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Bioavailability assessment of essential and toxic metals in edible nuts and seeds

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ABSTRACT

Bioavailability of essential and toxic metals in edible nuts and seeds has been assessed by using an *in vitro* dialyzability approach. The samples studied included walnuts, Brazil nuts, Macadamia nuts, pecans, hazelnuts, chestnuts, cashews, peanuts, pistachios and seeds (almond, pine, pumpkin and sunflower). Metals were measured by inductively coupled plasma-mass spectrometry in dialyzates and also in samples after a microwave assisted acid digestion pre-treatment. Low dialyzability percentages were found for Al, Fe and Hg; moderate percentages were found for Ba, Ca, Cd, Co, Cu, K, Li, Mg, Mn, Mo, P, Pb, Se, Sr, Tl and Zn; and high dialyzability ratios were found for As, Cr and Ni. The highest dialyzability percentages were found in raw chestnuts and raw hazelnuts. Metal dialyzability was found to be negatively affected by fat content. Positive correlation was found between carbohydrate content and metal dialyzability ratios. Protein and dietary fibre content did not influence metal bioavailability. Predicted dialyzability for some metals based on fat and protein content could also be established.

1. Introduction

Nuts and edible seeds are recognized for their nutritional value and potential contribution to health promotion. Nuts contain exceptionally high levels of bioactive compounds, such as antioxidants, essential fatty acids, vitamins, essential amino acids, and essential minerals (Anzano & González, 2000; Cabrera, Lloris, Giménez, Olalla, & López, 2003; Chang, Gutenmann, Reid, & Lisk, 1995; Glew et al., 2006; Gómez-Ariza, Arias-Borrego, & García-Barrera, 2006; Juranovic, Breinhoelder, & Steffan, 2003; Lavedrine, Ravel, Villet, Ducros, & Alary, 2000; Momen, Zachariadis, Anthemidis, & Stratis, 2007; Moodley, Kindness, & Jonnalagadda, 2007; Naozuka, Marana, & Oliveira, 2010; Rodushkin, Engstrom, Sorlin, & Baxter, 2008; Simsek & Aykut, 2007; Wuilloud, Kannamumarath, & Caruso, 2004). Some of the reported health benefits derived from nut and seed consumption are control of body weight and blood pressure, reduction of coronary heart disease, and reduction of levels of blood cholesterol and triacylglycerols. In addition, nuts and edible seeds provide antioxidant, anti-microbial, anti-inflammatory, anti-mutagenic, anti-cancer, anti-diabetic and glucoregulatory (Gentile et al., 2012; Ip & Lisk, 1994; Kendall, Josse, Esfahani, & Jenkins, 2010; Rajaei, Barzegar, Mobarez, Sahari, & Esfahani, 2010) properties.

Several studies have been performed for assessing the levels of bioactive compounds (and also the levels of toxic substances) in foodstuff (nuts and edible seeds included). Knowing the total content of essential/toxic substances in food, however, does not provide enough information regarding the beneficial effects or the human dietary risks resulting from its consumption. Information regarding the amount of the substances that reach the systemic circulation after ingestion, and is available to perform its biological activity or toxic effect (bioavailability) is therefore necessary. The total content of essential and/or non-essential substances in foodstuff must be based on the knowledge of the amount of substances released during the gastric and intestinal digestion/absorption stages (Ruby et al., 1999).

Several studies on bioavailability and bioaccessibility (maximum fraction of a substance in food that is theoretically released from its matrix in the gastrointestinal tract and thus becomes available for intestinal absorption) of essential minerals and toxic metals in foodstuff have been performed (Intawongse & Dean, 2006; Moreda-Piñeiro et al., 2011). However, only a few studies

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have focused on nuts (Arpadjan, Momchilova, Venelinov, Blagoeva, & Nikolova, 2013; Dumont, De Pauw, Vanhaecke, & Cornelis, 2006; Gomes da Silva, Verola Mataveli, & Zezzi Arruda, 2013; Nascimento, Naozuka, & Oliveira, 2010; Ovca, van Elteren, Falnoga, & Šelih, 2011; Suliburska & Krejpcio, 2014). Those studies were conducted mainly to determine bioaccessibility of essential metals.

The current paper focuses on the development and application of an *in vitro* dialyzability method to assess the bioavailability ratios of essential/toxic elements in twelve different types of raw nuts and seeds purchased at local supermarkets. Also, since toxic and essential metal bioavailability ratios in nuts and seeds could depend on nut processing/cooking, several toasted and fried nuts available at local supermarkets were included in the study. Since nut/seed constituents can affect bioavailability ratios, the effect of major nutrient constituents on the bioavailability of the investigated elements was also performed.

2. Materials and methods

2.1. Instrumentation

Metal content was measured with a Perkin Elmer Nex-Ion 300X ICP-MS instrument (Waltham, MA, USA) equipped with a SeaFast SC2 DX autosampler (Elemental Scientific, Omaha, NB, USA). A Boxcult incubator situated on a Rotabit orbital-rocking platform shaker (J.P. Selecta, Barcelona, Spain) was used for temperature control of the enzymolysis procedure. Dialyzability was performed using Cellu Sep® H1 high grade regenerated cellulose tubular membranes (molecular weight cut-off 10 kDa, 50 cm length, 25.5 mm diameter dried and a volume to length ratio of 5.10 ml cm^{-1}) obtained from Membrane Filtration Products Inc. (Seguin, TX, USA). A domestic Taurus blade grinder (Taurus, Barcelona, Spain) was used to grind and homogenize nut/seed samples. A Conterm drying oven (J.P. Selecta) was used for trace water removal from samples. An Ethos Plus microwave lab-station (Milestone, Sorisole, Italy) with 100 ml closed Teflon vessels and Teflon covers, HTC adapter plate and HTC safety springs (Milestone) was used for assisting the acid digestion procedure. The chemometrics package used for data analysis was STATGRAPHICS Plus 5.1 (Manugistics Inc., Rockville M.D., USA).

