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RESEARCH ARTICLE

Extrusion cooking of food-to-food fortified wholegrain sorghum-based porridges enhances Caco-2 ferritin formation

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Abstract

Background and Objectives: Iron deficiency is still a major public health concern in sub-Saharan Africa, and this is in part due to a monotonous diet of cereals often low in bioavailable minerals and high in mineral bioavailability inhibitors, notably phytate and polyphenols. Sorghum is a major food crop across the semi-arid tropics in Africa because of its tolerance to high temperature and low rainfall. Extrusion cooking is a process that applies high heat, pressure, and shear to raw food materials to produce ready-to-eat products. The application of high heat, pressure, and shear can destroy anti-nutrients in plant foods and hence enhance the bioavailability of minerals. Food-to-food fortification (FtFF) is a strategy where micronutrient-rich food combinations are used to promote the bioavailability of essential micronutrients by increasing the content of micronutrient bioavailability enhancers. The objective of this study was to determine the effects of extrusion cooking of wholegrain sorghum-based porridges fortified with baobab fruit powder and moringa leaf powder on iron bioaccessibility.

Findings: Although extrusion reduced bioaccessible iron content (BIC) and percentage bioaccessible iron (PBI), it enhanced ferritin-formation in Caco-2 cells (by 38%) compared to conventional cooking, most probably because extrusion reduced contents of phenolics and phytate, hence freeing more iron. Fortification with baobab increased PBI by 14%–34% whether extruded or conventionally cooked, probably due to its organic acids. Fortification with moringa reduced BIC and PBI (by 30% and 71%, respectively) whether extruded or conventionally-cooked, probably due to its high calcium and phytate contents.

Conclusion: Extrusion cooking has the potential to help alleviate iron deficiency in sorghum-based foods because it reduces the content of anti-nutrients.

Significance and Novelty: This study highlights the potential of extrusion cooking coupled with fortification with tropical foodstuffs high in organic acids to improve iron bioavailability in wholegrain-based starchy staple foods.

KEYWORDS
bioaccessibility, extrusion, ferritin-formation, food-to-food fortification, iron, sorghum, wholegrain

1 | INTRODUCTION

Extrusion cooking is a food processing technology that can be applied to produce a variety of convenience-type products from diverse plant foods (Guy, 2001). It is a continuous cooking process that applies high heat, pressure, and shear to raw food materials to produce ready-to-eat products (Fellows, 2000). Due to rapid urbanization in Africa, there is increasing demand for ready-to-eat foods (Tiuganji et al., 2020), especially in families with working mothers. Another advantage of extrusion cooking is that it can destroy anti-nutrients in plant foods and hence enhance the digestibility of their macronutrients (Nikmaram et al., 2017). For example, it has been reported to improve iron availability in peas and kidney beans (Alonso et al., 2001) and in rice and maize-based protein-enriched snacks (Wani & Kumar, 2016).

Iron deficiency is still a major public health concern in sub-Saharan Africa (Bouis et al., 2017; Lemoine & Tounian, 2020). Deficiency of micronutrients such as iron, while seldom causing death, adversely affects health. Iron deficiency accounts for 30-50% of anemia reported in children and women (Pasricha et al., 2021). These deficiencies are in part due to a monotonous diet of cereals (Gibson et al., 2018), roots, and tubers (Gregory & Wojciechowski, 2020). These starchy foods are often low in bioavailable minerals and cereals are specifically high in mineral bioavailability inhibitors, notably phytate and polyphenols (Gibson et al., 2018). Notwithstanding these nutritional drawbacks, sorghum is a major food crop across the semi-arid tropics of Africa because of its tolerance to high temperature and low rainfall (Taylor, 2019).

To help prevent mineral deficiencies, at-risk communities are encouraged to diversify their diets by including vegetables rich in essential minerals and fruits rich in promoters of mineral bioavailability (World Health Organization, 2017). Food-to-food fortification (FtFF) is a strategy where micronutrient-rich food combinations are used to promote the bioavailability of essential micronutrients by increasing the content of micronutrients and enhancers of their absorption and decreasing the levels of inhibitors of micronutrient bioavailability (Kruger, 2020). FtFF with moringa leaves and baobab fruit pulp has been shown to improve iron and zinc bioaccessibility in pearl millet (Adetola et al., 2019) and maize (Adetola et al., 2022).

Hence, the objective of this study was to determine the effects of extrusion cooking of sorghum-based porridges FtF fortified with baobab fruit powder and moringa leaf powder on iron bioaccessibility, as measured by both dialyzability and Caco-2 cell assay.

2 | EXPERIMENTAL

2.1 | Materials

Red non-tannin sorghum was procured from Mpumalanga Province, South Africa. The grain was milled using a hammer mill fitted with a 500 μm mesh size screen. The wholegrain flour was stored at 4°C in sealed plastic buckets. Baobab fruit powder was from Nautica Organic Trading, Durban, South Africa. Dried moringa leaf powder was from Supa Nutri, Cape Town, South Africa.

Digestive enzymes and bile salts used were pepsin (P-7000, CAS number: 9001-75-6), pancreatin (P-1750, CAS number: 8049-47-6), and bile extract (B-8631, CAS number: 8008-63-7; Sigma-Aldrich). Dialysis tubing Spectra/Por 7 (Ø = 20.4 mm) with a molecular weight cutoff of 10 kDa was used (G.I.C. Scientific).

2.2 | Porridge formulations

The following formulations of sorghum-based flours with fortificants were prepared (corn starch was included as a filler to maintain a constant final percentage weight for all formulations, figures in brackets represent the ratios of the ingredients):

A. Wholegrain sorghum flour+corn starch (85:15)
B. Wholegrain sorghum flour+ferrous sulfate+corn starch (85:0.02:14.98) as a conventional iron fortification standard
C. Wholegrain sorghum flour+ferrous sulfate+corn starch +ascorbic acid+citric acid (85:0.02:14.35:0.01:0.62) as a conventional iron fortification gold standard, with organic acids being added before processing. The organic acids added were based on the amount present in the baobab fruit pulp and moringa leaf powders.
D. Wholegrain sorghum flour+ferrous sulfate+corn starch +ascorbic acid+citric acid (85:0.02:14.35:0.01:0.62) as a

conventional iron fortification gold standard, with organic acids being added after processing.

E. Wholegrain sorghum flour+baobab fruit pulp powder+corn starch (85:6:9), with baobab being added before processing.
F. Wholegrain sorghum flour+baobab fruit pulp powder+corn starch (85:6:9), with baobab being added after processing.
G. Wholegrain sorghum+moringa leaf powder+corn starch fortified (85:6:9).
H. Wholegrain sorghum+moringa leaf powder+baobab fruit pulp powder+corn starch (85:6:6:3), with baobab being added before processing.
I. Wholegrain sorghum+moringa leaf powder+baobab fruit pulp powder+corn starch (85:6:6:3), with baobab being added after processing.

All formulations were made to meet approximately 25% of the recommended dietary intake for iron at low bioavailability of an adult woman (32.4 mg) in the total formulation (Saunders et al., 2013), with the exception of the formulations containing only baobab fruit pulp powder. This is because the iron content of the baobab was low.

