

Evaluation of the Bioaccessibility of Ca, Fe, Mg and Mn in Ground Coffee Infusions by *in vitro* Gastrointestinal Digestion

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Determinou-se a bioacessibilidade de Ca, Mg, Mn e Fe em infusões de café em pó através de digestão gastrointestinal *in vitro* com soluções de suco estomacal e intestinal (pepsina, pancreatina e sais biliares). A absorção de metais nas vilosidades intestinais foi simulada por ultrafiltração com uma membrana semi-permeável de 3 kDa de massa molar de corte. As concentrações de Ca, Fe, Mg e Mn em infusões de café e permeados de incubados gastrointestinais foram medidas por espectrometria de absorção atômica com chama. Estabeleceu-se que Mg, com uma contribuição de 62% à fração bioacessível, foi o elemento mais bioacessível. As bioacessibilidades de Ca (42%) e Fe (43%) foram similares, mas os resultados individuais de ambos os metais variaram amplamente. O metal menos bioacessível foi o Mn, com uma contribuição média de 27%. Beber uma xícara de café diariamente cobre minimamente os valores diários de referência de Ca, Fe, Mg e Mn (menos que 3%).

The bioaccessibility of Ca, Mg, Mn and Fe in ground coffee brews was assessed through *in vitro* gastrointestinal digestion with stomach and intestinal juice solutions (pepsin, pancreatin and bile salts). Absorption of metals in the intestinal villi was simulated by ultrafiltration over a 3 kDa molecular weight cut-off semi-permeable membrane. Concentrations of Ca, Fe, Mg and Mn in coffee infusions and permeates of gastrointestinal incubates were measured by flame atomic absorption spectrometry. It was established that Mg, with a 62% contribution to the bioaccessible fraction, was the most bioaccessible. The bioaccessibilities of Ca (42%) and Fe (43%) were similar but individual results for both metals varied widely. The least bioaccessible metal was Mn, with an average contribution of 27%. Drinking one cup of coffee daily covers dietary reference intakes of Ca, Fe, Mg and Mn to a small degree (less than 3%).

Keywords: ground coffee, bioaccessibility, *in vitro* gastro-intestinal digestion, calcium, iron, magnesium, manganese, FAAS

Introduction

A great many people are habitual consumers of ground coffee.¹ Information about total concentrations of different elements, i.e., minerals, micronutrients and trace elements, in ground coffee is commonly used to evaluate its quality and safety.²⁻⁴ Total concentrations of elements in ground coffees are considered in evaluating their nutritional value and the ability of coffee brews to cover daily dietary intake requirements of various elements.⁵⁻⁷ However, this is not entirely justified when considering the real uptake of elements from coffee or its role in human nutrition. Elements, especially metals, may exist in ground coffee infusions as either simple or complexing ions of different

stability. Various low, moderate and high molecular mass endogenous bioligands, e.g., phenolic acids and flavonoids, may willingly bind metal ions through hydroxyl, carboxylate and phenolate *O*-donor groups.⁸ In particular, the presence of the latter species can affect the extent of transfer of metals from coffee powder into infusions during brewing because they have different solubilities.⁹ Having different bioavailabilities, these species may also modify the uptake of elements from coffee by the human body. Surprisingly, the issue of the bioavailability and the bioaccessibility of elements from coffee and the extent of their absorption through the gastrointestinal tract is not firmly established in Polish and foreign literature.

