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Mineral and trace element content in legumes (lentils, chickpeas and beans): Bioaccesibility and probabilistic assessment of the dietary intake

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ABSTRACT

Three samples of legumes widely consumed in Spain (lentils, beans and chickpeas) were selected in order to assess the total and bioaccessible content of trace elements. The influence of dietary components and the effect of processing on elements bioaccessibility were also evaluated. Raw lentils presented medium contents of 84, 34, 13, 9.1, 450 and 922 μg g^{-1} for Fe, Zn, Mn, Cu, Ca and Mg respectively. Raw chickpeas and beans presented the following contents, 45, 37, 26, 8.3, 828, 1176 μg g^{-1} and 45, 24, 12, 6.9, 977, 1166 μg g^{-1}. Results showed that element total content decreased in cooked samples with respect to soaked ones, due to a solubilization of the inorganic elements into the water during cooking treatments at different rates. Thus, trace element concentrations differed, resulting in the following: lentils (10, 5.2, 1.8, 1.8, 219, 155 μg g^{-1}); chickpeas (9.1, 7.2, 4.3, 2.9, 297, 273 μg g^{-1}) and beans (11, 5, 2.1, 1.8, 312, 268 μg g^{-1}). However, elements initially present are considerably more bioaccessible probably due to a destruction of some antinutritional components as a consequence of processing. Finally, according to a probabilistic assessment used to determine the contributions to Dietary References Intakes (DRI), legumes were proper sources of Fe, Cu, Mn and Zn.

1. Introduction

Legumes have been, and in some areas still are, one of man's basic foodstuffs. These basic crops have been an integral part of the human diet for millennia, but today legumes are not experiencing anywhere near the same increase in production as corn, wheat, rice and soybeans, and its consumption has undergone a slow, but constant, decline in both developed and developing countries (FAO, 2016). From a nutritional point of view, they are a good source of vegetable proteins and amino-acids, fiber, and at the same time, are low in fats (Torija and Díez, 1999; Olmedilla et al., 2010; Iqbal et al., 2006; Khattab and Arntfield, 2009; Tharanathan and Mahadevamma, 2003). Moreover, legumes may also constitute an appropriate source of proteins for cattle (Jezierny et al., 2010) and similarly, a good source of minerals and trace elements such as Fe and Zn (Jodral-Segado and Navarro-Alarcón, 2003; Campos-Vega et al., 2010). As a result of the above reasons, the General Assembly of the United Nations, at its 68th session, proclaimed 2016 as the International Year of Pulses (A / RES / 68/231). This year it was proposed to raise the public's awareness about the nutritional benefits of legumes as part of sustainable food production with the aim of achieving food security, combating malnutrition, reducing poverty, improving human health and increasing agricultural sustainability (FAO, 2016).

In Spain, three species of legumes stand out for their high consumption rates: beans (*Phaseolus vulgaris* L.), chickpeas (*Cicer arietinum* L.) and lentils (*Lens culinaris* L.). The most notable consumption is associated with chickpeas (1.3 kg per person per year), while beans and lentils reach 0.9 kg per person in each case (Mercasa, 2015). Most legumes of dietary significance (such as beans, chickpeas and lentils) are widely marketed in dried and/or processed forms (ready-to-eat). In the case of the dried form, the traditional way of legume preparation includes soaking them in water followed by cooking, a treatment expected to alter their macro- and micronutrient composition. They are usually consumed boiled as part of a stew. In relation to processed forms - ready-to eat products - they are previously cooked in industrial machinery and packed in glass jars. Subsequently, they can be consumed as part of a salad, side dish or stew.

From a nutritional point of view, it would be very useful to know the concentrations of several micronutrients, such as mineral or trace elements, present in this food group. In this regard, previous studies have already been developed with different types of legumes (Akinyele and Shokunbi, 2015; Campos-Vega et al., 2010; Iqbal et al., 2006; Cabrera et al., 2003). However, only determining the content of minerals or trace elements present in a food may not be sufficient to evaluate its nutritional quality. It is much more appropriate to assess the portion of

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this element, initially present in the food, which is solubilized and absorbed in the intestinal lumen. Later, this fraction will be used by the body for the physiological function for which it is intended, thus resulting in the concept of bioaccessibility (Cámara-Martos et al., 2015).

In this sense, studies to determine bioaccessibility of inorganic elements in legumes mainly focus on Fe, Zn and Ca (Singh et al., 2016; Ramírez-Cárdenas et al., 2010; Viadel et al., 2006a,b; Lombardi-Boccia et al., 2003). However, to our knowledge, no study has been conducted to analyze the bioaccessibility of other elements such as Mn, Cu and Mg, which are in abundance in pulses. These micronutrients are also needed for proper development of an organism and their deficiency leads to a number of pathologies (Avila et al., 2016; Bertinato and Finglas, 2016; Mazur and Maier, 2016). Furthermore, the bioaccessibility of these elements may be altered by several dietary components and the effect of processing (Ramírez-Ojeda et al., 2016; Cámara et al., 2007; Viadel et al., 2006a,b; Cámara et al., 2005; Sebastiá et al., 2001).

Given the above, the objectives of this article were i) to determine the bioaccessibility and the total content of Fe, Zn, Mn, Cu, Ca and Mg present in three varieties of legumes (lentils, beans and chickpeas) widely consumed by the Spanish population, sold in two formats (soaked raw and ready-to-eat); ii) to study the influence of processing and other food components such as protein and fat in the bioaccessibility of these elements; iii) to assess the contributions to the Dietary Reference Intakes (DRIs) of these micronutrients, from data obtained by consumption of these legumes, through a probabilistic approach.

