Effects of different souring methods on the protein quality and iron and zinc bioaccessibilities of non-alcoholic beverages from sorghum and amaranth

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Summary

Souring by lactic acid fermentation and lactic acid acidification as well as inclusion of amaranth were explored as ways of improving the protein quality and iron and zinc bioaccessibilities of non-alcoholic sorghum-based beverages. The bioaccessible iron and zinc increased by 128–372%, 24–194%, respectively, in the fermented and chemically acidified beverages compared to the beverages without fermentation or acidification. The protein digestibility, reactive lysine, and bioaccessible iron in sorghum-amaranth beverages increased by 14–58%, 24–52% and 34–64%, respectively, compared with the 100% sorghum beverages. Both fermentation and acidification with lactic acid have the potentials for improving the nutritional quality of cereal-based foods as a means of combating protein malnutrition and iron and zinc deficiencies.

Keywords: Acidification, amaranth, back-slopped inoculum, beverages, bioaccessible iron and zinc, fermentation, *Lactobacillus plantarum*, protein digestibility, reactive lysine, sorghum.

Introduction

Protein malnutrition and iron and zinc deficiencies continue to be a major nutritional problem in developing countries, especially in Africa (FAO, 2006; Platel & Srinivasan, 2016; Temba *et al*., 2016). In most of these countries, foods of plant origin form an important part of the diets (Galati *et al*., 2014). Sorghum, being a hardy crop, is capable of thriving in harsh environments like semi-arid tropics of Africa, characterised by poor soils, high temperature and low rainfall (Awika, 2017). As a result, sorghum-based foods have become an integral part of the diets of people living in the semi-arid tropics of Africa, thus providing food for millions of people in this region (Nedumaran *et al*., 2012; Awika, 2017). Apart from being an energy source, sorghum also contributes protein, essential fatty acids, fibre, minerals and vitamins to the diet (Temba *et al*., 2016).

A major nutritional challenge with sorghum is that it has poor protein quality for two reasons. First, it is very limiting in lysine, only approx. 2 g per 100 g protein (Taylor & Schüssler, 1986). According to Henley *et al*. (2010), lysine in sorghum is between 35% and 90% lower compared to other cereals like wheat, barley and pearl millet. Second, there is a significant reduction in the digestibility of sorghum protein upon wet cooking (Duodu *et al*., 2003), which further intensifies the problem of sorghum's poor protein quality. Another

problem is that sorghum, like all plants, contains non-haem iron, which has a very low bioavailability compared to that of haem iron in meat (Zimmermann *et al*., 2005). Moreover, sorghum and other cereal-based foods contain high levels of anti-nutritional compounds like phytate and polyphenols which further lower the bioavailability of non-haem iron as well as zinc in sorghum-based foods (Hunt, 2003). To address the nutritional problem of sorghum's poor protein quality and low bioavailability of iron and zinc, compositing with other grain types with better protein quality and also lactic acid fermentation can be applied.

Amaranth, a pseudocereal is an ancient crop that is being strongly promoted in arid and semi-arid areas of East Africa as an alternative crop to support food security (Alemayehu *et al*., 2014). This is because like sorghum, amaranth is a hardy crop and therefore has a very high tolerance to high temperature, drought and poor soil conditions (D'Amico & Schoenlechner, 2017). The fact that both its leaves and grains can be utilised for food purpose also makes amaranth a particularly useful crop for challenging growing conditions (Alemayehu *et al*., 2014). Amaranth has some superior nutritional properties compared with cereal grains such as sorghum. The protein content of amaranth (13.1–21.0%) (Venskutonis & Kraujalis, 2013) is higher than sorghum (8.6–15.6%) (Awika, 2017).Amaranth has higher levels of indispensable (essential) amino acids such as lysine, methionine + cystine and histidine than cereals (D'Amico & Schoenlechner, 2017). Lysine contents of up to 6.48 g/100 g protein (Chávez-Jáuregui *et al*., 2000) and 5.2 g/100 g protein (Venskutonis & Kraujalis, 2013) have been reported for amaranth. These values are within the range of lysine requirement for infants, children and older children according to the Food and Agriculture Organization (FAO 2013). Moreover, the total mineral content of amaranth is higher than cereal grains (Alvarez-Jubete *et al*., 2010).