From the in vitro iron dialyzability results, porridge formulations A, E, G, and H were used for the ferritin ELISA assay with Caco-2 cells to study the effect of extrusion cooking compared to conventional cooking as well as the effect of fortification with moringa and baobab on ferritin formation:

2.3 | Conventional wet cooking

Deionized water was added to each wholegrain sorghum-based flour in a ratio of 3:10, flour: water (w/w). The slurry was heated to boiling temperature (95°C) and maintained with constant stirring for 25 min. The slurry was left to cool at ambient temperature, after which it was placed in plastic containers and frozen to −20°C and freeze-dried in an Instruvac freeze-dryer model RFR 3878 (Air and Vacuum Technologies). Freeze-dried porridge flour was crushed to a particle size that passes through a 500 μm opening screen before further analysis. The pre-cooked porridge flour was stored at 4°C in double-sealed, airtight plastic bags.

2.4 | Extrusion cooking

A co-rotating twin-screw extrusion cooker model TX 32 (CFAM Technologies; $L/D = 21.5{:}1$) was used. Porridge formulations prepared as above were extruded separately. The barrel comprised of five heating zones toward the die was set at 60/70/80/140/140°C, respectively. Water was fed into the system at a dosing rate of 3 L/h (to obtain a final moisture content of 20% calculated based on the moisture content of the flours) and the feed rate was 10 kg/h. A die opening of 3 mm was used and the screw speed was maintained at 250 rpm. Extrudates were collected three times after every 30 min interval to produce triplicates. They were dried immediately in a force draught oven at 50°C for 5 min to a moisture content below 10%. The cooled extrudates were milled using an air-cooled analytical mill to a maximum particle size of 500 μm. The milled extrudates were stored at 4°C in double-sealed, airtight plastic bags.

2.5 | Determination of phytate content

Phytate was determined using the extraction and indirect quantitative assay of Fruhbeck et al. (1995). The method is based on spectrophotometric determination of organic phosphate present in sample that has been acid extracted and purified to remove the inorganic phosphate. Dowex1-anion-exchange resin-AG 1 × 4 (4% Cross-linkage, chloride form, 100–200 mesh, 74–149 μm) in glass barrel Econo-columns, 7 × 5 mm was used for purification of the extracts. The standard, sodium phytate (P-8810, Sigma-Aldrich) and purified extracts were reacted with Wade reagent, after which absorbance was measured at 500 nm.

2.6 | Determination of total phenolic content

The Folin–Ciocalteu method according to Waterman and Mole (1994) was used to quantify total phenolic content (mainly free total phenolics) and reported in catechin equivalents (CEs).

2.7 | Determination of organic acids

The extraction and quantification of organic acids were by reversed phase-HPLC according to Tembo et al. (2017) with modifications as per Adetola et al. (2019).

2.8 | Determination of iron content

Acid digestion of the plant foods was performed using conc. nitric acid plus hydrogen peroxide according to EPA method 3051A (U.S. EPA, 2007). Iron contents of the

digested flour samples were analyzed by EPA method 200.7 (U.S. EPA, 1996) using inductively coupled plasma atomic emission spectrometry (ICP-AES; iCAP 6000 series, Thermo Fisher Scientific). Iron was analyzed at 239.5 nm. To ensure accuracy, samples were analyzed against National Institute for Standards and Technology traceable standards and independent quality control solutions. A calibration acceptance criterion of $R^2 > 0.9995$ was used and an internal standard technique was used to check result accuracy.

2.9 | Determination of iron bioaccessibility using the in vitro dialyzability assay

The in vitro dialyzability method of Miller et al. (1981) was used. Digestive enzymes and bile salts used were pepsin (P-7000), pancreatin (P-1750), and bile extract (B-8631). Dialysis tubing Spectra/Por 7 (Ø 20.4 mm) with a molecular weight cutoff of 10 kDa was used (G.I.C. Scientific). Mineral contents of the dialysates were measured using ICP-AES as described. Iron bioaccessibility was calculated as the percentage of the mineral in the dialysate relative to the total mineral content in the porridge sample.

2.10 | In vitro digestion for Caco-2 cells: Solubility method

The in vitro digestion method of Glahn et al. (1998) with modifications according to Perales et al. (2005) was used and comprised sequential gastric and intestinal stages. The modifications mainly involved demineralizing the pepsin and pancreatin-bile salt solutions. In addition, instead of using dialysis tubing, aliquots of 20 g sample were transferred to polypropylene centrifuge tubes (50 ml) and centrifuged at 3500*g* for 1 h at 4°C and supernatants were used to determine the iron bioaccessible content. The bioaccessible fraction (soluble fraction) was used in the Caco-2 cell ferritin assay.

2.11 | Culturing of Caco-2 cells and determination of ferritin synthesis by Caco-2 cells

Cell culturing and determination of ferritin were done as described by Viadel et al. (2007) with modifications. Caco-2 cells were obtained from Separation Scientific (Cellonex Cell Line) and were used between passage numbers 8 and 20. The cells were subcultured and maintained at 37°C in an incubator (under a 5% CO_2/95% air atmosphere at constant humidity). Growth medium of Dulbecco's minimum essential media (Sigma-Aldrich) with Earle's salts, L-glutamine, sodium bicarbonate, and sodium pyruvate supplemented with 10% v/v fetal bovine serum, 1% v/v nonessential amino acids (Sigma-Aldrich), and 1% v/v antibiotic-antimycotic solution (Sigma-Aldrich) was used. At 80% confluence, cells were seeded at a density of 5×10^4 cells/cm^2 in six-well plates and maintained at 37°C in an incubator under a 5% CO_2/95% air atmosphere at constant humidity. The seeded cells were grown under low iron conditions, minimum essential medium (Sigma-Aldrich) supplemented with 10% demineralized FBS, and 1% v/v antibiotic-antimycotic solution (Gibco). Culture medium was changed every 2 days. The iron uptake assays were performed with differentiated cells 14–16 days after seeding. At the end of each assay, the cell monolayers were washed three times with buffer solution, and the cells were detached with a cell scrapper.

Cell monolayers were collected with 2 ml deionized water at 4°C and sonicated at 30 kHz for 30 s at 4°C. About 10 µl aliquots of the sonicated Caco-2 monolayer were used in ferritin measurement (Human Ferritin ELISA Kit for serum, plasma, cell culture supernatant, and urine; Sigma-Aldrich). The cell protein content was determined using a TaKaRa Bradford Protein Assay Kit Bicinchoninic Acid (Separations). Ferritin contents were expressed as ng ferritin/mg protein.

2.12 | Statistical analyses

Each experiment was performed thrice (with the exception of dialyzability and ferritin formation which was repeated six times) and one-way analysis of variance was used to determine the differences between treatments. Fisher's LSD test at a .05 level of significance was applied. Statistica 10 (StatSoft Inc.) was used.

