Received: 3 May 2018 | Revised: 3 August 2018 | Accepted: 14 August 2018

DOI: 10.1002/cche.10094

RESEARCH ARTICLE

Bioaccessibility and total content of iron, zinc, copper, and manganese in rice varieties (*Oryza sativa* L.): A probabilistic assessment to evaluate their contribution to dietary reference intake

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Abstract

Background and objectives: Fe, Zn, Cu, and Mn contents as well as bioaccessible fractions of eight rice varieties were analyzed in order to evaluate them nutritionally.

Findings: Total trace element concentrations ranged between 5.90 and 15.3 mg/kg for Fe, 8.82–12.9 mg/kg for Zn, 1.45–5.59 mg/kg for Cu, and 2.45–13.6 mg/kg for Mn. Brown rice presented the highest trace element contents and at the same time the lowest bioaccessibility. A negative significant statistical correlation was found between dietary fiber and Mn bioaccessibility ($r = -0.872$; $p < 0.01$). Significant statistical negative correlations were found between vegetable proteins of rice and trace elements bioaccessibility.

Conclusions: A probabilistic assessment (@Risk) used to determine the contribution of DRI for Fe, Zn, Mn, and Cu through the intake of 150–200 g of boiled rice showed that this food can be a proper source of Cu and to a lesser extent Mn but not in the case of Fe and Zn.

Significance and novelty: The present study develops a probabilistic model to evaluate the contributions to the DRIs of these micronutrients, from data of rice varieties consumption and their bioaccessibility—total content.

KEYWORDS

bioaccessibility, copper, iron, manganese, rice, zinc

1 | INTRODUCTION

Rice (*Oryza sativa* L.) is a crop of great economic and social importance worldwide. It is one of the most important cereal crops in the world, supplying a staple source of energy, protein, and other nutrients to half of the world's population (Borresen & Ryan, 2014). Because of its ability to adapt to various climatic conditions, there are thousands of rice varieties which have diverse quality features such as size and shape grain, pigmentation, translucency among others (Juliano, 2016).

About 8.20 billion people in developing countries are suffering from micronutrient deficiencies, of which 2 billion are suffering from Fe deficiency (Stein, 2010). In this sense, rice is also an important source of energy, vitamins, amino acids, trace elements, and other micronutrients for humans. The mineral and trace element content of rice are highly influenced by the variety and the degree of polishing or milling. During this process, the bran is removed from the brown rice resulting in white rice grains (Hansen et al., 2012). Since the outer grain layers are richer in trace elements than the inner core, significant lower trace element content is usually observed in the polished/milled grains (Pinto, Almeida, & Ferreira, 2016). Furthermore, another treatment that can also cause nutritional modifications in trace element concentration is cooking (Hemalatha, Platel, & Srinivasan, 2007; Praveena & Omar, 2017). For that reason, it is interesting to analyze the nutritional components of a food in the way it is consumed and not in its raw state.

Moreover, in order to evaluate the real nutritional value of a food, it is important to know not only the trace element total amount initially present but also the concentration of this element which is solubilized in intestinal lumen and subsequently will be absorbed, thus resulting, in the concept of bioaccesibility (Cámara-Martos, Marval-León, & Moreno-Rojas, 2015). In this sense, trace element bioaccesibility can be affected by a wide variety of nutritional components such as proteins (Hemalatha, Gautam, Platel, & Srinivasan, 2009; Velasco-Reynold, Navarro-Alarcón, López-G de la Serrana, Pérez-Valero, & López-Martínez, 2008), fat (Moreda-Piñeiro, Herbello-Hermelo, Dominguez-, Bermejo Barrera, & Morera-Piñeiro, 2016), dietary fiber (Moreda-Piñeiro et al., 2012), and phytic acid (Burgos, Binaghi, Ronayne de Ferrer, & Armada, 2018).

While Fe bioaccesibility and Zn bioaccesibility in cereal samples have been already studied (Gabaza, Abraha, Muchuweti, & Vandamme, 2018; Hemalatha, Platel, & Srinivasan, 2007; Singh, Prasad, & Aalbersberg, 2016), less attention has been paid to other trace elements such as Cu or Mn. The nutritional relevance of these micronutrients has been already widely reported (Cabrera-Vique & Bouz, 2009; Navarro-Alarcón, Gil-Hernandez, & Gil-Hernández, 2005; Velasco-Reynold et al., 2008;). On the other hand, it is especially important to know the effect of other dietary components (such as proteins and dietary fiber) upon these trace elements bioaccesibility.

Given the above, the objectives of this article were (a) to determine the bioaccessibility (solubility and dialysability) of trace elements (Fe, Zn, Cu, and Mn) present in eight different varieties of cooked rice cultivated in different regions around the world, in order to evaluate them nutritionally.); (b) to study the influence of processing and other food components such as protein and dietary fiber in the bioaccessibility of these elements; and (c) to assess the contributions to the dietary reference intakes (DRIs) of these micronutrients, from data obtained by consumption of these rice varieties through a probabilistic approach.