Lactic acid-fermented foods and beverages are popular in many sub-Saharan African countries (Galati *et al*., 2014; Taylor, 2016). This is probably due to the social, religious and therapeutic value associated with such beverages in African countries (Aka *et al*., 2014). Cereals are used to produce indigenous fermented foods such as non-alcoholic and alcoholic beverages in most African countries (Aka *et al*., 2014). At a household level, back-slopping, whereby a small portion of the previous successful fermentation liquid is used to inoculate a new batch of flour is normally used to prepare soured food products (Galati *et al*., 2014). Commercial soured products are also produced using special lactic starter cultures (Holzapfel, 2002) and there is also a growing trend of using chemical acidification with food-grade acids to produce soured beverages (Onyango & Wanjala, 2018). In the present work, compositing with amaranth, together with souring using fermentation and lactic acid acidification are explored as ways of improving sorghum protein quality and iron and zinc bioaccessibilities.

Materials and methods

Materials

A red non-tannin sorghum (variety MMSH 625) obtained from the Golden Valley Research Station, Chisamba, Zambia and amaranth grain supplied by Advance Seed, Krugersdorp, South Africa were used in this work. For one of the fermentation treatments, a presumed probiotic strain of *Lactobacillus plantarum* (Code FS2), which had been previously isolated from traditionally fermented African maize gruel (Fayemi *et al*., 2017) was used. The

bacterial cells were preserved in MRS broth containing 25% glycerol in 2 mL cryo tubes and stored at −80 °C.

Methods

Preparation of sorghum flour, amaranth flour and their composite

Whole grain sorghum and amaranth were separately milled into flour using a hammer mill (Falling Number Laboratory Mill 3100, Perten Instruments, Stockholm, Sweden) fitted with a 500-µm opening screen to give wholegrain flour (100% sorghum and 100% amaranth flours). Composite flour was prepared by thorough mixing of sorghum and amaranth flours in a 50:50 (w/w) ratio. The composite flour together with the 100% sorghum and 100% amaranth flours were separately packed into air- and light-tight plastic containers and stored at 4 °C prior to further processing and/or analyses.

Preparation of **L. plantarum** *starter culture and back-slopped inoculum*

L. plantarum strain (FS2) was resuscitated in MRS broth for 18 h at 37 °C for three consecutive generations (Fayemi *et al*., 2017). The resulting cell suspension was centrifuged at 1095 × ***g*** for 15 min at 4 °C, after which the supernatant was decanted off. The bacteria cells were washed twice in 0.1% sterile buffered peptone water (BPW) and centrifuged again. The supernatant was decanted off and the bacteria cells suspended in 0.1% sterile BPW. The bacteria suspension was standardised (determination of bacterial concentration) using McFarland densitometer (DEN-1 Grant Instruments, Royston, UK), which has been previously calibrated with McFarland standard. An inoculum for back-slopping was prepared by firstly mixing 30 g of each flour (100% sorghum, 100% amaranth, and sorghum: amaranth 50:50) separately with 500 mL distilled water in a plastic container to forma slurry. The slurry was covered and allowed to ferment at 30 °C to pH 3.5. The fermented slurry was used as a starter culture (10% v/v i.e. 10 mL of the fermented slurry plus 90 mL of fresh slurry) to initiate the fermentation for the back-slopped beverages.

Preparation of fermented/acidified beverages before cooking

Wholegrain flour (100% sorghum) (60 g) was mixed with distilled water (1 L) in four different containers to form a slurry. The slurry in one of the containers was inoculated with *L. plantarum* starter culture to a final inoculum level of 10^6 cfu mL^{-1} (determined using a McFarland densitometer), whereas the slurry in the second container was inoculated with 10% (v/v) of the previously fermented slurry (100% sorghum flour). The inoculated slurries were incubated at 30 °C and fermented. The pH was monitored until a final pH of 3.5 was attained within 24–36 h. Lactic acid was added to the slurry in one of the remaining two containers and hydrochloric acid was added to the slurry in the last container. Acidification of the slurry both with lactic acid and HCl was monitored with a pH meter and initially stopped at pH 5.0 and later continued until pH 3.5 was reached after incubation at 50 °C for 2 h. The purpose of incubation for 2 h was to allow time for the endogenous phytase in the grain to hydrolyse the phytate. The fermented and acidified treatments were cooked on an electric hot plate with constant stirring and allowed to boil for 10 min, temperature approximately 96 °C. The resulting beverages were frozen at −20 °C and designated as fermented/acidified and cooked. Fermented and acidified 100% sorghum beverages without

cooking were also prepared as described above and frozen immediately at −20 °C. This process was repeated with 100% amaranth flour and the composite flour.