3 | RESULTS AND DISCUSSION

3.1 | Antinutritional components (phytate, total phenolics, and dietary fiber) and organic acids (ascorbic acid and citric acid)

3.1.1 | Mineral absorption enhancers (organic acids—Ascorbic acid and citric acid)

Due to their role as enhancers of mineral bioavailability in foods (Iyengar et al., 2010; Lönnerdal, 2000), the content of organic acids (ascorbic acid and citric acid) in the wholegrain sorghum-based porridges was of interest. Baobab had higher contents of ascorbic and citric acids

(50 times more than moringa with none in sorghum for ascorbic acid, and 1.3 times more than moringa and 112 times more than sorghum for citric acid; Table 1). Similar trends in organic acid contents of baobab compared to moringa have been reported previously (Adetola et al., 2019; Adetola et al., 2022).

3.1.2 | Phytate and total phenolics

Moringa leaf powder had the highest phytate content, 1.6 times higher than wholegrain sorghum and 4.1 times higher than baobab fruit pulp powder (Table 1). The values reported here are similar to those reported by Kruger et al. (2014) for wholegrain sorghum, for baobab fruit pulp (Adetola et al., 2022), and moringa leaf powder (Leone et al., 2015).

The wholegrain sorghum-based porridges prepared by extrusion cooking consistently had a lower phytate content than their conventionally wet-cooked counterparts (on average 16% lower) (Table 2). This lower phytate content was probably due to thermal dephosphorylation of the phytate into lower inositol phosphates (Watson et al., 2019) by extrusion cooking. In fact, it has been observed that with extrusion cooking of kidney beans and peas there was an increase in inositol-penta/tetra/tri-phosphates accompanied by a corresponding decrease in inositol hexphosphate content. Reduction in phytate content by extrusion cooking has also been reported in wholegrain sorghum-based composite flours by Tadesse et al. (2019) and Arun Kumar et al. (2018). Concerning the effects of fortification, generally, none of the various fortification treatments reduced phytate content.

Table 1 shows that baobab fruit pulp powder had the highest TPC, 6% more than moringa leaf powder and 90% more than wholegrain sorghum. Braca et al. (2018) similarly reported high values of TPC in baobab. Similarly, Leone et al. (2015) reported high TPC values of moringa leaf. The TPC values for wholegrain sorghum (Table 1) are also within range reported by Kruger et al. (2012). Table 2 shows that the wholegrain sorghum porridges prepared by extrusion cooking had lower TPC than the conventionally cooked porridges. The combination of high temperatures, shear, and pressure during extrusion cooking leads to degradation of phenolic compounds or alteration of their chemical activity (Sharma et al., 2012) which might alter their ability to chelate iron. Fortification with baobab fruit pulp and moringa leaf powder alone or in combination resulted in increased TPC (15%–64% higher), due to their high TPC contents. A principal component analysis (PCA) plot projecting the dependent variables and treatments on a

TABLE 1 Organic acids, phytate, total phenolic content (TPC), insoluble dietary fiber (IDF), soluble dietary fiber (SDF), and iron contents of wholegrain sorghum, baobab fruit pulp powder, and moringa leaf powder determined on dry basis

Food material	Citric acid (mg/100 g)	Ascorbic acid (mg/100 g)	Phytate (mg/100 g)	TPC (mg CE/100 g)	IDF (g/100 g)	SDF (g/100 g)	Iron content (mg/100 g)
Wholegrain sorghum	284 ± 15[a]	ND[b]	1078 ± 136	404 ± 19	8.66 ± 0.51	1.51 ± 0.48	4.84 ± 0.80
Baobab fruit pulp powder	3506 ± 148	164 ± 27	418 ± 7	4119 ± 7	12.95 ± 0.77	42.61 ± 1.43	2.32 ± 0.44
Moringa leaf powder	2722 ± 358	2 ± 0	1721 ± 61	3852 ± 0	38.17 ± 0.85	4.32 ± 1.07	100.93 ± 7.52

[a]Values are the means ± SD of at least two samples of each plant food analyzed independently in triplicate ($n = 6$).
[b]Not detected.

TABLE 2 Effects of processing (conventional and extrusion cooking) and addition of organic acids, baobab fruit pulp, and moringa leaf powder to wholegrain sorghum porridge on phytate and total phenolic content (TPC; dry basis) of sorghum-based porridge formulations

Formulation	Phytate (mg/100 g)			TPC (mg CE/100 g)		
	Conventionally cooked	Extrusion cooked	Average effect of porridge formulation[1,2]	Conventionally cooked	Extrusion cooked	Average effect of porridge formulation
A. Wholegrain sorghum	$808^{bBC} \pm 133$[3,4,5]	$679^{aB} \pm 18$	$744^{BC} \pm 76$	$340^{bB} \pm 46$	$250^{aC} \pm 16$	$295^{B} \pm 31$
B. Wholegrain sorghum + $FeSO_4$	$955^{bCD} \pm 67$	$672^{aB} \pm 15$ (−31%)[6]	$813^{CD} \pm 186$	$394^{bBC} \pm 8$	$204^{aB} \pm 3$ (−18%)	$299^{B} \pm 104$
C. Wholegrain Sorghum + $FeSO_4$ + Ascorbic Acid + Citric Acid (added before processing)	$783^{bAB} \pm 11$ (−3%)	$699^{aB} \pm 7$ (−28%)	$714^{BC} \pm 104$	$303^{bA} \pm 10$ (−12%)	$131^{aA} \pm 14$ (−48%)	$217^{A} \pm 95$ (−26%)
D. Wholegrain Sorghum + $FeSO_4$ + Ascorbic Acid + Citric Acid (added after processing)	$846^{bBC} \pm 23$	$670^{aAB} \pm 68$ (−31%)	$758^{BC} \pm 104$	$342^{bAB} \pm 43$	$142^{aA} \pm 5$ (−43%)	$242^{A} \pm 113$ (−18%)
E. Wholegrain Sorghum + Baobab (Baobab added before processing)	$800^{bAB} \pm 21$	$705^{aB} \pm 39$ (−27%)	$752^{B} \pm 57$	$465^{bD} \pm 45$ (+37%)	$278^{aCD} \pm 28$	$372^{C} \pm 108$ (+26%)
F. Wholegrain Sorghum + Baobab (Baobab added after processing)	$765^{bAB} \pm 36$ (−15%)	$603^{aA} \pm 45$ (−38%)	$684^{AB} \pm 94$	$401^{bBC} \pm 12$	$452^{aF} \pm 20$ (+81%)	$427^{D} \pm 31$ +45%)
G. Wholegrain Sorghum + Moringa	$960^{bD} \pm 41$ (+19%)	$845^{aC} \pm 12$ (−13%)	$903^{C} \pm 67$	$563^{bE} \pm 49$ (+66%)	$316^{aD} \pm 17$ (+26%)	$439^{D} \pm 139$ (+49%)
H. Wholegrain Sorghum + Moringa + Baobab (Baobab added before processing)	$1058^{bE} \pm 130$ (+31%)	$682^{aB} \pm 101$ (−29%)	$870^{D} \pm 33$ (+17%)	$664^{bF} \pm 51$ (+95%)	$405^{aE} \pm 50$ (+62%)	$535^{E} \pm 149$ (+81%)
I. Wholegrain Sorghum + Moringa + Baobab (Baobab added after processing)	$893^{aBC} \pm 22$ (−8%)	$885^{aC} \pm 45$ (+30%)	$889^{E} \pm 34$ (+19%)	$547^{aE} \pm 33$ (+61%)	$536^{aG} \pm 29$ (+114%)	$542^{E} \pm 27$ (+84%)
Average effect of cooking method[7,8]	$904^{b} \pm 47$	$747^{a} \pm 41$ [−17%][9]		$454^{b} \pm 47$	$302^{a} \pm 41$ [−25%]	

[1]For each overall effect of porridge formulation, means of each formulation with different superscript uppercase letters in a column differ significantly ($p < .05$).

