

Application of Extrusion Cooking to Enhancing Mineral Bioaccessibility in Millet Flour Enriched with Natural Fortificants for Vulnerable Populations

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Abstract

Background: Minerals bioaccessibility of food products could be increased by enhancing the apparent absorption of most minerals with the reduction of anti-nutritional factors (phytates) through extrusion cooking. The aim of the study was to increase the mineral bioaccessibility in co-extruded millet flours enriched with Moringa and Baobab for vulnerable populations. **Methods:** Three extruded instant formulas were developed using pearl millet, Moringa and Baobab powders: FA (90% Millet + 10% Baobab); FB (90% Millet + 10% Moringa); FC (80% Millet + 10% Baobab + 10% Moringa). Non-extruded formulations of FA, FB and FC were used as controls. Then treatments and controls were analyzed to determine their percent mineral bioaccessibility using the *in vitro* equilibrium dialysability method (Miller *et al.*, 1981) and their total amounts bioaccessible according to the Burgos *et al.*, 2018 method. Phytates in all samples were also determined using the Method of Fruhbeck *et al.*, 1995. **Results:** Extrusion cooking significantly improved iron bioaccessible percentages in co-extruded flours respectively in FB and FC ($p < 0.05$). Whereas, in FA, a reduction was noticed ($p < 0.05$). Extrusion cooking significantly improved magnesium and phosphorus bioaccessible percentages in co-extruded flours respectively in FA and FB and significantly reduced their percentages in FC ($p < 0.05$). A comparison of non-extruded to extruded samples showed an improvement in amounts of bioaccessible iron, magnesium and phosphorus in all extruded flours ($p < 0.05$). Extrusion significantly reduced the percentages

and amounts of bioaccessible zinc and calcium in all extruded flours compared to their controls ($p < 0.05$). Importantly, phytate levels in co-extruded products were reduced by 29.4%, 11.5%, and 9% respectively in FB, FA and FC. **Conclusion:** Extrusion cooking reduced the chelation effect of phytates by thermal degradation, which led to a modification in the bioavailability of minerals. Food-to-food fortification and extrusion cooking displayed a positive effect on the bioaccessibility of iron, magnesium and phosphorus. For calcium and zinc, extrusion has no positive effect on their bioaccessibilities. However, with daily consumption, co-extruded fortified flours could be used as a way to fight against malnutrition in vulnerable populations.

Keywords

Iron, Zn, Mg, P, Calcium, Extrusion, Bioaccessibility, Dialysability

1. Introduction

There are 49 essential nutrients needed for the good functioning of the human body. These are composed of macronutrients (carbohydrates, lipids and proteins) responsible for the energy intake and micronutrients (fibers, vitamins, minerals...), vital for supporting the human body. Minerals are divided into macro-minerals (calcium, magnesium, phosphorus) and essential micro-minerals (iron, zinc), where each element has different functional properties in its isolated form or part of a structure [1]-[3]. These essential micronutrients in diets could be related to deficiencies linked to many disorders or diseases, mainly in Africa [4]. Biofortification consists of deliberately increasing the contents of essential micronutrients, where their bioavailability is crucial [5].

Through biofortification strategies, micronutrients could be incorporated in crops, in plants by adding fertilizers and in food by using food called food-to-food fortification. In order to increase the content of several micronutrients in a basic food product, enrichment with one or more foods (food-to-food fortification) could be a natural strategy using local plant materials rich in micronutrients [5]. However, enrichment using plant material sources may not provide bioaccessible or bioavailable targeted micronutrients [6]. Van der Merwe's study, which was carried out in 2019, showed the potential of including tropical plant materials rich in iron and zinc (Moringa leaves and Hibiscus calyxes) or availability enhancers. A study on a mineral (Baobab fruit pulp) in a millet-based food matrix with a provitamin A source from plant origin was conducted to prevent iron and zinc deficiencies. Mineral bioaccessibility, assessed by a dialysability test, showed that Moringa, Hibiscus, and Baobab significantly increased the bioaccessibility of iron and zinc when added at 10% in millet (dry basis) [6]. Moreover, it has been noticed that Moringa (high content of iron) added at 30 per 100 parts of millet decreased bioaccessibility of iron and zinc. Similarly, study carried out by [4] showed that an addition of Moringa leaves at 15% to whole millet porridge reduced the bioaccessibility of iron and zinc. Negative effects of Moringa leaves were attributed

primarily to the formation of calcium-phytate-mineral complexes due to their very high calcium content (2 - 4 g/100 g, dry basis) [7]. Besides, two studies showed that addition of Baobab fruit pulp (*Adansonia digitata*) as a source of ascorbic and citric acids (promoters of iron absorption) could improve the bioaccessibility of iron [4] [7]. As Baobab is widely available in many parts of Africa, its use for improving iron bioavailability in other staple cereals could be an opportunity [7].

To increase bioaccessibility or bioavailability of fortified foods rich in micronutrients, many techniques such as mechanical (fractionation, hulling and milling of grains), chemical (redox potential modification of pH, addition of enzymes), biological (fermentation, soaking, germination, etc.) methods alone or combined with thermal processes (cooking at high pressure) exist [8]. Extrusion process, a high temperature short time (HTST) processing, is one of the thermal mentioned methods that could food matrix into desirable products [2] [8]. The process is described as an important technique for the modification and manufacture of a wide variety of foods, as well as traditional and new food mixtures [9]. The transmitted heat to food products has a greater impact on mineral bioavailability in legumes and cereals, as well as their stability [2] [8]. Extrusion is efficient in reducing microbial contamination and inactivating enzymes [10]-[12]. The thermal energy generated by the process allows the raw mixture to be quickly cooked so that the material properties are modified by the physicochemical changes of the polymers derived from biomass or biopolymers (natural fibers, proteins, nucleic acid, polysaccharide fibers or carbohydrates cellulose, complex carbohydrates) [13]. However, it is widely recognized that the relationship between the extruder and the effects of extrusion parameters on the properties of the resulting extrudates is highly dependent on the individual machine used [9]. In this study, extrusion cooking technology is used as a modifier for the absorption of most minerals by reducing antinutritional factors such as phytates [1] [2] [14]. Therefore, in this present study, we are interested in three plant crops (millet, Moringa leaf powder and Baobab powder) for making three instant nutritional products. As pearl millet a major staple food in arid and semi-arid regions of Africa, Baobab powder is rich in organic acids for improving the absorption of minerals, and Moringa leaf powder, rich in essential minerals including iron, calcium and magnesium, are interesting raw materials. However, these plant materials contain substantial amounts of antinutrients, such as phytate [15]. The objective of this present study was to enhance mineral bioaccessibility of iron, zinc, magnesium, phosphorus and calcium in instant functional foods developed through food-to-food fortification of millet with Moringa and Baobab powders via extrusion for targeting vulnerable populations.

2. Materials and Methods

2.1. Formulation of Extruded and Traditional Mixtures

Three extruded instant formulas from mixtures of pearl millet, Moringa and Baobab powders were developed: FA (90% Pearl millet + 10% Baobab); FB (90%

Pearl millet + 10% Moringa); FC (80% Pearl millet + 10% Baobab + 10% Moringa). Non-extruded formulations F1, F2 and F3 were used respectively as controls of FA, FB and FC. The traditional flours were obtained by mixing millet flour with Moringa and Baobab powders according to the same ration of extruded instant formula. Comparisons between the co-extruded and controls were expressed as FA/F1 (Millet-Baobab), FB/F2 (Millet-Moringa), and FC/F3 (Millet-Moringa-Baobab).

2.2. Extrusion Cooking Processing

A single-screw mini-extruder (Technochem model), with electric motor 7.5 HP and two variable frequency drives (one for 220 V and the other for 380 V), was used. Our experiments were conducted with 220 V. The extruder parameters were set at a frequency of 50 Hz and a temperature between 110°C - 130°C. Three shear rings of 1.47 mm in diameter each, followed by a bigger one (1.59 mm), were respectively inserted along and the end of the screw. The rotational speed of the screw was set at 900 rpm and total liquid content was 30%. The three formulations were transformed continuously and in a short time and shaped within the inner barrel/screw system. The hopper was used to introduce each mixture into the machine. Then, the screw swallows and advances the mixture to the end of the extruder where the extruded product exits through the 6 mm diameter die. The expulsion of the extruded product is due to the axial thrust of the material, which is caused by the pressure reaction during transport. The heat is generated both by transfer in contact of the product with the internal wall of the sheath and the mechanical shear exerted by the screw. At the end of the extrusion, the extrudates obtained are dried in the oven at 60°C for 7 hours then ground in a mobile hammer mill (1 mm sieve) to obtain the flours.