2 | MATERIAL AND METHODS

2.1 | Materials and reagents

All reagents were of analytical-reagent grade. Ultrapure water (18 MΩ/SCF) prepared with a Milli-Q Reference Water Purification (Millipore, Madrid, Spain) was used throughout experiments. All glassware was soaked in 10% nitric acid overnight and rinsed three times with deionized water prior to use. Nitric acid (HNO_3; 65%) and hydrochloric acid (HCl; 35%) were obtained from Panreac (Barcelona, Spain). Sodium bicarbonate ($NaHCO_3$; 97%) was obtained from Scharlau (Barcelona, Spain).

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

Enzymes used for fiber assays (α-amylase heat stable; protease from *Bacillus licheniformis*; amyloglucosidase from *Aspergillus niger*) were obtained from Sigma-Aldrich Co. Standard solutions for measuring the elements Fe, Zn, Cu, and Mn were prepared immediately before use by dilution with distilled deionized water of 1,000 mg/L standards (Scharlau Chemie).

2.2 | Samples

A total of eight rice varieties (*Oryza sativa* L.) belonging to the most consumed rice varieties in Argentina and Spain have been studied (see Table 1). They were purchased in shopping centers or small markets in the cities of Corrientes (Argentina) or Cordoba (Spain). Three different packets (about 1 kg per sample) belonging to different batches of each variety were purchased during 2017 in order get a representative sample. Samples were analyzed such as they are consumed in order to determine the real nutritional value. For this purpose, rice samples (50 g) were boiled in 150 ml of deionized water during 20 min, and then, water was discarded and rice samples were ground, freeze-dried and packed in polypropylene vacuum bags, until required for analyses.

2.3 | Procedure for in vitro gastrointestinal digestion

2.3.1 | Solubility assay

The procedure described by Cámara, Amaro, Barberá, and Clemente (2005) with slight modifications was used to estimate soluble trace element solubility. The simplified

TABLE 1 Brief description of studied rice varieties

Variety name	Subspecie	Origin	Description[a]
Long grain	*Indica*	Argentina	Long, slender grain rice. It is widely commercialized in Argentina
Bomba	*Japonica*	Spain	Medium grain. Grain shorter and thicker than long grain rice long. It has a soft and tender texture when cooked. It is the most variety consumed in Spain
Brown rice	*Japonica*	Spain	Medium grain. It is darker than refined because it retains part of the bran. Rich in fiber and minerals
Double Carolina	*Japonica*	Argentina	Round short grain. It does not shatter easily. It has stickiness texture
Basmati	*Indica*	India	Long grain. It has a special aroma due to a high concentration of a substance called acetylpyrroline
Parboiled	*Indica*	Spain	Husked or wholly milled rice obtained from paddy rice or husked rice steeped in water and subjected to heat treatment
Redondo	*Japonica*	Spain	Short, rounded grain rice. It contains a large amount of starch that provides a creamy texture to the medium in which it is made
Long grain	*Indica*	Spain	Long, slender grain rice. It is cultivated and grown in Spain

[a]Source: Federación Española de Nutrición (FEN). Arroz (*Oryza sativa*). http://www.fen.org.es/mercadoFen/pdfs/arroz.pdf.

process is based on mimicking the physiological conditions of the gastrointestinal tract, that is, chemical composition of digestive fluid, pH, and typical residence time for each step of the digestion process. Thus, four samples consisting of 5 g each of lyophilized sample of rice were homogenized with 22 ml of 0.1 N HCL. To acidify, the pH was adjusted to two with 6 N HCL.

To carry out pepsin–HCl digestion, 0.5 g of pepsin solution per 100 g of homogenized was added (corresponding to 0.208 g of porcine pepsin by 5 g of lyophilized sample). The mixture was then incubated for 2 hr at 37°C in a shaking water bath (HSB-2000 Shaking Bath; E-Chrom Tech CO., LTD, Taipei, Taiwan). After this time, to stop gastric digestion, the sample was maintained for 10 min in an ice bath. Following, the pH was adjusted to five by adding 1 M $NaHCO_3$ to continue with intestinal digestion step. Then, 10.3 ml of a mixture of pancreatin and bile salts (corresponding to 0.042 g of pancreatin and 0.266 g of bile salts by 5 g of lyophilized sample) was added to each test tube, which was incubated for 2 hr more. After intestinal digestion, tubes are submerged for 10 min in an ice bath to stop the action of this mixture of enzymes.

Finally, the pH was adjusted to 7.2 with 0.5 M NaOH. Aliquots of the digested sample were transferred to polypropylene centrifuge tubes (50 ml; Costar Corning Europe, Badhoevedorp, The Netherlands), and these were centrifuged for 1 hr at 3,000 *g* and 3°C (Eppendorf Centrifuge 5810 R). Then, the supernatant (soluble fraction) was collected, its organic matter was destroyed, and the trace element content was measured by atomic absorption spectrometry.

