J Food Sci Technol (November 2021) 58(11):4083–4090
https://doi.org/10.1007/s13197-021-04966-0

REVIEW ARTICLE

Bioaccessibility of minerals in combinations of biofortified foods with Fe, Zn and vitamin A

Priscila Brigide[1] · Larissa Catelli Rocha Torres[2] · Solange Guidolin Canniati Brazaca[2] · Renata Silva Figliuzzi[3] · Neuza Maria Brunoro Costa[1]

Revised: 22 December 2020 / Accepted: 6 January 2021 / Published online: 2 February 2021

Abstract Initiatives to improve the nutritional quality of staple foods, such as beans and cassava by bio fortification should be encouraged as an alternative to overcome the deficiencies of iron and vitamin A. The evaluation of the bio accessibility of the minerals in these foods is also important, since the composition of nutrients does not necessarily correspond to the amount absorbed and metabolized in the body. Thus, the present work aims to evaluate the bio accessibility of iron (Fe) and zinc (Zn) in the presence of β-carotene in combinations of bio fortified food sources of Fe and Zn (cowpea cooked with and without maceration: CM/CW, respectively) and β-carotene (cooked cassava and cassava flour: CC/CF, respectively). The mixtures, after cooking, were analyzed for the centesimal composition and minerals, phytates, the percentage of iron and zinc bio accessibility by in vitro method and molar ratio. The mixtures presented significant amounts of proteins, carbohydrates and fibers. The β-carotene content showed no statistical difference in processing methods. The Fe content showed lower levels in the controls with cassava flour and its bio accessibility was also lower for treatment with cassava flour with CM, while for Zn or higher content it was used for treatment CW/CF, differing only from the treatment CW/CC, although their bio accessibilities were not different, except to iron in CMCC treatment. Both the IP6 fraction and the IP5 fraction did not show a significant difference ($p > 0.05$) between the treatments, it suggests no interference in bio accessibility. A diet rich in iron and vitamin A in adequate amounts with minimal content of absorption inhibitors can be effective in controlling iron deficiency. Bio fortified mixtures must be encouraged in different forms of consumption.

Keywords Minerals · β-carotene · Bio fortification · Cowpeacassava

✉ Priscila Brigide
pbrigide@yahoo.com.br

Larissa Catelli Rocha Torres
larissa.catelli.rocha@usp.br

Solange Guidolin Canniati Brazaca
sgcbraza@usp.br

Renata Silva Figliuzzi
renatafigliuzzi@gmail.com

Neuza Maria Brunoro Costa
neuzambc@gmail.com

[1] Post-Graduation Program On Food Science and Technology, Federal University of Espírito Santo (UFES), Alto Universitário s/n, Alegre, ES CEP 29500-000, Brazil

[2] Post-Graduation Program On Food Science and Technology, School of Agriculture "Luiz de Queiroz", University of Sao Paulo, Piracicaba, SP, Brazil

[3] Graduation of Biology, Universityof Espírito Santo (UFES), Alto Universitário s/n, Alegre, ES CEP 29500-000, Brazil

Introduction

According to the World Health Organization (WHO), deficiencies of iron, iodine and vitamin A are the most common forms of malnutrition, leading to serious public health problems. Deficiencies of iron and zinc can cause anemia, compromising the physical capacity of physical work, growth retardation and changes in neurological and immune function (WHO 2014).

Strategies to combat nutritional deficiencies have been implemented, one of them is of great importance, is's

biofortification, which is based on the genetic improvement of conventional plants to increase the density of nutrients in staple foods, appears as an alternative choice. The cultures produced have higher levels of micronutrients recognized as limiting by the World Health Organization, especially iron, zinc and vitamin A (HARVEST PLUS 2016).

In this scenario in Brazil, the Harvest Plus program started in 2004, aiming to develop varieties of rice, beans, cassava, corn, wheat and sweet potatoes with higher concentrations of Fe, Zn and β-carotene. In addition, it is possible to produce significant increases in iron concentrations in beans, even containing high levels of antinutritional factors, such as phytic acid and tannins, which can decrease their availability and absorption (Sperotto and Ricachenevskyet al. 2017). The program is coordinated by the Brazilian Agricultural Research Corporation (EMBRAPA), constituting the BIOFORT network.

Among these foods, beans are considered one of the most important food crops in the world due to their nutritional quality, which provides nutrients such as proteins, iron, zinc and vitamins, in addition to fibers (Hayat et al. 2014) and the cassava root is a rich source of carbohydrates (Carvalho et al. 2017).

Regarding cassava, due to the high moisture content of freshly harvested cassava (*Manihotesculenta*), around 60%, the roots are classified as perishable. One way to increase the shelf life is dehydration to produce different types of flour, are widely used for human consumption (Souza et al. 2008).