2.3. Freeze Drying of Traditional Flours

Before dialysability tests, traditional flours F1, F2 and F3, as well as extruded ones were transformed into porridge. A volume of 100 ml of deionized water was added to 25 or 30 g of traditional flour. Each slurry was heated at 100°C and maintained for 10 to 15 minutes with constant stirring. The cooked porridge was cooled to room temperature (25°C), frozen at -20°C and freeze-dried. It is then dried and then finely ground using the IKA A11 grinder (Staufen, Germany) and stored at -20°C before analysis. While for the extruded flours, 80 ml for 20 mg of flour was used. The slurry was stirred until having a homogenous porridge. Then, the obtained porridge is freeze-dried and stored at -20°C.

2.4. Phytate Content

Phytates were extracted from the flours using a hydrochloric acid solution (2.4% w: v) of at pH 0.6, in which the filtrate is dissociated from the mineral and protein complexes. Dowex 1 (anion-exchange resin, AG 1 x 4, 4% cross-linkage, chloride form, 100 - 200 mesh) resin (0, 25 à 0, 3 g) was added into glass barrel Econo-

columns, 7 × 5 mm for anion-exchange purification of the extracts to exclude the inorganic phosphate (Frühbeck *et al.*, 1995). Amounts of phosphate in the sample, purified extracts with 1 ml of Wade reagent, were measured using a spectrophotometry set at a wavelength of 500nm [16] [17].

2.5. Molar Ratio

The molar ratio phytate/mineral was used to predict the inhibitory effect on mineral bioavailability. The number of moles in phytate and mineral was respectively determined by dividing phytate content and mineral content by their atomic weight (phytate: 660.04 g/mol; Fe: 55.845 g/mol; Zn: 65.38 g/mol; Ca: 40.078 g/mol). The molar ratio between phytate and mineral was obtained after dividing the mole of phytate by the mole of minerals [18] [19].

$$\text{Molar ratio} = \frac{(\text{Phytate content}/\text{atomic weight of phytate})}{(\text{mineral content}/\text{atomic weight of mineral})}$$

2.6. Ascorbic Acid

The extraction and quantification of ascorbic acid content in the flours were carried out according to the method of [20] with a slight modification.

2.7. Minerals Contents

Flour Acid digestion was performed using concentrated nitric acid and hydrogen peroxide as described in EPA method 3051A [21]. The sample and the acidic solution are placed in a quartz microwave vessel. The vessel is sealed and heated using a laboratory microwave. After cooling, the vessel contents were filtered, centrifuged and then diluted to volume. Mineral contents (Fe, Zn, Mg, P, and Ca) of digested flours were analyzed by EPA Method 200.7 [22] using inductively coupled plasma atomic emission spectrometry (ICP-OES-AES) (iCAP 6000 series, Thermo Fisher Scientific, Waltham, USA). Elements were analyzed using wavelengths 239.5 nm for Fe, 206.7 nm for Zn, 285.2 nm for Mg, 214.9 nm for P and 315.8 nm for Ca.

2.8. *In Vitro* Dialysability Mineral Bioaccessibility

The flours were subject to *in vitro* digestion to simulate human gastric and intestinal digestion. The *in vitro* dialysability method of Miller *et al.* (1981) was used [23]. The gastric stage was done in duplicate with 1.8 g of pepsin solution (16 g de pepsin + 100 ml de HCL 0.1 M) at pH 2. The intestinal stage was repeated 3 times using dialysis tubing Spectra/Por 7 (Ø = 20.4 mm) with a molecular mass cut-off of 10,000 Da (G.I.C. Scientific, Johannesburg, South Africa) and 3 g of pancreatin-bile extract mixture solution consisting of 4 g of pancreatin (Sigma, P-1750, from porcine pancreas), 25 g of bile extract (Sigma, B-8631, porcine) and 1 liter of 0.1 M NaHCO₃ at pH 7.5. Mineral contents of the dialysates were determined by ICP-AES as described above, but without the digestion step. Two independent dialysability assay experiments were performed, with the intestinal step being each time

performed in triplicate. Mineral bioaccessibility (%) was calculated as the percentage of the mineral in the dialysate as compared to the total mineral content in the digest:

$$\text{Mineral bioaccessibility (\%)} = \frac{\text{concentration of mineral in the dialysate} \times 100}{\text{concentration of mineral in pepsin digest}}$$

2.9. Potential Contribution

The amount of bioaccessible mineral in 100 g food sample was determined according to [24]:

$$\begin{aligned} & \text{Amount of bioaccessible mineral in 100 g food} \\ & = \frac{\% \text{ bioaccessible mineral} \times \text{mineral content of food sample (mg/100g)}}{100} \end{aligned}$$

2.10. Contribution of Bioaccessible Iron to Absolute Requirements (AR) and of Bioaccessible Zinc to Physiologic Requirements (PR)

The percentage contribution of bioaccessible iron and zinc in 50 g and 75 g of porridge formulations to the absolute requirements (AR) and physiologic requirements (PR) for the children (1 - 3 years old) and lactating women. Therefore, these requirements were calculated and expressed as a percentage of AR and PR [6] [15]:

% Contribution of bioaccessible Fe in developed flours to the AR

$$= \frac{\text{Absolute bioaccessible Fe per portion}}{\text{Absolute requirements per day}} \times 100$$

% Contribution of Zinc bioaccessible in developed flours to the PR

$$= \frac{\text{Absolute bioaccessible Zinc per portion}}{\text{Physiologic requirements per day}} \times 100$$

2.11. Statistical Analyses

For each flour, the biochemical composition was determined in triplicate. Minerals experiments were done in duplicate with two repetitions. Bioaccessibility experiments were done in duplicate with three replicates for intestinal stage. The mean \pm standard deviation calculations for minerals, bioaccessibility and the amount of bioaccessible minerals were performed by using descriptive statistics with XLSTAT 6.1.9 software to determine significant differences between specific means at 5% confidence level ($p < 0.05$). Almost all composition values from this study were expressed on a dry basis.

3. Result

3.1. Effect of Extrusion Cooking on Mineral Contents

The co-extruded samples FA (millet-Baobab), FB (millet-Moringa) and FC (millet-Moringa-Baobab) contained significantly more mineral contents compared to non-extruded formulas F1, F2, and F3 (Table 1). There were increases of iron for 53% comparing F1/FA, 13% F2/FB and 60% F3/FC for. The co-extruded flours

FA and FC showed significantly higher increases of iron ($p < 0.05$) after extrusion cooking (compared respectively to their controls F1 and F3). While for FB, there is a non-significant difference ($p < 0.05$) in the iron content compared to F2. After heat treatment by extrusion, the Zn, Mg and P contents were significantly increased in each co-extruded flour. The significant increases of zinc, in comparing co-extruded and related control (non-extruded) were 10% (F1/FA), 19% (F2/FB) and 14% (F3/FC) ($p < 0.05$). Significant increases ($p < 0.05$) of magnesium were 19% (F1/FA), 7% (F2/FB), and 10% (F3/FC). This same trend was observed for phosphorus with significant increases ($p < 0.05$) of 22% (F1/FA), 13% (F2/FB) and 21% (F3/FC). For calcium, a non-significant increase ($p < 0.05$) of 11% in FA/F1, and a decrease of 10% and 5% in FB and FC.