[2]Average effect of porridge formulation refers to the average of each treatment (fortification) across the rows regardless of whether the formulation was conventionally wet-cooked or extruded.

[3]Values are the means ± SD of at least two samples of each formulation analyzed independently in triplicate ($n = 6$).

[4]For each dependent variable (phytate, TPC), means of each treatment (conventionally cooked, extruded) with different superscript lowercase letters in a row differ significantly ($p < .05$) by pairwise comparison.

[5]Means of each treatment (conventionally cooked, extruded) with different superscript uppercase letters in a column differ significantly ($p < .05$).

[6]Figures in curved brackets are the average percentage difference of extruded porridges compared to conventionally wet-cooked sorghum porridges were statistically significant.

[7]For each overall effect of processing technology (cooking or extruded), means of each formulation with different superscript lowercase letters in a row differ significantly ($p < .05$).

[8]Average effect of cooking method refers to the average of each cooking method along the column regardless of the treatment performed.

[9]Figures in square brackets are the average percentage difference between extrusion cooked and conventionally cooked were statistically significant.

two-dimensional factor plane (Figure 1a,b) showed that the extruded treatments, except those containing moringa, were in different quadrants to total phenolic content and phytate content. This illustrates clearly the effect of extrusion cooking of reducing phytate and phenolics content.

3.2 | Total iron content, iron bioaccessibility, and ferritin formation in Caco-2 cells

3.2.1 | Total iron content, percentage iron bioaccessibility, and bioaccessible iron (dry basis)

Moringa contained 25 times more iron than sorghum and 44 times more iron than baobab (Table 1). While varying data for the iron content of moringa leaf powder have been reported, 19 mg/100 g by Adetola et al. (2019) and 58.4 mg/100 g by Van Der Merwe et al. (2019), it consistently has a higher iron content than sorghum. Adetola et al. (2019) reported iron content of 3.7 mg/100 g in baobab, close to what we reported in this study, and Kayodé et al. (2006) reported iron content (3.0–11.3 mg/100 g) in sorghum within range of what was reported in this study.

Regarding the total iron content (TIC), extrusion cooking significantly increased the iron content of the sorghum-based porridges (by 9%; Table 3). This is probably due to the abrasive action of the plant materials resulting in some iron from the extruder parts, probably in the form of iron oxide (rust), being incorporated into the extrudates (Alonso et al., 2001). Extrusion cooking when compared to conventional wet cooking had no significant ($p > .05$) effect on the bioaccessible iron content (BIC) of the porridges (Table 3). However, extruded porridge fortified with moringa and with moringa plus baobab had significantly lower, BIC, by 59% and 53%, respectively compared to the conventionally wet-cooked porridge treatments. The reduction in BIC could be as a result of the iron incorporated in the formulation following extrusion not being bioaccessible.

Fortification with ferrous sulfate increased BIC by overall 143% but reduced the percentage bioaccessible iron (PBI) by overall 19% (Table 3). Fortification with ferrous sulfate plus ascorbic and citric acids whether before or after cooking (conventional or extrusion cooking) increased both BIC and PBI (by 540% and 99% before processing and by

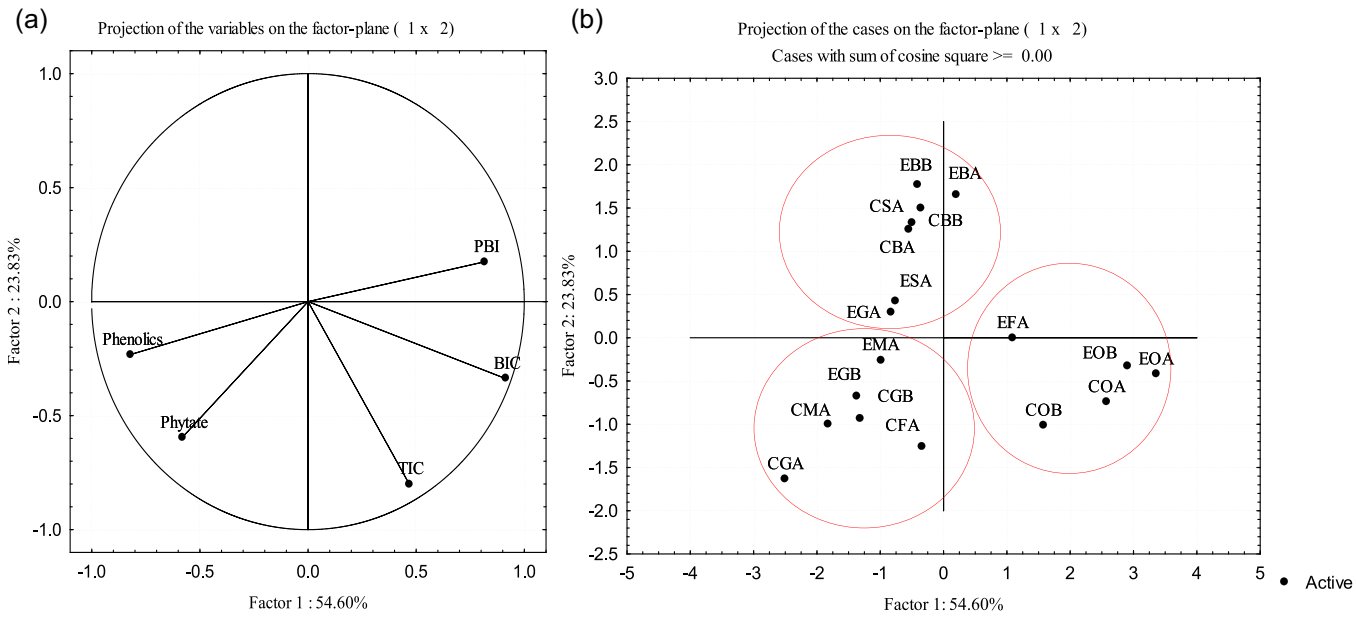

FIGURE 1 Principal component analysis showing 1 × 2 factor coordinate plots of dependent variables (phytate, phenolics, total iron content—TIC, bioaccessible iron content—BIC and percentage bioaccessible iron—PBI) (a), and independent variables (fortification and processing technique-wet-cooking and extrusion) (b). Key: CSA—Cooked Sorghum, CFA—Cooked Sorghum+Fe, COA— Cooked Sorghum +Fe+Organic Acids co-cooked, COB—Cooked Sorghum+Fe+Organic Acids added after, CBA—Cooked Sorghum+Baobab co-cooked, CBB—Cooked Sorghum+Baobab added after, CMA—Cooked Sorghum+Moringa, CGA—Cooked Sorghum+Moringa+Baobab co-cooked, CGB—Cooked Sorghum+Moringa+Baobab added after, ESA—Extruded Sorghum, EFA—Extruded Sorghum+Fe, EOA—Extruded Sorghum+Fe+Organic Acids co-cooked, EOB—Extruded Sorghum+Fe+Organic Acids added after, EBA—Extruded Sorghum+Baobab co-cooked, EBB—Extruded Sorghum+Baobab added after, EMA—Extruded Sorghum+Moringa, EGA—Extruded Sorghum+Moringa +Baobab co-cooked, EGB—Extruded Sorghum+Moringa+Baobab added after.