2.3.2 | Dialyzability assay

For the measurement of the dialyzable fraction, a procedure similar to solubility assay was followed to the stage of gastric digestion with pepsin. Prior to the intestinal digestion step, a dialysis bag (molecular mass cutoff 12–14,000 Å) containing 25 ml of deionized water and an amount of $NaHCO_3$ equivalent to the titratable acidity previously measured (Cámara et al., 2005) was placed in the flasks. Incubation was continued for 45 min, the pancreatic–bile salt mixture was added, and incubation was continued up to 2 hr. After incubation, the dialysis membranes were removed from the flasks and rinsed with desionized water. The dialyzable fraction was transferred to porcelain crucibles, its organic matter was destroyed, and the trace element content was measured by atomic absorption spectrometry.

2.4 | Analytical determinations

To determine total trace element content of rice samples, 0.5 g of lyophilized sample was weighed in a porcelain crucible. Each sample was analyzed four times. The samples were incinerated in a muffle furnace at 460°C for 15 hr. The ash was bleached after cooling by adding 2.5 ml of 2 N HNO_3, drying on thermostatic hotplates, and maintaining in a muffle furnace at 460°C for 1 hr more. Ash recovery was performed with 1 ml of HCl 6 N, making up to 10 ml with deionized water.

To determine trace element content in soluble and dialyzable fraction obtained in previous section, a similar procedure was followed. All tests (total content, solubility and dialyzability assays) were performed four times for each variety.

Elemental analyses were performed by flame absorption atomic spectroscopy (FAAS) with a Varian Spectra AA—50B model, equipped with standard air-acetylene flame, and single element hollow cathode lamps. The instrumental conditions for the determination are shown in Table 2.

TABLE 2 Instrumental conditions, limit of detection, limit of quantification, and analysis of certified references materials

					Certified references material (mg/kg)					
					Rice flour NIST—1568a			Mussel tissue ERM—CE278k		
Element	Wavelength (nm)	Slit width (nm)	LOD (mg/L)	LOQ (mg/L)	Certified	Found	Recovery (%)	Certified	Found	Recovery (%)
Fe	248.3	0.2	0.084	0.28	7.42 ± 0.44	7.58 ± 0.52	102	161 ± 8	174 ± 7	108
Zn	213.9	0.7	0.168	0.56	19.42 ± 0.26	20.38 ± 0.24	105	71 ± 4	74 ± 4	105
Mn	279.5	0.2	0.013	0.043	19.20 ± 1.80	18.48 ± 4.20	96	4.88 ± 0.24	4.92 ± 0.16	101
Cu	324.8	0.7	0.014	0.05	2.35 ± 0.16	2.26 ± 0.34	96	5.98 ± 0.27	6.38 ± 0.66	107

LOD: limit of detection; LOQ: limit of quantification.

The detection limit was calculated as the mean value of 30 measurements of the blanks plus three times their standard deviation. Regarding the quantification limit, it was calculated as the mean value of 30 measurements of the blanks plus 10 times their standard deviation. The accuracy and precision of the different analytical techniques used in determining trace element concentrations were validated by recovery experiments using CRMs (Table 2).

AOAC methods (2005) were used to determine protein (Micro-Kjeldahl) and dietary fiber. The protein concentration was calculated from the nitrogen values using a conversion factor of 5.95. Regarding total dietary fiber, it was determined by an enzymatic gravimetric method using amylase, protease, and amyloglucosidase. Finally, ethanol is added to precipitate the soluble fiber.

2.5 | Statistics and probabilistic assessment

The data were analyzed using SPSS 15.0 (IBM, Armonk, NY, USA). In order to validate the normality of the data obtained, the Shapiro–Wilks test was used. Later, Pearson's correlations (parametric conditions) were used for determining the dependence between variables. Significant differences were considered when $p < 0.05$.

A probabilistic model was developed to estimate the intake level for Fe, Zn, Mn, and Cu derived from consumption of these rice varieties. The model here developed followed a probabilistic approach in which variables were described by probability distributions. They were fitted to concentration data obtained in our study for each element (total element concentration and bioaccessible element concentration). Furthermore, in order to estimate the intake level, we assumed a serving of boiled rice ranging from 150 to 200 g, which was defined by an uniform distribution in the probabilistic model; meaning that all values in that range had the same probability to occur.

The probability distributions describing the Fe, Zn, Cu, and Mn concentration data were fitted by using @Risk v7.5 (Palisade, Newfield, NY, USA). Goodness of fit was assessed by using different statistical tests such as chi-square test and Akaike information criterion (AIC). These statistical tests allow researchers to give a guess of how well the fitted distribution described the observed data. In addition, the visual analysis was equally considered to assess the fit of the probability distributions to intake data. The simulation was run using 100.000 iterations for each element.

3 | RESULTS AND DISCUSSION

Total and bioaccessible concentrations for Fe, Zn, Cu, and Mn in rice samples are shown in Table 3. Moisture, protein, and dietary fiber in rice varieties are shown in Table 4.