Table 1. Mineral contents of raw materials, extruded and traditional flours

Raw material, extruded and traditional flours	Fe (mg/100 g)	Zn (mg/100 g)	Mg (mg/100g)	P (mg/100 g)	Ca (mg/100 g)
Millet flour	9.217 ^a ± 0.651, ²	3.634 ^b ± 0.0419	97.746 ^a ± 2.926	231.783 ^b ± 6.326	14.420 ^a ± 0.182
Moringa powder	42.810 ^b ± 5.934	1.883 ^a ± 0.0483	506.276 ^c ± 6.898	317.300 ^c ± 4.08	2273.389 ^c ± 35.448
Baobab powder	10.704 ^a ± 1.0131	1.832 ^a ± 0.0439	149.108 ^b ± 1.14	51.448 ^a ± 0.342	301.359 ^b ± 2.899
F1	5.397 ^A ± 0.167 ^{3,4}	2.312 ^B ± 0.0189	56.411 ^A ± 1.115	120.734 ^A ± 1.91	31.119 ^A ± 0.763
FA	8.243 ^C ± 0.730	2.545 ^C ± 0.0104	67.326 ^B ± 1.91	147.605 ^C ± 3.03	34.459 ^A ± 0.612
F2	9.226 ^D ± 1.104	2.289 ^{AB} ± 0.0264	86.299 ^C ± 0.591	144.867 ^C ± 1.54	210.79 ^C ± 2.40
FB	10.432 ^B ± 0.0898	2.728 ^D ± 0.264	92.255 ^D ± 2.5	163.900 ^D ± 3.5	188.763 ^B ± 4.45
F3	7.334 ^B ± 0.0919	2.117 ^A ± 0.031	92.292 ^D ± 1.85	133.111 ^B ± 3.18	225.539 ^D ± 5.04
FC	11.763 ^F ± 0.0169	2.420 ^{BC} ± 0.024	101.643 ^B ± 0.000	161.144 ^D ± 1.59	214.173 ^C ± 3.21

¹Values are the means ±1 SD at least two sample of each raw material analyzed independently in duplicate (n = 4) for minerals.

²Means with different superscript lowercase letters in a column differ significantly according to the Fischer's LSD Test ($p < 0.05$). ³Values (dry weight basis) are the means ±1 SD of samples (conventionally cooked, extruded) analyzed in duplicate (n = 4) for minerals. ⁴Means with different superscript uppercase letters in a column differ significantly according to the Fischer's LSD Test ($p < 0.05$). F1: non-extruded millet/Baobab powder; F2: non-extruded millet/moringa powder; F3: non-extruded millet/moringa powder/Baobab powder; FA: extruded millet/Baobab powder; FB: extruded millet/moringa powder; FC: extruded millet/moringa powder/Baobab powder.

3.2. Effect of Extrusion Cooking on Phytate Contents

The effect of extrusion cooking on phytate reduction, in comparing co-extruded flours FA, FB and FC with their non-extruded controls, was studied. The results, in **Figure 1**, showed that extrusion processing significantly reduced phytate levels by 11.46%, 29.34%, and 9.16% respectively in FA, FB, and FC. In comparison before extrusion, phytate contents were at 3231.9 mg/100 g for F1, 4457.7 mg/100 g for F2 and 4698.2 mg/100 g for F3. After extrusion cooking process, the actual levels were 2861.5 mg/100 g, 3149.7 mg/100 g and 4267.8 mg/100 g respectively for FA, FB and FC flours. Other authors such as [24] and [25] also observed a slight reduction of phytic acid content after application of extrusion cooking. The

results obtained from phytate contents in our study were higher than those reported by [4] in their mixture of millet/Baobab, millet/Moringa and millet/Baobab/Moringa.

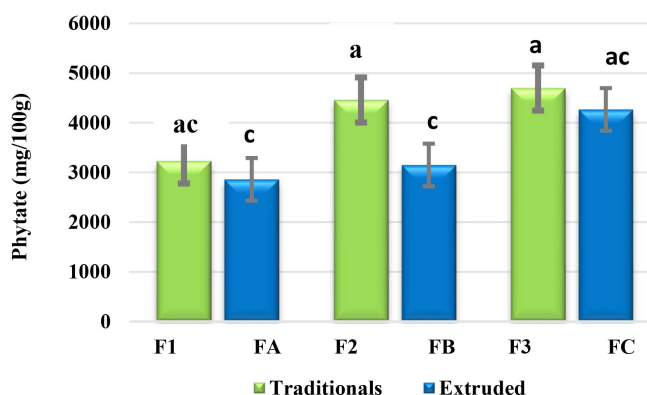


Figure 1. Effect of extrusion cooking on phytate levels of extruded flours. Moringa leaf powder and Baobab fruit pulp was added before the processing. Values are the means \pm 1 SD of two independent samples analyzed in duplicate ($n = 4$). Different letters indicate significant differences according to Fischer's LSD test ($p < 0.05$). Error bars indicate standard deviation.

3.3. Effect of Extrusion Cooking on Vitamin C Contents

In our study, the effect of extrusion cooking on the vitamin C contents in flours was evaluated (Figure 2). Before extrusion, vitamin C contents of traditional flours were 27.5 ± 1.2 , 3.5 ± 0.4 and 28.4 ± 0.1 mg/100 g, respectively for F1, F2 and F3. We found them higher than those obtained by [4] did in blends of millet/Baobab (18 mg/100 g) did, millet/Moringa (1 mg/100 g) and millet/Moringa/Baobab (17 mg/100 g) did. Vitamin C contents were reduced by 73% in FA (7.5 ± 0.5 mg/100 g), by 40% in FB (2.1 ± 0.2 mg/100 g), and by 58% in FC (12 ± 0.8 mg/100 g) after extrusion cooking.

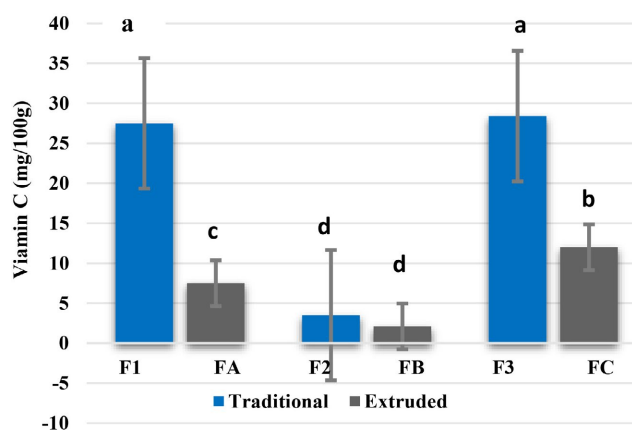


Figure 2. Effect of extrusion cooking on the vitamin C content in extruded flours. Values are the means \pm 1 SD of two independent samples analyzed in duplicate ($n = 4$). Different letters indicate significant differences according to Fischer's LSD test ($p < 0.05$). Error bars indicate standard deviation.

3.4. Mineral Bioaccessibility

3.4.1. Percentages of Minerals Bioaccessibility in Different Studied Flours

Plant foods, including fruits, vegetables and processed foods, contain many types of micronutrients such as minerals with many human health benefits. To achieve beneficial health effects, these minerals must be bioavailable after being efficiently absorbed in the intestine and delivered to the appropriate target location [26]. Moreover, the bioaccessibility/bioavailability of any food mineral is a function of its total content, absorption proportions and involved inhibitory substances [27]. After determining the mineral contents in the dialysates and flours by *in vitro* dialysability test and acid digestion respectively, we calculated the percentages of total bioaccessible minerals in all studied flours. **Table 2** summarizes the percentages of minerals bioaccessibility of the flours before and after extrusion.

Table 2. Percentages of mineral bioaccessibility in millet flour, extruded and traditional flours.