Preparation of fermented/acidified beverages after cooking

Wholegrain flour (100% sorghum) (60 g) was made into a slurry by mixing with distilled water (1 L) in five different containers. The slurries were cooked separately on an electric hot plate, as described. The resulting beverages were cooled to ambient temperature, after which one of them was frozen immediately at −20 °C and designated as cooked non-fermented. Another one was inoculated with *L. plantarum* starter culture to a final inoculum level of 10^6 cfu mL^{-1}, whereas the third beverage was inoculated by back-slopping with 10% (v/v) of the previously fermented slurry. The inoculated beverages were incubated at 30 °C and fermented to pH 3.5 within 24–36 h, after which they were frozen at −20 °C. The remaining beverages were acidified separately with lactic acid and hydrochloric acid as described and then frozen at −20 °C. This process was repeated with 100% amaranth flour and the composite flour.

All the beverages (the different treatments) were prepared in duplicate, freeze-dried and then crushed to a particle size that passed through a 500-µm opening screen before further analyses.

Analyses

Microbiological analysis of fermented beverages

The microbial population of the total aerobic bacteria, lactic acid bacteria and yeasts and moulds was determined using plate count agar (PCA) (Merck Millipore, Johannesburg, South Africa), MRS (De Man *et al*., 1960) and potato dextrose agar (PDA) (Merck Millipore), respectively, as described by Fayemi *et al*. (2017). The PDA was amended with 50 mg chloramphenicol (Fluka Biochemika, Buchs, Switzerland) in a litre to inhibit the growth of bacteria. The yeast and mould plates were incubated at 25 °C for 3–5 days, whereas the MRS agar plates were incubated anaerobically at 37 °C for 48 h using an anaerobic jar together with Anaerocult system (Merck Millipore). The colonies were counted using a colony counter (Anderman and Company Ltd, Surrey, UK).

Determination of pH and titratable acidity

The pH and titratable acidity of the fermenting samples were determined at 6-h intervals until the pH dropped to around 3.5.The pH was determined using a microprocessor pH meter. Total titratable acidity was determined by titration with 0.1 m NaOH solution as described by Wakil & Kazeem (2012) and expressed as percentage lactic acid equivalent.

Protein content

The Dumas combustion method (American Association of Cereal Chemists International, 2000), Method 46-30 was used to determine the protein content ($N \times 6.25$).

In vitro *protein digestibility*

A pepsin digestion method for determination of *in vitro* protein digestibility based on Hamaker *et al*. (1986) was used. Freshly prepared pepsin (Cat. No. P-7000, Sigma-Aldrich, Johannesburg, South Africa) solution with activity of 714 units per mg protein was used to digest 200 mg sample for 2 h at 37 °C in a shaking water bath. The residual protein in the sample after pepsin digestion was determined by the Dumas combustion method. Protein digestibility was calculated as the difference between the total protein and the residual protein after pepsin digestion expressed as a percentage of the total protein.

Reactive lysine

A rapid dye-binding capacity (DBC) method (Kim *et al*., 2007) using Crocein Orange dye (70% dye content) (Cat. No. 27965, Sigma-Aldrich) was used to determine the reactive lysine content. In principle, the food sample is first shaken with the dye and the amount of dye bound (DBC) is determined by measuring the extinction of the dye at 482 nm. This measures the sum of total histidine, arginine and reactive lysine. The food sample is then treated with propionic anhydride which masks the reactive lysine groups by neutralising the basicity of free ε-NH_2 groups of reactive lysine in a propionylation reaction. The DBC of the food sample treated with propionic anhydride measures histidine and arginine. Thus, the difference between the DBC of the food not treated with the propionylation agent and that of the food treated with it gives a measure of the reactive lysine content. Reactive lysine content was expressed as g/100 g protein.

Phytate content

Phytate content was determined spectrophotometrically at 500 nm by measuring the absorbance of the product of the reaction between phytate, ferric chloride and sulphosalicylic acid as described by Frühbeck *et al*. (1995). Phytate extracted from the freeze dried samples with 0.66 m HCl was purified by anion exchange chromatography using a Dowex 1 anion-exchange resin before reaction with the ferric chloride and sulphosalicylic acid reagent. The phytate content was determined from a standard curve prepared with sodium phytate (Sigma-Aldrich) solution and expressed as mg g^{-1} sample.