Assessing the bioaccessibility of elements in different food products and beverages *in vivo* seems to be the best avenue, but such an approach is expensive, demanding

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and sometimes questionable.¹⁰ More frequently, *in vitro* simulations of processes occurring in the gastrointestinal tract, i.e., the digestion of food product and beverage components and absorption of the species released by intestinal villi, are preferred. Several approaches to such *in vitro* simulations have been described in the literature.^{11,12} The most popular is a two-step incubation that simulates stomach and intestinal digestions using enzyme-based solutions including amylase, pepsin, pancreatin, bile salts and inorganic compounds,¹³ or, if accessible, normal gastric juices.¹⁴ Samples are incubated under conditions selected to mimic gastric and intestinal peristalsis, e.g., a temperature of 37 °C with gentle shaking. After *in vitro* digestion, absorption of elements in the villi is simulated by ultrafiltration over membranes having molecular weight cut-offs (MWCOs) of 1.3 or 5 kDa.¹⁴ The process can also be mimicked by extraction with cell culture biomembranes^{13,15} or dialysis.¹⁶⁻¹⁸ To date, the bioaccessibility of essential elements has been assessed in a number of different food products, beverages and stimulants, including fruit juices,^{15,19} tea,¹⁴ herbal decoctions,¹³ different milks,^{16-18,20} baby foods,²¹ wines,²² cereals,¹² seafood and fishes,¹² cocoa powders,¹² wheat,²³ white cheese and bread,²⁴ fruits and vegetables,^{12,24} whole meals and fast-foods,²⁵ different kinds of meat,²³ and honey.²⁶

It is recognized that coffee constituents, mostly phenolics and melanoidins, chelate some nutrients, particularly Fe and Zn, and are responsible for an increased inhibition of the absorption of these metals from diet in which coffee is included.^{27,28} On the other hand, coffee brews represent an important source of dietary Mg.²⁸ A statistically significant association between coffee consumption and a lower risk of type 2 diabetes mellitus reinforces the role of coffee and an intake of Mg with this beverage.²⁹ However, it is difficult to opine that the total amount of Mg determined in the coffee brew is entirely available to the human body as no such research has been carried out so far.

For all reasons stated above, the information on the bioaccessibility of physiologically important nutrients in coffee infusions seems to be important. The objective of this study was to determine the contributions of the bioaccessible fractions of four important micronutrients, i.e., Ca, Fe, Mg and Mn, in brews of 13 roasted and ground coffees commercially available in the Lower Silesia region of Poland under gastrointestinal conditions. Towards this aim, a two-step extraction procedure simulating digestion by gastric and intestinal juices followed by separation of the smallest molecular weight fraction (< 3 kDa) of elemental species by filtration of the gastrointestinal digests of coffee infusions through a semi-permeable membrane with a 3 kDa MWCO was used.

Experimental

Reagents and samples

ACS grade solutions of 30% (m/m) H₂O₂, 37% HCl and 65% (m/m) HNO₃ were supplied by J. T. Baker (Deventer, the Netherlands). Pepsin from porcine gastric mucosa (800-2500 units mg⁻¹ of protein), pancreatin from porcine pancreas, bile salts, NaHCO₃, as well as TraceCERT® single-element 1000 mg L⁻¹ stock standard solutions of Ca, Fe, Mg and Mn were provided by Sigma-Aldrich Chemie GmbH (Steinheim, Germany). For gastrointestinal digestion, solutions of simulated gastric (SGJ) and intestinal (SIJ) juices were prepared using solid reagents. They contained 0.20 g L⁻¹ of pepsin in a 0.10 mol L⁻¹ HCl solution (SGJ) and 0.15 g L⁻¹ of pancreatin with 1.50 g L⁻¹ of bile salts in a 0.10 mol L⁻¹ NaHCO₃ solution (SIJ). The content of the simulated gastrointestinal juices was selected based on procedures previously used for bioaccessibility assays of selected metals in milk,²⁰ wines,²² and honey.²⁶ Because coffee is consumed as a fluid, which is not broken down in the oral cavity, it was decided to omit any simulation of digestion in the mouth by saliva, as proposed by other authors in the case of fruit juices.¹⁹

Stock standard solutions of Ca, Fe, Mg and Mn were used to prepare and matrix-match (in reference to levels in SGJ and SIJ solutions) four-element standard solutions for calibration. Re-distilled water was used in all preparations.

Eleven of the most popular ground roasted coffees (C1-C11) and two roasted bean coffees (C12 and C13) commercially available in Poland were selected for the study.

