Journal of Food Composition and Analysis 119 (2023) 105291

Contents lists available at ScienceDirect

Journal of Food Composition and Analysis

journal homepage: www.elsevier.com/locate/jfca

Revealing the extended effect of biofortification on seed of cowpea cultivars

Ronaldo Cunha Coelho [a,b], Darlisson Slag Neri Silva [a], Hudson de Carvalho Silva [a], Maurisrael de Moura Rocha [c], Roberto Carlos Fernandes Barsotti [d], Heloisa França Maltez [d], Clecio Dantas [e], Cícero Alves Lopes Júnior [a,*], Herbert de Sousa Barbosa [a,*]

[a] Grupo de Estudos em Bioanalítica – GEBIO, Department of Chemistry, Federal University of Piauí, 64049-550 Teresina, PI, Brazil
[b] Teacher Training Department, Federal Institute of Piauí, Cep: 64000-040 Teresina, Piauí, Brazil
[c] Embrapa Meio-Norte, 64008-780 Teresina, PI, Brazil
[d] Centro de Ciências Naturais e Humanas, Universidade Federal do ABC, Avenida dos Estados, 5001, Bairro Santa Terezinha, 09210-580 Santo André, SP, Brazil
[e] Laboratório de Química Computacional Inorgânica e Quimiometria (LQCINMETRIA), State University of Maranhão – UEMA, Campus Caxias, 65604-380 Caxias, MA, Brazil

A R T I C L E I N F O

Keywords:
Beans
Biofortification
ICP-MS
Nutrient bioavailability
In vitro digestion
Multivariate analysis

A B S T R A C T

The present research reveals the extended effect of iron and zinc biofortification in recently introduced cowpea cultivars, considering several nutritional factors. Results showed that the manganese concentration in biofortified cultivars was higher than in common beans, and bioaccessibility was *ca.* 51.6% higher. Also, the magnesium content was higher, reaching > 50% bioaccessibility and > 21% dialyzability. The availability of minerals in cooked biofortified cultivars significantly increased, and the evaluated cowpea cultivars presented high lipid contents and energy values. Moreover, we found unknown synergistic correlations between copper and magnesium, and between manganese and the lipid content. This antagonistic correlation of copper and manganese with zinc may explain their limited contents in the biofortified cowpea beans. PCA analysis discriminated cowpea and common bean cultivars and indicated the prevalence of the effects of biofortification. Overall, the new biofortified cowpea cultivars presented a nutritional quality compatible with the demands of the human diet.

1. Introduction

Micronutrient malnutrition, also known as hidden hunger, is a serious public health problem that affects about 2 billion people worldwide, particularly preschool children and pregnant women in low and middle-income countries (Okwuonu et al., 2021; Silva et al., 2021). This problem has become worse because it is estimated that 800 million people are food insecure due to factors such as the COVID-19 pandemic (Blanco-Rojo and Vaquero, 2019; FAO, 2021). The countries with micronutrient malnutrition are predominantly in Africa, Asia and Latin America. Micronutrient intakes in developing countries are commonly from edible plant tissues, cereals, and grains. In these countries, cowpea is the most consumed staple food crop. So, increasing the nutritional quality of crops plays a crucial role in combating human micronutrient malnutrition. Biofortification has demonstrated feasibility for improving the nutritional status of the human diet (Coelho et al., 2021). This approach consists of the production of micronutrient-enhanced crops by applying conventional plant breeding and biotechnology techniques (Bouis and Saltzman, 2017; Singh et al., 2016). Biofortified crops can reach poor rural people more susceptible to micronutrient malnutrition without altering their diet (Mejia et al., 2017).

Beans, mainly cowpea [*Vigna unguiculata* (L.) Walp.], are consumed by more than 200 million people in developing countries. They are a rich source of protein (20–32%), carbohydrates and a wide range of

Abbreviations: Ac, Bioaccessible concentration in cooked; Ar, Bioaccessible concentration in raw; AOAC, Association of Official Analytical Chemists; ARA, Cowpea BRS Aracê; BLA, Common bean Black; CAR, Common bea Carioca; Dc, Dialyzable concentration in cooked seed; Dr, Dialyzable concentration in raw seed; FAO, Food and Agriculture Organization; GRE, Common bean Green; GUA, Cowpea BRS Guariba; ICP-MS, Inductively Coupled Plasma Mass Spectrometry; LOD, Limit of detection; LOQ, Limit of quantitation; NIST, National Institute of Standards and Technology; PCA, Principal Component Analysis; *r*, Pearson's correlation coefficients; Tc, Total concentration in cooked seed; Tr, Total concentration in raw seed; TUM, Cowpea BRS Tumucumaque; XIQ, Cowpea BRS Xiquexique; WHI, Common bean White.

* Corresponding authors.
E-mail addresses: ciceroalj@ufpi.edu.br (C.A. Lopes Júnior), hbarbosa@ufpi.edu.br (H.S. Barbosa).

https://doi.org/10.1016/j.jfca.2023.105291
Received 2 January 2023; Received in revised form 10 March 2023; Accepted 20 March 2023
Available online 21 March 2023

micronutrients (Adebooye and Singh, 2008; Adjei-Fremah et al., 2019; Kumar and Dhaliwal, 2022; Mbuma et al., 2022). Cowpea shows high adaptability and production stability under stress conditions such as high temperature, drought, and low soil fertility (Silva et al., 2022). In this scenario, cowpea has been targeted by the biofortification breeding program by the Brazilian Agricultural Research Corporation (Embrapa) and collaborators, who produced and recently released for family and business farmers some cultivars biofortified with iron (Fe) and zinc (Zn) (Gomes et al., 2022). Previous reports have shown that biofortified cowpea cultivars including BRS Aracê , BRS Tumucumaque and BRS Xiquexique have higher total and bioaccessible concentrations of Fe and Zn than common beans (Coelho et al., 2021). Also, cooked cowpea seeds presented an improvement in nutritional quality considering the evaluated micronutrients, Fe and Zn. Generally, cowpea seeds have a higher protein concentration than common beans (Teka et al., 2020).

Although cowpea biofortification has been a successful approach to producing genotypes enriched with Fe and Zn, its effects on other nutrients are unknown. In general, biofortified cowpea cultivars also have high protein concentrations (Freire Filho, 2011). The agronomic biofortification of wheat with Zn simultaneously increased the Zn, Fe and protein content in the grains (Zhang et al., 2022). In addition to these interactions, Zn and Mn have a synergistic relationship as demonstrated by Liu et al. (2014) when evaluating the extended effect of Zn enrichment in wheat grain. The interactions and external relationships between micronutrients need to be better understood to produce foods with high nutritional value because the human diet requires at least 18 mineral elements as well as vitamins and other nutrients (Silva et al., 2019). Furthermore, the antinutritional factors of the staple food crop must be low to minimize the negative interference with nutrient availability (Díaz-Gómez et al., 2017; Huertas et al., 2022).

An approach based on the investigation of total nutrient metal concentration is limited in evaluating the effect of biofortification on the nutritional quality of produced foods. In this sense, *in vitro* bioavailability studies are more appropriate, because they allow the assessment of bioaccessibility, which represents the maximum fraction of the substance or nutrient metal in the food that is theoretically released in the gastrointestinal tract and then becomes available for intestinal absorption. Furthermore, a more realistic simulation protocol based on a dialyzability approach can be applied in which a dialysis membrane is used during the simulated intestinal digestion stage and the bioavailable (dialyzable) fraction is associated with the portion of the substance that crosses the membrane (Herbello-Hermelo et al., 2018; Coelho et al., 2021). Recent dialysis-based *in vitro* bioavailability studies for nutrient metals have been reported investigating biofortified cowpea and common bean (Coelho et al., 2021).

Considering the questions related to biofortification and the influence on the absorption capacity of other mineral nutrients and other chemical components present in the seeds, it is essential to evaluate the effect of biofortification on mineral nutrients. Furthermore, it is known that correlations between total, bioaccessible and dialyzable concentrations of elements, and centesimal composition remain largely unclear. Therefore, the objective of the present study was to unravel the associated effects of biofortification with a focus on the centesimal composition and availability of Cu, Mn and Mg. It also aimed to investigate the cooking effects on the grains and evaluate common and non-biofortified bean cultivars.

2. Materials and methods

2.1. Reagents, standards, and materials

All the reagents used in this study were analytical grade or better. We purchased 1000 mg L^{-1} Cu, Mg, and Mn stock standard solutions from PerkinElmer (Jardim das Laranjeiras, SP, Brazil) and used them for the calibration curve. Sigma-Aldrich (St. Louis, MO) supplied the enzymes (α-amylase – 10065, pepsin – P7000, pancreatin – P1715), bile salts, $NaHCO_3$, NaOH and cellulose dialysis membranes (14.0 kDa MWCO). Moreover, we purchased 30% hydrogen peroxide, nitric acid, and hydrochloric acid distilled in a Savillex DST-1000 system (Eden Prairie, MN) from Synth (São Paulo, SP, Brazil) to improve the purity and the reagents used in the analysis of centesimal composition from Dinâmica (Indaiatuba, SP, Brazil) and Vetec (Duque de Caxias, RJ, Brazil). We also used ultrapure water (≥ 18.2 MΩ cm) from a Millipore RiOs-DI™ purification system, bought from Milli-Q (Billerica, MA) to prepare all solutions and clean materials such as plastic pots, conical tubes, and glassware, which remained immersed in a 10% (*v/v*) HNO_3 bath for 24 h.