Flour is the main derivative of cassava for human consumption in Brazil, as it is consumed throughout the country, principally in the North and Northeast regions, being the main source of energy (Souza et al. 2008). According to our previous unpublished study, cassava flour showed higher values β-caroteneof than cooked cassava. It is known that the two forms of processing are widely consumed by the Brazilian population.

Some studies suggest that there is a synergism between the metabolism of Fe, Zn and vitamin A (Sant´ana et al. 2019; Antunes et al. 2019; Corrêa et al. 2020). Thus, the mixtures of these foods were analyzed for chemical composition, minerals and their bioaccessibility, phytates and phytate/mineral molar ratio.

Materials and methods

Foods with high Fe content, cowpea (*Vignaunguiculata* L Walp.) Cultivar BRS Aracê, and β-carotene, cassava BRS Jari, supplied by EMBRAPA, were evaluated.

Sample preparationThe preparation was carried out, according to normal household processing habits, that is, all foods were subjected to cooking up to the point of food consumption, in stainless steel utensils, using MiliQwater in all stages of preparation. For the formulation of cassava flour, the oven was used at 105 °C for 3 h and then the blender was used until the flour consistency. Cassava was cooked in a pan with enough water to cover them at minimum cooking time. The beans were macerated in the proportion of (1: 3 water), then the water was discarded and replaced by water in the proportion of (1: 2 w:v) for cooking, after what was dried in an oven at 105 °C for 24 h. While the beans without maceration, water was placed in the proportion of (1: 2w:v) and oven dried at 105 °C for 24 h. All treatments were prepared 1: 1 (w: w). And the following procedures were obtained:Treatments.

a) biofortified cowpea with maceration + cooked cassava (CM/CC)
b) biofortified cowpea with maceration + cassavaflour (CM/CF)
c) biofortified cowpea without maceration + cooked cassava (CW/CC)
d) biofortified cowpea without maceration + cassava flour (CW/CF)

Centesimal composition

Chemical analyzes of moisture content, crude protein, ether extract, dietary fibers (soluble and insoluble), ash and minerals were carried out according to AOAC (2005). Carbohydrates were obtained by difference.

Mineral content

Digestion was performed with 250 mg of sample, and 6 mL of 20% nitric acid and 2 mL of 30% (v:v) hydrogen peroxide were added, and after digestion, the volume was increased to 25 mL with ultrapure water. The determination was made in an ICP-OES by Optical Emission Spectrometry in Plasma Coupled at the Center for Nuclear Energy in Agriculture—CENA University of São Paulo—USP.

Phytates

A food sample of 0.5 g was macerated and diluted in 10 mL 0.5 M HCl. Then the sample was homogenized under constant stirring for two hours. After this period, the samples were centrifuged at 5000 rpm at 4 °C for 30 min and the supernatants were removed and frozen overnight. The next day, the samples were centrifuged at 5000 rpm at 4 °C for 15 min and filtered through a 0.45 micron filter. The filtered volume was diluted to a final volume of 25 mL.

The samples were purified using a SAX column. The column was washed successively with 10 mL of 0.05 M HCl and 25 mL of the sample, the eluates were discarded and 2 mL of 2 N HCl were eluted in the column. The eluates were lyophilized and taken up into 1.0 mL in MiliQ water and subjected to quantitative analyzes by HPLC.

The solution was filtered with nylon membrane filters (0.45 μm) and the HPLC working conditions LC-20A Prominence, RID-20A; LC-20A; CTO-20A; SIL-20A, CBM-20A) were: oven temperature 35 °C, flow rate of 0.8 mL.min-1, isocratic mode and injection volume of 20 μL.The peak area data of the standards was used for the calculation of the calibration curve, from which the concentration of phytic acid in the samples was obtained (Frontela et al. 2009).

Extraction of carotenoids

Carotenoid analysis was performed according to Rodriguez-Amaya (1999), with some modifications. Briefly, 3 g of the sample was mixed with50 mL of acetone in a mortar to promote the extraction of the pigments. The mixture was filtered through a Büchner funnel and the residue was taken back to the mortar. The extraction and filtration were repeated until the residue became colorless. The extract was collected in a kitassato. The carotenoids were gradually transferred to approximately 30 mL of petroleum ether in a separating funnel, followed by the addition of water, separation of the phases and disposal of the lower phase of water–acetone after each addition. When all the carotenoids were transferred to petroleum ether, the ethereal phase was washed three or four times with water to completely remove the acetone, and placed in an Erlenmeyer flask. This mixture was taken to a separating funnel and distilled water was added, observing the formation of two phases: the upperethereal phase and the lower aqueous phase. The aqueous phase was collected and the ethereal phase washed with water until there was no more alkali residue.