Iron and zinc contents

The whole grain flour samples were digested with nitric-perchloric acid according to Zasoski & Burau (1977). The digested samples were analysed for iron and zinc by Inductively Coupled Plasma–Atomic Emission Spectrometry (ICP-AES) at emission wavelengths of 259.939 nm and 206.200 nm respectively.

Iron and zinc bioaccessibility assay

The dialysis method described by Luten *et al*. (1996) was used to determine bioaccessible iron and zinc of the beverages. A dialysis tubing Spectra/Por 7 (Ø = 20.4 mm) with a molecular weight cut-off of 10 kDa (G.I.C. Scientific, Johannesburg, South Africa) was used. In order to keep the minerals soluble in solution, 0.45 mL of 65% nitric acid was added to the dialysate. The mineral content (iron and zinc) in the dialysate was analysed by ICP-AES

and the mineral bioaccessibility was calculated as the percentage of the mineral in the dialysate compared to the total mineral content in the digest expressed as mg/100 g food sample.

Statistical analysis

All analyses were carried out in triplicate and data were analysed by one-way analysis of variance (anova) at $P \leq 0.05$ using IBM SPSS statistical for Windows version 20.0 (IBM, Armonk, NY, USA). Turkey's HSD Post hoc test was applied to determine differences between specific means. Principal component analysis (PCA) was also carried out to determine if there were any relationships between the various sample treatments in terms of the measured parameters.

Results and discussion

Effect of different souring methods on pH, titratable acidity and microbial populations of sorghum, amaranth and their composite beverages

As expected, there was a decrease in pH of the fermenting samples from an initial range of 5.5 to 6.8 (*L. plantarum* and back-slopped fermentations) to around 3.5 at the end of fermentation. This was mirrored by an increase in titratable acidity from a range of 0.02 to 0.06% lactic acid to 0.20 to 0.37% lactic acid at the end of fermentation. This can be related to the microbial populations in the fermenting samples. At the end of the fermentations (to pH around 3.5), the population of total aerobic bacteria and lactic acid bacteria (LAB) were $>10^9$ cfu mL^{-1} in both *L. plantarum* and back-slopped fermented treatments (Table 1). Generally, the populations of total aerobic bacteria and lactic acid bacteria were similar throughout the fermentation process and increased with increasing fermentation time (leading to increasing production of lactic acid).

Similarly, yeast and mould count also increased from 10^3 to 10^7 cfu mL^{-1}. The ability of LAB and yeasts to dominate the grain non-alcoholic fermentation process is probably due to the acidic environment created by LAB which favours the proliferation of yeasts, whereas yeasts, on the other hand, provide growth factors (vitamins and soluble nitrogen compounds) which stimulate the growth of LAB (Nout & Sarkar, 1999).

With all three flour types (100% sorghum, 100% amaranth and their composite), the microbial populations (LAB, yeasts and moulds) at 12 h fermentation time were significantly ($P \leq 0.05$) higher with the back-slopping fermentation treatment compared to the *L. plantarum* fermented treatment (Table 1). This was probably because the inoculum used for back-slopping contained a population of well-adapted microorganisms, particularly LAB and yeasts and hence they would grow more rapidly than the *L. plantarum,* which would require time to adapt to the new environment (Nout & Sarkar, 1999). This is also reflected by the observation that the final pH of 3.5 was attained earlier for cooked followed by back-slopped samples than for cooked followed by *L. plantarum* fermented samples (Table 1).

Table 1 Effects of fermentation with *Lactobacillus plantarum* starter culture and by back-slopping on microbial counts (log_{10} cfu mL^{-1}) of sorghum, amaranth and their composite flour beverages before cooking and after cooking