2.2. Bean samples

The seed samples of the biofortified cowpea cultivars BRS Aracê (ARA), BRS Xiquexique (XIQ), BRS Tumucumaque (TUM) and non-biofortified BRS Guariba (GUA) were obtained from the same cultivation conducted under sprinkler irrigated conditions from April to June 2019 in the experimental field at Embrapa Mid-North (Teresina, Piauí, Brazil, –5° 5″ S, 42° 48″ W). The soil was of the quartz sand type with predominantly sandy texture. The soil was prepared using a harrow. No fertilizer was used. Planting was carried out in furrows with 8 seeds/meter and a distance of 0.5 m between them. After planting, pre-emergent herbicide was used for weed control. During plant growth, two sprays of insecticides were applied to control aphids, cowflies and bed bugs. The harvest was carried out 70 days after planting. Furthermore, the study investigated the commercial cultivars of the common bean (*Phaseolus vulgaris* L.) Black (BLA), White (WHI), Carioca (CAR), and Green (GRE) from the same batch, producer, and in the same popular supermarket located in the city of Teresina, Piauí , Brazil. The selected common bean cultivars are the ones that Brazilians produce and consume the most.

2.3. Sample preparation

Following Coelho et al. (2021), we finely ground the raw samples of dry seeds from different bean cultivars using an A 11BS1 grinder (IKA, Staufen, Germany) operating at 50/60 Hz. For cooking, we placed *ca.* 10 g of sample in a Teflon tube containing 20 mL of deionized water. Then we closed the tubes and left them in a digestion block (EasyDigest ®, Analab, Wantzenau, France) for 1.5 h at 100 °C. This closed system simulates the typical cooking process applied to beans with temperature control and minimizing contamination and leaching. After that, we ground the cooked seeds in a domestic grinder (Cadence, Balneário Piçarras, SC, Brazil) equipped with stainless steel blades operating at 60 Hz. This step was to simulate the phase of chewing the food. Next, we froze and lyophilized the partially pulverized samples for 48 h (L101, LIOTOP®, São Carlos, SP, Brazil). After drying, we stored the raw and cooked seeds in hermetically sealed propylene tubes and kept them in a desiccator.

2.4. Determination of total Cu, Mn, and Mg content in raw and cooked beans

The total concentration of Cu, Mn, and Mg was determined in raw and cooked seeds of bean cultivars as described by Coelho et al. (2021). First, 2.0 mL of sub-distilled HNO_3 concentrate and 200 μL of 30% (*w/w*) H_2O_2 were added to 200 mg of dried and pulverized samples. Then the tubes were closed and left at 85 °C for 3.0 h in a digestion block. After decomposition, we cooled the solutions down and made them up to 30 mL using deionized water. We prepared the samples in triplicates and a blank for each run. Also, we analyzed in the same run the Standard Rice Flour 1568b reference material from the National Institute of Standards and Technology (NIST) to ensure protocol accuracy.

2.5. *In vitro* gastrointestinal digestion of raw and cooked beans

The bioaccessibility and bioavailability (dialyzability) of Cu, Mn, and Mg in raw and cooked seeds of different bean cultivars were evaluated by applying the simulated *in vitro* gastrointestinal digestion protocol described in detail by Coelho et al. (2021). Briefly, 200 mg of the powdered dry sample received an addition of 200 μL of 1% (*w/v*) α-amylase solution in 0.1 mol L^{-1} $NaHCO_3$ buffer pH 6.8. Then we added 3.0 mL of a 0.5% (*w/v*) pepsin solution pH 1.2 and incubated the mixture for 2.0 h at 37 °C under 70 rpm shaking. We used HCl concentrate and NaOH 1.5 mol L^{-1} to adjust the pH. For the final stage, intestinal digestion, we added 3.0 mL of a 3.0% (*w/v*) solution of pancreatin and 2.5% (*w/v*) bile salts in 0.1 mol L^{-1} $NaHCO_3$ buffer pH 7.4. In addition, we inserted a dialysis membrane (14 kDa MWCO – 3 cm) filled with $NaHCO_3$ solution into the tube. After incubation for 2.0 h at 37 °C under 70 rpm shaking, we placed and kept the tubes in an ice bath for 15 min to stop the intestinal enzymatic activity. At the end of the *in vitro* digestion, we transferred the internal solution (dialyzable fraction) contained in the dialysis membrane to conical tubes and added ultrapure water until the volume reached 15 mL. Finally, we centrifuged the gastrointestinal suspension at 3000 rpm for 10 min and pumped the supernatant (bioaccessible fraction) in a 50 mL conical tube, increasing the volume to 30 mL using ultrapure water. We performed the protocol in triplicate and prepared a blank for each run.

2.6. Instrumentation

The concentrations of Cu, Mg, and Mn were measured using an Agilent 7900 quadrupole ICP-MS (Hachioji, TY, Japan). Thus, the total, bioaccessible and dialyzable concentrations in samples were determined. For Mg, we carried out the analysis without an internal standard, so we used Ge (*m/z* 72) as an internal standard to minimize likely interferences on the Cu and Mn measurements and filled the collision cell (KED mode) with a flow of He (5.0 L min^{-1}) to remove potential isobaric interferences on the monitored masses of the evaluated isotopes. Also, we performed an external calibration to quantify the nutrient metals by combining the standard solutions of each element with the different matrices as follows: bioaccessible (gastrointestinal solution), dialyzable ($NaHCO_3$ buffer pH 7.4), and a total fraction (diluted HNO_3 solution 1:15). Moreover, we performed the sample introduction using a concentric Mira Mist® nebulizer (flow of 300 μL min^{-1}) connected to a Scott double-pass nebulization chamber and a peristaltic pump. In addition, we used a sampling cone and a nickel skimmer at the interface and optimized the ICP-MS daily for optimal sensitivity and stability. Table S1 shows the radio-frequency power, lens voltage, air and He fluxes, and other operating conditions of the ICP-MS.

2.7. Analysis of the centesimal composition in raw beans

The lipid, moisture, and ash contents were determined using the AOAC methods (Association of Official Analytical Chemists, 2005) and the total protein concentration was quantified using the Kjeldahl method with a nitrogen conversion factor of 6.25. Then we found the total carbohydrate content by difference (Bramorski et al., 2011; Primitivo et al., 2022). In addition, we estimated the calorific value of beans using the Atwater conversion factors: 4 kcal g^{-1} for proteins, 4 kcal g^{-1} for carbohydrates, and 9 kcal g^{-1} for lipids (Sánchez-Peña et al., 2017).

2.8. Statistical analysis

We expressed the results as mean ± standard deviation. We evaluated the significance of the differences between the results using ANOVA, paired *t*-test, and Tukey considering the levels $p < 0.05$ and $p < 0.001$. Also, we estimated the Pearson's coefficients (r) to evaluate the correlation between percentage composition, and total, bioaccessible and dialyzable concentrations of Cu, Mn, and Mg in different bean cultivars. We considered significant the values for $r > 0.70$ and $p < 0.05$. Moreover, the correlation between measured characteristics was investigated using the unsupervised pattern recognition technique, principal component analysis (PCA). We arranged the original data in an *X*-matrix (24 × 36) where the rows represent the eight studied cultivars in triplicate, and columns correspond to the 36 variables related to the centesimal composition and contents of Cu, Mn, and Mg of raw and cooked samples. Before PCA modeling, we self-scaled the data matrix to equalize the ranges of variability and ran the statistical analysis using the PAST software (version 4.03).

3. Results and discussion

3.1. Quality control of element determinations

Although ICP-MS has desirable characteristics and is superior to other analytical techniques including high sensitivity, wide linear dynamic range, multi-element analysis, ability to discriminate, and simple sample preparation, its measurements can suffer from matrix effect and spectral interference. For example, the isotopes $^{65}Cu^{+}$, $^{24}Mg^{+}$, and $^{55}Mn^{+}$ have overlapping isobaric species $^{32}S^{16}O_2H^{+}$, $^{12}C_2^{+}$, and $^{40}Ar^{14}NH^{+}$, respectively. In this sense, we decomposed the Rice Flour NIST SRM 1568a and measured ($n = 3$) the total concentration of the elements. The measured concentrations of Cu, Mn, and Mg were 2.92 ± 0.16 mg kg^{-1} compared to 2.35 ± 0.16 mg kg^{-1} (certified), 18.0 ± 1.4 mg kg^{-1} compared to 19.2 ± 1.8 mg kg^{-1} (certified), and 399 ± 5 mg kg^{-1} compared to 559 ± 10 mg kg^{-1} (certified). The differences between the measured and certified concentrations were significant for neither Cu nor Mn applying paired *t*-test at $p < 0.05$ nor for Mg with $p < 0.001$.

After the confirmation of accuracy, the precision for mineral measurements in the NIST SRM 1568a was less than 7.9%. We calculated the limits of detection (LOD) and quantification (LOQ) as 3 × and 10 × the standard deviation of the blanks. Results showed the following LOD and LOQ values: 0.01 and 0.05 μg kg^{-1}, 0.01 and 0.04 μg kg^{-1}, and 0.98 and 3.28 μg kg^{-1}, for Cu, Mn, and Mg, respectively. The reasonable values of the figures of merit indicate that the applied analytical strategies (sample preparation, collision cell with He gas, use of an external calibration curve with a combined matrix, and use of an internal standard (^{72}Ge) were effective, which ensures the reliability of our results.