The ethereal phases were mixed and passed through a funnel containing anhydrous sodium sulfate to remove any residual water.The final extract obtained was concentrated in a rotary evaporator and taken to dryness with nitrogen and then recovered in the same amount of acetone and filtered in a filter unit (0.45 mm).The purity of the solutions was verified by HPLC, and the quantification was performed by spectrophotometry, based on the maximum absorbance, according to Beer's Law. The absorption coefficient used 2.592 for β-carotene. The maximum absorption wavelength was β: 450 nm, in petroleum ether (Rodriguez-Amaya 1999).

Quantification

The quantification of carotenoids was performed by external standardization, using calibration curves constructed with five points.To obtain these curves, solutions of the standards, in the proportions corresponding to those found in the samples, were mixed in a volumetric flask and the volume was completed with petroleum ether, to obtain 50 mL of final mixture.Then, 1, 2, 3, 4 and 5 mL aliquots were taken in triplicate, taken to dryness with nitrogen, diluted in 1 mL of HPLC grade acetone and injected into the chromatograph.

The final extracts of the samples, were dried in nitrogen, were also diluted in 1 mL of acetone grade HPLC and injected in the chromatograph.To calculate the concentration of the carotenoids in the samples, the standard curve was used, in which the peak area of each carotenoid corresponds to a value in μg/mL.Then, the value obtained for each sample, in μg/mL, was divided by its initial weight (in g), obtaining a value in μg/g of carotenoid in the sample.

Chromatographic conditions

The chromatograph used was the Shimadzu 20A model, equipped with a pumping system model LC-20AT, automatic sample injector model SIL-20AHT, column oven model CTO-20A, communicator model CBM-20A and UV detector (270 and 325 nm) model SPD-20A. And following the chromatographic conditions according to Pinheiro-Sant'Ana et al. (1998), which include: Kinetex chromatographic column (Phenomenex) C18, 50 × 2.1 mm, 2.6 μm; UV–visible detector; mobile phase—methanol: ethyl acetate: acetonitrile (1: 1: 8); mobile phase flow: 0.60 mL/min L^{-1}), in isocratic elution. Chromatograms were read at 450 nm.

Phytate/mineral molar ratio

The molar ratio of phytate to iron was calculated as the millimoles of phytate present in the sample divided by the molecular mass of 660.80 and atomic mass of 55.80 for iron, 65.28 for zinc.

Bioaccessibility of iron and zinc

The in vitro digestion procedure was carried out according to Megías et al. (2009), to simulate gastrointestinal digestion.

The 2 g of each sample was weighed in triplicate. Deionized water (40 mL) was used as negative control group (Blank) and ferrous sulfate as a positive control (0.00208 g). To simulate the gastric digestion, the samples and controls were placed in Erlenmeyer and 38 mL of

water were added, the pH was adjusted to nearly 2.0 with 1 N HCl solution. Then, 0.003 g of pepsin was added to the medium, based on the protein content, in the samples and were incubated in a water bath at 37 °C under agitation for 2 h. Then, for the intestinal digestion, the pH of the medium was adjusted to nearly 7.5 with NaOH 0.1 N and then added 0.0075 g of pancreatin, based on the protein content of the samples. The samples were again incubated in a water bath at 37 °C, under agitation for 2 h.

After gastrointestinal digestion, the enzymes used were inactivated by thermal treatment at 75 °C for 20 min in a water bath. The samples were cooled, and then centrifuged at 5500 RPM at 4 °C for 25 min, to separate the soluble and residual fraction. Mineral analysis was carried out on the soluble fraction.

Analysis of iron and zinc

After digesting the sample in the microwave, iron and zinc were read in triplicate in order to obtain the quantity of minerals that would be available to be absorbed by the body.

All glassware and instruments that came into contact with the samples were previously demineralized with 10% nitric acid (DINAMICA) for 24 h and then washed with ultra pure water (Ultra purifier THERMO Easypure II 18.2 MΩ x cm).

$$\text{Bioaccessibility } (\%) = \frac{\text{Mineral content } (\text{mg}/100\ \text{g}) \text{ in the intestinal fraction}}{\text{Total mineral content}(\text{mg}/100\ \text{g})} \times 100$$

Statistical analysis

The results are reported as the means ± standard deviation (SD) of treatments. After the treatments were tested for normality and equal variances, the means were compared through a one-way analysis of variance (ANOVA) with a Tukey posttest for multiple comparisons. Values of $p < 0.05$ were considered significant. Statistical evaluation of the data was performed with the software SAS.