TABLE 3 Effects of processing (conventional and extrusion cooking) and addition of organic acids, baobab fruit pulp, and moringa leaf powder to wholegrain sorghum porridge on total iron content (TIC), bioaccessible iron content (BIC) and percentage bioaccessible iron (PBI) of sorghum-based porridge formulations

	TIC (mg/100 g db)			BIC (mg/100 g db)			PBI (%)		
Formulations	**Conventionally cooked**	**Extrusion cooked**	**Average effect of porridge formulation[1,2]**	**Conventionally cooked**	**Extrusion cooked**	**Average effect of porridge formulation**	**Conventionally cooked**	**Extrusion cooked**	**Average effect of porridge formulation**
A. Wholegrain Sorghum	$3.7^{aA} \pm 0.4$[3,4,5]	$5.3^{bB} \pm 0.1$	$4.5^{B} \pm 0.9$	$0.4^{aA} \pm 0.1$	$0.4^{aB} \pm 0.1$	$0.4^{B} \pm 0.1$	$9.2^{aD} \pm 2.4$	$7.1^{aCD} \pm 1.8$	$8.1^{E} \pm 2.4$
B. Wholegrain Sorghum + $FeSO_4$	$12.9^{aD} \pm 0.1$ (+250%)[6]	$14.2^{bE} \pm 0.2$ (+170%)	$13.5^{E} \pm 0.7$ (+202%)	$0.9^{dC} \pm 0.0$ (+143%)	$0.9^{aD} \pm 0.0$ (+143%)	$0.9^{D} \pm 0.0$ (143%)	$6.9^{aC} \pm 1.4$ (−24%)	$6.0^{aC} \pm 1.1$ (−15%)	$6.6^{D} \pm 1.3$ (−19%)
C. Wholegrain Sorghum + $FeSO_4$ + Ascorbic Acid + Citric Acid (added before processing)	$13.4^{aE} \pm 0.1$ (+264%)	$15.0^{bF} \pm 0.9$ (+185%)	$14.2^{F} \pm 1.1$ (+218%)	$2.4^{aE} \pm 0.1$ (+549%)	$2.4^{aE} \pm 0.1$ (+535%)	$2.4^{F} \pm 0.1$ (+540%)	$16.7^{aH} \pm 0.7$ (+82%)	$15.7^{aG} \pm 0.7$ (+121%)	$16.2^{H} \pm 0.9$ (+99%)
D. Wholegrain Sorghum + $FeSO_4$ + Ascorbic Acid + Citric Acid (added after processing)	$13.8^{aF} \pm 0.2$ (+273%)	$15.4^{bF} \pm 0.0$ (+193%)	$14.6^{G} \pm 0.9$ (+227%)	$1.8^{fD} \pm 0.1$ (+395%)	$2.0^{gE} \pm 0.3$ (+441%)	$1.9^{E} \pm \pm 0.2$ (+419%)	$13.7^{aG} \pm 0.7$ (+49%)	$13.0^{aF} \pm 1.9$ (+84%)	$13.3^{G} \pm 1.4$ (+64%)
E. Wholegrain Sorghum + Baobab (Baobab added before processing)	$3.6^{aA} \pm 0.2$	$4.8^{bA} \pm 0.0$ (−10%)	$4.2^{AB} \pm 0.6$	$0.5^{aB} \pm 0.0$ (+22%)	$0.5^{aC} \pm 0.1$ (+27%)	$0.5^{C} \pm 0.1$ (+27%)	$12.1^{bF} \pm 0.8$ (+32%)	$9.7^{aE} \pm 1.1$ (+37%)	$10.9^{F} \pm 1.6$ (+34%)
F. Wholegrain Sorghum + Baobab (Baobab added after processing)	$3.6^{aA} \pm 0.2$	$5.0^{bAB} \pm 0.2$	$4.3^{AB} \pm 0.8$	$0.4^{aAB} \pm 0.1$	$0.4^{aB} \pm 0.1$	$0.4^{B} \pm 0.0$	$11.3^{bEF} \pm 1.8$ (+23%)	$7.3^{aCD} \pm 1.2$	$9.3^{E} \pm 2.5$
G. Wholegrain Sorghum + Moringa	$10.4^{aB} \pm 0.1$ (+183%)	$11.2^{bD} \pm 0.3$ (+116%)	$10.8^{C} \pm 0.5$ (+142%)	$0.4^{bA} \pm 0.0$	$0.2^{aA} \pm 0.0$ (−59%)	$0.3^{A} \pm 0.0$ (−30%)	$3.4^{bAB} \pm 0.4$ (−63%)	$1.3^{aA} \pm 0.1$ (−81%)	$2.4^{AB} \pm 1.1$ (−71%)

TABLE 3 (Continued)

Formulations	TIC (mg/100 g db)			BIC (mg/100 g db)			PBI (%)		
	Conventionally cooked	Extrusion cooked	Average effect of porridge formulation[1,2]	Conventionally cooked	Extrusion cooked	Average effect of porridge formulation	Conventionally cooked	Extrusion cooked	Average effect of porridge formulation
H. Wholegrain Sorghum + Moringa + Baobab (Baobab added before processing)	$10.8^{aC} \pm 0.3$ (+194%)	$11.4^{bD} \pm 0.2$ (+115%)	$11.1^{D} \pm 0.4$ (+148%)	$0.3^{bA} \pm 0.1$	$0.2^{aA} \pm 0.0$ (−59%)	$0.2^{A} \pm 0.1$ (−38%)	$3.0^{bA} \pm 0.4$ (−68%)	$1.3^{aA} \pm 0.2$ (−81%)	$2.2^{A} \pm 0.9$ (−74%)
I. Wholegrain Sorghum + Moringa + Baobab (Baobab added after processing)	$10.8^{aC} \pm 0.2$ (+193%)	$10.7^{aC} \pm 0.0$ (+106%)	$10.7^{C} \pm 0.2$ (+140%)	$0.5^{aB} \pm 0.0$ (+27%)	$0.5^{aC} \pm 0.0$ (+22%)	$0.5^{C} \pm 0.1$ (+24%)	$4.4^{aB} \pm 0.2$ (−52%)	$4.3^{aB} \pm 0.8$ (−40%)	$4.3^{C} \pm 0.5$ (−47%)
Average effect of cooking methods[7,8]	$9.2^{a} \pm 4.2$	$10.3^{b} \pm 4.1$ [+10%][9]		*$0.8^{a} \pm 0.7$*	$0.8^{a} \pm 0.8$		*$9.2^{b} \pm 4.8$*	$7.5^{a} \pm 4.8$ [−19%]	

[1]For each average effect of porridge formulation, means of each formulation with different superscript lowercase letters in a column differ significantly ($p < .05$).