3.2. Total, bioaccessible, and dialyzable concentration of mineral nutrients in raw beans

3.2.1. Copper (Cu)

As presented in Fig. 1, the total Cu concentration in the seeds of the studied cultivars ranged between 4.4 and 10.7 mg kg^{-1}. On average, the Cu concentration in the seeds of the cowpea cultivars was *ca.* 42% smaller than in the seeds of the common bean cultivars. Although the cultivar Tumucumaque was the richest, the concentration of Cu found in its seeds was lower than *ca.* 11% and 32% of the common bean cultivars Carioca and Black, respectively. Furthermore, the concentration of Cu in the seeds of the Aracê , Xiquexique, and Guariba cowpea cultivars was similar to that of common Green beans ($p < 0.05$). Fontenele et al. (2012) determined the Cu concentration in Tumucumaque, Xiquexique, and Guariba seeds and 27 other Brazilian cowpea cultivars and found values ranging from 20 to 22 mg kg^{-1}. On the other hand, Feitosa et al. (2018) reported a high Cu concentration in seeds of the common bean cultivar Black, *ca.* 12.5 mg kg^{-1}. The Cu concentrations of the studied cowpea cultivars were lower than the ones presented by Fontenele et al. (2012). These lower concentrations may be related to endo-climatic conditions and plant cultivation that interfere with the uptake, transport, and storage of mineral nutrients in grains.

In comparison, the bioaccessible Cu concentration was proportional to its total concentration, in which the values found in the seeds of the cowpea cultivars were *ca.* 35% lower than the ones found in common beans. The Carioca and Black cultivars presented the highest

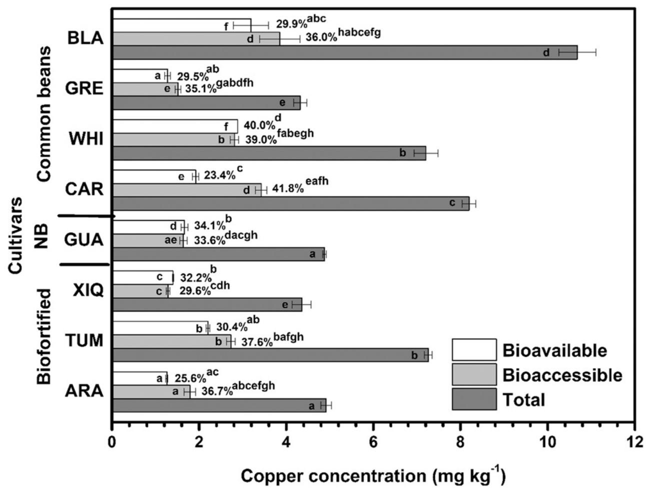

Fig. 1. Total, bioaccessible and dialyzable concentrations (mg kg^{-1}, $n = 3$) of Cu and fractions (%) in raw common bean seeds, biofortified and non-biofortified cowpea cultivars. The different lowercase letters inside or on top of columns mean that concentration or percentage means are statistically different by Tukey's test ($p < 0.05$).

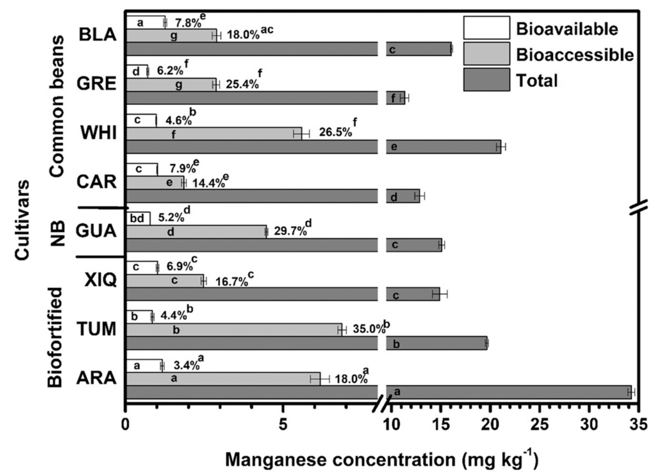

Fig. 2. Total, bioaccessible and dialyzable concentrations (mg kg^{-1}, $n = 3$) of Mn and fractions (%) in raw common bean seeds, biofortified, and non-biofortified cowpea cultivars. The different lowercase letters inside or on top of columns mean that concentration or percentage means statistically differ by the Tukey's test ($p < 0.05$).

bioaccessible Cu concentrations, which significantly differed from the cowpea cultivars by the Tukey test ($p < 0.05$). However, in percentage terms, the bioaccessible fraction of Cu was in the range of 29.6–37.6% and 35.1–41.8% in seeds of cowpea cultivars and common beans, respectively (Fig. 1).

Regarding the dialyzability of Cu, the concentration found in seeds of common bean cultivars was also 42% higher than that found in seeds of cowpea cultivars. Fig. 1 presents a positive correlation between the total and dialyzable Cu concentrations. However, the Carioca cultivar showed a significant reduction in micronutrient bioavailability. The bioavailable fraction of Cu in cowpea seeds ranged from 25.6% to 34.1% while for common beans it ranged from 23.4% to 40.0%. The bioavailable fraction of Cu in the Guariba cultivar (34.1%) was slightly lower than that of the White bean (40.0%), but the difference was significant ($p < 0.05$).

Results show that the genetic improvement focused on Fe and Zn biofortification promoted a negligible effect on the total Cu concentrations in seeds of new cowpea cultivars. This behavior may be associated with the interaction between micronutrients in physiological processes such as uptake, translocation, and storage in plants. Cu, Fe, and Zn share carrier biomolecules and accumulation structures. Therefore, Fe and Zn biofortification may have limited the Cu content in the seeds of new cowpea cultivars. However, Cu availability was similar in the seeds of the different studied bean cultivars.

3.2.2. Manganese (Mn)

When comparing Figs. 1 and 2, it is clear that Mn is more abundant than Cu in the seeds of the different cultivars. Their concentrations were in the range of 11.3 and 34.3 mg kg^{-1}, respectively. Considering the average, the total concentration of Mn in seeds of the cowpea cultivars was 2.7–7.0 times higher than the concentration of Cu, while the common bean cultivars presented concentrations *ca.* 1.5–2.9 times higher. Pereira et al. (2020), who evaluated three genotypes of the Black common bean, also observed this smaller difference between Mn and Cu concentrations in common bean seeds.

Furthermore, the cowpea cultivar Aracê presented the highest total concentration of Mn and significantly differed from the other beans by the Tukey test ($p < 0.05$). The Mn content of Aracê was also higher than the contents of 30 cowpea cultivars studied by Carvalho et al. (2012). Also, Gerrano et al. (2019), studying several cowpea cultivars (*ca.* 22 genotypes), found Mn concentrations lower than the Aracê , Tumucumaque, and Xiquexique cultivars evaluated here. Our results are also higher than those reported by Kumari and Platel (2017), who found a total concentration of Mn *ca.* 10.1 and 9.1 mg kg^{-1} for cowpea and common beans, respectively. Considering the common bean cultivars, only the White and Black had a similar high total concentration of Mn as the biofortified cowpea cultivars. Although not biofortified, the Guariba cultivar had a higher Mn content than the common beans, except for White.

These results indicate that the genetic improvement process, aimed at the biofortification of cowpea cultivars with Fe and Zn, increases the Mn content. The enrichment of basic dietary cultures with Mn plays an important role in innate immunity and the human nervous system (Neves et al., 2020).

As presented in Fig. 2, the bioaccessible concentration of Mn in seeds of cowpea cultivars was *ca.* 51.6% higher than in seeds of common bean cultivars. Cultivars Tumucumaque and Aracê showed the highest bioaccessible concentrations of Mn and the difference is significant concerning the other cultivars, according to the Tukey test ($p < 0.05$). The bioaccessible concentration in seeds of the non-biofortified cultivar Guariba was 35.8%, 35.5%, and 58.6% higher than in seeds of the common beans Green, Black, and Carioca, respectively. The bioaccessible fraction of Mn in the seeds of the cowpea cultivars ranged from 16.7% to 35.0% while in the common bean cultivars it ranged from 14.4% to 26.5%. Kumari and Platel (2017) also found a higher bioaccessible concentration of Mn in cowpea beans (43.5 ± 3.5%) than in common beans (35.1 ± 1.5%).

Although the Tukey test ($p < 0.05$) showed that the dialyzability of Mn differs between some cultivars of cowpea and common bean, the difference was small. Aracê and Black cultivars had the highest concentrations of dialyzable Mn. Considering the dialyzable fraction of Mn, the percentage values were in the range of 3.4–6.9% and 4.6–7.9% for cultivars of cowpea and common beans, respectively. Nosratpour and Jafari (2018) claim that the availability of Mn in edible plant matrix tissues is *ca.* 5%, thus the newly introduced cowpea cultivars Xiquexique and Guariba as well as the most common bean cultivars provide a higher fraction of available Mn.

In addition, Figs. 1 and 2 show that the dialyzability of Cu is *ca.* 3.7 times more than Mn. This difference is related to the interaction between micronutrients and other compounds in edible plant tissues. Some studies have demonstrated the strong interaction of Mn with Ca, fibers, and phytates resulting in insoluble complexes that decrease the soluble fraction of the micronutrient available for absorption in the human intestinal tract (Nosratpour and Jafari, 2018).