Results and discussion

The food processes of maceration, cooking orflour production showed little effect on the centesimal composition (Table 1), although the beans without maceration and cooked cassava were more effective to preserve the ash contents ($p < 0.05$).

Regarding themoisture content, treatments with cooked lyophilized cassava (CMCC and CWCC) reported higher levels (9.53 and 8.51%), compared tothe mixtures with cassava flour (CMCF and CWCF) ranging from 7.10 to 7.20%.Nevertheless, thehe moisture content of both cassava flour and cooked cassava was within the limit established by Brazilian legislation (maximum of 14%), which helps to control microbial deterioration of the product.

Regarding the protein content, the treatments do not differ between the treatments, varying from 13.46 to 15.04%. Silva et al (2002) determined the protein content of 45 cowpea genotypes (*Vignaunguiculata* (L.) Walp.) 20.29–29.29%. Souza et al. (2008) evaluated the content of protein in cassava flours with an average of 0.90%. Taking into account the procedures and their proper mixtures and individual values, the data are close to the literature.

Antunes et al (2019) found the lipid content of 0.30% for biofortified cassava and cowpea from 1.90 to 2.00%, lower than the present study.Emphasizing that both cassava and beans are not abundant sources of this macronutrient. According to Nair and Agustine (2018), the importance of fat is related to synergism for the absorption of vitamin A.

Regarding the ash content, all values are recovered within the limit set by the Brazilian legislation (maximum content of 2.0%).This good characteristic of these raw materials is due to the fact that plant foods are endowed with micronutrients (Nair and Augustine 2018).

According to BRASIL / ANVISA (1998), to be a source of dietary fiber, the food must contain at least 3% of fibers and to be fiber-rich, the food must contain at least 6% fiber. Thus, the mixtures can be considered fiber-rich foods.Fibers are more common in plant foods, such as beans.

The yellow cassava, BRS Jarí, was one of the first biofortified agricultural products developed by Embrapa. Natural source of energy and little fibrous, BRS Jarí is rich in vitamin A, in addition to conserving its nutritional properties after cooking (which is lost in more traditional varieties) (Souza et al. 2015).

Many authors report the importance of carotenoids, especially pro-vitamin A (α and β-carotene) and, in addition, the antioxidant capacity of some of them. Currently, more than 600 carotenoids are known and characterized by their chemical structures. In vegetables, common pro-vitamin A carotenoids include β-carotene and its isomers 9, 13 and 15, α-carotene and β-cryptoxanthin. Other common carotenoids such as lycopene, lutein and zeaxanthin have no pro-vitamin A activity, but serve as natural antioxidants. The carotenoid content of plant sources contributes approximately 68% of the world vitamin A diet and 82% in developed countries (Carvalho et al. 2017).

The levels of β-carotene are shown in Table 2 and there was no significant difference, which shows that both cassava flour and cooked cassava are similar concerning vitamin A content.

Table 1 Centesimal composition of biofortified mixtures (g.100 g^{-1}) % dry matter

Treatments	Moisture	Protein	Lipids	Ashes	Carbohydrates	Insoluble fiber	Soluble fiber	Total fiber
CMCC	9.53 ± 0.33[a]	15.04 ± 0.8[a]	4.59 ± 0.75[a]	2.02 ± 02[b]	47.30	17.19	4.2	21.51
CMCF	7.10 ± 0.32[b]	14.52 ± 0.45[a]	4.44 ± 0.15[a]	2.01 ± 0,10[b]	50.90	16.94	4.09	21.03
CWCC	8.51 ± 0.11[a]	13.46 ± 0.82[a]	4.57 ± 0.39[a]	2.07 ± 0.25[b]	49.60	15.92	5.87	21.79
CWCF	7.20 ± 0.37[b]	14.69 ± 0.32[a]	4.44 ± 0.26[a]	2.52 ± 0.10[a]	52.63	15.20	3.32	18.52

Means + standard deviation. Different letters in vertical indicate a significant difference between treatments at 5% probability in the Tukey test. CMCC: biofortified cowpea with maceration + cassava, CMCF: biofortified cowpea with maceration + cassava flour; CWCC: biofortified cowpea without maceration + cassava, CWCF: biofortified cowpea without maceration + cassava flour

Table 2 Content of ß- carotene

Treatments	mg.100 g^{-1}
CMCC	34.92 ± 0.84[a]
CMCF	32.81 ± 0.18[a]
CWCC	36.88 ± 0.50[a]
CWCF	37.40 ± 0.63[a]