3.1 | Iron

Fe content of studied rice samples ranged between 5.90 mg/kg of Basmati Rice and 15.3 mg/kg of Brown Rice (Table 3). As expected, the highest content of this trace element was found in Brown Rice. In fact, significant statistical differences were found ($p < 0.05$) between this and other varieties with the exception of Carolina and Redondo. These elevated concentrations for some trace elements found in rice varieties with bran, as opposed to refined varieties, have also been reported by previous studies (Pinto et al., 2016; Praveena & Omar, 2017). However, a high content in Fe does not necessarily mean a high nutritional value since Brown Rice was also the variety with the lowest Fe soluble concentration (3.79 mg/kg) as opposed to Redondo with the highest content (10.7 mg/kg). This decrease in Fe bioaccessibility may be due to its chemical composition with high levels of antinutritional factors present in bran (Madsen & Brinch-Pedersen, 2016) which negatively influence the Fe absorption (Garcia-Casal, 2006; Guansheng et al., 2005; Hunt, 2003; Hurrell, 2004; Lestienne, Caporiccio, Besançon, Rochette, & Treche, 2005)

TABLE 3 Content of Fe, Zn, Cu, Mn total, soluble, and dialyzable (mg/kg dry matter) in the rice varieties studied (mean ± standard deviation)

	Fe			Cu			Zn			Mn		
Rice variety	Total	Soluble	Dialyzable	Total	Soluble	Dialyzable	Total	Soluble	Dialyzable	Total	Soluble	Dialyzable
Long grain Argentina	7 ± 2	6.3 ± 0.9	4.75 ± 0.14	5.6 ± 1.5	1.8 ± 0.5	0.35 ± 0.03	11.2 ± 0.8	1.8 ± 0.5	0.33 ± 0.13	8.2 ± 0.9	2.2 ± 0.6	0.36 ± 0.08
Brown rice	15 ± 3	4 ± 1	4 ± 3	4 ± 2	1.0 ± 0.5	0.5 ± 0.2	13 ± 2	0.87 ± 0.06	0.26 ± 0.05	14 ± 2	1.00 ± 0.15	0.15 ± 0.03
Double Carolina	13.4 ± 1.4	9 ± 3	5 ± 3	2.8 ± 1.4	1.4 ± 0.5	0.9 ± 0.6	10.6 ± 1.3	2.1 ± 0.4	0.3 ± 0.2	11 ± 2	2.5 ± 0.4	0.64 ± 0.02
Basmati	5.9 ± 1.4	4.7 ± 1.2	7 ± 2	3.4 ± 1.5	0.9 ± 0.2	0.5 ± 0.3	10.1 ± 1.2	1.2 ± 0.3	0.15 ± 0.09	11 ± 3	0.38 ± 0.02	0.37 ± 0.09
Parboiled	6.5 ± 0.8	6 ± 2	8 ± 4	2.0 ± 0.3	1.3 ± 0.1	0.7 ± 0.2	8.8 ± 1.4	1.3 ± 0.3	0.22 ± 0.02	2.5 ± 0.2	0.40 ± 0.10	0.57 ± 0.10
Bomba	7.6 ± 0.3	6.4 ± 1.1	8 ± 3	1.5 ± 0.4	1.2 ± 0.5	0.6 ± 0.4	10 ± 3	3.25 ± 0.11	1.2 ± 1.1	4.5 ± 0.2	0.77 ± 0.12	0.47 ± 0.11
Redondo	13 ± 7	10.7 ± 0.3	6 ± 3	1.6 ± 0.6	1.1 ± 0.9	0.06 ± 0.04	11 ± 2	2.5 ± 0.3	1.3 ± 1.2	11 ± 3	1.6 ± 0.6	0.15 ± 0.09
Long grain Spain	9 ± 2	8 ± 3	5.6 ± 1.2	2.1 ± 1.2	0.6 ± 0.3	0.19 ± 0.07	10 ± 2	2.12 ± 0.06	1.1 ± 0.3	10.0 ± 1.4	0.53 ± 0.12	0.28 ± 0.08

TABLE 4 Content of protein, dietary fiber (mg/100 g dry matter) and moisture (%) in the rice varieties studied (mean ± standard deviation)

Rice variety	Moisture	Protein	Total dietary fiber
Long grain Argentina	73.5 ± 0.1	7.2 ± 0.1	5.2 ± 1.0
Brown rice	58.9 ± 0.4	7.4 ± 0.4	8.2 ± 0.2
Double Carolina	71.7 ± 0.8	5.4 ± 0.4	5.5 ± 1.0
Basmati	69.8 ± 0.6	8.5 ± 1.0	8.3 ± 0.9
Parboiled	50.8 ± 0.7	6.9 ± . 0.3	10.7 ± 0.1
Bomba	50.1 ± 0.6	7.2 ± 0.1	10.9 ± 1.0
Redondo	70.6 ± 0.5	7.5 ± 0.1	5.8 ± 0.9
Long grain Spain	50.8 ± 0.9	7.3 ± 0.4	5.9 ± 0.4

However, with the exception of Brown Rice, the rest of the samples studied showed a proper Fe solubility percentage with average values around 80%. These high solubility percentages may be caused by cooking because rice samples were properly boiled. Processing techniques like boiling reduce the levels of antinutritional organic factors present in grain, which include oxalates, phenols, tannins, enzyme inhibitors, and phytates (with strong trace element binding properties, thereby decreasing their bioavailability; Mohan, Tresina, & Daffodil, 2016).