3.2.3. Magnesium (Mg)

Unlike Cu and Mn, Mg is a macro-element with a similar abundance to potassium and phosphorus in beans (Pereira et al., 2020). Fig. 3 shows the concentrations of total, bioaccessible and dialyzable Mg in seeds of common, biofortified, and non-biofortified cowpea cultivars. On average the total Mg concentration in cowpea cultivars was slightly higher than in common bean cultivars. The genetic improvement did not cause an increase in Mg concentration in the seeds of cowpea cultivars. The Tukey test ($p < 0.05$) showed that the high total Mg concentration presented by cowpea cultivars was reached only by Carioca and Green beans. Avanza et al. (2013) determined the total Mg concentration in cowpea cultivars cultivated in northeastern Argentina and found values ranging between 620 and 760 mg kg^{-1}. Their values were lower than the ones found in this study for cowpea cultivars. On the other hand, our results were inferior to those of the Southeast African cowpea cultivars (Naiker et al., 2019), which presented Mg contents from 4540 to 4960 mg kg^{-1}. Comparing the Mg contents in common beans reported in the literature, Lovato et al. (2018) found Mg concentrations in Carioca and Black beans of 2110 ± 86 and 1870 ± 12 mg kg^{-1}, respectively, similar to our results. On the other hand, Ribeiro and Kläsener (2020), investigating 17 common bean genotypes, found a mean concentration of Mg of 2300 mg kg^{-1}.

Fig. 3 shows that bioaccessible Mg concentrations in cowpea cultivars are slightly higher than in common bean cultivars. Cultivars Tumucumaque and Guariba presented the highest values, but the difference is only significant considering the bioaccessible concentration of Mg in common beans Carioca and Black and in the biofortified cultivar Xiquexique, according to Tukey's test ($p < 0.05$). The bioaccessible fraction of Mg in seeds of the different studied bean cultivars was higher than 50%, except for the cultivars Xiquexique (46%) and Carioca (42%). It is important to mention the high percentage of bioaccessible Mg in White bean seeds (63%). The results of Mg bioaccessibility found for cowpea and common bean cultivars were higher than those found by Kafaoglu et al. (2016) for hazelnuts, almonds, peanuts, cashew nuts, Brazil nuts, and walnuts (ranging from 16% to 28%). On the other hand, Mingroni et al. (2018) evaluated fresh edible vegetables such as apples, bananas, and papaya and found values ranging from 60% to 66%, which were slightly higher than the ones obtained in this study.

In addition, the dialyzable concentration of Mg was similar between the cowpea and common bean cultivars, ranging from 402 to 494 mg kg^{-1} (Fig. 3). The biofortified cultivar Xiquexique presented the highest dialyzable Mg concentration. The dialyzable fraction of Mg in the seeds of the common bean cultivars was higher than 20%. The Tukey test ($p < 0.05$) showed that there are no significant differences between the percentages, except for the White bean, which reached the highest value (*ca.* 29.2%). In comparison with other edible vegetables, the absorption of Mg when consuming the biofortified (23.1%) and non-biofortified (24.5%) cowpea cultivars is similar to or higher than the intake of nut (17.5%), chestnut (16.3%), pistachio (20.4%), hazelnut (17.9%), pumpkin seed (23.6%), and sunflower (6%) (Moreda-Piñeiro et al., 2016). These results show that the consumption of cowpea cultivars contributes significantly to meeting the daily need for Mg absorption in the human diet.

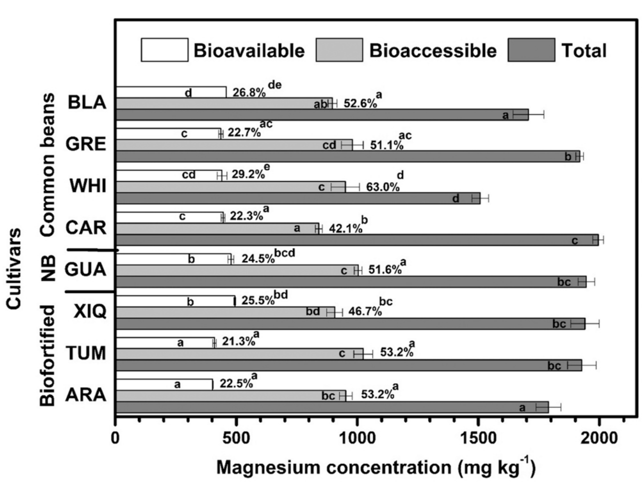

Fig. 3. Total, bioaccessible and dialyzable concentrations (mg kg^{-1}, $n = 3$) of Mg and fractions (%) in raw common bean seeds, biofortified and non-biofortified cowpea cultivars. The different lowercase letters inside or on top of columns mean that concentration or percentage means statistically differ by Tukey's test ($p < 0.05$).

3.3. Total, bioaccessible, and dialyzable concentration of mineral nutrients in cooked beans

Beans, usually consumed as whole seeds, are cooked using mild or harsh methods to overcome the "hard to cook" defect (Phillips, 2013). Cooking methods also interfere with the nutritional aspects of beans (Avanza et al., 2013; Pereira et al., 2014). Recently, Coelho et al. (2021) revealed that the bioaccessibility and dialyzability of Fe and Zn are higher in cooked cowpea and common bean seeds. The authors observed a low percentage of leaching and contamination. Therefore, we used the same cooking procedure and cooking system in this study.

Fig. S1 shows the total concentration and retention factor of Cu, Mn and Mg in the cooked beans of the different bean cultivars studied. The retention factor was estimated to assess the effect of cooking on the concentration of mineral nutrients in the seeds of bean cultivars (Coelho et al., 2021; Pereira et al., 2014). For Cu, the retention factor for cowpea cultivars was higher than 120%, except for Tumucumaque (108%), while the Cu retention factor was low for the cultivars Black (74.4%) and Green (82.2%). Pereira et al. (2020) also found low values of Cu retention factor in seeds of common bean cultivars cooked in a system similar to the one used in this study. Regarding the Mn retention factor, the average values were 98.8 ± 9.7% and 97.1 ± 5.3% for the cowpea and common bean cultivars, respectively. These results indicate that the cooking effects on the concentration of Mn in bean grains are not significant. On the other hand, Pereira et al. (2020) demonstrated a high reduction in the concentration of Mn in cooked seeds of common bean cultivars, finding a low mean retention factor of *ca.* 37.3 ± 4.7%.

In addition, the Mg retention factor of cowpea cultivars was *ca.* 103 ± 6% while for common beans was in the range of 80–109%. The cultivar Black presented the lowest value of the retention factor for Mg (80%) as well as for Cu (74%), which can be attributed to the slight leaching of nutrients. Avanza et al. (2013) found a reduction in the total Mg content in seeds of some cowpea genotypes only after 40 min of cooking in an open pan. These findings show that cowpea cultivars can maintain mineral nutrient contents after commonly applied cooking methods.

Table 1 shows the bioaccessible and dialyzable concentration of mineral nutrients in cooked seeds of different cultivars of cowpea and common bean. The cowpea cultivars had lower bioaccessible Cu concentrations than the common ones, except for the cultivar Green, and the difference was significant, according to the Tukey test ($p < 0.05$). Cu bioaccessibility was higher than 34.2% in cooked seeds of common bean cultivars. White beans presented the highest bioaccessible Cu fraction *ca.* 49.6%. Cooking significantly increased the bioaccessibility of Cu in the cowpea cultivars Aracê, Xiquexique, and Guariba *ca.* 39%, 59%, and 29%, respectively. In contrast, cooked Carioca bean seeds presented a 20% lower bioaccessible Cu concentration than the raw ones. Considering the bioaccessibility of Mn, the mean concentration found in the cooked seeds of cowpea cultivars was 1.7 times higher than the one found in common bean. The biofortified cowpea Aracê (6.4 mg kg^{-1}) and Tumucumaque (5.8 mg kg^{-1}) cultivars showed the highest concentrations of bioaccessible Mn. The highest percentage of bioaccessible Mn was also found in cooked seeds from Tumucumaque (32%), indicating that biofortification promotes an increase in the bioaccessibility

Table 1
Concentration (mg kg^{-1}, $n = 3$) bioaccessible and dialyzable fractions (%) of Cu, Mn and Mg in cooked seeds of common bean, biofortified and non-biofortified cowpea cultivars.