Means + standard deviation. There was no significant difference between treatments at the 5% level by the Tukey test. CMCC: biofortified cowpea with maceration + cassava, CMCF: biofortified cowpea with maceration + cassava flour; CWCC: biofortified cowpea without maceration + cassava; CWCF: biofortified cowpea without maceration + cassava flour

Table 3 Content of minerals by biofortified mixtures (mg.kg^{-1})

Treatments	Fe	Zn
CMCC	39.60 ± 0.18[a]	21.03 ± 0.72[ab]
CMCF	28.50 ± 0.50[b]	22.14 ± 0.24[a]
CWCC	36.80 ± 0.10[a]	20.42 ± 0.94[b]
CWCF	28.90 ± 0.70[b]	21.54 ± 0.83[ab]

Means + standard deviation. Different letters in vertical indicate a significant difference between treatments at 5% probability in the Tukey test. CMCC: biofortified cowpea with maceration + cassava, CMCF: biofortified cowpea with maceration + cassava flour; CWCC: biofortified cowpea without maceration + cassava; CWCF: biofortified cowpea without maceration + cassava flour

Oliveira et al (2010) evaluated seven raw and cooked yellow cassava to identify total carotenoids, α and β-carotene and their isomers in new varieties that could contribute to improving nutritional quality in populations with malnutrition problems located in the tropics and, mainly, in Northeast Brazil. The total carotenoids ranged from 14.15 to 2.64 μg g^{-1} in raw roots, and the total β-carotene from 10.32 to 1.99 μg g^{-1}. The highest percentage of total carotenoid retention was found in two varieties (99.49%) and, in total, β-carotene (94.31%), both after cooking.

The analysis of the mineral content of the treatments is shown in Table 3. Significant differences were observed between the treatments, because the iron content independent of the processing given to the beans, the treatment that presented the cassava in the cooked form, presented greater contents differing from the others. Regarding Zn, except for CWCC treatment, the content was lower.

More than 1000 commonbeans were evaluated by researchers from the International Center for Tropical Agriculture (CIAT), and they found variations between 34 and 89 mg.kg^{-1} for the levels of Fe in the grains and between 21 and 54 mg.kg^{-1} for Zn concentrations (Beebe et al 2000). In Peru, genotypes with high levels of Fe, greater than 100 mg.kg^{-1} were found (Gregorio 2002). The improvement of cultivars with high levels of Zn and Fe is an effective tool in the fight against anemia and in strengthening the immune system (Rocha 2008).

Faria et al (2018) evaluated four different types of beans (red, black, white and cowpea) cooked under different conditions (homemade and industrially processed canned product) regarding Fe content ranged from 3.6 to 6.4 mg.100 g^{-1}; Zn 1.6 to 3.4 mg.100 g^{-1} and the Ca content 0.54 to 0.94 mg.100 g^{-1}.

Dias and Leonel (2006) report that in the North region of Brazil, flour is extremely important for the supply of energy (20–50% of the total) and iron (30–40% of the total, but of low bioavailability) ingested by rural populations and low-income urban, being consumed more in the form of porridges and kind specific dishes like beijuand farofas. In the Northeast, the flour is widely consumed in the form of mush, or accompanying beans, dried meat, coffee and panela, in addition to being part of typical dishes of the region, what highlights the importance of these two basic foods present in the Brazilian diet as sources of iron and zinc.

According to Israr et al (2013), phytate is known to be effective in chelating minerals, reducing the availability of minerals that form complexes in the body and making oxalate more bioavailable.The contents of inositol hexaphosphate (IP6), inositol pentaphosphate (IP5) (mg.

$100.g^{-1}$) for cultivars submitted to different dry processing are shown in Table 4

The InsP6—inositol phosphate is the most abundant in vegetables and is considered an anticancer agent but also an antinutritional component. Its presence is marked in black beans (88% of the total of inositol phosphates). However, procedures that can differentiate between the 6 forms of InsP have been developed only recently. InsP6 represents an average of 88% of the total inositol phosphates in black beans (Campos Vega et al. 2010). In the case of biofortified beans, IP6 predominated over IP5, with IP6 reaching 98% of the total phosphates of the samples. It is worth mentioning that both IP5 and IP6 are known to inhibit the bioavailability of iron and zinc in food.Extremely higher value was found by Vilakati et al (2016) in hulled cowpea with 1220 mg.100 g^{-1}, while Kruger et al (2014) found the values from 0.55 to 0.80 mg.100 g^{-1}.