Instrumentation

A Perkin Elmer (Waltham, MA, USA) single-beam flame atomic absorption spectrometer (FAAS), model 1100B, was used to measure concentrations of Ca, Fe, Mg and Mn in sample solutions, i.e., coffee digests, infusions and permeates. It was equipped with a single-slot burner head for a very sharp air-acetylene-lean flame and a premix burner chamber with a standard nebulizer, an end cup, and a drain interlock assemblage. A flow spoiler immersed into the chamber was applied to discriminate the size of the droplets formed in solution aerosol. All instrumental settings, including flow rates of air and acetylene, analytical wavelengths, slits and lamp currents, were consistent with the recommendations of the instrument manufacturer given for determinations of Ca, Fe, Mg and Mn. Absorbances were measured using a time-average integration mode: three readings were integrated at 0.1 s intervals over a 3 s

integration time and averaged. Five multi-element standard solutions were used for calibration. The concentrations of metals in these solutions were in the following ranges: 0.05-2.00 mg L⁻¹ (Ca, Fe), 0.01-2.00 mg L⁻¹ (Mg), and 0.05-1.00 mg L⁻¹. Instrumental detection limits (IDLs) of 0.012, 0.018, 0.001 and 0.006 mg L⁻¹ were assessed for Ca, Fe, Mg and Mn, respectively.

A Sartorius AG Vivaflow 50 (Goettingen, Germany) disposable plug and play laboratory cross-flow cassette equipped with a polyethersulfone (PES) membrane with a 3 kDa MWCO was used to separate the bioaccessible fractions (< 3 kDa) of Ca, Fe, Mg and Mn from incubates of coffee infusions by ultrafiltration. A standard Cole-Parmer (Vernon Hill, IL, USA) 2-channel MasterFlex L/S peristaltic pump was used to maintain a flow rate of 200-400 mL min⁻¹ (max. 4 bars) through the cassette.

An Elpin (Lubawa, Poland) thermostatic water bath shaker (type 357) was used in incubation experiments. An MPW-350 centrifuge (MPW Medical Instruments, Warsaw, Poland) was used to separate incubates of ground coffees treated with SGJ and SIJ solutions.

Preparation of coffee brews

To prepare brews of ground roasted coffees, 7.2 g samples (two spoons on average) were placed in 400 mL Pyrex glass beakers, on which were poured 200 mL of boiling re-distilled water with stirring. After 10 min of brewing, the infusions were separated from the settled grounds by filtrating them through qualitative cellulose filter papers (thickness 150 mm). In the case of roasted bean coffees (C12 and C13), the beans were ground first in a manual coffee mill. Three independent infusions were prepared from each coffee brand. In addition, procedural/reagent blanks were prepared. The infusions were split; 20 mL portions were analysed by FAAS for total concentrations of Ca, Fe, Mg and Mn, another 40 mL aliquots were subjected to *in vitro* simulated gastrointestinal digestion prior to evaluating the contribution of the bioaccessible fraction of each studied metal.

Total concentrations in ground coffees and coffee brews

Total concentrations of Ca, Fe, Mg and Mn in analysed ground coffees were determined as described elsewhere with a small modification.³⁰ Accordingly, samples of ground coffees (1.0 g) were placed in 250 mL Pyrex glass beakers to which were added 5.0 mL of a concentrated HNO₃ solution for predigestion (12 h). Then, 5.0 mL of HNO₃ were added and the samples were digested at 80-90 °C on a temperature-controlled hot plate. After evaporating half

the volume of each sample solution, 5.0 mL of a 30% H₂O₂ solution were added and digestion was continued. Finally, the sample solutions were evaporated to near dryness, after which the digests were reconstituted with 10 mL of water, transferred quantitatively to 25 mL Pyrex glass volumetric flasks and topped with water to the mark.