Bioaccessibility						
Cultivars	Cu		Mn		Mg	
	[Cu][1]	%	[Mn][1]	%	[Mg][1]	%
ARA	2.48 ± 0.07[a*]	38.7 ± 0.5[a]	6.41 ± 0.35[a]	17.3 ± 0.2[a]	1184 ± 48[a*]	58.9 ± 0.8[a*]
TUM	2.75 ± 0.05[b]	35.2 ± 1.6[bd]	5.75 ± 0.11[b*]	31.6 ± 0.2[b*]	1074 ± 27[b]	55.6 ± 0.0[b*]
XIQ	2.05 ± 0.09[c*]	38.0 ± 0.1[a*]	1.96 ± 0.02[c*]	14.9 ± 0.2[c]	1288 ± 64[a*]	66.6 ± 2.0[c*]
GUA	2.11 ± 0.06[c*]	34.2 ± 0.5[b]	3.58 ± 0.22[d*]	22.4 ± 0.9[d*]	1358 ± 15[a*]	69.9 ± 0.1[d]
WHI	2.96 ± 0.16[d]	40.8 ± 0.1[c]	3.25 ± 0.04[d*]	17.0 ± 0.5[a*]	973 ± 13[c]	59.2 ± 0.7[a]
CAR	2.73 ± 0.04[b*]	35.8 ± 0.8[d]	1.90 ± 0.05[c]	14.4 ± 0.1[e]	876 ± 28[d]	52.0 ± 0.4[e*]
BLA	3.94 ± 0.14[e]	49.6 ± 0.5[e*]	2.77 ± 0.03[e]	18.0 ± 0.2[f]	886 ± 20[d]	65.1 ± 0.8[c*]
GRE	1.47 ± 0.03[f]	41.5 ± 1.1[c]	2.39 ± 0.09[f*]	21.4 ± 1.1[d]	1030 ± 7[b]	58.7 ± 0.2[a*]
Dialyzability						
Cultivars	Cu		Mn		Mg	
	[Cu][1]	%	[Mn][1]	%	[Mg][1]	%
ARA	2.04 ± 0.09[a*]	31.8 ± 1.0[ae*]	2.00 ± 0.06[a*]	5.38 ± 0.06[a*]	457 ± 25[a*]	22.8 ± 0.0[a]
TUM	1.86 ± 0.03[b]	23.8 ± 1.1[b*]	0.89 ± 0.04[b]	4.87 ± 0.09[b*]	475 ± 5[a*]	24.6 ± 0.3[b*]
XIQ	1.90 ± 0.06[ab*]	35.4 ± 0.3[c]	0.75 ± 0.04[c*]	5.71 ± 0.17[c*]	536 ± 18[b*]	27.7 ± 0.4[c*]
GUA	1.86 ± 0.10[ab*]	30.1 ± 1.2[a*]	0.57 ± 0.01[d*]	3.58 ± 0.01[d*]	501 ± 8[c*]	25.8 ± 0.2[d]
WHI	2.01 ± 0.13[ab*]	27.7 ± 1.6[d*]	0.79 ± 0.02[c*]	4.14 ± 0.09[e*]	337 ± 16[d*]	20.5 ± 0.4[e*]
CAR	2.50 ± 0.07[c*]	32.7 ± 0.3[e*]	0.94 ± 0.04[b*]	7.07 ± 0.11[f*]	520 ± 5[c*]	30.8 ± 0.9[f*]
BLA	2.61 ± 0.03[c]	32.8 ± 0.4[e]	1.13 ± 0.03[e*]	7.33 ± 0.18[f]	369 ± 1[e*]	27.1 ± 0.9[c]
GRE	1.38 ± 0.03[d]	38.8 ± 0.8[f*]	0.67 ± 0.01[f]	6.04 ± 0.44[c]	360 ± 15[de*]	20.5 ± 0.6[e*]

* Different in relation to the grains of the raw bean cultivars.

of this micronutrient. In addition, cooking caused a significant reduction of 16%, 21%, and 20% in the bioaccessibility of Mn in seeds of the cowpea cultivars Tumucumaque, Xiquexique, and Guariba, respectively. It also caused a 42% and 17% reduction in White and Green bean seeds, respectively.

The bioaccessible Mg concentration in cooked seeds of cowpea cultivars was higher and significantly different from those of common bean cultivars, according to the Tukey test ($p < 0.05$). Mg bioaccessibility was higher in the cultivars Xiquexique (66.6%) and Guariba (69.9%). The cooking effect promoted an increase of 24%, 42%, and 35% in the bioaccessibility of Mg in cooked seeds of Aracê, Xiquexique, and Guariba, respectively. This behavior presented by cowpea cultivars may be related to the form and interactions of Mg in the sample matrix, indicating that this macro-nutrient is weakly linked to nutritional inhibitors such as polyphenols, phytates, and tannins (Oliveira and Naozuka, 2017). The results indicate that cowpea cultivars further improve the nutritional value when cooked compared to raw beans.

Regarding the dialyzability of mineral nutrients in the cooked seeds (Table 1), the cowpea cultivars presented a slightly lower dialyzable concentration of Cu than the common beans. However, the difference is significant according to the Tukey test ($p < 0.05$). The dialyzability of Cu in seeds of cowpea cultivars ranged between 23.8% and 35.4% while in seeds of common beans they ranged from 27.7% to 38.8%. Cooking significantly increased the dialyzability of Cu in the seeds of the biofortified cowpea cultivars Aracê (62.3%) and Xiquexique (35.9%). This behavior was also observed for the Carioca cultivar (30.3%) while the Black and White cultivars presented a reduction of 18% and 30%, respectively, in the concentration of bioavailable Cu when compared to the raw seeds. The biofortified Aracê cultivar showed the highest concentration of dialysable Mn ($p < 0.05$). The dialyzable percentage of Mn in seeds of cowpea cultivars was lower than in seeds of common cultivars, which is related to the low total concentration of Mn or even the greater influence of substances that negatively influence the release of minerals (*e.g.* anti-nutritional compounds). Cooking promoted a 70% increase in the dialyzability of Mn in the seeds of the Aracê cultivar, while for the Xiquexique and Guariba cultivars there was a 27% reduction when compared to the raw samples. Cooked white beans also presented 19% less bioavailable Mn compared to their raw seeds.

Moreover, considering the Mg absorption, cooked seeds of cowpea cultivars can provide *ca.* 24% more dialyzable Mg than common beans. Besides, the average dialyzability of Mg in cowpea beans was slightly higher than in common beans. Thus, considering the absorption of Mg, cooked seeds of cowpea cultivars can provide *ca.* 24% more dialyzable Mg than regular beans. The cooking method interfered significantly, increasing by 13.7%, 8.6%, and 15.7% the absorption of Mg from the biofortified cultivars Aracê, Xiquexique, and Tumucumaque, respectively. On the other hand, most of the cooked common bean cultivars had lower Mg dialyzability, except for the Carioca bean, which showed an increase of 16.7% in macronutrient absorption.

The results revealed that cooked cowpea cultivars provide higher mineral content for absorption in the human gastrointestinal tract than common cultivars. For example, the cooking method increased the dialyzability of Cu, Mn, and Mg in biofortified Aracê cowpea seeds. Indeed, the genetic improvement process focused on Fe and Zn biofortification is also effective in promoting the improved availability of mineral nutrients investigated in this study.

3.4. *Centesimal composition in raw beans*

The genetic improvement process has interfered with the chemical composition of seeds of new cultivars of staple crops. In this sense, we determined the concentration of proteins, lipids, carbohydrates, ash, moisture content, and energy value in the seeds of newly introduced biofortified cowpea cultivars and common bean genotypes. Table 2 shows the results.

In this study, cowpea cultivars presented a lower percentage of moisture content than the common cultivars. Avanza et al. (2013) and Kan et al. (2018) evaluated cowpea cultivars and found values higher than those we found. On the other hand, Naiker et al. (2019) showed similar moisture content and Frota et al. (2008) presented a lower one. Considering the ash content, the values found in the biofortified cowpea cultivars were higher than the ones found in the bean cultivars, and they were also higher than the values reported in other studies (Frota et al., 2008; Kan et al., 2018; Naiker et al., 2019). Notably, higher ash content may be related to a higher amount of metal oxides, indicating a higher amount of minerals present in biofortified cowpea cultivars.

The total protein content in seeds of cowpea cultivars was slightly higher than in seeds of common bean cultivars, but the difference was not significant. Previous studies showed that biofortified cowpea cultivars have total protein contents ranging from 24% to 40% (Avanza et al., 2013; Frota et al., 2008; Naiker et al., 2019). On the other hand, Kan et al. (2018) presented protein concentration *ca.* 1.3 times smaller than biofortified cowpea cultivars when studying cowpea cultivars.

The biofortified cultivar Aracê presented the highest lipid

Table 2
Values found (mean ± standard deviation, $n = 3$) for centesimal composition in seeds of cowpea and common bean cultivars *.

cultivars	moisture (%)	ashes (%)	proteins (%)	lipids (%)	carbohydrates (%)	energetic value (kcal 100 g^{-1})
ARA	8.37 ± 0.25^{a}	3.87 ± 0.09^{a}	27.13 ± 1.54^{a}	3.00 ± 0.17^{a}	58.63 ± 2.07^{ad}	361 ± 8^{ab}
TUM	8.55 ± 0.30^{a}	3.47 ± 0.09^{b}	26.25 ± 0.04^{b}	1.26 ± 0.03^{b}	60.88 ± 1.11^{ab}	356 ± 2^{ab}
XIQ	8.97 ± 0.49^{a}	4.55 ± 0.06^{c}	23.27 ± 0.64^{cf}	1.83 ± 0.04^{c}	63.90 ± 2.12^{b}	365 ± 11^{b}
GUA	10.5 ± 0.5^{b}	3.20 ± 0.08^{d}	24.72 ± 0.23^{d}	1.03 ± 0.03^{d}	61.93 ± 1.37^{ab}	353 ± 8^{abc}
WHI	12.4 ± 0.2^{c}	2.95 ± 0.07^{e}	28.10 ± 1.06^{a}	1.49 ± 0.06^{e}	55.69 ± 0.38^{c}	342 ± 4^{cfe}
CAR	17.0 ± 0.5^{d}	3.63 ± 0.07^{b}	20.79 ± 1.02^{e}	1.99 ± 0.10^{c}	56.60 ± 0.43^{d}	327 ± 2^{d}
BLA	13.4 ± 0.2^{e}	3.84 ± 0.0^{a}	21.79 ± 0.88^{cef}	1.32 ± 0.01^{b}	60.07 ± 0.45^{a}	335 ± 3^{f}
GRE	11.0 ± 0.5^{b}	3.27 ± 0.07^{d}	23.86 ± 1.12^{fd}	1.05 ± 0.03^{d}	60.83 ± 1.63^{ab}	348 ± 2^{ace}

* Different letter in the same column means that the means are statistically different by the Tukey test ($p < 0.05$).

concentration and was significantly different from the other beans according to the Tukey test ($p < 0.05$). Kan et al. (2018) presented lipid concentration in the studied cultivars that ranged from 4.2% to 5.6%. A high concentration of lipids is a nutritional quality factor for the grains, as it provides a possible increase in substances such as unsaturated linoleic and oleic acids (Frota et al., 2008; Lovato et al., 2018).