[2]Average effect of porridge formulation refers to the average of each treatment (fortification) across the rows regardless of whether the formulation was conventionally wet-cooked or extruded.

[3]Values are the means ± SD of at least two samples of each formulation analyzed independently in triplicate ($n = 6$).

[4]For each dependent variable (TIC, BIC, and PBI), means of each treatment (conventionally cooked, extruded) with different superscript lowercase letters in a row differ significantly ($p < .05$) by pairwise comparison.

[5]Means of each treatment (conventionally cooked, extruded) with different superscript uppercase letters in a column differ significantly ($p < .05$).

[6]Figures in curved brackets are the average percentage difference compared to sorghum porridge were statistically significant.

[7]For each average effect of processing technology (cooking or extruded), means of each formulation with different superscript lowercase letters in a row differ significantly ($p < .05$).

[8]Average effect of cooking method refers to the average of each cooking method along the column regardless of the treatment performed.

[9]Figures in square brackets are the average percentage difference between extrusion cooked and conventionally cooked were statistically significant.

419% and 64% after, respectively). The increase in both BIC and PBI was due to the enhancing effects of the organic acids. Ascorbic and citric acids are known mineral bioaccessibility enhancers as they chelate minerals and keep them in a soluble and absorbable form (Iyengar et al., 2010; Lönnerdal, 2000). The PCA projecting the BIC and PBI and fortification treatments on a two-dimensional factor plane (Figure 1a,b) showed that all the treatments containing organic acids were associated with PBI and BIC, further supporting the enhancing effect of organic acids on mineral bioaccessibility.

FtFF with baobab before processing significantly increased BIC, while baobab FtFF both before and after processing significantly increased PBI, by 23%–37%. This was supported by the PCA plot where all treatments containing baobab, observed along factor 1, were associated with PBI (Figure 1a,b), suggesting an enhancing role of baobab in iron bioaccessibility. While the content of ascorbic and citric acids in the baobab was equivalent to the organic acids added in the ferrous sulfate treatment, the effect of baobab on the bioaccessible iron was far less. This can be attributed to the phenolic compounds in baobab binding the iron. The increase in PBI following FtFF with baobab fruit powder can therefore be attributed to the presence of organic acids (mainly citric acid) in the baobab fruit powder. Similar results for BIC and PBI were reported by Adetola et al. (2019) baobab fruit powder FtFF of pearl millet porridges.

FtFF with moringa whether alone or in combination with baobab, increased the iron content of the sorghum-based porridges by approximately three times due to its high iron content (Tables 1 and 3). However, moringa caused an overall significant reduction in both the BIC and percentage of bioaccessible iron, by 30% and 71%. This is likely due to its high phytate and calcium contents (Adetola et al., 2019). This is supported by the PCA plot which revealed that all treatments containing moringa were associated with high phytate and phenolics contents and were in the opposite quadrant and plane to the BIC and PBI when projected on a two-dimensional factor plane (Figure 1a,b). When complexes are formed between phytic acid with calcium and iron, they are more stable and less soluble than complexes of iron with phytic acid making complexes formed in the presence of high calcium content far less available (Rousseau et al., 2020).

3.2.2 | Ferritin formation in Caco-2 cells

While in vitro dialyzable iron assesses gastric/upper intestinal digestive release and solubility of iron and is an indicator of iron availability for absorption, ferritin formation by Caco-2 cells provides information about how much of the soluble iron may be available for utilization by enterocytes (Ferruzzi et al., 2020). This is because when Caco-2 cells fully differentiate, they express transport and metabolizing systems critical to iron absorption (such as divalent metal ion transporter-1 and ferroportin transporters). As the storage form of cellular iron is ferritin, higher levels of available iron induce greater ferritin formation by Caco-2 cells and hence are an indicator of iron bioavailability (Glahn et al., 1998). In fact, quantitative determination of iron bioaccessibility using the in vitro digestion/Caco-2 cell culture model has been well correlated with human data (Glahn et al., 1998; Mahler et al., 2009) and as such presents a method of predicting iron bioavailability in vivo.

Figure 2 shows that when wholegrain sorghum was extrusion cooked there was a 46% increment in ferritin synthesis when compared to conventionally cooked wholegrain sorghum porridge. In this regard, it is notable that extrusion cooking caused a significant decrease in both total phenolics and phytate (Table 2). As these compounds decrease iron availability (Gabaza et al., 2018) their reduction by extrusion cooking is the likely cause of the increase in ferritin formation. FtFF of co-extruded sorghum with baobab and moringa and their combination showed a reduction in ferritin synthesis, by 18%, 34%, and 26%, respectively when compared to sorghum alone. This is apparently somewhat contradictory to the dialyzable iron results, where there was an increase in bioaccessible iron with baobab inclusion (Table 3).

The in vitro iron dialyzability assay assesses iron bioaccessibility in terms of the amount of iron that is released by simulated digestion processes and soluble to cross a semi-permeable membrane of a particular threshold, in this case, 10 kDa. In contrast, ferritin formation by Caco-2 cells measures how much iron can be taken up by the cells to form ferritin (Ferruzzi et al., 2020). Therefore, while with in vitro iron dialyzability analysis of the extrusion cooked and conventionally cooked porridges there was the same amount of iron crossing the dialysis membrane, more iron was available for ferritin formation in the extruded porridges. This was probably due to the fact that the contents of the iron-binding phenolics and phytate were reduced by extrusion cooking.

It is also possible that another reason for the observed difference in trends between the in vitro iron dialyzability assay and the Caco-2 ferritin formation assay is that the dialyzability assay used in this research involved the utilization of an older in vitro gastrointestinal food digestion instead of more advanced techniques such as INFOGEST 2.0 method described by Brodkorb et al. (2019).