Concentrations of Ca, Fe, Mg and Mn in the coffee infusions were determined using a recently developed simplified method of analysis.³⁰ Ten millilitre samples of coffee infusions were acidified with 1.0 mL of a concentrated HNO₃ solution and directly analysed by FAAS. For each coffee brand, three independently prepared solutions of digested ground coffee samples and infusions of this coffee were analysed by FAAS. Simple standard solutions and reagent blanks were used for calibration. Concentrations of Fe and Mn were measured in undiluted sample solutions. In the case of Ca and Mg, the sample solutions were diluted either 10 and 20 fold (Ca) or 50 and 100 fold (Mg) prior to measurements.

In vitro gastrointestinal digestion of coffee brews

Aliquots of coffee infusions (40 mL) were placed in 100 mL polypropylene (PP) sample tubes and 5.0 mL of an SGJ solution were added for *in vitro* simulation of gastric digestion. Sample tubes were capped with polyethylene (PE) screw caps and placed in a temperature-controlled shaking water bath. Incubation of coffee infusions was carried out at 37 °C with agitation (250 rpm) and lasted 2 h. After that, the enzymatic reaction was stopped by placing the sample tubes in an ice bath. To mimic intestinal conditions, the pH of gastric digests was increased to 7.0 (close to intestinal pH) by adding few drops of a 0.10 mol L⁻¹ NaHCO₃ solution. Then, 5.0 mL of an SIJ solution were added. The sample tubes were capped and incubation was continued at 37 °C with agitation (250 rpm) for the next 2 h. When the incubation was over, the enzymatic reaction was stopped by placing the sample tubes in an ice bath. The final incubates were filtered over 0.45 µm cellulose membrane filters and then through 3 kDa Vivaflow 50 cross-flow ultrafiltration cassettes. The initial portions of the permeates (about 5 mL), corresponding to the dead volumes of the ultrafiltration cassettes and delivery tubes, were discarded. Next, three 5.0 mL portions of permeates were collected. This is schematically shown in Figure 1. Concentrations of Ca, Fe, Mg and Mn were measured in these permeates after subsequent acidification with a concentrated HNO₃ solution (0.5 mL of acid *per* 5.0 mL of permeate). The bioaccessible fraction contribution was calculated as $100 \times B / T$, where B is the concentration of element (in mg L⁻¹) in the bioaccessible fraction (the ultrafiltration

permeate resulting from passing gastrointestinal digests through 3 kDa ultrafiltration cassettes) and T is the concentration of element (in mg L^{-1}) in the infusion. In a similar way, the bioaccessibility of the selected elements was previously assessed for other beverages, i.e., tea infusion¹⁴ and orange juice,¹⁹ where concentrations of elements found in the 3 kDa ultrafiltrates were related to their total concentrations determined in both beverages.

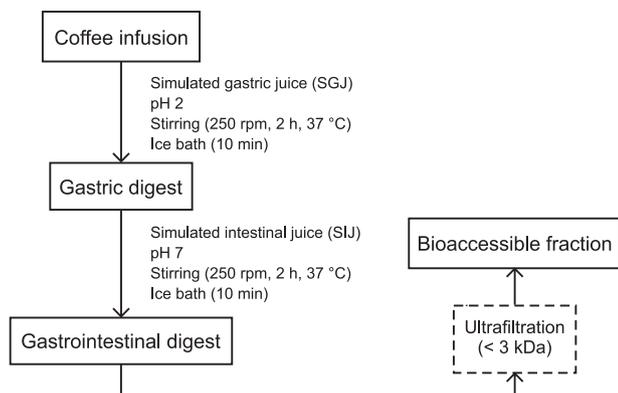


Figure 1. A scheme of the *in vitro* gastrointestinal digestion of coffee infusions and the determination of the bioaccessible fraction of Ca, Fe, Mg and Mn.

