccoli, and garlic, are able to accumulate Te, as well. In fact, most studies focusing on Te refer to tellurite, tellurate or a handful of organic tellurides, and it can be seen as a "forgotten" element in biology [12]. However, in recent years, Te-containing metabolites besides tellurate (such as methyltellurocysteine oxide (MeTeCysO) and telluromethionine oxide (TeMetO)) were observed in garlic [13]; in the cases of these metabolites, Te is presumed to share a metabolic pathway with S and Se [13].

Although no information has been obtained to date regarding Te in Brazil nuts, the theory preconized by Cowgill [11] alluded to the idea that a high content of Se [3] may indicate the presence of Te in such samples. The biological and toxicological effects of Te in plants have not been fully elucidated, and the evaluation of its presence, as well as its bioaccessibility, in Brazil nuts opens up new perspectives for research advances in this direction.

To this end, our proposal was to provide new information regarding

Abbreviations: ICP-MS, Inductively Coupled Plasma Mass Spectrometry; UBM, unified bioaccessibility method; MeTeCysO, methyltellurocystein oxide; TeMetO, telluromethionine oxide; DRC, dynamic reaction and collision cell system; BARGE, Bioaccessibility Research Group of Europe; SRM, standard reference material; RSD, relative standard deviation; REL, recommended exposure limit; NIOSH, National Institute for Occupational Safety and Health; LD_{50}, lethal dose for 50% of population

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the total content of Te in Brazil nuts through the development of an accurate analytical method, as well as to evaluate its bioaccessibility through a validated UBM [14]. Bioaccessibility refers to the quantity of a nutrient or toxicant released from the food matrix and solubilized in the body fluids during gastrointestinal digestion (bioaccesible fraction), thereby becoming potentially available for absorption in the small intestine [15,16]. As Te is considered non-essential and harmful to humans and animals [17,18], such evaluations are of utmost importance. Quality control for the bioaccessibility results was attained through mass balance validation based on the total Te content and the sum of the bioaccessible and residual fractions.

2. Materials and methods

2.1. Instrumentation

All analyses were performed inside a Class 10,000 clean room. To quantify the total content of Te in Brazil nuts, as well as evaluating the oral bioaccessibility data, an inductively coupled plasma quadrupole mass spectrometer – ICP-MS (model ELAN DRC-e, Perkin Elmer, Norwalk, CT, USA), equipped with a dynamic reaction and collision cell (DRC) system, was employed. The solutions were introduced using a concentric nebulizer and cyclonic spray chamber. All of the operational conditions for the ICP-MS are presented in Table 1.

The microwave-assisted acid sample decomposition was carried out in a microwave oven (model DGT-100, Provecto Analitica, Jundiaí, Brazil) equipped with magnetron of 2450 ± 13 MHz and operated at 1200 W (nominal power) using closed Teflon vessels.

2.2. Reagents and solutions

Solutions were prepared with deionized water (≥ 18.2 MΩ cm) from a Millipore Direct-Q 3UV water purification system (Bedford, USA). For microwave-assisted acid decomposition and standard/sample dilutions, HNO_3 (sub-boiling) and 30% (w/v) H_2O_2 (Merck, Darmstadt, Germany) were used. Before use, all of the glassware was cleaned with 10% (v/v) HNO_3 and rinsed three times with deionized water.

For the bioaccessibility evaluation, Tables 1S and 2S (Supplementary material) list all reagents used in the unified bioaccessibility method (UBM) testing assays, including salts, enzymes and organic chemicals, for mimicking salivary, gastric, duodenal and biliary fluids.

Daily ICP-MS performance was checked using a multielemental standard (Perkin Elmer, Shelton, USA) containing Ce, In, Mg and U, all at concentrations of 1 μg L^{-1}, and Ba at 10 μg L^{-1}.

Table 1
Optimized instrumental operational conditions and measurement.