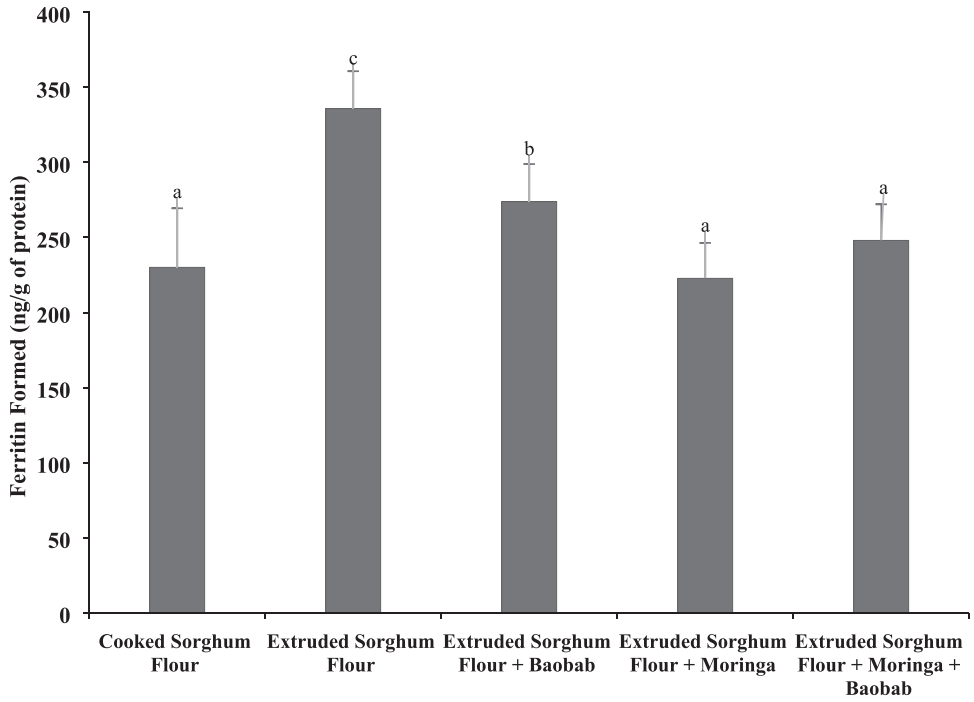

FIGURE 2 Effects of processing (conventional and extrusion cooking), FtFF with baobab fruit pulp and moringa leaf powder (added before processing) to wholegrain sorghum porridge on ferritin formation in Caco-2 cells grown under low iron condition. Data are the mean ± standard deviation of three independent experiments carried out in triplicate (n = 9). Different letters indicate significant differences at $p < .05$. Error bars indicate standard deviation. FtFF, Food-to-food fortification.

4 | CONCLUSIONS

Extrusion cooking increases ferritin formation in Caco-2 cells when compared to conventional wet cooking, which is indicative of the enhancing effect of extrusion cooking on iron bioaccessibility. This is largely due to its ability to reduce the content of phytate (probably by dephosphorylation) and phenolic content (probably by degradation). However, extrusion did not affect the amount and percentage dialyzable iron. The apparent contradiction between dialyzability and ferritin formation data is probably because they measure different aspects of bioaccessibility. By in vitro iron dialyzability analysis of the extrusion cooked and conventionally cooked porridges, there was the same amount of iron crossing the dialysis membrane. However, more of this iron was available for ferritin formation in the extruded porridges due to the reduction in phytate and phenolics. While an acute iron uptake study in animal or human subjects is required to provide more definitive data regarding the effect on iron bioavailability of wholegrain sorghum fortification with tropical foodstuffs, this study highlights the potential of extrusion cooking to improve iron bioavailability in wholegrain-based starchy staple foods.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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REFERENCES

Adetola, O. Y., Kruger, J., Ferruzzi, M. G., Hamaker, B. R., & Taylor, J. R. N. (2022). Potential of moringa leaf and baobab fruit food-to-food fortification of wholegrain maize porridge to improve iron and zinc bioaccessibility. *International Journal of Food Sciences and Nutrition*, *73*(1), 15–27.

Adetola, O.Y., Kruger, J., White, Z., & Taylor, J. R. N. (2019). Comparison between food-to-food fortification of pearl millet porridge with moringa leaves and baobab fruit and with

adding ascorbic and citric acid on iron, zinc and other mineral bioaccessibility. *LWT*, *106*, 92–97.

Alonso, R., Rubio, L. A., Muzquiz, M., & Marzo, F. (2001). The effect of extrusion cooking on mineral bioavailability in pea and kidney bean seed meals. *Animal Feed Science and Technology*, *94*, 1–13.

Arun Kumar, T. V., Mani, I., Pramod, A., Samuel, D., Jha, S., Sahoo, P., Sinha, J., & Kar, A. (2018). Effect of extrusion technique on antinutritional factors of sorghum-soya blends. *Indian Journal of Agricultural Sciences*, *88*, 81–89.

Bouis, H., Saltzman, A., Low, J., Ball, A., & Covic, N. (2017). An overview of the landscape and approach for biofortification in Africa. *African Journal of Food, Agriculture, Nutrition and Development*, *17*, 11848–11864.

Braca, A., Sinisgalli, C., DE. Leo, M., Muscatello, B., Cioni, P. L., Milella, L., Ostuni, A., Giani, S., & Sanogo, R. (2018). Phytochemical profile, antioxidant and antidiabetic activities of *Adansonia digitata* L. (Baobab) from Mali, as a source of health-promoting compounds. *Molecules*, *23*, 3104.

Brodkorb, A., Egger, L., Alminger, M., Alvito, P., Assunção, R., Ballance, S., Bohn, T., Bourlieu-Lacanal, C., Boutrou, R., & Carrière, F. (2019). INFOGEST static in vitro simulation of gastrointestinal food digestion. *Nature Protocols*, *14*, 991–1014.

Fellows, P. J. (2000). In P. J. Fellows (Ed.), *Food Processing Technology: Principles and Practice* (3rd ed., pp. 294–308). Woodhead Publishing Limited.

Ferruzzi, M. G., Kruger, J., Mohamedshah, Z., Debelo, H., & Taylor, J. R. N. (2020). Insights from in vitro exploration of factors influencing iron, zinc and provitamin A carotenoid bioaccessibility and intestinal absorption from cereals. *Journal of Cereal Science*, *96*, 103126.

Fruhbeck, G., Alonso, R., Marzo, F., & Santidrian, S. (1995). A modified method for the indirect quantitative analysis of phytate in foodstuffs. *Analytical Biochemistry*, *225*, 206–212.

Gabaza, M., Shumoy, H., Muchuweti, M., Vandamme, P., & Raes, K. (2018). Baobab fruit pulp and mopane worm as potential functional ingredients to improve the iron and zinc content and bioaccessibility of fermented cereals. *Innovative Food Science & Emerging Technologies*, *47*, 390–398.

Gibson, R. S., Raboy, V., & King, J. C. (2018). Implications of phytate in plant-based foods for iron and zinc bioavailability, setting dietary requirements, and formulating programs and policies. *Nutrition Reviews*, *76*, 793–804.

Glahn, R. P., Lee, O. A., Yeung, A., Goldman, M. I., & Miller, D. D. (1998). Caco-2 cell ferritin formation predicts nonradiolabeled food iron availability in an in vitro digestion/Caco-2 cell culture model. *The Journal of Nutrition*, *128*, 1555–1561.

Gregory, P. J., & Wojciechowski, T. (2020). Root systems of major tropical root and tuber crops: Root architecture, size, and growth and initiation of storage organs. *Advances in Agronomy*, *161*, 1–25.

Guy, R. (2001). Raw materials for extrusion cooking. In R. Guy (Ed.), *Extrusion cooking: Technologies and Applications* (1st ed., pp. 5–27). Woodhead Publishing Limited.