For each coffee brand, gastrointestinal extraction was repeated twice using freshly prepared coffee infusions. The collected portions ($n = 6$) of permeates (the “< 3 kDa” fraction) were analysed by FAAS. As before, the concentrations of Fe and Mn were measured in undiluted portions of the permeates using matrix-matched standard solutions. The concentrations of Ca and Mg were measured in diluted permeates, and simple standard solutions were used for the calibration. The results were averaged and corrected for values of procedural blanks. The contributions by the bioaccessible fractions of Ca, Fe, Mg and Mn were calculated as ratios of the concentration of each metal that passed through the 3 kDa ultrafiltration membrane (the “< 3 kDa” fraction) to its concentration in the infusion.

Results and Discussion

Optimisation of *in vitro* gastrointestinal digestion of coffee brews

At the outset, we tried to find minimal concentrations of reagents in the solutions of simulated gastric and intestinal juices that would provide the best performance in both digestion steps and reduce the risk of contamination and possible interference during measurements of Ca, Fe, Mg and Mn by FAAS. In addition, the effect of incubation time was studied. Accordingly, three different SGJ and SIJ

solutions were used, i.e., 0.20, 0.40 and 1.0 g L^{-1} pepsin in 0.10 mol L^{-1} HCl (SGJ1, SGJ2 and SGJ3, respectively), 0.15 g L^{-1} pancreatin with 1.5 g L^{-1} bile salts in 0.10 mol L^{-1} NaHCO_3 (SIJ1), 0.30 g L^{-1} pancreatin with 3.0 g L^{-1} bile salts in 0.10 mol L^{-1} NaHCO_3 (SIJ2) and 0.75 g L^{-1} pancreatin with 7.5 g L^{-1} bile salts in 0.10 mol L^{-1} NaHCO_3 (SIJ3). Incubations with each digestion step lasted 1, 2 or 3 h. All experiments were carried out using a single ground coffee, i.e., C10. Infusions of this coffee were prepared and subjected to gastrointestinal digestion. Concentrations of Ca, Fe, Mg and Mn were determined by FAAS in permeates (the “< 3 kDa fraction”) obtained after ultrafiltration of incubates and were referred to as the bioaccessible fraction.

It was found that except for Ca, differences in the contributions of the bioaccessible fractions of Ca, Fe, Mg and Mn assessed using SGJ and SIJ solutions containing different concentrations of enzymes and bile salts were all within the range from -7.5 to $+6.7\%$. Accordingly, contributions of the bioaccessible fraction of studied elements were varied in the following ranges: 33.2–46.9% (Fe), 55.1–62.6% (Mg) and 27.0–37.7% (Mn). In the case of Ca, it was observed that the most concentrated SGJ and SIJ solutions decreased the contribution by the bioaccessible fraction of this metal by about 28%. This was possibly related to complexation of Ca by enzymes. The precision of the results (expressed as relative standard deviation, RSD) was fair, and varied from 8.6 to 22% for Ca, from 21 to 62% for Fe, from 1.3 to 21% for Mg and from 19 to 59% for Mn. This could be associated with the low concentrations of the measured metals, particularly Fe and Mn, in the collected permeates. The effect of different particle sizes of coffee powder may also contribute to the noted variations in the concentrations of metals in the prepared infusions. However, to imitate domestic conditions, all ground coffees were analysed as consumed; they were not additionally ground and sieved to select a uniform narrower mass fraction. Taking into account the precision of the determinations of Ca, Fe, Mg and Mn, it was concluded that increasing the concentrations of reagents in the SGJ and SIJ solutions was practically insignificant for extraction of these metals. It was further established that a shorter incubation time (1 h) for each digestion step was insufficient to extract Fe and Mn. Contributions of the bioaccessible fractions of these metals were about 20% lower compared to those obtained during 2 h incubations, i.e., 40.2% for Fe and 34.1% for Mn. When each digestion step was longer, i.e., 3 h, it was found that bioaccessibilities of Ca, Fe and Mn are slightly changed (respectively by -9 , $+6$ and -7%), but the bioaccessibility of Mg is diminished by 41%.