Flours	% Bioaccessibility				
	Iron	Zinc	Magnesium	Phosphorus	Calcium
Millet*	2.26 ± 0.075	13.31 ± 0.363	63.46 ± 2.877	85.60 ± 1.903	43.65 ± 1.616
F1	4.68 ^c ± 0.516 ^{1,2}	56.89 ^{ab} ± 3.85	54.96 ^a ± 1.109	77.1 ^c ± 4.86	49.01 ^a ± 3.31
FA	4.01 ^b ± 0.434	50.57 ^a ± 1.34	61.91 ^{bc} ± 4.96	84.08 ^d ± 5.80	42.22 ^d ± 3.87
F2	3.6 ^{ab} ± 0.355	77.88 ^c ± 10.17	59.49 ^b ± 1.022	57.35 ^a ± 1.29	16.05 ^{ab} ± 1.45
FB	4.52 ^c ± 0.52	60.68 ^{ab} ± 28.53	63.90 ^c ± 4.78	68.15 ^b ± 5.29	13.96 ^a ± 1.67
F3	3.29 ^a ± 0.347	69.66 ^{bc} ± 3.57	60.92 ^{bc} ± 0.894	72.45 ^{bc} ± 1.88	20.63 ^c ± 0.22
FC	3.46 ^a ± 0.094	49.54 ^a ± 1.72	59.02 ^b ± 2.20	68.52 ^b ± 2.46	17.02 ^b ± 1.04

¹Values (dry weight basis) are the means ± 1 SD of two completely independent dialysability experiments, with the intestinal step being each time performed in triplicate and each dialysate is analyzed for its dialyzable minerals (n = 6).

²Means with different superscripts letters in a column differ significantly (p < 0.05). *Millet. Values on the same line are the percentage of minerals bioaccessibility of millet before food fortification F1: non-extruded millet/Baobab powder; F2: non-extruded millet/moringa powder; F3: non-extruded millet/moringa powder/Baobab powder; FA: extruded millet/Baobab powder; FB: extruded millet/moringa powder; FC: extruded millet/moringa powder/Baobab powder.

3.4.2. Effect of Extrusion Cooking on the Mineral Bioaccessibility

Iron bioaccessibility

The initial percentages of bioaccessible iron in traditional flours were 4.68% for F1, 3.6% for F2 and 3.29% for F3. While the percentages of bioaccessible iron calculated for the extruded flours were 4.01% for FA, 4.52% for FB and 3.46% for FC (**Table 2**). The amounts of bioaccessible iron in the dialysis tubes were 0.25 ± 0.028 mg/100 g for F1, 0.33 ± 0.033 mg/100 g for F2 and 0.24 ± 0.025 mg/100 g for F3. Those compared to co-extruded flours of 0.33 ± 0.36 mg/100 g in FA, 0.47 ± 0.055 mg/100 g in FB and 0.41 ± 0.011 mg/100 g in FC. Fortified millet with Moringa and Baobab powders could improve the iron bioaccessible. Thus, after cooking-extrusion, we observe a very significant increase in bioaccessible iron of 32%

between F1/FA ($p = 0.000$), 42% between F2/FB ($p < 0.0001$) and 70.8% between F3/FC ($p < 0.0001$) (**Table 2**). Through these outcomes, we noticed that heat treatment improved the bioaccessible iron percentages in FB and FC co-extruded flours and reduced it in FA extruded flour. Extrusion cooking combined with food-to-food fortification improved the amount of bioaccessible iron in all the extruded flours obtained compared to their controls (**Figure 3**).

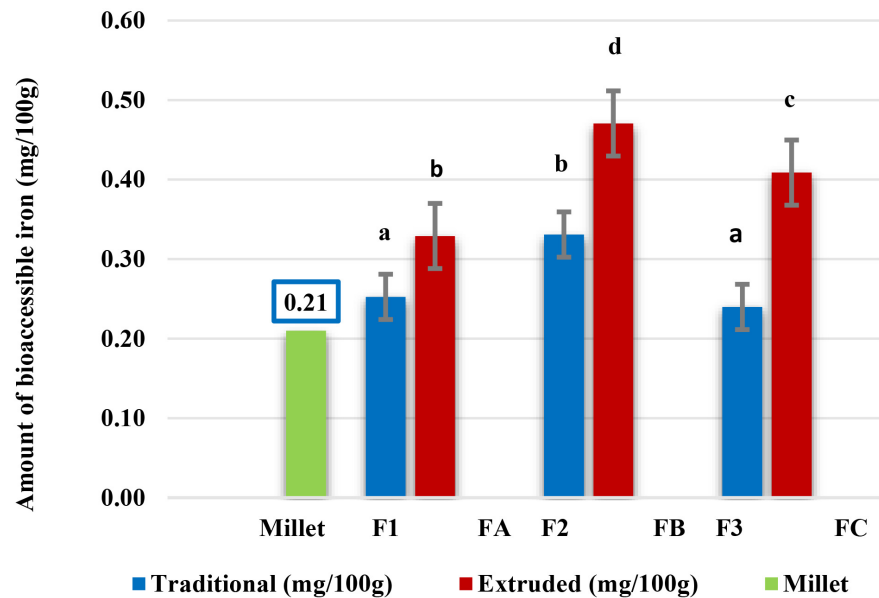


Figure 3. Effect of processing (traditional and cooking-extrusion) on the amount of bioaccessible iron in developed flours. Moringa leaf powder and Baobab fruit pulp was added before cooking extrusion. Each formulation was subjected to two gastric stages, with the intestinal step being each time performed in triplicate and each dialysate is analyzed for its dialyzable minerals ($n = 6$). Different letters indicate significant differences according to Fischer's LSD test ($p < 0.05$). Error bars indicate standard deviation.

Zinc bioaccessibility

The percentages of bioaccessible zinc were 56.89%, 77.88% and 69.66% respectively in traditional flours F1, F2 and F3. As for the co-extruded flours, percentages of bioaccessible zinc of 50.57%, 60.68% and 49.54% were found respectively in FA, FB and FC (**Table 2**). In terms of amounts of bioaccessible zinc, we found 1.31 ± 0.089 mg/100 g in F1, 1.79 ± 0.234 mg/100 g in F2 and 1.46 ± 0.075 mg/100 g in F3. While in the co-extruded samples, amounts of bioaccessible zinc were 1.26 ± 0.033 mg/100 g, 1.64 ± 0.77 mg/100 g and 1.19 ± 0.041 mg/100 g respectively in FA, FB and FC. Food-to-food fortification of millet with Moringa and Baobab powders improved the amount of bioaccessible zinc in all traditional flours (**Figure 4**). These results were the same as those obtained by [15] with an increase in amounts of bioaccessible zinc. Non-significant reductions of 3.8%, 8.4%, and 18.5% ($p < 0.05$) were also found in comparing F1/FA, F2/FB, and F3/FC. The extrusion process reduced the percentages of amounts of bioaccessible zinc in all extruded flours compared to their controls (**Figure 4**).

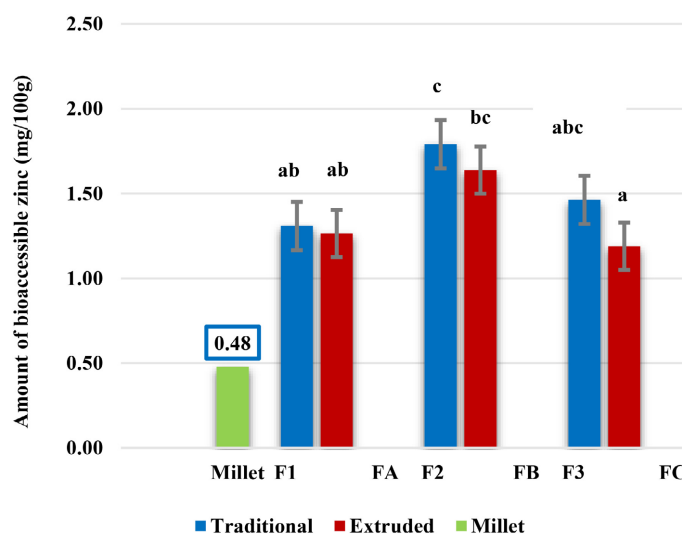


Figure 4. Effect of processing (traditional and cooking-extrusion) on the amount of bioaccessible zinc in developed flours. Moringa leaf powder and Baobab fruit pulp was added before cooking extrusion. Each formulation was subjected to two gastric stages, with the intestinal step being each time performed in triplicate and each dialysate is analyzed for its dialyzable minerals ($n = 6$). Different letters indicate significant differences according to Fischer's LSD test ($p < 0.05$). Error bars indicate standard deviation.