The prediction of the mineral bioavailability of foods containing phytates is complicated by the complex interactions between minerals and phytic acid, phytase activity in the food and / or in the intestine, conditions in food processing, food digestibility as well as the individual's physiological state, suggested that the phytate: mineral molar ratio is the best tool to predict mineral bioavailability (Ramirez Cardenas 2006). To assess the effect of the phytate result present in food on the bioavailability of minerals (Fe and Zn), the phytate/mineral molar ratio has been investigated (Ma et al.2005). Phytate starts losing its inhibition effect on iron when the phytate/Fe molar ratios are below 1 (Hallberg et al. 1989); whereas if the phytate/Zn molar ratio exceeds 5, the bioavailability of Zn could be reduced by 50% (Turnlund et al. 1984). This suggests that the phytate present in beans does interfere with the absorption of iron and zinc and contribute to the deficiency of these minerals. Emphasizing that the presence of phytates is more common in cereals and legumes and not in roots (Table 5).

Table 4 Phytates contents (IP5 + IP6) of biofortified mixtures (mg.100 g^{-1})

Treatments	IP5	IP6	Phytates(IP5 + IP6)
CMCC	14.99 ± 0.14	30.78 ± 0.45	45.78
CMCF	14.14 ± 0.42	32.69 ± 0.47	46.84
CWCC	17.6 ± 0.52	32.68 ± 0.32	50.28
CWCF	14.41 ± 0.13	29.94 ± 0.53	43.25

Means + standard deviation. There was no significant difference between treatments at the 5% level by the Tukey test. CMCC: biofortified cowpea with maceration + cassava, CMCF: biofortified cowpea with maceration + cassava flour; CWCC: biofortified cowpea without maceration + cassava; CWCF: biofortified cowpea without maceration + cassava flour

Table 5 Molar ratio of phytate to iron and zinc

Treatments	Phyt/Fe	Phy/Zn
CMCC	0.70	1.54
CMCF	0.71	1.58
CWCC	0.77	1.69
CWCF	0.66	1.45

Table 6 Fe and Zn bioaccessibility of biofortified mixtures (%)

Treatments	Fe	Zn
CMCC	8.41 ± 1.24[b]	50.73 ± 2.96[a]
CMCF	14.30 ± 1.74[a]	49.54 ± 1.74[a]
CWCC	14.34 ± 1.78[a]	65.03 ± 1.86[a]
CWCF	14.89 ± 1.46[a]	51.53 ± 2.35[a]

Means + standard deviation. Different letters in vertical indicate a significant difference between treatments at 5% probability in the Tukey test. CMCF: biofortified cowpea with maceration + cassava, CMCC: biofortified cowpea with maceration + cassava flour; CWCC: biofortified cowpea without maceration + cassava; CWCF: biofortified cowpea without maceration + cassava flour

Once phytate is degraded, legumes can become good sources of iron and zinc due to their bioavailability.

It is clear that the total mineral content does not correspond to the amount that will be absorbed. Thus, bioaccessibility is important to determine the fraction of the nutrient that is released from its food matrix, in the gastrointestinal tractduring digestion and with intestinal availability for intestinal absorption. And, bioaccessibility depends mainly on food synergy (Kruger et al. 2014; Faria et al. 2018). Table 6 shows thebioaccessible fraction (%) after intestinal and intestinal digestion. The mineral Zn has greater values of bioaccessibilitythan Fe. No difference was observed between treatments concerning Zn bioacessibility; while for Febioacessibility, only the CMCC treatment showed lower value.

The bioaccessibility values are higher in relation to the study by Singh et al (2016) that assess the bioaccessibility of Fe and Zn in different legumes in raw and cooked form. As Ramírez-Ojeda et al (2018) determined the total and bioaccessible content. It should be noted that the present research uses biofortified raw materials and one of the biofortification purposes, in addition to increasing nutrient density and increasing bioavailability as reported in studies (Tako et al. 2015; Haas et al. 2016).

Conclusion

The biofortified mixtures showed high contents of protein, regardless of the processing of beans (with or without maceration) and treatment of cassava, as well as

carbohydrates and fibers.Thismay be of great importance for nutrition in developing countries, which depend on plant foods as source of protein, energy and minerals.

The β-carotene content showed no statistical difference in the processing methods.

Various forms of consumption should be encouraged, avoiding food monotomy.

Regarding the Fe content, the treatments in which the cassava was in the form of flour showed the lowest values regardless of the bean processing, however the Zn showed the highest content for the treatment of macerated beans and cassava flour, differing only from the treatment with beans without maceration with freeze-dried cooked cassava.

Concerning Fe bioaccessibility, only the treatment with macerated beans and cassava flour had a lower percentage, while for Zn they all showed high levels without differing from each other. It has been suggested that cassava treatments can be used without suffering interference from processing and that beans donot necessarily need the macerating step.