For all these reasons, the solutions of lowest concentration, i.e., SGJ1 (0.20 g L^{-1} pepsin in 0.10 mol L^{-1} HCl) and SIJ1 (0.15 g L^{-1} pancreatin and 1.5 g L^{-1} bile

salts in 0.10 mol L⁻¹ NaHCO₃), were used for enzymatic digestion. An incubation time of 2 h seemed to be the most optimal, as previously reported by Hur *et al.*³¹ In addition, the validity of the results obtained with the aforementioned *in vitro* gastrointestinal digestion was confirmed. Standard solutions of Ca, Fe, Mg and Mn (all at 1.0 mg L⁻¹) were subjected to the complete procedure and the collected permeates were analysed by FAAS. It was found that average recoveries (n = 3) of Ca, Fe, Mg and Mn were 97.8 ± 5.0, 101 ± 1, 108 ± 12 and 96.7 ± 4.1%, respectively.

Bioaccessibility of Ca, Fe, Mg and Mn from coffee brews

As a plant-origin product, coffee has a complex biological matrix. It contains phenolic compounds,¹ high and low molecular weight melanoids,^{32,33} caffeine, trigonelline, nicotic, acetic, formic, malic, citric, lactic and quinic acids, soluble fibres, pectins and small amounts of phytic acid.¹ Hence, the possibility of release of these compounds during gastrointestinal digestion of a coffee brew is great; the aforementioned groups of compounds may also alter the uptake of metals, e.g., render them unavailable for absorption.^{34,35}

Table 1 presents concentrations of Ca, Fe, Mg and Mn in infusions of ground coffees and bioaccessible fractions

(“< 3 kDa”) as determined by gastrointestinal digestion. This is the first time such data have been presented. The outcomes achieved were compared to those reported for other beverages (milk, tea) and dietary products. In general, considering the sum of the contributions by the bioaccessible fractions of Ca, Fe, Mg and Mn in each case, it appears that these metals are the least bioaccessible in coffee C3 and the most available in coffee C12.

The average concentration of Ca in the coffee infusions was 17.2 mg L⁻¹ with a coefficient of variation (CV) of 18%. The contribution of the bioaccessible fraction of Ca varied from 23.3% (coffee C1) to 72.8% (coffee C8). The average contribution of this fraction of Ca was moderate, i.e., 42.0% with a CV of 37%. Although Ca is soluble at pH 1-8,¹² there are possible inhibitors of oral bioaccessibility of this metal in coffee, i.e., oxalic and phytic acids.¹ Similar results for Ca were obtained in the case of tea infusions, i.e., 48% bioaccessibility.¹⁴ It is worth noting that the bioaccessibility of Ca from coffee infusions is about two fold higher than from milk, dairy products and infant formulas.¹⁸ Nevertheless, concentrations of Ca in the latter food products are much higher compared to coffee brews, and they are better sources of this metal.

The concentration of Fe in the coffee infusions varied over a small range, i.e., from 0.11 to 0.24 mg L⁻¹ with a CV

Table 1. Concentrations of Ca, Fe, Mg and Mn in coffee infusions and in the bioaccessible fraction resulted from the gastrointestinal digestion