Calcium bioaccessibility

The percentages of bioaccessible calcium were 49.01%, 16.05% and 20.63% respectively in traditional flours F1, F2 and F3. While in co-extruded, percentages were 42.22%, 13.96% and 17.02% respectively in FA, FB, and FC (**Table 2**). In terms of the amount of bioaccessible calcium, these percentages corresponded to 15.24 ± 1.03 mg/100 g in F1, 33.83 ± 3.06 mg/100 g in F2, and 46.53 ± 0.49 mg/100 g in F3. In co-extruded flours, the amounts of bioaccessible calcium were 14.57 ± 1.34 mg/100 g, 26.36 ± 3.16 mg/100 g and 36.46 ± 2.22 mg/100 g respectively for FA, FB, and FC. From the results mentioned above, the incorporation of Baobab powder improved the percentage of bioaccessible calcium in F1. Moringa powder and the combination of two powders reduced respectively the percentages of bioaccessible calcium in F2 and F3. Food-to-food fortification of millet flour with Moringa and Baobab powders improved the amount of bioaccessible calcium in all traditional flours (**Figure 5**). These results were similar to those obtained by [4] [15], who observed an increase in bioaccessible calcium amounts after fortifying their millets with Moringa and Baobab powders. We also obtained a significant reduction of amounts of bioaccessible calcium of 22% and 21.6% ($p < 0.05$) respectively in FB and FC. In FA, the amount of bioaccessible calcium was slightly reduced by 4.4% ($p = 0.587$) (**Figure 5**).

Magnesium bioaccessibility

Bioaccessible Mg percentages in traditional flours were 54.96%, 59.49% and 60.92% respectively for F1, F2 and F3 (**Table 2**). Bioaccessible magnesium amounts corresponding to these percentages were 31 ± 0.626 mg/100 g in F1, 51.34 ± 0.883 mg/100 g in F2, and 56.23 ± 0.825 mg/100 g in F3. Bioaccessible Mg

percentages in co-extruded flours (FA, FB, and FC) were 61.91%, 63.90% and 59.02% respectively (**Table 2**). An increase in bioaccessible magnesium percentages was noticed by extrusion cooking in FA and FB. While a reduction of the percentage in FC was noticed (**Table 2**). In terms of bioaccessible magnesium amounts, we found that the extruded flours FA, FB and FC contained respectively 41.67 ± 3.338 mg/100 g, 58.98 ± 4.41 mg/100 g, and 59.97 ± 2.234 mg/100 g. After extrusion cooking, a significant increase of bioaccessible magnesium amount ($p < 0.05$) of 34% and 15% were noticed respectively in comparing F1/FA and F2/FB. For F3/FC, a significant increase of 6.65% bioaccessible magnesium amount was noticed ($p < 0.05$). The use of millet food-to-food fortification with Moringa and Baobab powders reduced the percentages and the amounts of bioaccessible magnesium in all traditional flours (**Figure 6**). These results are contrary to those obtained by [4] [15] who observed an increase of bioaccessible magnesium amounts after fortifying millet with Moringa and Baobab powders.

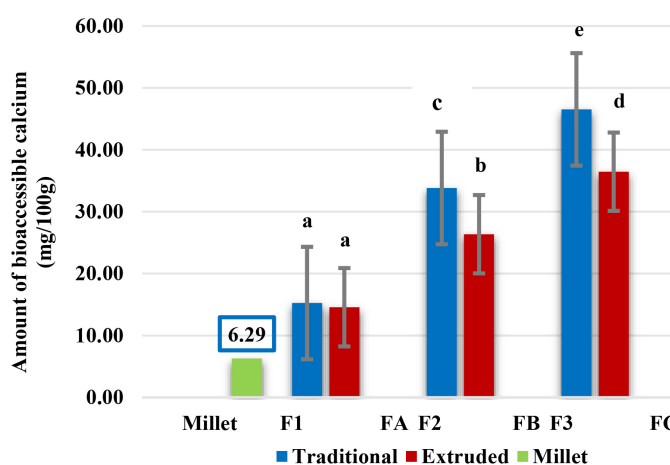


Figure 5. Effect of processing (traditional and cooking-extrusion) on the amount of bioaccessible calcium in developed flours. Moringa leaf powder and Baobab fruit pulp was added before cooking extrusion. Each formulation was subjected to two gastric stages, with the intestinal step being each time performed in triplicate and each dialysate is analyzed for its dialyzable minerals ($n = 6$). Different letters indicate significant differences according to Fischer's LSD test ($p < 0.05$). Error bars indicate standard deviation.

Phosphorus bioaccessibility

Traditional flours (F1, F2 and F3) showed percentages of bioaccessible phosphorus of 77.1%, 57.35% and 72.45% respectively. While for co-extruded flours, percentages of bioaccessible phosphorus were 84.08%, 68.15% and 68.52% respectively for FA, FB and FC (**Table 2**). The amount of bioaccessible phosphorus corresponding to percentages of traditional were 93.05 ± 5.87 mg/100 g in F1, 83.1 ± 1.87 mg/100 g in F2, and 96.43 ± 2.5 mg/100 g in F3. For co-extruded flours, the amount of bioaccessible phosphorus was 124.10 ± 8.56 mg/100 g in FA, 111.70 ± 8.68 mg/100 g in FB and 110.38 ± 3.97 mg/100 g in FC. Through food-to-food fortification of millet with Moringa and Baobab powders, a reduction in percentages and amounts of bioaccessible phosphorus in all traditional flours (**Figure 7**)

was noticed. In comparing the co-extruded and controls, we found significant increases ($p < 0.05$) of 33.37% for F1/FA, 34.42% for F2/FB, and 14.47% for F3/FC. Therefore, we could notice that extrusion cooking improved bioaccessible phosphorus percentages in FA and FB and reduced the percentage in FC, compared to their controls (Table 2).

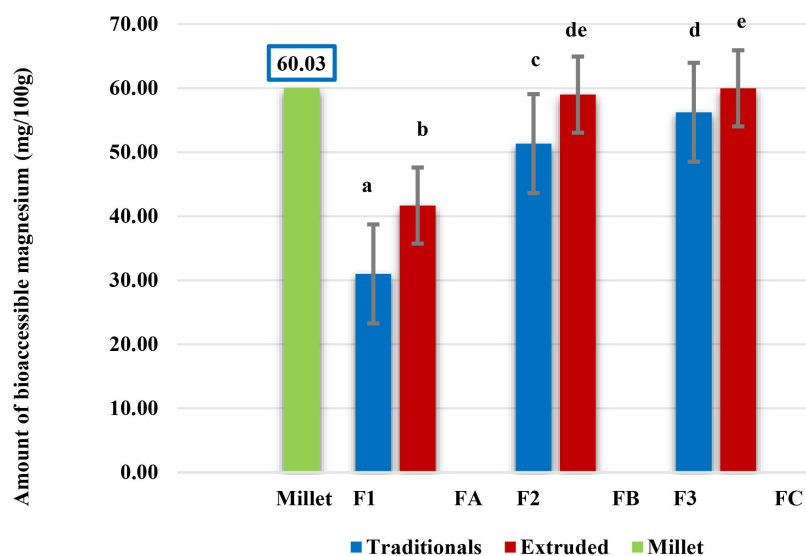


Figure 6. Effect of processing (traditional and cooking-extrusion) on the amount of bioaccessible magnesium in developed flours. Moringa leaf powder and Baobab fruit pulp was added before cooking extrusion. Each formulation was subjected to two gastric stages, with the intestinal step being each time performed in triplicate and each dialysate is analyzed for its dialyzable minerals ($n = 6$). Different letters indicate significant differences according to Fischer's LSD test ($p < 0.05$). Error bars indicate standard deviation.