Flour type	Treatment	Fermentation time (h)	Total aerobic bacteria	Lactic acid bacteria	Yeasts and moulds
Sorghum (100%)	Raw flour fermented using *L. plantarum*	0	$6.52^{bcde} \pm 0.04$	$6.59^{abcde} \pm 0.16$	$3.24^{b} \pm 0.34$
		12	$7.00^{gh} \pm 0.06$	$6.90^{cdefg} \pm 0.08$	$4.76^{de} \pm 0.02$
		24	$9.36^{no} \pm 0.05$	$9.59^{lm} \pm 0.16$	$6.56^{f} \pm 0.02$
	Cooked and fermented flour using *L. plantarum*	0	$6.23^{ab} \pm 0.07$	$6.08^{a} \pm 0.05$	$2.69^{a} \pm 0.12$
		12	$6.39^{abcd} \pm 0.12$	$6.24^{ab} \pm 0.34$	$3.90^{c} \pm 0.08$
		36	$9.65^{nopq} \pm 0.02$	$9.77^{lm} \pm 0.10$	$6.41^{f} \pm 0.02$
	Raw flour fermented by back-slopping	0	$7.31^{hij} \pm 0.12$	$7.74^{i} \pm 0.02$	$4.97^{de} \pm 0.03$
		12	$8.41^{lm} \pm 0.07$	$8.57^{k} \pm 0.03$	$6.76^{fghi} \pm 0.02$
		24	$9.85^{q} \pm 0.15$	$9.69^{lm} \pm 0.12$	$7.02^{i} \pm 0.02$
	Cooked and fermented flour by back-slopping	0	$7.06^{ghi} \pm 0.03$	$7.16^{fgh} \pm 0.06$	$4.62^{d} \pm 0.04$
		12	$8.25^{l} \pm 0.03$	$8.32^{jk} \pm 0.06$	$6.63^{fg} \pm 0.03$
		30	$9.34^{n} \pm 0.03$	$9.39^{l} \pm 0.12$	$6.98^{ghi} \pm 0.02$
Amaranth (100%)	Raw flour fermented using *L. plantarum*	0	$6.65^{def} \pm 0.07$	$6.77^{bcdef} \pm 0.10$	$3.39^{b} \pm 0.12$
		12	$6.84^{efg} \pm 0.09$	$6.90^{cdefg} \pm 0.08$	$4.72^{de} \pm 0.02$
		24	$9.32^{n} \pm 0.06$	$9.84^{lm} \pm 0.09$	$6.61^{f} \pm 0.02$
	Cooked and fermented flour using *L. plantarum*	0	$6.24^{abc} \pm 0.29$	$6.43^{abc} \pm 0.01$	$2.54^{a} \pm 0.09$
		12	$6.39^{abcd} \pm 0.12$	$6.54^{abcd} \pm 0.09$	$3.99^{c} \pm 0.06$
		30	$9.44^{nop} \pm 0.03$	$9.90^{lm} \pm 0.08$	$6.48^{f} \pm 0.02$
	Raw flour fermented by back-slopping	0	$7.64^{jk} \pm 0.01$	$7.80^{ij} \pm 0.01$	$4.99^{e} \pm 0.01$
		12	$8.57^{lm} \pm 0.02$	$8.66^{k} \pm 0.03$	$6.67^{fghi} \pm 0.03$
		24	$9.79^{q} \pm 0.02$	$9.81^{lm} \pm 0.05$	$7.00^{i} \pm 0.02$
	Cooked and Fermented flour by back-slopping	0	$7.22^{hi} \pm 0.06$	$7.39^{ghi} \pm 0.01$	$4.62^{d} \pm 0.05$
		12	$8.34^{lm} \pm 0.03$	$8.43^{k} \pm 0.05$	$6.61^{f} \pm 0.02$
		24	$9.69^{opq} \pm 0.03$	$9.97^{m} \pm 0.52$	$6.98^{ghi} \pm 0.01$
Sorghum:Amaranth (50:50)	Raw flour fermented using *L. plantarum*	0	$6.57^{cde} \pm 0.02$	$6.97^{cdefg} \pm 0.10$	$3.15^{b} \pm 0.21$
		12	$7.15^{ghi} \pm 0.04$	$7.09^{efg} \pm 0.07$	$4.80^{de} \pm 0.01$
		24	$9.74^{pq} \pm 0.06$	$9.87^{lm} \pm 0.12$	$6.70^{fghi} \pm 0.02$
	Cooked and fermented flour using *L. plantarum*	0	$6.11^{a} \pm 0.05$	$6.22^{a} \pm 0.06$	$2.59^{a} \pm 0.16$
		12	$6.97^{fgh} \pm 0.10$	$7.02^{defg} \pm 0.09$	$3.97^{c} \pm 0.10$
		36	$9.87^{q} \pm 0.12$	$9.93^{lm} \pm 0.04$	$6.77^{fghi} \pm 0.16$
	Raw flour fermented by back-slopping	0	$7.82^{k} \pm 0.02$	$7.71^{hi} \pm 0.02$	$5.03^{e} \pm 0.01$
		12	$8.64^{m} \pm 0.02$	$8.72^{k} \pm 0.04$	$6.74^{fghi} \pm 0.04$
		24	$9.65^{nopq} \pm 0.05$	$9.69^{lm} \pm 0.12$	$6.97^{ghi} \pm 0.03$
	Cooked and fermented flour by back-slopping	0	$7.38^{ij} \pm 0.09$	$7.02^{defg} \pm 0.09$	$4.71^{de} \pm 0.05$
		12	$8.48^{lm} \pm 0.02$	$8.60^{k} \pm 0.02$	$6.65^{fgh} \pm 0.05$
		24	$9.55^{nopq} \pm 0.06$	$9.59^{lm} \pm 0.16$	$7.00^{hi} \pm 0.01$