Coffee	Concentration / (mg L ⁻¹)							
	Infusion ^a				Bioaccessible fraction ^b			
	Ca	Fe	Mg	Mn	Ca	Fe	Mg	Mn
C1	17.5 ± 0.2	0.202 ± 0.083	47.0 ± 5.2	0.317 ± 0.014	4.07 ± 0.63	0.097 ± 0.054	31.9 ± 1.9	0.036 ± 0.011
C2	23.0 ± 1.1	0.198 ± 0.040	23.0 ± 1.1	0.554 ± 0.043	8.67 ± 0.70	0.036 ± 0.022	8.68 ± 0.69	0.256 ± 0.018
C3	19.0 ± 1.0	0.230 ± 0.040	59.7 ± 3.6	0.475 ± 0.014	4.50 ± 0.25	0.086 ± 0.022	31.9 ± 1.9	0.054 ± 0.011
C4	15.2 ± 0.3	0.176 ± 0.036	46.2 ± 0.9	0.256 ± 0.007	4.32 ± 0.37	0.061 ± 0.032	31.8 ± 1.8	0.068 ± 0.014
C5	20.0 ± 0.3	0.173 ± 0.025	56.0 ± 0.6	0.450 ± 0.011	5.08 ± 0.22	0.072 ± 0.029	31.9 ± 1.6	0.065 ± 0.011
C6	14.5 ± 0.1	0.155 ± 0.036	45.0 ± 0.9	0.299 ± 0.011	4.06 ± 0.32	0.047 ± 0.014	32.0 ± 1.9	0.065 ± 0.011
C7	19.7 ± 0.8	0.169 ± 0.025	56.1 ± 1.7	0.576 ± 0.040	9.12 ± 1.27	0.094 ± 0.061	35.2 ± 3.5	0.128 ± 0.022
C8	10.9 ± 0.1	0.209 ± 0.029	40.9 ± 0.4	0.266 ± 0.011	7.93 ± 0.67	0.101 ± 0.040	28.0 ± 2.2	0.108 ± 0.011
C9	17.9 ± 0.7	0.194 ± 0.018	58.7 ± 1.8	0.428 ± 0.011	10.1 ± 0.8	0.072 ± 0.029	38.2 ± 2.7	0.101 ± 0.014
C10	15.3 ± 0.6	0.220 ± 0.054	43.9 ± 2.2	0.155 ± 0.029	7.99 ± 0.99	0.079 ± 0.022	27.6 ± 1.9	0.068 ± 0.025
C11	14.3 ± 0.3	0.245 ± 0.076	42.9 ± 0.9	0.205 ± 0.014	7.63 ± 1.19	0.162 ± 0.061	25.8 ± 3.1	0.061 ± 0.018
C12	17.9 ± 1.2	0.119 ± 0.043	51.7 ± 3.6	0.464 ± 0.047	9.31 ± 2.67	0.061 ± 0.011	34.3 ± 1.4	0.133 ± 0.036
C13	18.5 ± 1.0	0.112 ± 0.036	59.9 ± 3.0	0.569 ± 0.040	8.54 ± 0.22	0.065 ± 0.029	40.1 ± 1.6	0.155 ± 0.036
Mean	17.2	0.185	48.5	0.386	7.02	0.079	30.6	0.100
Max.	10.9	0.112	23.0	0.155	4.06	0.036	8.68	0.036
Min.	23.0	0.245	59.9	0.576	10.1	0.162	40.1	0.256
CV / %	18.1	21.6	21.1	37.2	32.2	39.4	25.2	58.8

^aAverage value (n = 3) ± SD; ^baverage value (n = 6) ± SD.

of 22%. The variation in the concentration of this metal in the bioaccessible fraction was higher, i.e., between 0.04 and 0.16 mg L⁻¹ with a CV of 39%. The average contribution of this fraction of Fe was 43.3%, with low and high values of 18.2% (coffee C2) and 66.2% (coffee C11), respectively. It could be presumed that the main inhibitors of Fe in coffee infusions are phytic acid and polyphenols,³⁶ which are abundant in coffee. In the case of phytic acid, the formation of insoluble Fe complexes occurs when large quantities of phytates are consumed.³⁶ As reported by Powell *et al.*¹⁴ for tea infusions, polyphenols readily bind trivalent Fe ions, inhibiting absorption of this metal. The presence of Ca may also reduce the absorption of Fe.¹ On the other hand, there are substances that may enhance its uptake, e.g., ascorbic acid due to its reduction of Fe(III) to Fe(II).³⁷ Other organic acids with OH groups, i.e., citric, lactic and tartaric, may increase absorption of Fe as well.³⁸ Hence, the great variability in the bioaccessibility of Fe observed here for coffee infusions is likely associated with differences in the composition of the coffees.