On the other hand, the dialyzable Fe fraction ranged from 3.98 mg/kg for Brown Rice to 8.26 mg/kg for Bomba. Once again, Brown Rice showed the lowest bioaccessible percentage. A significant statistical negative correlation was found between soluble Fe and protein content ($r = -0.409$; $p < 0.05$) but not between dialyzable Fe and protein content. Although several studies have already documented a positive effect of animal protein upon Fe bioaccessibility (Ramírez-Ojeda, Moreno-Rojas, Sevillano-Morales, & Cámara-Martos, 2017; Storcksdieck, Bonsmann, & Hurrell, 2007), the effect with other protein sources is less clear (such as vegetable proteins). There are some studies reporting a negative effect (Berner & Miller, 1985) while other authors have also shown this positive effect on Fe bioaccessibility (Joshi, Thatte, Prakash, & Jyothi, 2014; Lombardi-Boccia, Carbonaro, Di Lullo, & Carnovale, 1994,). In our study, we only found this negative correlation between vegetable proteins of rice and Fe solubility.

Finally, a significant statistical positive correlation was found between soluble Fe (mg/kg)—soluble Zn (mg/kg) ($p < 0.01$; $r = 0.576$). Although, in this case, this correlation between both variables is low, similar interactions have already been found in several studies with lentils and beans (Ramírez-Ojeda, Moreno-Rojas, & Cámara-Martos, 2018) and may justify the way that many dietary factors that promote or impair Fe bioaccessibility do so with Zn.

Similarly, another significant statistical positive correlation was also found between dialyzable Fe (mg/kg) and dialyzable Cu (mg/kg; $p < 0.05$; $r = 0.452$). This synergistic effect between both elements has also been reported in other in vitro studies with a food matrix such as legumes (lentils and chickpeas) (Ramírez-Ojeda et al., 2018), weaning foods (Ramírez-Ojeda et al., 2017), hospital meals (Velasco-Reynold et al., 2008), and school menus (Cámara, Barberá, Amaro, & Farré, 2007). The mechanism for this positive interaction remains unclear; however, this strong correlation between both elements has just been reported for in vivo models. This is due to the influence of Cu on Fe metabolism and hemoglobin biosynthesis (Collins, Prohaska, & Knutson, 2010; Gulec & Collins, 2014; Ha, Doguer, Wang, Flores, & Collins, 2016). Decreased Cu status has been shown to reduce holo-ceruloplasmin production and impair ferroxidase activity, leading, in a number of cases, to decreased tissue Fe release and the generation of anemia that is responsive to dietary supplementation with Cu but not Fe (Sharp, 2004)

3.2 | Zinc

Total Zn content in samples ranged between 8.82 mg/kg for Parboiled Rice and 12.9 mg/kg for Brown Rice (Table 3). Concentration values obtained in the present study are in agreement with those reported in previous studies for Brown (15.9 mg/kg) and Parboiled Rice (6.1 mg/kg) (Pinto et al., 2016). Similarly to Fe, Brown variety presented the highest content for Zn, revealing how the cereal milling process implies trace element losses (Liu, Zheng, & Chen, 2017; Persson, Hansen, Laursen, Husted, & Schjoerring, 2011). It has been shown that bran cereals contain considerable amounts of Zn (around 60 mg/kg) (Kamal–Eldin, 2016) which can contribute considerably to daily recommended intake (DRI) of this micronutrient. Nonetheless, this high Zn content present in Brown Rice involved at the same time, the lowest Zn concentration soluble (0.87 mg/Kg), as opposed to Bomba variety which showed the highest one (3.25 mg/kg). Once again, cereal bran is a rich source of antinutritional components (Madsen & Brinch-Pedersen, 2016) which can form strong complexes with divalent cations during processing (Gharibzahedi & Jafari, 2017) impairing Zn bioaccesibility.

The dialyzable Zn fraction ranged from 0.15 mg/kg for Basmati Rice to 1.24 mg/kg for Bomba Rice. A significant statistical negative correlation was found between Zn bioaccessibility (soluble) and vegetable proteins ($r = -0.409$; $p < 0.05$) in studied rice varieties. In general, Zn bioaccessibility percentages were not high, with values that did not exceed 15% (solubility assay) and 3% (dialyzability assay) for most studied varieties.

3.3 | Copper

Regarding Cu, the highest total contents of this trace element corresponded to Long grain Argentina (5.59 mg/kg) and Brown Rice (4.22 mg/kg). In fact, significant statistical differences were found ($p < 0.05$) between these two varieties and others that were studied with the exception of Carolina and Basmati. The variety with the lowest total Cu content was Bomba (1.45 mg/kg; see Table 3). These values are in agreement with those reported by previous research such as 0.50–3.3 mg/kg (Islam, Ahmed, & Habibullah-Al-Mamun, 2014) and 1.90 mg/kg (Kumari & Platel, 2017).

Bioaccessibility of Cu varied significantly among the rice varieties examined, ranging from 24% (Brown Rice) to 80% (Bomba Rice) in solubility assay and from 4% (Redondo Rice) to 41% (Bomba Rice) in dialyzability assay. In comparison with other vegetable foods, rice presented a high Cu bioaccessibility for most studied varieties which has already been found in previous studies (Kumari & Platel, 2017).