Values are mean ± standard deviation Mean values in the same column with different superscript letters are significantly different ($P \leq 0.05$).

Effects of different souring methods and amaranth inclusion on the protein content, pepsin digestibility and reactive lysine contents of the beverages

The mean protein content of amaranth-only flour and beverages (14.5 g/100 g) was higher compared to the sorghum-only flour and beverages (9.3 g/100 g), whereas that of the composite flour and beverages (12.3 mg/100 g) was intermediate (Table 2). In virtually all the treatments prepared from 100% sorghum flour, chemical acidification with lactic acid as well as fermentation with *L. plantarum* starter culture and by back-slopping did not have any significant effect ($P \leq 0.05$) on protein content (Table 2). However, for beverages prepared from 100% amaranth flour, there was a significant reduction ($P \leq 0.05$) in protein content (by 14.5%, 15.25% and 17.0% for fermentation with *L. plantarum*, fermentation by back-slopping and chemical acidification respectively) after fermentation without cooking and chemical acidification without cooking. There was also a significant ($P \leq 0.05$) reduction in protein content of the composite flour (by 11%) after fermentation by back-slopping without cooking. The reduction in protein content after fermentation can be attributed to the conversion of amino acids into ammonia and flavour compounds by fermenting microorganisms (Pranoto *et al*., 2013). This might also be responsible for the reduction observed after chemical acidification, since the samples were firstly acidified to pH 5 and

held at 50 °C for 2 h. During this period, the endogenous microflora in the grains could have possibly had sufficient time to convert the amino acids into ammonia and flavour compounds.

Table 2 Effects of fermentation and acidification on protein content, *in vitro* pepsin digestibility, and available lysine of non-alcoholic beverages from sorghum, amaranth and their composite flour