Magnesium was established as the most bioaccessible among studied metals in the brewed coffees. The average concentration of Mg in the coffee infusions was 48.5 mg L⁻¹ with a CV of 21%, while the average contribution of the bioaccessible fraction of this metal was 62.2%, with the lowest CV value, i.e., 18%. This suggests that the bioaccessibility of Mg in coffee is not strongly affected by matrix constituents, although this metal may be willingly bound by melanoids.³⁵ The lowest bioaccessibility of Mg was found in coffee C2 (37.8%), the highest in coffee C6 (70.8%). In all coffees, the bioaccessibility of Mg from infusions was relatively high and quite comparable. These results were in good agreement with those previously described for black tea by Powell *et al.*,¹⁴ where a 66.4% contribution of the bioaccessible fraction of Mg to its concentration was reported for its brew.

Data about the absorption and excretion of Mn has revealed that uptake of this metal in the gastrointestinal track is low.³⁹ Indeed, compared to the results obtained for Ca, Fe and Mg, Mn was the least bioaccessible in the coffee infusions. The average concentration of this microelement in the analysed coffee infusions was 0.39 mg L⁻¹ (with a CV of 37%), about twice as high as the average concentration of Fe. However, the average contribution of the bioaccessible fraction of this metal was low, i.e., 26.8%. In addition, the bioaccessibility of Mn from infusions of different coffees varied over a high range, i.e., from 11.4% (coffee C1) to 46.1% (coffee C2). Its CV of 43% reflected this fact. It can be supposed that such variability in the results achieved for Mn could be related to differences in the compositions of the analysed coffees and the chemical nature of this metal.

Accordingly, the bioaccessibility of Mn could be affected by the presence of phytic and ascorbic acids as well as pectins.³⁴ These compounds may bind Mn and impair its uptake. In addition, being an acid-soluble metal like Fe, Mn could form insoluble hydroxide precipitates under conditions of gastrointestinal digestion.¹⁴

Dietary reference intakes (DRIs) of Ca, Fe, Mg and Mn through coffee brews

The extent of coverage of adequate intakes (AIs) and recommended dietary allowances (RDAs) of the studied metals was evaluated considering their normative values established by the National Research Council for 10-30 year life stage group,⁴⁰ i.e., 1000 mg day⁻¹ of Ca (AI for males and females), 8 and 18 mg day⁻¹ of Fe (RDAs for males and females, respectively), 400 and 310 mg day⁻¹ of Mg (RDAs for males and females, respectively), and 2.3 and 1.8 mg day⁻¹ of Mn (AIs for males and females, respectively). Considering that one cup of the different coffees (7.2 g of ground coffee brewed in 200 mL of boiling water) is drunk *per* day, it appears that the respective DRIs of Ca, Fe, Mg and Mn are marginally covered, i.e., 0.08-0.20% (Ca), 0.04-0.41% (Fe), 0.4-2.6% (Mg) and 0.3-2.8% (Mn). These findings may be compared with previous results^{6,41} reporting that one cup of coffee may contribute about 0.3% to RDIs of Ca and Fe, about 1.0-5.0% to the RDI of Mg and 2.2-3.4% to the RDI of Mn. The cited values are more than 2-3 fold higher than those assessed in the present study. It should be noted, however, that they were based on the total concentrations of Ca, Fe, Mg and Mn determined in coffee infusions without considering the bioaccessibility of these metals.

Conclusions

The present study reports for the first time on the bioaccessibility of selected macro- and microelements, i.e., Ca, Fe, Mg and Mn, in brews of ground coffees. It was found that the most bioaccessible metal in coffee is Mg, with the bioaccessible fraction contributing on average 62% in reference to the Mg content in the infusions. The least bioaccessible is Mn, with an average contribution of 27% in reference to the Mn content in the infusions. The bioaccessibility of Ca and Fe were comparable; the bioaccessible fractions of these metals contributed on average 42 and 43%, respectively.

It was also discovered that consumption of a single cup of coffee does not cover more than 3% of the DRI for each studied metal. However, in real life situations, coffee is commonly drunk with additives like sugar, milk

or both and may accompany eating a meal. This makes the bioaccessibility of metals much more complicated, as can be expected.

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