3.4 | Manganese

Total Mn concentration in samples showed values from 2.45 mg/kg in Parboiled Rice to 13.6 mg/kg for Brown Rice (Table 3). In fact, a significant statistical difference ($p < 0.05$) was found between the highest concentration and the rest of studied varieties. Results reported in this study are in agreement with those found in previous studies such as 6.20 mg/kg (Manjusha, Dash, Karunasagar, & Arunachalam, 2008) or 6.24 mg/kg (Kumari & Platel, 2017). Furthermore, it was also found for this trace element that the varieties which presented the highest Mn concentrations also showed high concentration for the rest of analyzed trace element. Thus, positive correlations were found between total Mn content (mg/kg) and total Fe content (mg/kg; $p < 0.01$; $r = 0.475$); total Mn content (mg/kg) and total Zn content (mg/kg; $p < 0.05$; $r = 0.434$); and finally total Mn content (mg/kg) and total Cu content (mg/kg; $p < 0.05$; $r = 0.402$).

Regarding bioaccessible concentrations, in the case of soluble Mn, the values ranged between 0.35 mg/kg for Basmati Rice and 2.52 mg/kg for Carolina Rice, with significant statistical differences ($p < 0.05$) between the highest value and all the rest of variety values. In relation to dialyzable Mn, the values obtained were from 0.15 mg/kg for Brown Rice to 0.64 mg/kg for Double Carolina. Although, in the present study, a negative interaction was found between fiber content in studied rice varieties and trace element bioaccessibility for Fe and Zn, this interaction was statistically significant for soluble Mn (mg/kg) and fiber content (g/100 g) ($r = -0.747$; $p < 0.01$). Similarly, a significant statistical negative correlation ($r = -0.475$; $p < 0.01$) was also found between soluble Mn (mg/kg) and vegetable protein content (g/100 g).

3.5 | Probabilistic assessment

A probabilistic model approach was developed to estimate the intake level of Fe, Zn, Cu, and Mn, which derive from consumption of a serving of boiled rice (150–200 g). For this purpose, trace element concentrations were moved from dry matter to fresh matter. Dietary reference intakes (DRI) for Spanish and American adult population (20–59 years old) were considered for Fe (men, 9 mg/day; women, 18 mg/day), Zn (men, 9.5 mg/day; women, 7 mg/day), Mn (men, 2.3 mg/day; women, 1.8 mg/day) (FESNAD, 2010), and Cu (900 µg/day) (United States Department of Agriculture (USDA), 2011). The models were developed from values of total and bioaccessible (soluble) trace element content. It should also be noted that the present statistical tool was completed using the variability of inorganic element present in food as well as the variability of the rice variety ingested. Furthermore, as indicated in the materials and methods section, we assumed a serving of boiled rice ranging from 150 to 200 g, which was defined by an uniform distribution in the probabilistic model. Both aspects determine the total amount of inorganic element ingested.

Regarding Fe, total trace element concentrations were fit to gamma distribution (AIC = 140,079.8; $\chi^2 = 51.14$; Figure 1a). Thus, results derived from the simulation of the probabilistic model indicated that the intake level of Fe through the consumption of a serving of boiled rice would be below 1.16 mg for the 95% of adult population (13% of DRI for men and 6.4% of DRI for women) and does not reach 0.58 mg for the 50% of population (3.2% of DRI for women). This shows that studied rice varieties are not a good source of Fe and it would be advisable to eat this cereal with other ingredients rich in Fe such as meat (Czerwonka & Tokarz, 2017) or some fish species (Bilandžić et al., 2018). Regarding Fe bioaccesible concentrations (Fe soluble and available to be absorbed by the enterocytes), data were fit to Weibull distribution (AIC = 133,806.0; $\chi^2 = 77.20$; Figure 2a). According to this model, 95% of adult population would not exceed Fe intake of 0.80 mg (4.4% of DRI for women at best).