Furthermore, the carbohydrate concentration of the seeds of the biofortified cowpea cultivars was higher than the one of the common bean cultivars. Avanza et al. (2013) and Frota et al. (2008) also evaluated the concentration of carbohydrates in the seeds of some cowpea cultivars and found values lower than those of biofortified cultivars. The energy values of the biofortified cowpea cultivars were higher and significantly different from the common bean cultivars and the cowpea cultivar BRS-Milênio studied by Frota et al. (2008), which indicates that the metabolic needs of the human organism can be met with the consumption of a smaller portion of the cowpea cultivars recently introduced by Embrapa Meio-Norte.

3.5. Correlation and principal component analysis

The metabolism of elements in a plant tissue depends on the existing intra-and interspecific relationship in the matrix. We used Pearson's coefficients to evaluate the correlation between the concentrations of Cu, Mn, and Mg and the other studied components in bean seeds. Table S2 shows the estimated Pearson's correlation coefficients (r). We considered significant the values with $r > 0.7$ and $p < 0.05$. Although not significant, the total Cu concentration correlates positively with its bioaccessible fraction. The correlation between the total Cu concentration and the other components was also low. Nevertheless, it is worth noting that the inverse relationship between Cu and Zn ($r = -0.641$). As expected, these elements compete for transporter proteins for uptake and transport in higher plants, which may have limited the Cu content in seeds of biofortified cowpea cultivars (Ajeesh Krishna et al., 2020).

The absorption of Cu in the gastrointestinal tract showed a reasonable positive correlation with the bioaccessible ($r = 0.744$, $p < 0.05$) and dialyzable Mg fractions ($r = 0.754$, $p < 0.05$). This correlation had not been revealed by previous ionomic studies on plant matrices (Baxter, 2009). Thus, the availability of Cu significantly interferes with the absorption of macronutrients. Pearson's coefficients were negative for the correlation between the total Mg concentration with its bioaccessible ($r = -0.853$, $p < 0.05$) and dialyzable ($r = -0.783$, $p < 0.05$) fractions. This negative correlation demonstrates that Mg uptake is associated with other components of the bean seed matrix such as Cu. In contrast, Cu bioaccessibility was negatively correlated with carbohydrate content ($r = -0.891$, $p < 0.05$). Studies report that carbohydrates strongly interact with Cu, reducing its absorption and inducing its deficiency, aggravating the metabolic crisis in patients with pathologies related to micronutrient metabolism (Song et al., 2018).

Pearson's coefficient was significant and negative for the correlation between the total Mn concentration and its dialyzable fraction ($r = -0.754$, $p < 0.05$), indicating that other nutritional factors interfere with the absorption of Mn. The correlations between Mn dialyzability and bioaccessible Fe and protein contents were also significant and negative, $r = -0.855$ and -0.939, respectively ($p < 0.05$). The antinutritional effect of proteins and Fe on the absorption of Mn from plant foods is poorly known. Ca, fiber, and phytates are the most common components for promoting the reduction of the available soluble fraction of the micronutrient for absorption in the human intestinal tract due to the insoluble complexes formed (Nosratpour and Jafari, 2018).

Considering the interspecific correlations, the total concentration of Mn and the dialyzable Zn fraction presented a negative and significant Pearson coefficient ($r = -0.736$, $p < 0.05$). This finding may contribute to the efficiency of the biofortification process, indicating a barrier in the production of cultures with high Zn and Mn contents. On the contrary, the correlation between Mn and the lipid content was positive ($r = 0.752$, $p < 0.05$). This behavior may be specific to beans, as Moreda-Piñeiro et al. (2016), evaluating the dialyzability of Mn in fresh nuts and seeds, did not observe any interactions with lipids.

To expand the information on the observed correlations, we applied principal component analysis (PCA) using a dataset that brought together the studied variables for the eight bean cultivars and included the reported total, bioaccessible, and dialyzable concentrations of Fe and Zn by Coelho et al. (2021), as the bean matrices they used were the same (Granato et al., 2018).

The four components can explain 85.0% of the total variance described in PC1 and PC2, 38.0% and 23.0%, respectively. Fig. 4 shows the biplot graph for PC1 *vs* PC2 and PC3 *vs* PC4. Considering PC1 (Fig. 4A), it was possible to observe the discrimination between cowpea and common bean cultivars. The positive PC1 score for cowpea cultivars can be attributed to the effect of biofortification, which increased the total concentration of Zn and Mg and the bioaccessibility of Fe and Zn, compared to common beans (Table S3). PC2 revealed a distinction between cowpea cultivars, a consequence of the interaction between cooking and biofortification factors. This distinction is mainly related to the total and dialyzable concentration of Mn, the total and bioaccessible concentration of Cu, and the dialyzable concentration of Zn in the seeds of the biofortified cultivars Aracê and Tumucumaque. Although PC2 has demonstrated a slight similarity between cowpea and common bean cultivars, biofortification is a more significant factor than cooking. Cooking beans in a closed system must have reduced the effect of this factor on the nutritional composition of the samples.

Major constituents have a low influence on bean species discrimination. Ash, protein, and lipids were significant for PC3 and PC4, which describe 25% of the total variance, as Fig. 4B shows. The dialyzability of Fe, Cu, Mg, and Zn still has a significant effect on PC3 and PC4. Lipid concentration makes the seeds of biofortified Aracê different from other cowpea seeds.

4. Conclusions

Our results revealed the effect of the genetic improvement process, focused on Fe and Zn biofortification, on several nutritional factors of recently introduced cowpea cultivars. Mn concentration in the seeds of the biofortified cowpea cultivars was significantly higher than in the

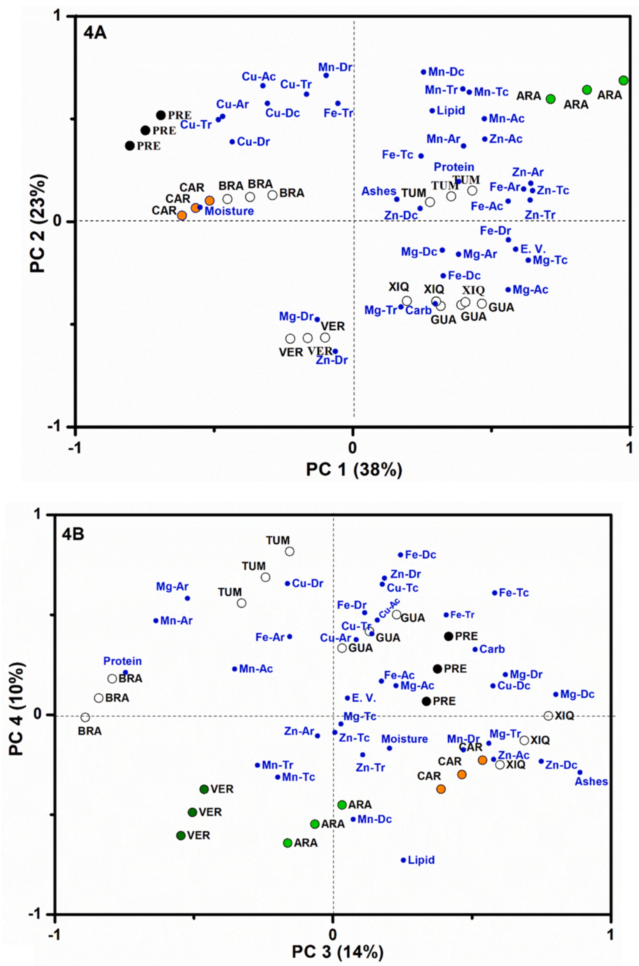

Fig. 4. Biplot PCA graph for (A) PC1 *vs* PC2 and (B) PC3 *vs* PC4 considering the nutritional factors evaluated in raw and cooked bean cultivar seeds. The letters A, D and T means the bioaccessible, dialyzable and total concentrations of Fe, Zn, Cu, Mn and Mg in the raw (r) and cooked (c) samples.

seeds of the common bean cultivars. Also, the bioaccessibility was *ca.* 51.6% higher. Similarly, the Mg content of cowpea cultivars was higher, reaching bioaccessibility and dialyzability superior to 50% and 21%, respectively. Cu levels were not very sensitive to the effects of biofortification. Cooking slightly altered the Cu, Mn, and Mg contents of the seeds of the cowpea cultivars.