2. Materials and methods

2.1. Materials and reagents

Deionized water obtained with a Milli-Q system (Millipore, Madrid, Spain) was used exclusively. To eliminate the risk of contamination, all polyethylene material and glassware, after each use, were washed with tap water, soaked in a 20% HNO_3 solution (at least overnight), and rinsed with deionized water three times.

All reagents were of analytical-reagent grade. To obtain working standards, standard solutions of Cu, Zn, Mn, Fe, Ca and Mg (1000 mg/L) (Scharlau Chemie, Barcelona, Spain) were used and diluted as necessary. High quality concentrated nitric acid (HNO_3) 65%, and hydrochloric acid (HCl) 35% (Panreac, Barcelona, Spain) were used for sample mineralization. Sodium bicarbonate ($NaHCO_3$) 97% was supplied by Scharlau (Barcelona, Spain). Lanthanum chloride ($LaCl_3$) was obtained from Perkin Elmer (Madrid, Spain).

Sigma-Aldrich Co. (St. Louis, MO) provided the digestive enzymes (pepsin; pancreatin) and bile salts. 3.2 g of pepsin (P-7000 from porcine gastric mucosa) were dissolved in 20 mL of HCl (0.1 M) to prepare the pepsin solution. The solution of pancreatin and bile salts was obtained by dissolving in 150 mL of 0.1 M $NaHCO_3$, 0.6 g of pancreatin (P-3292 from porcine pancreas) and 3.9 g of bile salts (B-8756 of porcine origin). The working solutions were prepared immediately before use. The dialysis membranes, with a pore size (MWCO) of 12–14,000 Å (Size 6 Inf Dia 27/32″–21.5 mm, 30 m, Bestlno. 1063F09, Medicell Int. LTD, London, UK), were rinsed several times with distilled deionized water before use.

2.2. Samples

Lentils, chickpeas and beans of three different brands, widely sold in Spain, were chosen. Each different brand was selected in both a raw as well as ready-to-eat format. In the latter case, legumes were directly analyzed (previous preparation of sample), while in the case of the raw samples, they were soaked in deionized water, at room temperature overnight, before use in the study. Three different packets from different supermarkets, representing both raw and ready-to-eat formats of pulse, were used in several periods of this study (September 2015 to March 2016). In total, the number of legumes used in the laboratory was 54 (3 legumes × 3 brands × 3 batches × 2 formats available for purchase). All samples were poured onto Petri dishes, freeze-dried and packed in polypropylene vacuum bags, until required for analyses.

2.3. Total mineral content

1 g of a ground and lyophilized sample was ashed for 15 h in a muffle furnace at 460 °C, to determine the total content of mineral (Moreno-Rojas et al., 1994). Once the ash was cooled, this was bleached with 2.5 mL of HNO_3 2 N, dried on thermostatic hotplates, until complete mineralization, for 1 h in a muffle furnace at 460 °C. Afterwards, the ash was dissolved in a 1 mL solution of HCl 20% (v/v) and made up to a known volume (10 mL) with deionized water. Each sample was analyzed in quintuplicate. Therefore the number of samples analyzed was 270 (54 legumes · 5)

Atomic absorption spectrometry (FAAS) with a Varian SpectraAA – 50B model (Palo Alto, California, USA), equipped with standard air-acetylene flame and single element hollow cathode lamps, was used to determine Zn, Fe, Ca and Mg content. In the case of Ca and Mg, $LaCl_3$ was added to the mineral solution at a final concentration of 2%, to avoid interference by phosphate. Electrothermal atomic absorption spectrometry (ETAAS) was used for the determination of Cu and Mn in a soluble and dialyzable fraction by a Perkin-Elmer model Analyst 600 (Waltham, Massachusetts, USA) with graphite furnace and an autosampler. The instrumental conditions for the determination are shown in Tables 1 and 2. The detection limit (LOD) was calculated as the mean value of 30 measurements of the blank plus three times their standard deviation. Regarding the quantification limit (LOQ), it was calculated as the mean value of 30 measurements of the blanks plus 10 times their standard deviation. These parameters are registered in Table 1. Furthermore, CRM (NIST, Gaithersburg, Maryland, USA) were also analyzed (under the same conditions as samples) to evaluate the accuracy

Table 1
Instrumental conditions, limit of detection, limit of quantification and analysis of certified references materials.

Element	Wavelength (nm)	Slit Width (nm)	LOD (mg L^{-1})	LOQ (mg L^{-1})	Certified references material (mg kg^{-1})					
					Rice flour NIST – 1568a			Bovine liver BCR – 185R		
					Certified	Found	Recovery (%)	Certified	Found	Recovery (%)
Fe	248.3	0.2	0.084	0.28	7.42 ± 0.44	7.58 ± 0.52	102	–	–	
Zn	213.9	0.7	0.168	0.56	19.42 ± 0.26	20.38 ± 0.24	105	138.6 ± 2.1	130.4 ± 17.1	94
Mn	279.5	0.2	0.013	0.043	19.20 ± 1.80	18.48 ± 4.20	96	11.07 ± 0.29	11.32 ± 2.87	102
Cu	324.8	0.7	0.014	0.05	2.35 ± 0.16	2.26 ± 0.34	96	277 ± 5	264 ± 38	95
Ca	422.7	0.7	0.315	1.05	118.4 ± 3.1	116.7 ± 2.1	98	–	–	
Mg	285.2	0.7	0.011	0.036	559 ± 10	556 ± 18	99	–	–	

LODLimit of detection.
LOQLimit of quantification.