Iyengar, V., Pullakhandam, R., & Nair, K. M. (2010). Dietary ligands as determinants of iron–zinc interactions at the absorptive enterocyte. *Journal of Food Science*, *75*, H260–H264.

Kayodé, A. P. P., Linnemann, A. R., Hounhouigan, J. D., Nout, M. J. R., & Van Boekel, M. A. J. S. (2006). Genetic and environmental impact on iron, zinc, and phytate in food sorghum grown in Benin. *Journal of Agricultural and Food Chemistry*, *54*, 256–262.

Kruger, J. (2020). Potential of food-to-food fortification with cowpea leaves and orange-fleshed sweet potato, in combination with conventional fortification, to improve the cellular uptake of iron and zinc from ready-to-eat maize porridges. *Food Science & Nutrition*, *8*, 3190–3199.

Kruger, J., Taylor, J. R. N., & Oelofse, A. (2012). Effects of reducing phytate content in sorghum through genetic modification and fermentation on in vitro iron availability in whole grain porridges. *Food Chemistry*, *131*, 220–224.

Kruger, J., Oelofse, A., & Taylor, J. R. N. (2014). Effects of aqueous soaking on the phytate and mineral contents and phytate: Mineral ratios of wholegrain normal sorghum and maize and low phytate sorghum. *International Journal of Food Sciences and Nutrition*, *65*, 539–546.

Lemoine, A., & Tounian, P. (2020). Childhood anemia and iron deficiency in sub-Saharan. *Archives de Pediatrie: Organe officiel de la Societe francaise de Pediatrie*, *27*(8), 490–496.

Leone, A., Spada, A., Battezzati, A., Schiraldi, A., Aristil, J., & Bertoli, S. (2015). Cultivation, genetic, ethnopharmacology, phytochemistry and pharmacology of *Moringa oleifera* leaves: An overview. *International Journal of Molecular Sciences*, *16*, 12791–12835.

Lönnerdal, B. (2000). Dietary factors influencing zinc absorption. *The Journal of Nutrition*, *130*, 1378S–1383S.

Mahler, G. J., Shuler, M. L., & Glahn, R. P. (2009). Characterization of Caco-2 and HT29-MTX cocultures in an in vitro digestion/cell culture model used to predict iron bioavailability. *The Journal of Nutritional Biochemistry*, *20*, 494–502.

Miller, D. D., Schricker, B. R., Rasmussen, R. R., & Van Campen, D. (1981). An in vitro method for estimation of iron availability from meals. *The American Journal of Clinical Nutrition*, *34*, 2248–2256.

Nikmaram, N., Leong, S. Y., Koubaa, M., Zhu, Z., Barba, F. J., Greiner, R., Oey, I., & Roohinejad, S. (2017). Effect of extrusion on the anti-nutritional factors of food products: An overview. *Food Control*, *79*, 62–73.

Pasricha, S.-R., Tye-Din, J., Muckenthaler, M. U., & Swinkels, D. W. (2021). Iron deficiency. *The Lancet*, *397*(10270), 233-248.

Perales, S., Barberá, R., Lagarda, M. J., & Farré, R. (2005). Bioavailability of calcium from milk-based formulas and fruit juices containing milk and cereals estimated by in vitro methods (solubility, dialyzability, and uptake and transport by Caco-2 cells). *Journal of Agricultural and Food Chemistry*, *53*, 3721–3726.

Rousseau, S., Kyomugasho, C., Celus, M., Hendrickx, M. E. G., & Grauwet, T. (2020). Barriers impairing mineral bioaccessibility and bioavailability in plant-based foods and the perspectives for food processing. *Critical Reviews in Food Science and Nutrition*, *60*, 826–843.

Saunders, A. V., Craig, W. J., Baines, S. K., & Posen, J. S. (2013). Iron and vegetarian diets. *The Medical Journal of Australia*, *199*, S11–S16.

Sharma, P., Gujral, H. S., & Singh, B. (2012). Antioxidant activity of barley as affected by extrusion cooking. *Food Chemistry*, *131*, 1406–1413.

Tadesse, S. A., Beri, G. B., & Abera, S. (2019). Chemical and sensory quality of sorghum-based extruded product supplemented with defatted soy meal flour. *Cogent Food & Agriculture*, *5*, 1653617.

Taylor, J. R N. (2019). Sorghum and millets: Taxonomy, history, distribution, and production. In J. R. N Taylor, & K. G Duodu (Eds.), *Sorghum and millets: Chemistry, technology and nutritional attributes* (2nd ed., pp. 1–19). Woodhead Publishing.

Tembo, D. T., Holmes, M. J., & Marshall, L. J. (2017). Effect of thermal treatment and storage on bioactive compounds, organic acids and antioxidant activity of baobab fruit (*Adansonia digitata*) pulp from Malawi. *Journal of Food Composition and Analysis*, *58*, 40–51.

Tiuganji, N. M., Nehme, P., Marqueze, E. C., Isherwood, C. M., Martins, A. J., Vasconcelos, S., Cipolla-Neto, J., Lowden, A., Skene, D. J., & Moreno, C. R. C. (2020). Eating behavior (duration, content, and timing) among workers living under different levels of urbanization. *Nutrients*, *12*, 375.

U.S. EPA. (1996). Determination of metals and trace elements in water and wastes by inductively coupled plasma-atomic emission spectrometry. In T. D. Martin, C. A. Brockhoff, J. T. Creed, & S. E. Long (Eds.), *Methods for the determination of metals in environmental samples*. Noyes Publications.

U.S. EPA. (2007). *Method 3051 A microwave assisted acid digestion of sediments, sludges, soils, and oils. Revision 1*. Washington, DC.

Van Der Merwe, R., Kruger, J., Ferruzzi, M. G., Duodu, K. G., & Taylor, J. R. N. (2019). Improving iron and zinc bioaccessibility through food-to-food fortification of pearl millet with tropical plant foodstuffs (moringa leaf powder, roselle calyces and baobab fruit pulp). *Journal of Food Science and Technology*, *56*, 2244–2256.

Viadel, B., Perales, S., Barbera, R., Lagarda, M., & Farre, R. (2007). Ferritin synthesis by Caco-2 cells as an indicator of iron bioavailability: Application to milk-based infant formulas. *Food Chemistry*, *102*, 925–931.

Wani, S. A., & Kumar, P. (2016). Effect of extrusion on the nutritional, antioxidant and microstructural characteristics of nutritionally enriched snacks. *Journal of Food Processing and Preservation*, *40*, 166–173.

Waterman, P. G., & Mole, S. (1994). *Analysis of phenolic plant metabolites*. Blackwell Scientific Publications.

Watson, F. T., Smernik, R. J., & Doolette, A. L. (2019). Thermal degradation of phytate produces all four possible inositol pentakisphosphates as determined by ion chromatography and 1H and 31P NMR spectroscopy. *Phosphorus, Sulfur and Silicon and the Related Elements*, *194*, 1140–1148.

World Health Organization. (2017). *Nutritional anaemias: Tools for effective prevention and control*; Licence: CC BY-NC-SA 3.0 IGO (pp. 2–10).

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