Flour type	Treatment	Protein (g/100 g)	IVPD (%)	R lysine (g/100 g protein)
Sorghum (100%)	Raw flour	$9.57^{bc} \pm 0.09$	$74.0^{f} \pm 0.1$	$2.34^{abc} \pm 0.02$
	Cooked flour	$9.57^{bc} \pm 0.01$	$51.9^{b} \pm 1.8$	$2.30^{ab} \pm 0.01$
	Fermented using *L. plantarum*) (uncooked)	$9.41^{bc} \pm 0.29$	$77.3^{f} \pm 0.2$	$2.45^{cde} \pm 0.04$
	Fermented using *L. plantarum* and cooked	$9.61^{bc} \pm 0.15$	$56.4^{cd} \pm 1.8$	$2.43^{cde} \pm 0.02$
	Cooked and fermented using *L. plantarum*)	$9.19^{abc} \pm 0.15$	$51.9^{b} \pm 0.6$	$2.45^{cdec} \pm 0.02$
	Fermented by back-slopping (uncooked)	$9.34^{abc} \pm 0.13$	$77.5^{f} \pm 0.8$	$2.49^{de} \pm 0.04$
	Fermented by back-slopping and cooked	$9.76^{b} \pm 0.22$	$57.5^{d} \pm 0.9$	$2.28^{ab} \pm 0.02$
	Cooked and fermented by back-slopping	$9.38^{bc} \pm 0.19$	$67.5^{e} \pm 0.3$	$2.46^{cde} \pm 0.01$
	Acidified with lactic acid (uncooked)	$8.72^{a} \pm 0.04$	$76.0^{f} \pm 0.4$	$2.53^{e} \pm 0.04$
	Acidified with lactic acid and cooked	$9.28^{abc} \pm 0.07$	$53.2^{bc} \pm 1.6$	$2.26^{a} \pm 0.05$
	Cooked and acidified with lactic acid	$9.05^{ac} \pm 0.14$	$46.8^{a} \pm 1.0$	$2.39^{bcd} \pm 0.04$
	Mean	9.35 ± 0.29	62.7 ± 11.8	2.40 ± 0.09
Amaranth (100%)	Raw flour	$15.02^{c} \pm 0.06$	$89.3^{cd} \pm 1.7$	$3.95^{d} \pm 0.03$
	Cooked flour	$15.44^{c} \pm 0.02$	$91.3^{d} \pm 0.4$	$3.10^{a} \pm 0.04$
	Fermented using *L. plantarum* (uncooked)	$12.85^{a} \pm 0.17$	$84.7^{ab} \pm 0.8$	$3.94^{d} \pm 0.04$
	Fermented flour using *L. plantarum* and cooked	$14.98^{c} \pm 0.34$	$89.8^{cd} \pm 0.7$	$3.54^{b} \pm 0.03$
	Cooked and fermented using *L. plantarum*)	$14.96^{c} \pm 0.02$	$86.2^{bc} \pm 1.3$	$3.94^{d} \pm 0.01$
	Fermented by back-slopping (uncooked)	$12.73^{a} \pm 0.14$	$81.1^{a} \pm 1.8$	$3.93^{d} \pm 0.03$
	Fermented by back-slopping and cooked	$15.38^{c} \pm 0.19$	$87.4^{bc} \pm 0.3$	$3.51^{b} \pm 0.01$
	Cooked and fermented by back-slopping	$18.24^{d} \pm 0.11$	$85.9^{bc} \pm 0.3$	$3.83^{cd} \pm 0.03$
	Acidified with lactic acid) (uncooked)	$12.46^{a} \pm 0.24$	$85.1^{b} \pm 0.8$	$3.77^{c} \pm 0.03$
	Acidified with lactic acid and cooked	$13.62^{b} \pm 0.04$	$92.1^{d} \pm 0.5$	$3.01^{a} \pm 0.04$
	Cooked and acidified with lactic acid	$13.52^{b} \pm 0.03$	$89.6^{cd} \pm 0.8$	$3.86^{cd} \pm 0.04$
	Mean	14.47 ± 1.68	87.5 ± 3.3	3.67 ± 0.34
Sorghum: Amaranth (50:50)	Raw flour	$12.12^{bcd} \pm 0.22$	$83.5^{d} \pm 0.1$	$3.87^{e} \pm 0.03$
	Cooked flour	$12.61^{d} \pm 0.15$	$73.7^{a} \pm 0.1$	$3.50^{cd} \pm 0.04$
	Fermented using *L. plantarum* (uncooked)	$12.43^{d} \pm 0.10$	$82.5^{d} \pm 0.6$	$3.56^{d} \pm 0.02$
	Fermented using *L. plantarum* and cooked	$12.51^{d} \pm 0.05$	$79.7^{c} \pm 0.5$	$3.47^{cd} \pm 0.01$
	Cooked and fermented using *L. plantarum*	$12.74^{d} \pm 0.19$	$72.5^{a} \pm 0.9$	$3.05^{a} \pm 0.02$
	Fermented by back-slopping (uncooked)	$10.75^{a} \pm 0.00$	$82.8^{d} \pm 0.7$	$3.50^{cd} \pm 0.01$
	Fermented by back-slopping and cooked	$12.62^{d} \pm 0.11$	$78.9^{bc} \pm 0.4$	$3.22^{b} \pm 0.05$
	Cooked and fermented by back-slopping	$14.05^{e} \pm 0.48$	$76.9^{b} \pm 0.8$	$3.42^{c} \pm 0.04$
	Acidified with lactic acid (uncooked)	$11.34^{ab} \pm 0.14$	$84.5^{d} \pm 0.3$	$3.44^{cd} \pm 0.02$
	Acidified with lactic acid and cooked	$11.53^{abc} \pm 0.19$	$78.0^{bc} \pm 0.8$	$3.02^{a} \pm 0.01$
	Cooked and acidified with lactic acid	$12.23^{cd} \pm 0.10$	$74.0^{a} \pm 0.2$	$3.57^{d} \pm 0.05$
	Mean	12.27 ± 0.86	78.8 ± 4.2	3.42 ± 0.24

IVPD = *in vitro* protein digestibility; R lysine = Reactive lysine. Values are mean ± standard deviation on dry basis. Mean values in the same column with different superscript letters are significantly different ($P < 0.05$) for each sample type.