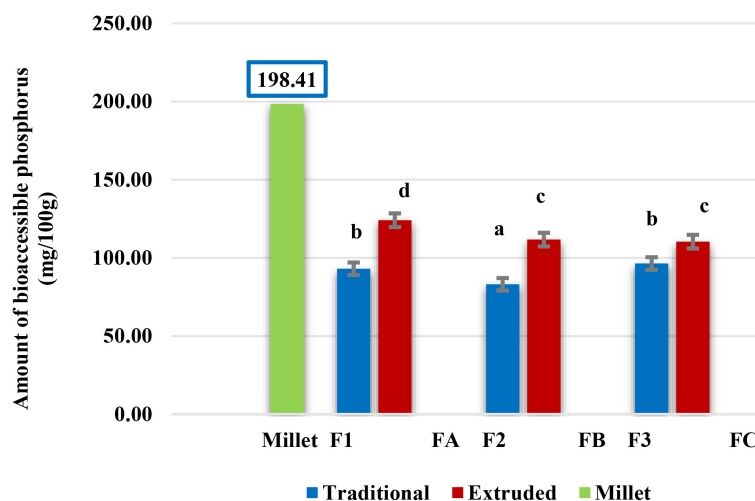


Figure 7. Effect of processing (traditional and cooking-extrusion) on the amount of bioaccessible phosphorus in developed flours. Moringa leaf powder and Baobab fruit pulp was added before cooking extrusion. Each formulation was subjected to two gastric stages, with the intestinal step being each time performed in triplicate and each dialysate is analyzed for its dialyzable minerals ($n = 6$). Different letters indicate significant differences according to Fischer's LSD test ($p < 0.05$). Error bars indicate standard deviation.

3.5. Molar Ratios of Phytate/Mineral Complexes

Table 3 summarizes the phytate/mineral molar ratios of the flours before and after extrusion. After extrusion cooking, a reduction, in the inhibitory effect of phytates on the bioaccessibility of minerals in all extruded flours, was observed. However, all phytate/mineral molar ratios were above critical values, except for the phytate*calcium/zinc molar ratios for F1 and FA flours, which therefore predicts low bioavailability of iron, zinc and calcium in our flours.

Table 3. Phytate/mineral molar ratios of extruded and traditional flours.

Flours	Molar ratio phytate/Fe > 1	Molar ratio phytate/Zn > 15	Molar ratio phytate/Ca > 0.24	Molar ratio phytate*Ca/Zn > 200
F1	50.7 ^a ± 0.784 ^{1,2}	138.5 ^d ± 0.563	6.3 ^a ± 0.077	107.5 ^e ± 1.012
FA	29.5 ^c ± 1.31	111.5 ^e ± 2.267	5.0 ^b ± 0.045	95.8 ^e ± 1.141
F2	41.3 ^b ± 2.46	192.9 ^b ± 1.119	1.3 ^c ± 0.007	1014.8 ^b ± 5.176
FB	25.5 ^d ± 0.109	115.2 ^e ± 5.573	1.0 ^d ± 0.012	543.4 ^d ± 32.559
F3	54.2 ^a ± 0.335	219.9 ^a ± 1.621	1.3 ^c ± 0.014	1237.3 ^a ± 9.692
FC	30.7 ^c ± 0.220	174.7 ^c ± 0.867	1.2 ^c ± 0.009	933.4 ^c ± 3.325

¹Values are the means ±1 SD of two independent samples analyzed in duplicate (n = 4) for phytate: iron, phytate: zinc, phytate: calcium and phytate*calcium: zinc molar ratios of extruded and traditional flours

²Means with different superscripts letters in a column differ significantly (p < 0.05).

3.6. Percentage Contribution of Bioaccessible Iron in Developed Flours to the Absolute Requirements (AR)

3.6.1. Children (1 - 3 Years Old)

The absolute requirement for bioaccessible iron for children aged 1 to 3 years is 0.46 mg/day [28]. For a 50 g portion, the contribution percentages of iron content in traditional flours to the absolute requirements for bioaccessible iron were 27.2% for F1, 35.9% for F2, and 26.1% for F3. Compared to traditional flours, co-extruded flours were higher in contribution percentages of iron content with 35.9% for FA, 51.1% for FB and 44.6% for FC (**Figure 8**).

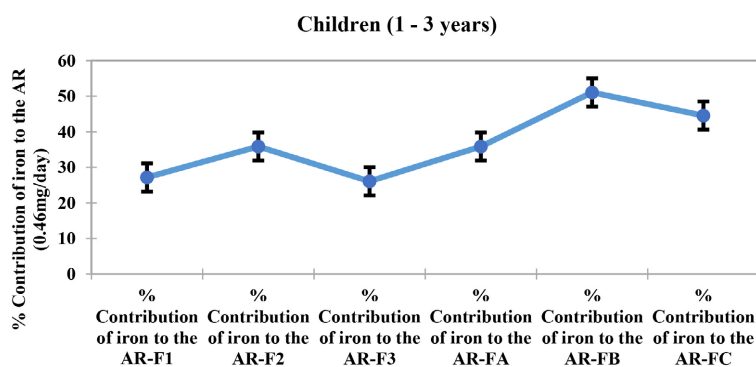


Figure 8. Percentages contribution of bioaccessible iron content in developed flours to the absolute iron needs for children aged 1 - 3 years. According to the absolute requirement for children consuming a plant-based diet with the lowest level of iron bioavailability (5%). Error bars indicate standard deviation.

3.6.2. Lactating Women

The absolute requirement for bioaccessible iron in lactating women is 1.15 mg/day [28]. For a 75 g portion, the contribution of iron percentages in traditional flours to the absolute requirements for bioaccessible iron was 16.3% for F1, 21.5% for F2, and 15.7% for F3. Compared to traditional flours, contribution of iron percentages in extruded flours was higher corresponding to 21.5% for FA, 30.7% for FB and 26.7% for FC (Figure 9).

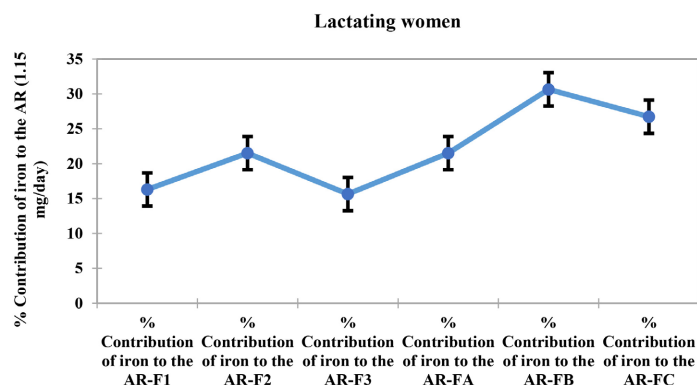


Figure 9. Percentages contribution of bioaccessible iron content in developed flours to the absolute iron needs for lactating women. According to the absolute requirement for lactating women consuming a plant-based diet with the lowest level of iron bioavailability (5%). Error bars indicate standard deviation.

3.7. Percentage Contribution of Bioaccessible Zinc in Developed Flours to the Physiologic Requirements (PR)

3.7.1. Children (1 - 3 Years Old)

The absolute physiologic requirements for bioaccessible zinc for children aged 1 to 3 years were 0.83 mg/day [29]. For 50 g portion, the contribution percentages of traditional flours to the absolute requirements for bioaccessible zinc were 78.3% for F1, 108.4% for F2, and 90.4% for F3. Contribution percentages for bioaccessible zinc were lower in extruded flours compared to traditional flours (Figure 10). Their percentage contributions were 75.9% for FA, 96.4% for FB, and 72.3% for FC.

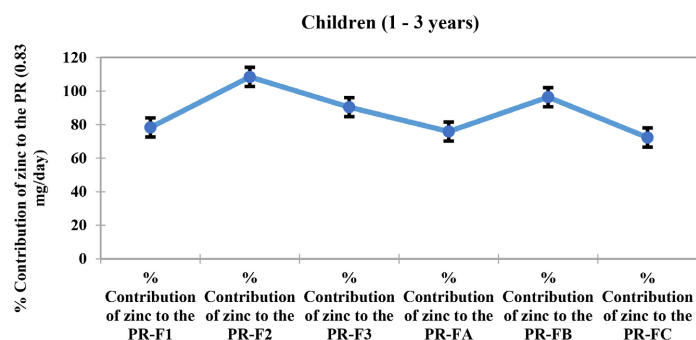


Figure 10. Percentages contribution of bioaccessible zinc content in developed flours to the physiologic requirements needs for children aged 1 - 3 years. According to the physiologic requirement for children consuming a plant-based diet with the lowest level of zinc bioavailability (15%). Error bars indicate standard deviation.