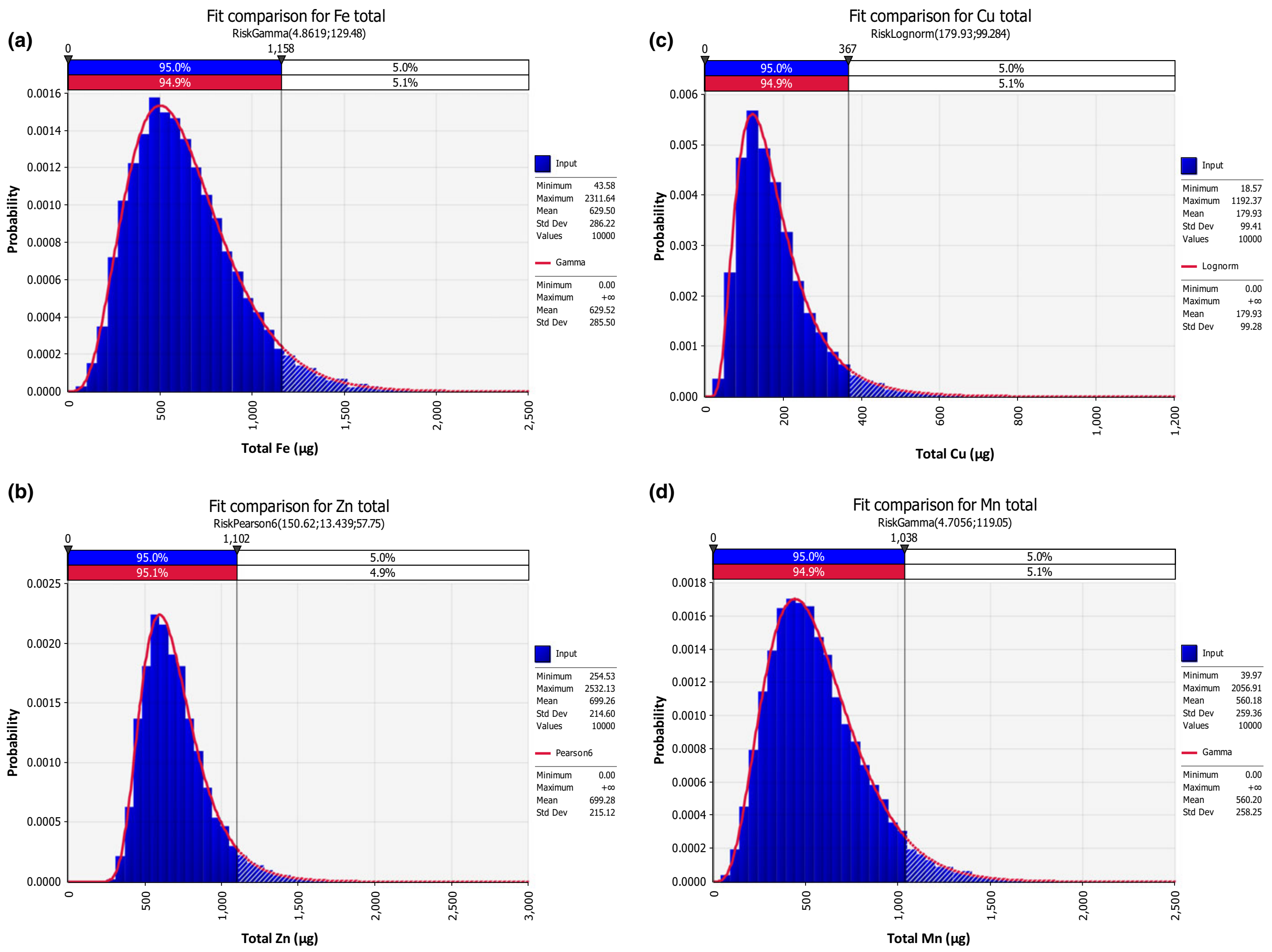

FIGURE 1 Simulated data and fitted probabilistic distribution for Fe (a); Zn (b); Cu (c); and Mn (d) total content [Color figure can be viewed at wileyonlinelibrary.com]