Both the IP6 and the IP5 fractions of phytates showed no significant difference between treatments According to the molar ratio, it is suggested that the phytate did not interfere with bioaccessibility.

A diet rich in iron and vitamin A in adequate amounts with minimal content of inhibitors can be an alternative choice to overcome iron deficiency.

Author contributions PB contributions to conception and design, acquisition of data, analysis and interpretation of data; RF carried out the experiments, LCRT, SGCB and RSF carried out the experiment, NMBC supervised the work and corrected the manuscript.

Funding The authors acknowledge CAPES for the scholarship granted to the first author, FAPES and the EMBRAPA for the donation of the bean and cassava.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

AntunesVazTostesSant'anaFariaToledoCosta PTMDGCTRARCLNBM (2019) Bioavailability of Iron and the Influence of Vitamin a in BiofortifiedFoods. Agro 9:1–15. https://doi.org/10.3390/agronomy9120777

AOAC (2005) Official method of analysis, 18th edn. Association of officiating analytical chemists, Washington DC

Beebe S, Gonzalez AV, Rengifo J (2000) Research on trace minerals in the common bean. Food Nutr Bull 21:387–391

BRASIL. MINISTÉRIO DA SAÚDE. AGÊNCIA NACIONAL DE VIGILÂNCIA SANITÁRIA. Portaria n° 27, de 13 de janeiro de 1998. Aprova o Regulamento Técnico referente à Informação Nutricional Complementar (declarações relacionadas ao conteúdo de nutrientes). Disponível em: http://www.anvisa.gov.br/e-legis/. Accessed on August 26, 2008

Campos Vega R, Loarca-Piña G, OomaH BD (2010) Minor components of pulses and their potential impact on human health. Food Res Inter 43:461–482

Carvalho LM, Corrêa MM, Pereira EJ, Nutti MR, Carvalho JL, Ribeiro EM, Freitas SC (2012) Iron and zinc retention in common beans (Phaseolus vulgaris L.) after home cooking. Food Nutr Res 56:15618. https://doi.org/10.3402/fnr.v56i0.15618

CarvalhoOrtizCarvalhoSmirdeleNeves LMJGMDJLVLFSC (2017) Carotenoids in yellow sweet potatoes, pumpkins and yellow sweet cassava. In: Goran SN (ed) Dragan J. IntechOpen, London

Corrêa SR, Brigide P, Vaz-Tostes MG, Costa NMB (2020) Cultivars of biofortified cowpea and sweet potato: Bioavailability of iron and interaction with vitamin A in vivo and in vitro. J FoodSci 85:816–823. https://doi.org/10.1111/1750-3841.15064

HARVEST PLUS. Crops. Available at: http://www.harvestplus.org/content/crops. Accessed in March 2016

Ramírez Cárdenas, L de los Á (2006) Biodisponibilidade de zinco e de ferro valor nutricional e funcional de diferentes cultivares de feijãocomum submetidos a tratamentos domésticos. Doctoral Thesis. Universidade Federal de Viçosa. Brazil

Dias LT, Leonel M (2006) Caracterização físico-química de farinhas de mandioca de diferentes localidades do Brasil. CiênAgrotec 4:692–700

Faria MA, Araújo A, Pinto E, Oliveira C, Oliva-Teles MT, Almeida A, Delerue- Matos C, Ferreira IMPLVO (2018) Bioaccessibility and intestinal uptake of minerals from different types of home-cooked and ready-to-eat beans. J FunctFoods 50:201–209. https://doi.org/10.1016/j.jff.2018.10.001

Frontela C, Scarino ML, Ferruzza S, Ros G, Martínez C (2009) Effect of dephynization on bioavailability of iron, calcium and zinc from infant cereals assessed in the Caco-2 cell model. World J Gastroenterol 15(16):1977–1984. https://doi.org/10.3748/wjg.15.1977

Gregorio GB (2002) Progress in breeding for trace minerals in staple crops. JNutr 132:500S-502S. https://doi.org/10.1093/jn/132.3.500S

Haas JD, Luna SV, Lung'ahoM G, Wenger MJ, Murray-Kolb LE, Beebe S, GahutuJ B, EgliI M (2016) Consuming iron biofortified beans increases iron status in Rwandan women after 128 days in a randomized controlled feeding trial. The J of Nutr 146:1586–1592. https://doi.org/10.3945/jn.115.224741

Hallberg L, Brune M, Rossander L (1989) Iron absorption in man: ascorbic acid and dose-dependent inhibition by phytate. Am J ClinNutr 49:140–144. https://doi.org/10.1093/ajcn/49.1.140