3.7.2. Lactating Women

The absolute physiologic requirements for bioaccessible zinc in lactating women were 2.89 mg/day [29]. For a 75 g portion, the contribution percentages of zinc content in traditional flours to the absolute requirements for bioaccessible zinc were 33.7% for F1, 46.7% for F2, and 38.9% for F3. Contribution percentages for zinc were lower in extruded flours (32.7% for FA, 41.5% for FB and 31.1% for FC) compared to traditional flours (Figure 11).

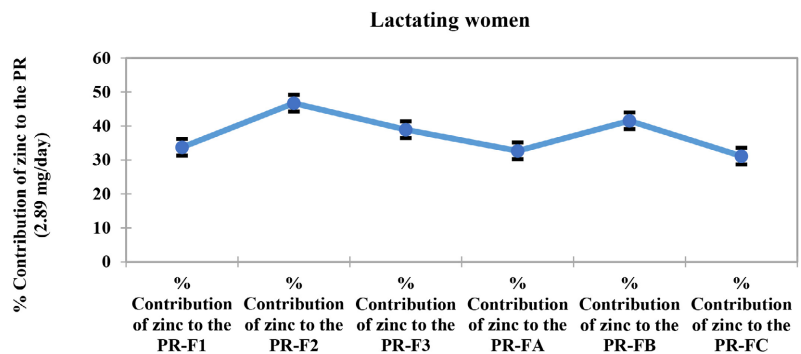


Figure 11. Percentages contribution of bioaccessible zinc content in developed flours to the physiologic requirements needs for lactating women. According to the physiologic requirement for lactating women consuming a plant-based diet with the lowest level of zinc bioavailability (15%). Error bars indicate standard deviation.

4. Discussion

4.1. Mineral

The iron contents obtained in the 6 flours of our study are slightly lower than those obtained by [4] using the same formulations. In fact, this difference can be explained by the incorporation rate of 15% Moringa and Baobab powders in their millet flour. The values for zinc, magnesium, phosphorus and calcium are much lower than those found by [4] for the same formulations. Those undoubtedly were due to 5% more incorporation of Moringa and Baobab powders in above mentioned author formulations. Therefore, the incorporation rate is related to a positive effect on the mineral contents. In addition, based on the obtained results, the extrusion could possibly contribute to the improvement of minerals content in co-extruded flours. The main reason for changes in mineral bioavailability during extrusion is the impact of extrusion conditions on mineral-binding components present in legumes and cereals such as phytic acid, phenolic compounds, fiber food and proteins [1] [2] [14]. Changes in flour components during extrusion that are linked to improved mineral absorption rely on thermal degradation of phytates, phenols, dietary fibers and proteins inside the extruder barrel. In general, the low moisture content of the feed and the high temperature of the barrel facilitate thermal degradation. Additionally, other extrusion conditions, such as high screw speed and low die and barrel temperatures have also been identified as variables that increase mineral absorption. These variables can enhance mineral absorption by creating non-thermal changes based on shear during extrusion. Of course,

changes in mineral absorption always depend on the composition of the raw material and the chemical form of the mineral element [2].

4.2. Phytic Acid

Phytic acid, or myo-inositol hexaphosphate, is a powerful chelating agent thanks to its poly-anionic phosphate groups. It is present in the form of phytates, salts mainly of calcium and magnesium, and contains 60% to 90% of the total phosphorus in the grain [8]. Extrusion causes dephosphorylation of phytic acid by the hydrolysis of inositol into low molecular weight forms including penta-, tetra and triphosphates [25]. During extrusion cooking, increasing temperature with high screw speed could influence phytate content in the extruded product [30].

4.3. Vitamin C

Vitamin C retention generally decreases with increasing temperature and/or residence time of the food material in the extruder [31]. Heating cooked foods can destroy or decrease ascorbic acid content and therefore potentially reduces iron absorption [32]. Similar cases in co-extruded flours FA, FB and FC were observed regarding vitamin C loss during heat treatment by extrusion cooking. We noted that using of mono-screw extruder could affect vitamin C in relation to the temperatures (as outcomes). Therefore, there was less loss of vitamin C in FB at 112 °C, compared to FC (116 °C) and FA (121 °C) with higher losses. The heat treatment certainly destroyed the vitamin C molecules, but the little that remained could help reduce plant ferric iron (Fe^{3+}) to ferrous iron (Fe^{2+}), the only form in which non-heme iron is transported across the intestinal membrane [4]. In fact, the reduction of Fe^{3+} promotes the formation of Fe^{2+} -ascorbic acid complex at acidic pH and maintains its form in the intestine at alkaline pH [8]. In practice, it is unlikely that extrusion parameters should be optimized to ensure minimal vitamin losses. Furthermore, few options for nutritional enrichment of extruded products with vitamins such as application of a more required amount to compensate for the losses during extrusion and storage or post-extrusion application, *e.g.*, by sprinkling, coating, spraying, coating, or filling are available [31].

4.4. Percentages of Minerals Bioaccessibility in Different Studied Flours

4.4.1. Iron Bioaccessibility

In the human body, iron exists in two oxidation states: ferrous iron Fe (II) and ferric iron Fe (III) [33]. In general, the removal of electrons or the addition of electrons to the atom influences the chemical activity and therefore the ability of metallic elements to interact with tissue targets (ligands). Iron is thermodynamically stable in hydrogen peroxide in its Fe^{3+} form (ferric iron) which unfortunately is only poorly soluble in the absence of organic chelation. Photochemical or biological reduction of Fe^{3+} can increase the biological availability of iron since the resulting ferrous iron Fe^{2+} is more soluble, has much faster ligand exchange kinetics, and forms much weaker complexes than Fe^{3+} . Ferrous iron (Fe^{2+}) is generally

more easily absorbed from the gastrointestinal tract than Fe^{3+} , probably due to its greater solubility [34]. In our study, the same results were found by [15] after incorporating Moringa and Baobab powders in millet mixtures with extrusion process. This improvement could be related to the presence of organic acids such as ascorbic acid in Moringa and Baobab powders as mineral absorption activators [35]. Ascorbic acid is known as a powerful enhancer for mineral bioavailability in foods. It chelates minerals and maintains them in a soluble and absorbable form [4]. Dietary iron, in ferric form, could be precipitated by phytic acid into insoluble ferric phytate at very acidic pH (1 - 3.5) [8] [36]. In the presence of ascorbic acid or vitamin C, iron in plant material is reduced to ferrous iron (Fe^{3+} to Fe^{2+}) with the formation of iron-ascorbic acid complexes [4] [8]. These complexes are present in the stomach at acidic pH and in the intestine at alkaline pH [8]. However, despite the soluble form of plant iron present in the intestine, its bioaccessibility remains low.

4.4.2. Zinc Bioaccessibility

These results are contrary to those obtained by [15], who showed an increase in the amount of bioaccessible zinc after extrusion of mixtures of millet with Moringa /and or Baobab powders. The results of bioaccessible zinc amounts obtained (in co-extruded and controls) were lower and higher than those respectively found by [4] and [15]. The reduction of bioaccessible zinc amounts in samples can be explained by the presence of calcium in the flours, which reinforces the inhibitory effect of phytate on zinc bioavailability and consequently on the bioaccessibility of the latter [37]. Indeed, phytate is the main nutritional inhibitor of zinc absorption by binding efficiently to the cation and forming stable complexes with low solubility and bioaccessibility. Zinc phytate is soluble below the pH range of 4.3 to 4.5 and insoluble at higher pH [8] [36]. Thus, at neutral pH, zinc phytate is much more resistant to hydrolysis [8]. Another important fact is the synergistic effect of secondary cations like Ca^{2+} . Indeed, two cations present simultaneously, can act together with increasing the amount of phytate precipitation. This is the case of Ca^{2+} ion, which improves the incorporation of Zn^{2+} ion into phytate by the formation of a phytate/calcium/zinc complex [36]. These complexes formed are stronger suggesting that calcium could worsen the inhibition of zinc absorption by phytates [38] by forming-by-forming calcium/phytic acid/zinc complexes that are even less soluble than zinc/phytic acid complexes [39]. Therefore, complexed zinc is not available for absorption and would be excreted in the stool. Beside of possible inhibitory impact of calcium cation on the intestinal bioavailability of zinc, several *in vivo* studies have also reported negative effects of non-heme iron on the absorption of zinc [38]. Magnesium also, in the presence of phytates, accentuates the precipitation of zinc during *in vitro* digestion, but its effect is less pronounced compared to calcium [36].