Model and brand	ELAN DRC-e, Perkin Elmer
Ciclonic nebulization chamber	Cyclonic
Nebulizer	Concentric (Meinhard)
RF power (W)	1200
Nebulizer gas flow – Ar (L min^{-1})	0.74–0.80 (optimized daily)
Auxiliary gas flow – Ar (L min^{-1})	1.0
Plasma gas flow (L min^{-1})	15
Read mode	Peak hopping
Dwell time (ms)	50
Detector dead time (ns)	60
Sweeps	20
Integration time (ms)	1000
RPq (V)	0.25
Replicatas	5
Dynamic reaction and collision cell (DRC) system	Non-used
Correction equation	Non-used
Monitored *m/z*	^{128}Te; ^{130}Te; ^{138}Ba

2.3. Samples, sample preparation and evaluation of the total Te content

Brazil nut samples, Bertholletia excelsa (Qualitá, São Paulo, Brazil, lot numbers 091215 and 150317), were acquired in a local market, frozen in liquid nitrogen and ground with a mortar and pestle. For the determination of total Te content, 20 nuts were macerated in liquid nitrogen, using a mortar and pestle, and dried at 40 °C to constant mass. Then 200 mg of nuts were weighted (9 replicates), microwave decomposed with 3.0 mL of sub-boiling HNO_3 and 2.0 mL of 30% (w/v) H_2O_2, with the resulting digests dried almost to desiccation, and the volume replaced up to 5 mL with 0.2% (v/v) HNO_3. The microwave-assisted decomposition conditions are as follows: 8 min @ 330 W; 5 min @ 590 W; 40 min @ 720 W.

For this task, several sample preparation optimization procedures were conducted. Those programs most representative of using microwave-assisted sample decomposition are visualized in Table 3S (Supplementary material). Additionally, external and analyte addition methods, as well as recovery tests, were also performed.

2.4. Bioaccessibility test

The in vitro physiologically based protocol for mimicking the gastrointestinal digestion of Brazil nuts was based on the UBM methodology developed by the Bioaccessibility Research Group of Europe [14]. All of the procedures were performed in triplicate for each gastric and gastrointestinal fraction sample, and the results are summarized in Fig. 1. Blank solutions were performed accordingly. Then, 6 flasks were used for the UBM methodology. Briefly, 300 mg of ground seeds, powdered in liquid nitrogen, was weighed in each polypropylene flask (50 mL), and 4.5 mL of salivary solution (at pH 6.5 ± 0.50) was added. The mixture was agitated manually for 10 s, after which 6.75 mL of gastric fluid (at pH 1.1 ± 0.1) was also added. The final pH was adjusted to 1.2 ± 0.050 using small volumes of concentrated HCl. The flasks were placed in the thermostatic bath for 60 min at 37 °C and shaken (end-over-end agitation mode) at ca. 150 rpm in order to guarantee the physiological conditions preconized by the UBM method (37 ± 2 °C).

From this step, the gastrointestinal fraction was later obtained using 3 of the 6 initial flasks, as follows (see also the left column of Fig. 1): 13.5 mL of duodenal and 4.5 mL of biliary fluids were added to both flasks from the previous steps, and the pH was adjusted to 6.3 ± 0.50 with 5 mol L^{-1} NaOH. The flasks were incubated in the thermostatic bath for 240 min at 37 °C and also shaken. After this period, the solution was centrifuged at 4500*g* for 15 min. After centrifuging, the supernatant (gastrointestinal bioaccessible fraction) was collected, and 500 μL of sub-boiling HNO_3 added.

To obtain the gastric fraction (see also the right column of Fig. 1), the other 3 flasks from the initial UBM methodology were used. Then, each flask containing the initial mock digestive portion was centrifuged at 4500*g* for 15 min. The supernatant (gastric bioaccessible fraction) was subsequently collected, and 250 μL of concentrated HNO_3 added.

Both gastric and gastrointestinal acidified supernatants were later filtered using Nylon syringe and filters of hydrophilic PVDF 0.2 μm pore size. The residual solid (the so-called nonbioaccessible fraction) was decomposed using a microwave-assisted acid decomposition process, similar to that described in Section 2.3. After decomposition, the resulting digested materials were dried almost to desiccation, and the volume was replaced up to 5 mL with 0.2% (v/v) HNO_3. All of the assays for oral bioaccessibility of Te were performed through the ICP-MS technique (see also Table 1).

3. Results and discussion

3.1. Evaluation of the total Te content in nuts

To obtain the bioaccessibility of Te, it is of utmost importance to