Hayat I, Ahmad A, Masud T, Ahmed A, Bashir S (2014) Nutritional and health perspectives of beans (Phaseolus vulgaris L.): an overview. Crit Rev Food SciNutr 54(5):580–592. https://doi.org/10.1080/10408398.2011.596639

Israr B, Frazier RA, Gordon MH (2013) Effects of phytate and minerals on the bioavailability of oxalate from food. FoodChem 3:1690–1693. https://doi.org/10.1016/j.foodchem.2013.04.130

Kruger J, Minnis-Ndimba R, Mtshali C, Minnaar A (2014) Novel in situ evaluation of therole minerals play in the development of the hard-to-cook (HTC) defect of cowpeas and its effect on the in vitromineral bioaccessibility. Food Chem 174:365–371. https://doi.org/10.1016/jfoodchem2014.10.134

Ma G, Jin Y, Piao J, Kok F, Guusje B, Jacobsen E (2005) Phytate, calcium, iron, and zinc contents and their molar ratios in foods commonly consumed in China. J of Agric and Food Chem 53:10285–10290. https://doi.org/10.1021/jf052051r

Megías C, Yust M, Pedroche J, Lquari H, Giron-Calle J, Alaiz M (2009) Purification of an ACE inhibitory peptide after hydrolysis

of sunflower (Helianthus annuus L.) protein isolates. J of Agric Food Chem 52:1928–1932. https://doi.org/10.1021/jf034707r

Nair KM, Augustine LF (2018) Food synergies for improving bioavailability of micronutrients from plant foods. Food Chem 238:180–185

Oliveira ARG, Carvalho LMJ, Nutti MR, Carvalho JL (2010) Assessment anddegradation study of total carotenoid and β-carotene in bitter yellow cassava varieties. AfrJ Food Sci 4:148–215

Ramírez-OjedaMoreno-RojasCámara-Martos AMRF (2018) Mineral and trace element content in legumes (lentils, chickpeas and beans): Bioaccesibility and probabilistic assessment of the dietary intake. J of Food Compos Anal 73:17–28. https://doi.org/10.1016/j.jfca.2018.07.007

Rocha M D M R (2008)Avaliação dos conteúdos de proteína, ferro e zinco em germoplasma elite de feijão-caupi. Embrapa Meio-Norte.Brasil. Technical Release

Rodriguez ADB (1999) A GuidetoCarotenoidAnalysis in foods. International Life Sciences Institute (ILSI) Press, Washington

Sant´AnaAntunesReisVazTostesMeiraCosta CTPTTCMGEFNMB (2019) Bio accessibility and bioavailability of iron in biofortified germinated cowpea. J Sci Food Agric 1:1–10

Silva SMS, Maia JM, Araújo ZB, Freire Filho FRl (2002) Composição Química de 45 Genótipos de Feijão-Caupi (Vignaunguiculata (L.) Walp.2 .Teresina, PI. Brasil. Technical release

Singh P, Prasad S, Aalbersberg W (2016) Bioavailability of Fe and Zn in selected legumes, cereals, meat and milk products consumed in Fiji. Food Chem 207:125–131

Souza CS, Oliveira IC,Rufino CPB, Flores PS, Lessa LS (2015)Avaliação de variedades de mandioca de mesa com polpa amarela, nas condições edafoclimáticas do Acre.In: 16 Congresso Brasileiro de Mandioca. Brasil

Sperotto RA, Ricachenevsky FK (2017) Common bean Fe biofortification using model species' lessons Front. Plant Sci 8:2187. https://doi.org/10.3389/fpls.2017.02187

TakoReedAnandaramanBeebeHartGlahnn ESASEJJRP (2015) Studies of cream seeded carioca beans (phaseolus vulgaris L.) from a Rwandan efficacy trial: in vitro and in vivo screening tools reflect human studies and predictbeneficial results from iron biofortified beans. PLoS ONE. https://doi.org/10.1371/journal.pone.0138479

TurnlundKingKeyesGongMichel JRJCWRBMC (1984) A stable isotope study of zinc absorption in young men: effects of phytate and alpha-cellulose. Am J Clin Nutr 40:1071–1077. https://doi.org/10.1093/ajcn/40.5.1071

Vilakati N, Taylor JRN, MacIntyre U, Kruger J (2016) Effects of processing and addition of a cowpea leaf relish on the iron and zinc nutritive value of a ready-to-eat sorghum cowpea porridge aimed at young children. LWT-Food Sci Tech. https://doi.org/10.1016/j.lwt.2016.06.022

WHO/CDC (2014) Methodological approaches to estimating global and regional prevalences of vitamin and mineral deficiencies report on the Joint World Health Organization/US centers for disease control and prevention technical consultation. Atlanta, USA, 7–9 December 2010. Geneva: World Health Organization