2.2. Reagents

Ultra-pure water of resistance 18 MΩ cm^{-1} was obtained from a Milli–Q purification device (Millipore Co., Billerica, MA, USA). Multi-element standard solutions were prepared by combining stock standard solutions (1.000 g l^{-1}) supplied by Merck (Poole, Dorset, UK). Digestive enzymes (porcine pepsin, p-7000, porcine pancreatin, P-1750), bile salts (approx. 50% sodium cholate and 50% sodium deoxycholate) and piperazine-NN-bis(2-ethane-sulfo nic acid) di-sodium salt (PIPES), were obtained from Sigma Chemicals (St. Louis, MO, USA). Sodium hydrogen carbonate was from Merck. AnalaR nitric acid 69%, hydrochloric acid 37% and hydrogen peroxide 33% (m/v) were from Panreac (Barcelona, Spain).

2.3. Nut and seed samples

Twelve different types of raw, toasted and fried nuts and seeds were analyzed. Samples included raw walnuts (*Juglans regia*), raw Brazil nuts (*Bertholletia excels*), raw Macadamia nuts (*Macadamia tetraphylla*), raw pecans (*Carya illinoinensis*), raw and toasted hazelnuts (*Corylus avellana*), raw chestnuts (*Castanea sativa*), fried cashews (*Anacardium occidentale*), toasted pistachios (*Pistacia vera*), raw and toasted *Largueta* almonds, fried *Marcona* almonds (*Prunus dulcis*), raw pine nuts (*Genus pine*), raw pumpkin seeds (*Curcubita maxima*), toasted sunflower seeds (*Helianthus annuus*) and toasted peanuts (*Arachis hypogaea*). All samples were obtained from a local supermarket. After shell removal, composite samples of raw pecans, raw hazelnuts, raw chestnuts, toasted peanuts and toasted pistachios (approximately 100 g) were homogenized (10 min) by mechanical blending and dehydrated in an oven at 40 °C (constant weight) for trace water removal. Finally, dried nut/seed samples were kept in polyethylene bottles with hermetic seals at −20 °C.

2.4. In vitro dialyzability procedure

The *in vitro* dialyzability procedure was performed in triplicate by weighing approximately 0.5 g of composite samples into 100 ml Erlemeyer flasks. The *in-vitro* dialyzability procedure is adapted from the protocol by Miller, Schricker, Rasmussen, and Van Campen (1981). Some modifications have previously been described (Moreda-Piñeiro, Alonso-Rodríguez, et al., 2012; Moreda-Piñeiro, Moreda-Piñeiro, et al., 2012); in brief, 20 ml of ultra-pure water was added, and after 20 min the pH was adjusted at 2.0 by adding small volumes of a 0.1 M hydrochloric acid solution. Then, 0.15 g of pepsin (approximately 150 µl of a 16.0% (m/v) pepsin solution prepared in 0.1 M hydrochloric acid) was added, and the simulated gastric digestion was performed under orbital – horizontal shaking (37 °C, 150 rpm) for 120 min, after which it was halted by immersing the flasks into an ice-bath. The procedure continued by adding 5 ml of 4.0% (m/v) pancreatin and 2.5% (m/v) bile salts solution prepared in 0.1 M sodium hydrocarbonate. At this point, dialysis membranes (10 kDa MWCO), filled with 20 ml of a 0.15 N PIPES solution (pH 7.5) were placed inside the flasks. Intestinal digestion took place under orbital – horizontal shaking (37 °C, 150 rpm) for 120 min, after which it was halted by immersing the flasks into an ice-bath. Dialyzates (solution contained in the dialysis membrane) and the residual or non-dialyzable fraction (slurries remaining in the flask) were transferred to polyethylene vials and weighed separately. Both the dialyzate and the residual fraction were kept at −20 °C before measurements. Reagent blanks were also obtained to control possible contamination (two blanks for each prepared set of samples).

2.5. Microwave assisted acid digestion

Raw samples (approximately 0.3 g) were subjected to a microwave assisted acid digestion procedure in triplicate (Moreda-Piñeiro et al., 2008) using 4 ml of ultrapure nitric acid, 2 ml of hydrogen peroxide, and 3 ml of ultrapure water. The mixtures were subjected to a four stage microwave irradiation program (power 1000 W): temperature was increased from room temperature to 90 °C in 2.5 min, from 90 °C to 140 °C in 6 min, and from 140 °C to 200 °C in 5.0 min; and finally maintained at 200 °C for 10 min. After cooling (venting time of 50 min.) the acid digests were made up to 25 ml with ultra–pure water. Two blanks were performed for each set of microwave conditions.

Non-dialyzable fractions (residues after the *in vitro* dialyzability procedure) were also subjected to a microwave assisted acid digestion procedure. In this case, the whole residue (approximately 25 g) was mixed with 4 ml of ultrapure nitric acid and 2 ml of hydrogen peroxide, but microwave irradiation at 200 °C (fourth stage) was maintained for 25 min. After cooling, the acid digests were diluted to 125 ml with ultrapure water.

2.6. ICP-MS measurements

Metals in acid digests were measured by ICP-MS under standard operating conditions (STable 1, Appendix A. Supplementary data). Acid digests from raw samples were 1:10 diluted before