4.4.3. Calcium Bioaccessibility

From our results, extrusion cooking reduced the percentages and amounts of bio-

accessible calcium in all extruded flours compared to their controls. These results are not similar to those found by [15], who showed an increase in bioaccessible calcium amounts after extrusion of their millet mixtures with Moringa and Baobab powders. Furthermore, bioaccessible calcium amounts in extruded and traditional flours remained higher than those in millet flour. These reductions might be due to phytate content loss during the extrusion cooking (Figure 1). In addition, the formation of insoluble complexes above pH = 6 with calcium by phytic acid could be the reason for low bioaccessibility by forming insoluble complexes in the intestine [39]. The amount of bioaccessible calcium found in comparing F1/FA was in agreement with those of [4] for the same millet/Baobab mixture and lower for the F2/FB and F3/FC mixtures. This may be due to the higher incorporation rates of Baobab and Moringa powders compared to those in our study.

4.4.4. Magnesium Bioaccessibility

Extrusion cooking increased the bioaccessible magnesium amounts in all extruded flours compared to their controls (Figure 6). The explanation could be related to the presence of mineral absorption activators (ascorbic acid) contained in Moringa and Baobab powders [4]. The increase in bioaccessible magnesium amounts in our samples after cooking-extrusion were the same as those obtained by [15]. Extrusion cooking and sufficient gastric acidity could explain the good digestive assimilation of magnesium in co-extruded and traditional flours. In fact, phytates also form complexes with magnesium and, during extrusion cooking, we obtained more soluble magnesium in the extruded flours (Table 1). The magnesium phytate formed is soluble below the pH range (7.2 - 8.0), and, therefore, can be absorbed at pH levels below 7 [36]. Thus, at neutral pH, the complex formed with phytate is the most sensitive for absorption [8]. However, the bioaccessible magnesium results obtained are lower than those of [4] for the same formulations.

4.4.5. Phosphorus Bioaccessibility

Extrusion cooking seems to increase the amount of bioaccessible phosphorus in all extruded flours. However, the amount of bioaccessible phosphorus in extruded flours remains lower compared to that of millet flour (Figure 7). The degradation of phytic acid during extrusion allows an increase in the bioavailability of phosphorus through the release of phosphates (Pi) and the formation of myo-inositol phosphates (IP5 to IP1) which have a lower binding capacity to metal ions (iron, zinc, mg and Ca) [8] [15] [39]. At physiological pH, phosphates behave as a buffer system present in two forms: (H_2PO^-) (20%) and (HPO_4^{2-}) (80%) [40].

4.5. Molar Ratios of Phytate/Mineral Complexes

Calcium has been shown negative effects on both non-heme and heme iron absorption compared to other inhibitors affecting only non-heme iron absorption. Initially, the inhibitory effect was suggested to occur during iron transport across

the basolateral membrane of the enterocyte to plasma. Because the absorption of both forms of iron is equally inhibited, it has been suggested that inhibition takes place during initial uptake [41]. High calcium contents combined with high phytate contents can reinforce the effect of the inhibitory power of phytate on the bioavailability of iron because calcium with phytate and iron forms insoluble complexes [7] [35]. Calcium content can also affect zinc absorption if the diet contains phytates. Indeed, calcium binds to phytate and zinc by forming insoluble poly-mineral-phytate complexes, which therefore cannot be absorbed [32] [39]. These complexes are stronger than phytate/zinc complexes [7] [35]. Studies in humans indicate that a meal's absorption of zinc and iron directly correlates with its phytate content. Phytate/mineral molar ratios are therefore used to predict its inhibitory effect on mineral bioavailability. The phytate/iron molar ratio > 1 is considered indicative of low iron bioavailability. The phytate/calcium molar ratio > 0.24 alters the bioavailability of calcium. Zinc absorption is greatly reduced with a negative zinc balance when the phytate/zinc molar ratio is more than 15. When diets are rich in phytate and calcium [18], the phytate molar ratio \times calcium/zinc > 200 is a more useful assessment of zinc bioavailability than the phytate/zinc molar ratio [19].

4.6. Contribution of Bioaccessible Iron to Absolute Requirements (AR) and of Bioaccessible Zinc to Physiologic Requirements

The absolute requirement for bioaccessible iron is the contribution of daily mineral basal losses (via stools, urine, skin and its appendages, milk, menstrual blood and semen) and iron quantities necessary for growth [28]. The percentage contributions of 50 and 75 g of the developed flours to the AR in iron bioaccessible were calculated according to [15]. Extruded flour FB (millet/Moringa powder) gives the highest percentage of bioaccessible iron contribution for children aged 1 to 3 years and for lactating women to the ARs. This indicates that the iron status of children aged 1 to 3 years and lactating women consuming such extruded food-to-food fortified flour could potentially increase the percentage contribution of iron to ARs, with a daily consumption [6] [15].

The absolute physiologic requirements for bioaccessible zinc are defined as the amount of zinc that must be absorbed to compensate for the amount of endogenous zinc lost from both intestinal and non-intestinal sites. For growing children, the zinc retained amount in newly accumulated tissues is considered to be the total physiologic requirement. In lactating women, transferred zinc into breast milk will be added to the requirements [29]. The percentage contributions of 50 and 75 g of the developed flours to physiologic requirements for bioaccessible zinc were calculated according to [15]. For physiologic requirement of zinc, extrusion cooking reduced the contribution percentages in all extruded flours. However, traditional flour F2 (millet/Moringa powder) showed the highest percentage of bioaccessible zinc contribution for children aged 1 to 3 years and for lactating women to the PRs. Thus, daily consumption of traditional flour F2 would improve the

zinc status of children aged 1 to 3 years and lactating women.

5. Conclusions

Nutrition helps provide quantitative and qualitative bioactive compounds for the body to improve health and well-being but also to prevent the risk of suffering from diseases (“optimal” diet). The potential of bioactive components in foods necessary for the body depends on their release from the food matrix, changes during digestion, absorption, metabolism and biodistribution. Among bioactive compounds, we have minerals that are more resistant to industrial production processes; however, despite the increase in safety provided by these procedures, they can have a substantial impact on the mineral bioaccessibility/bioavailability in foods. Food processing improves the bioaccessibility of micronutrients by decreasing the levels of anti-nutrients and making minerals in the food matrix more diffusible and bioaccessible/bioavailable. In this present study, focus was on the impact of food-to-food fortification combined with cooking-extrusion on the bioaccessibility of minerals (iron, zinc, magnesium, phosphorus and calcium) contained in the traditional foods (controls). After extrusion cooking, more iron, zinc, magnesium and phosphorus were released. This can be explained by the reduction of phytates in extruded flours. The dialysability tests subsequently carried out on the flours allowed us to predict the percentages of iron, zinc, magnesium, phosphorus and calcium that are bioaccessible before and after heat treatment. Extrusion cooking has a positive effect on the amounts of bioaccessible iron, magnesium and phosphorus and a negative effect on those of zinc and calcium. However, compared to other minerals, the quantities of bioaccessible iron in our samples were lower. Vegetable iron exists in ferric iron (Fe^{3+}). In the presence of vitamin C, there is a reduction of the ferric iron to ferrous iron (Fe^{2+}). The plant Fe^{2+} form is more bioavailable than the Fe^{3+} because of its solubility at physiological pH and low affinity with ligands that can inhibit its absorption. At luminal pH (6 - 7.4), phytate also could bind efficiently to calcium and zinc to form stable complexes with low solubility and bioaccessibility. Therefore, it seems necessary to find out another way to hydrolyze phytates. Thus, to best reduce phytate levels, it would be necessary to add exogenous phytases as food additives after cooking-extrusion or increase the quantity of sources rich in organic acids after cooking-extrusion.

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Conflicts of Interest

The authors declare no conflicts of interest.

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