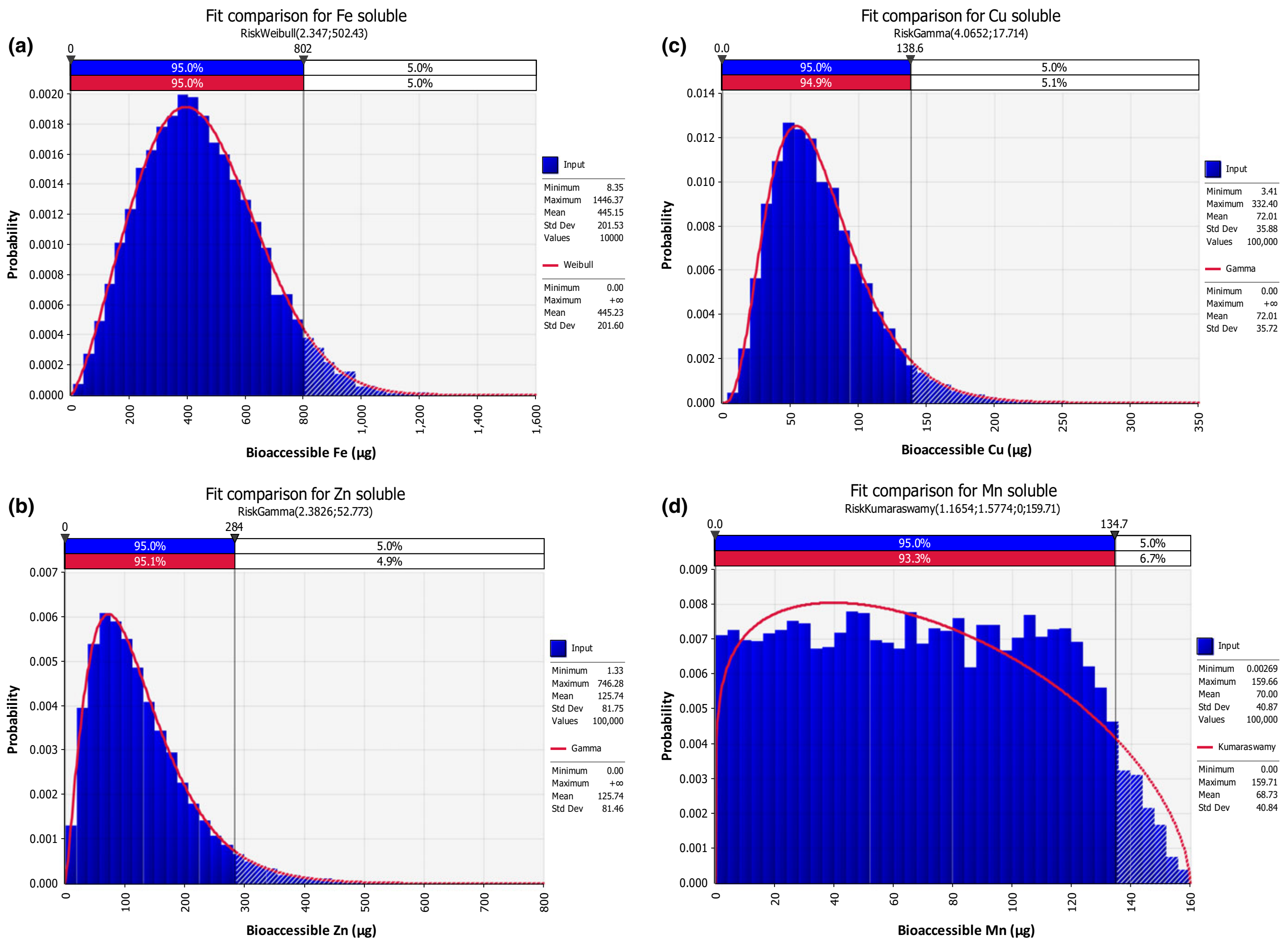

FIGURE 2 Simulated data and fitted probabilistic distribution for Fe (a); Zn (b); Cu (c); and Mn (d) bioaccessible content [Color figure can be viewed at wileyonlinelibrary.com]

Zn total concentrations were fit to risk Pearson distribution (AIC = 133,532.1; χ^2 = 56.30; Figure 1b). Results were very similar to the Fe model. Thus, Zn total intakes are below 0.66 mg for the 50% of population (50th percentile) and do not exceed 1.09 mg for the 95% (95th percentile). For this reason, rice should be accompanied with other ingredients such as meat or fish which are proper sources of this element (Bilandžić et al., 2018). In the same way, Zn soluble concentrations were fit to gamma distribution (AIC = 113,277.8; χ^2 = 44.21; Figure 2b) where Zn intakes do not pass 0.28 mg for the 95% of population (around 3% of DRI for men).

Better results were obtained to Cu, since total concentrations for this trace element were fit to Lognormal distribution (AIC = 116,293.2; χ^2 = 48.64; Figure 1c). It is found that intake between 150 and 200 g of these rice varieties provide 158 μg of Cu (18% DRI) and 367 μg of Cu (41% DRI) for 50th and 95th percentile, respectively. Regarding Cu bioaccessible, data were fit to gamma distribution (AIC = 98,208.6; χ^2 = 71.24; Figure 2c). According to this model, Cu intake for 95th percentile is 139.4 μg (around 16% of DRI at best). This value could be considered as acceptable for bioaccessibility measurements.

Finally, Mn total concentrations were fit to gamma distribution (AIC = 137,987.2; χ^2 = 59.53; Figure 1d). Results indicate that the intake level of Mn is 0.52 mg for 50th percentile and 1.04 mg for 95th percentile (in this last case covering around 60% DRI of this micronutrient for women). In this sense, rice varieties studied can be considered a proper dietary source of Mn. However, considering bioaccessibility values (soluble Mn), they were fit to betageneral distribution (AIC = 100,424.1; χ^2 = 300.37 (Figure 2d). Mn intake does not reach 0.14 mg to 95th percentile. This means that Mn present in rice varieties has a poor bioaccessibility and it would not be a proper source for this trace element.

4 | CONCLUSIONS

Trace element analysis of the eight rice varieties studied revealed that Brown Rice was the variety with the highest trace element content having at the same time the lowest bioaccessibility. This means that, although rice bran may be an important source of many trace elements, the presence of side components in bran may impair this nutritional value. On the other hand, a negative statistically significant interaction of rice fiber upon Mn bioaccessibility was observed. A negative effect caused by vegetable proteins was observed for most trace elements' bioaccessibility (Fe, Zn and Mn); however, this correlation was low. Finally, the probabilistic assessment used to determine the contribution of DRI for Fe, Zn, Mn, and Cu through the intake of 150–200 g of boiled rice showed that this food can be a proper source of Cu and to a lesser extent Mn but not in the case of Fe and Zn.

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How to cite this article: Ortiz ML, Cámara-Martos F. Bioaccessibility and total content of iron, zinc, copper, and manganese in rice varieties (*Oryza sativa* L.): A probabilistic assessment to evaluate their contribution to dietary reference intake. *Cereal Chem*. 2018;95:790–799. https://doi.org/10.1002/cche.10094