On the other hand, the cooked seeds presented higher bioaccessibility and dialyzability of minerals than the raw samples. The highlights evaluating the proximate composition consist of the high lipid content and energy value of cowpea seeds. Investigating the interaction between the studied nutritional factors, we unveiled an unknown ionomic correlation between Cu and Mg and a synergistic correlation between the Mn and lipids content. The findings also show that the antagonistic correlation of Cu and Mn with Zn can be a limiting factor for the increase in micronutrient content in the seeds of biofortified cowpea cultivars. PC1 was sufficient to discriminate cowpea and common bean cultivars based on the high loadings presented for Zn and Fe content, indicating that the effects of biofortification are superior to those of other factors, such as cooking.

Therefore, our results reveal that the positive effects of biofortification of cowpea cultivars recently introduced by Embrapa are beyond those reported by Coelho et al. (2021). The consumption of these biofortified cowpea beans is a potential instrument to reverse the deficiency and or maintain the micronutrient homeostasis in the human diet, considering the crucial role of these nutrients for vital functions such as innate immunity. However, further studies to investigate biomolecules such as metalloproteins and deeply understand the interaction of micronutrients in cowpea seeds are necessary. In addition, an *in vivo* trial can aid the evaluation of the effectiveness of new biofortified cowpea cultivars as nutrient vehicles.

CRediT authorship contribution statement

Ronaldo C. Coelho: Conceptualization, Methodology, Formal analysis, Writing – original draft. **Darlisson S.N. Silva**: Methodology, Formal analysis. **Hudson de C. Silva**: Methodology, Formal analysis. **Maurisrael de M. Rocha**: Investigation, Visualization. **Roberto C.F. Barsotti**: Methodology, Formal analysis. **Heloisa F. Maltez**: Methodology, Formal analysis, Investigation. **Clecio Dantas**: Formal analysis. **Cícero A. Lopes Júnior**: Conceptualization, Writing – original draft, Supervision, Visualization. **Herbert de S. Barbosa**: Conceptualization, Writing – original draft, Project administration, Funding acquisition, Supervision, Visualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgments

The authors thank to Fundação de Amparo à Pesquisa do Estado do Piauí (FAPEPI, Piauí, Brazil), Fundação de Amparo à Pesquisa do Estado do Maranhão (FAPEMA, Maranhão, Brazil, grant 160018), Coordenação de Aperfeiçoamento de Pessoal Nível Superior (CAPES), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, grant 409543/2016-0 and 200275/2020-8) for the financial support and fellowships. The authors also thank the Brazilian Agricultural Research Corporation - Mid North (Embrapa Meio-Norte) for providing the cowpea cultivars used in the study. The author RCC thanks to Instituto Federal do Piauí (IFPI, grant 23055.000976/2021-85).

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jfca.2023.105291.

References

Adebooye, O.C., Singh, V., 2008. Physico-chemical properties of the flours and starches of two cowpea varieties (Vigna unguiculata (L.) Walp). Innov. Food Sci. Emerg. Technol. 9 (1), 92–100. https://doi.org/10.1016/j.ifset.2007.06.003.

Adjei-Fremah, S., Worku, M., de Erive, M.O., He, F., Wang, T., Chen, G., 2019. Effect of microfluidization on microstructure, protein profile and physicochemical properties of whole cowpea flours. Innov. Food Sci. Emerg. Technol. *57*. https://doi.org/10.1016/j.ifset.2019.102207.

Ajeesh Krishna, T.P., Maharajan, T., Victor Roch, G., Ignacimuthu, S., Antony Ceasar, S., 2020. Structure, function, regulation and phylogenetic relationship of ZIP family transporters of plants. Front. Plant Sci. 11 (May), 1–18. https://doi.org/10.3389/fpls.2020.00662.

Avanza, M., Acevedo, B., Chaves, M., Añón, M., 2013. Nutritional and anti-nutritional components of four cowpea varieties under thermal treatments: Principal component analysis. LWT Food Sci. Technol. 51 (1), 148–157. https://doi.org/10.1016/j.lwt.2012.09.010.

Baxter, I., 2009. Ionomics: studying the social network of mineral nutrients. Curr. Opin. Plant Biol. 12 (3), 381–386. https://doi.org/10.1016/j.pbi.2009.05.002.

Blanco-Rojo, R., Vaquero, M.P., 2019. Iron bioavailability from food fortification to precision nutrition. A review. In: Innovative Food Science and Emerging Technologies, vol. 51. Elsevier Ltd, pp. 126–138. https://doi.org/10.1016/j.ifset.2018.04.015.

Bouis, H.E., Saltzman, A., 2017. Improving nutrition through biofortification: a review of evidence from HarvestPlus, 2003 through 2016. Glob. Food Secur. 12 (January), 49–58. https://doi.org/10.1016/j.gfs.2017.01.009.

Bramorski, A., da Rosa Cherem, A., Mezadri, T., Melo, S.S., Deschamps, F.C., Gonzaga, L. V., Rockenbach, I.I., Fett, R., 2011. Chemical composition and antioxidant activity of Gaylussacia brasiliensis (camarinha) grown in Brazil. Food Res. Int. 44 (7), 2134–2138. https://doi.org/10.1016/j.foodres.2010.09.033.

Carvalho, L.M.J., Corrêa, M.M., Pereira, E.J., Nutti, M.R., Carvalho, J.L.V., Ribeiro, E.M. G., Freitas, S.C., 2012. Iron and zinc retention in common beans (Phaseolus vulgaris L.) after home cooking. Food Nutr. Res. *56*. https://doi.org/10.3402/fnr.v56i0.15618.

Coelho, R.C., Carlos, R., Barsotti, F., França, H., Lopes, A., Sousa, H. de, 2021. Expanding information on the bioaccessibility and bioavailability of iron and zinc in biofortified cowpea seeds. Food Chem. 347 (January), 129027 https://doi.org/10.1016/j.foodchem.2021.129027.

Díaz-Gómez, J., Twyman, R.M., Zhu, C., Farré, G., Serrano, J.C., Portero-Otin, M., Muñoz, P., Sandmann, G., Capell, T., Christou, P., 2017. Biofortification of crops with nutrients: factors affecting utilization and storage. In: Current Opinion in Biotechnology, vol. 44. Elsevier Ltd, pp. 115–123. https://doi.org/10.1016/j.copbio.2016.12.002.

FAO, 2021. World Food and Agriculture – Statistical Yearbook 2021. World Food and Agriculture – Statistical Yearbook 2021. FAO. https://doi.org/10.4060/cb4477en.

Feitosa, S., Greiner, R., Meinhardt, A.K., Müller, A., Almeida, D.T., Posten, C., 2018. Effect of traditional household processes on iron, zinc and copper bioaccessibility in black bean (Phaseolus vulgaris L.). Foods 7 (8), 1–12. https://doi.org/10.3390/foods7080123.

Fontenele, A., Carvalho, U., Mateus, N., Sousa, D., Felipe, D., Clarissa, Lady, Maria, R., Viana, P., Thomaz, S., Saker, S., Barbosa, M., Sousa, D., Pacelli, G., Lima, G. de, Maia, S., Morais, D., Costa, C., Rodrigues, F., Filho, F., 2012. Nutritional ranking of 30 Brazilian genotypes of cowpeas including determination of antioxidant capacity and vitamins. J. Food Compos. Anal. 26, 81–88. https://doi.org/10.1016/j.jfca.2012.01.005.

Freire Filho, F.R., 2011. Feijão-Caupi no Brasil Produção, melhoramento genético, avanços e desafios, first ed. Embrapa Meio-Norte.

Frota, K.D.M.G., Soares, R.A.M., Arêas, J.A.G., 2008. Composição química do feijão caupi (Vigna unguiculata L. Walp), cultivar BRS-Milênio. Cienc. Tecnol. Aliment. 28 (2), 470–476. https://doi.org/10.1590/S0101-20612008000200031.

Gerrano, A.S., Jansen van Rensburg, W.S., Venter, S.L., Shargie, N.G., Amelework, B.A., Shimelis, H.A., Labuschagne, M.T., 2019. Selection of cowpea genotypes based on grain mineral and total protein content. Acta Agric. Scand. Sect. B Soil Plant Sci. 69 (2), 155–166. https://doi.org/10.1080/09064710.2018.1520290.

Gomes, M.J.C., Martino, H.S.D., Kolba, N., Cheng, J., Agarwal, N., de Moura Rocha, M., Tako, E., 2022. Zinc biofortified cowpea (Vigna unguiculata L. Walp.) soluble extracts modulate assessed cecal bacterial populations and gut morphology in vivo (Gallus gallus). Front. Biosci. Landmark 27 (5). https://doi.org/10.31083/j.fbl2705140.

Granato, D., Santos, J.S., Escher, G.B., Ferreira, B.L., Maggio, R.M., 2018. Use of principal component analysis (PCA) and hierarchical cluster analysis (HCA) for multivariate association between bioactive compounds and functional properties in foods: a critical perspective. In: Trends in Food Science and Technology, vol. 72. Elsevier Ltd, pp. 83–90. https://doi.org/10.1016/j.tifs.2017.12.006.

Huertas, R., William Allwood, J., Hancock, R.D., Stewart, D., 2022. Iron and zinc bioavailability in common bean (Phaseolus vulgaris) is dependent on chemical composition and cooking method. Food Chem. 387, 132900 https://doi.org/10.1016/j.foodchem.2022.132900.

Kafaoglu, B., Fisher, A., Hill, S., Kara, D., 2016. Determination and evaluation of element bioaccessibility in some nuts and seeds by in-vitro gastro-intestinal method. J. Food Compos. Anal. 45, 58–65. https://doi.org/10.1016/j.jfca.2015.09.011.

Kan, L., Nie, S., Hu, J., Wang, S., Bai, Z., Wang, J., Zhou, Y., Jiang, J., Zeng, Q., Song, K., 2018. Comparative study on the chemical composition, anthocyanins, tocopherols and carotenoids of selected legumes. Food Chem. 260 (December 2017), 317–326. https://doi.org/10.1016/j.foodchem.2018.03.148.

Kumar, B., Dhaliwal, S.S., 2022. Zinc biofortification of dual-purpose cowpea [Vigna unguiculata (L.) Walp.] for enhancing the productivity and nutritional quality in a semi-arid regions of India. Arch. Agron. Soil Sci. 68 (8), 1034–1048. https://doi.org/10.1080/03650340.2020.1868040.

Kumari, M., Platel, K., 2017. Bioaccessibility of trace elements and chromium speciation in commonly consumed cereals and pulses. Int. J. Food Prop. 20 (7), 1612–1620. https://doi.org/10.1080/10942912.2016.1215996.

Liu, N., Zhang, Y., Wang, B., Xue, Y., Yu, P., Zhang, Q., Wang, Z., 2014. Is grain zinc concentration in wheat limited by source? AJCS 8 (11), 1534–1541.

Lovato, F., Kowaleski, J., da Silva, S.Z., Heldt, L.F.S., 2018.). Composição centesimal e conteúdo mineral de diferentes cultivares de feijão biorfortificado (Phaseolus vulgaris L.). Braz. J. Food Technol. *21*. https://doi.org/10.1590/1981-6723.6817.

Mbuma, N.W., Gerrano, A.S., Lebaka, N., Labuschagne, M., 2022. Interrelationship between grain yield components and nutritional quality traits in cowpea genotypes. South Afr. J. Bot. 150, 34–43. https://doi.org/10.1016/j.sajb.2022.07.006.

Mejia, L.A., Dary, O., Boukerdenna, H., 2017. Global regulatory framework for production and marketing of crops biofortified with vitamins and minerals. Ann. N. Y. Acad. Sci. 1390 (1), 47–58. https://doi.org/10.1111/nyas.13275.

Mingroni, T.T., Hamada, J., Xavier, A.D.S., Cavalcantea, C., Nascimento, A.N. do, 2018. In vitro evaluation of Ca, Cu, and Mg bioaccessibility in fresh and dried fruits. J. Braz. Chem. Soc. 00 (1), 1–8. https://doi.org/10.21577/0103-5053.20180159.

Herbello-Hermelo, P., Lamas, J.P., Lores, M., Domínguez-González, R., Bermejo-Barrera, P., Moreda-Piñeiro, A., 2018. Polyphenol bioavailability in nuts and seeds by an in vitro dialyzability approach. Food Chem. 254, 20–25. https://doi.org/10.1016/j.foodchem.2018.01.183.

Moreda-Piñeiro, J., Herbello-Hermelo, P., Domínguez-González, R., Bermejo-Barrera, P., Moreda-Piñeiro, A., 2016. Bioavailability assessment of essential and toxic metals in edible nuts and seeds. Food Chem. 205, 146–154. https://doi.org/10.1016/j.foodchem.2016.03.006.

Naiker, T.S., Gerrano, A., Mellem, J., 2019. Physicochemical properties of flour produced from different cowpea (Vigna unguiculata) cultivars of Southern African origin. J. Food Sci. Technol. 56 (3), 1541–1550. https://doi.org/10.1007/s13197-019-03649-1.

Neves, D.S.C., Souza, A.S., de Lemos, L.R., 2020. Multivariate optimization of an aqueous two-phase extraction for determination of cadmium and manganese in food sample. Microchem. J. 159 (June), 105458 https://doi.org/10.1016/j.microc.2020.105458.

Nosratpour, M., Jafari, S.M., 2018. Bioavailability of minerals (Ca, Mg, Zn, K, Mn, Se) in food products. Encyclopedia of Food Chemistry. Elsevier, pp. 148–154. https://doi.org/10.1016/B978-0-08-100596-5.21618-1.

Okwuonu, I.C., Narayanan, N.N., Egesi, C.N., Taylor, N.J., 2021. Opportunities and challenges for biofortification of cassava to address iron and zinc deficiency in Nigeria. Glob. Food Secur. *28*. https://doi.org/10.1016/j.gfs.2020.100478.

Oliveira, A.P., Naozuka, J., 2017. Effects of iron enrichment of adzuki bean (Vigna angularis) sprouts on elemental translocation, concentrations of proteins, distribution of Fe-metalloproteins, and Fe bioaccessibility. J. Braz. Chem. Soc. 28 (10), 1937–1946. https://doi.org/10.21577/0103-5053.20170034.

Pereira, A.M., Bonemann, D.H., Scherdien, S.H., Ávila, B.P., Antunes, I.F., Ribeiro, A.S., Gularte, M.A., 2020. Evaluation of total and bioaccessible concentration of minerals in creole beans. J. Food Compos. Anal. 94 (April) https://doi.org/10.1016/j.jfca.2020.103622.

Pereira, E.J., Carvalho, L.M.J., Dellamora-Ortiz, G.M., Cardoso, F.S.N., Carvalho, J.L.V., Viana, D.S., Freitas, S.C., Rocha, M.M., 2014. Effects of cooking methods on the iron and zinc contents in cowpea (Vigna unguiculata) to combat nutritional deficiencies in Brazil. Food Nutr. Res. *58*. https://doi.org/10.3402/fnr.v58.20694.

Phillips, R.D., 2013. Cowpea processing and products. Dry Beans Pulses Prod. Process. Nutr. 235–259. https://doi.org/10.1002/9781118448298.ch10.

Primitivo, M.J., Neves, M., Pires, C.L., Cruz, P.F., Brito, C., Rodrigues, A.C., de Carvalho, C.C.C.R., Mortimer, M.M., Moreno, M.J., Brito, R.M.M., Taylor, E.J., Millson, S.H., Reboredo, F., Jorge Campos, M., Vaz, D.C., Ribeiro, V.S., 2022. Edible flowers of Helichrysum italicum: composition, nutritive value, and bioactivities. Food Res. Int. *157*. https://doi.org/10.1016/j.foodres.2022.111399.

Ribeiro, N.D., Kläsener, G.R., 2020. Physical quality and mineral composition of new Mesoamerican bean lines developed for cultivation in Brazil. J. Food Compos. Anal. 89 (November 2019), 103479 https://doi.org/10.1016/j.jfca.2020.103479.

Sánchez-Peña, M.J., Márquez-Sandoval, F., Ramírez-Anguiano, A.C., Velasco-Ramírez, S. F., Macedo-Ojeda, G., González-Ortiz, L.J., 2017. Calculating the metabolizable energy of macronutrients: a critical review of atwater's results. Nutr. Rev. 75, 37–48. https://doi.org/10.1093/nutrit/nuw044.

Silva, V.M., Boleta, E.H.M., Martins, J.T., dos Santos, F.L.M., da Rocha Silva, A.C., Alcock, T.D., Wilson, L., de Sá, M.E., Young, S.D., Broadley, M.R., White, P.J., dos Reis, A.R., 2019. Agronomic biofortification of cowpea with selenium: effects of selenate and selenite applications on selenium and phytate concentrations in seeds. J. Sci. Food Agric. 99 (13), 5969–5983. https://doi.org/10.1002/jsfa.9872.

Silva, V.M., Nardeli, A.J., Mendes, N.A. de C., Rocha, M. de M., Wilson, L., Young, S.D., Broadley, M.R., White, P.J., Reis, A.R. dos, 2021. Agronomic biofortification of cowpea with zinc: variation in primary metabolism responses and grain nutritional quality among 29 diverse genotypes. Plant Physiol. Biochem. 162 (January), 378–387. https://doi.org/10.1016/j.plaphy.2021.02.020.

Silva, V.M., Wilson, L., Young, S.D., Broadley, M.R., White, P.J., Reis, A.R. dos, 2022. Interaction between sulfur and selenium in agronomic biofortification of cowpea plants under field conditions. Plant Soil. https://doi.org/10.1007/s11104-022-05480-8.

Singh, U., Praharaj, C.S., Singh, S.S., Singh, N.P., 2016. Biofortification: introduction, approaches, limitations, and challenges. Biofortification Food Crops 1–490. https://doi.org/10.1007/978-81-322-2716-8.

Song, M., Vos, M.B., McClain, C.J., 2018. Copper-fructose interactions: a novel mechanism in the pathogenesis of NAFLD. Nutrients 10 (11), 1–24. https://doi.org/10.3390/nu10111815.

Teka, T.A., Retta, N., Bultosa, G., Admassu, H., Astatkie, T., 2020. Protein fractions, in vitro protein digestibility and amino acid composition of select cowpea varieties grown in Ethiopia. Food Biosci. *36*. https://doi.org/10.1016/j.fbio.2020.100634.

Zhang, Y., Liu, N., Hu, N., Zhang, W., Sun, Z., Wang, Z., 2022. Effect of Zn and sucrose supply on grain Zn, Fe and protein contents within wheat spike under detached-ear culture. Crop Pasture Sci. 73 (2), 13–21. https://doi.org/10.1071/CP21129.