For back-slopped treatments (100% amaranth), the cooked and fermented beverage had a higher protein content (18.2%) than the fermented and cooked beverage (15.4%). Similarly, the cooked and fermented beverage (back-slopped) from the sorghum-amaranth composite had a higher protein content (14.1%) than the fermented and cooked beverage (12.6%). Starch gelatinisation as a result of cooking prior to fermentation could have made the starch more easily digestible to produce more fermentable sugars. The higher levels of fermenting microorganisms associated with backslopping would produce more carbon dioxide which escapes from the system. The net effect would then be a decrease in the proportion of starch which makes the protein in the beverages more concentrated.

Concerning protein digestibility, the *in vitro* pepsin digestibility (IVPD) for 100% sorghum flour of 74% (Table 2) was within the range reported by other workers (Awadelkareem & Taylor, 2011; Taylor & Taylor, 2011) for different sorghum cultivars. Cooking caused a significant reduction by 30% in IVPD of the raw sorghum flour, which is a well-known phenomenon. The decrease in IVPD of sorghum after wet cooking is attributed to the

formation of disulphide bonds involving the γ- and β-kafirins, leading to formation of cross-linked protein species that are resistant to enzyme attack (Duodu *et al*., 2003).

The IVPD obtained for the raw amaranth flour (89.3%) was similar to that reported by Mlakar *et al*. (2010) (90%) and Amare *et al*. (2015) (82.4%).The 100% amaranth beverages had higher mean IVPD (87.5%) than the corresponding 100% sorghum beverages (62.7%) and mean IVPD of sorghum: amaranth composite beverages (78.8%) was higher than mean IVPD of 100% sorghum beverages (62.7%) (Table 2). Furthermore, contrary to that observed with sorghum flour, there was no significant change in protein digestibility of amaranth flour after cooking.

The uncooked sorghum: amaranth composite flour had higher IVPD (83.5%) than the 100% uncooked sorghum flour (74%). Although the IVPD of the composite flour was also reduced after cooking, the value obtained (73.7%), was much higher by about 41.8% when compared with cooked 100% sorghum flour. Generally, the IVPDs of all the beverages prepared from the composite flours (average 78.8%) were higher than corresponding beverages from 100% sorghum flour (average 62.7%). This can be attributed to the reduction in proportion of the less digestible sorghum kafirin protein and the increased proportion of more digestible amaranth protein.

For the 100% sorghum samples, beverages prepared by cooking after *L. plantarum* fermentation and back-slopping had higher IVPD (56.4% and 57.5% respectively) than the cooked beverage without fermentation (51.9%) (Table 2). According to Taylor & Taylor (2002), reduction in pH as a result of lactic acid produced during fermentation could cause structural modification of the sorghum proteins, thereby making them more accessible to proteolytic enzymes.

Unlike sorghum, fermentation and chemical acidification without cooking decreased IVPD in 100% amaranth beverages compared to the raw flour. This may be due to decrease in the amount of protein being exposed to pepsin digestion in the fermented and acidified flour as a result of possible microbial conversion of amino acids to volatiles such as ammonia and nitrogenous flavour compounds during fermentation and acidification as earlier discussed. In addition, after cooking fermented and chemically acidified beverages prepared from 100% amaranth flour, there was no significant difference in protein digestibility when compared with raw flour. This clearly indicates that unlike with sorghum, cooking had no adverse effect on the protein digestibility of amaranth. This can be attributed to the fact that highly digestible storage proteins (albumin and globulins) are the major components of amaranth seed protein (Venskutonis & Kraujalis, 2013), as compared to the relatively less digestible sorghum kafirin storage protein. This demonstrates the usefulness of compositing sorghum with amaranth for improved protein quality. This is also illustrated in Table 2 which shows that the sorghum: amaranth composite beverages had higher IVPD than the corresponding 100% sorghum beverages.

The reactive lysine content, an estimation of available lysine, was higher in the 100% amaranth beverages (by 53%) and the composite beverages (by 34%) than the 100% sorghum (Table 2). Again, this is because amaranth is rich in lysine-rich globulin protein. The value obtained for raw sorghum flour (2.34 g/100 g protein) was close to the range of 2.38–

2.97 g/100 g of protein reported for different sorghum types by Taylor & Taylor (2011) and within the range of 1.77–2.40 g/100 g protein reported by Anyango *et al*. (2011). Similarly, the reactive lysine content of the raw amaranth flour used in this study (3.95 g/100 g protein) was close to the 4.12 to 4.74 g/100 g reported by Lara & Ruales(2002) but somewhat lower than 5.8 g/100 g protein reported by Paredes-López & Mora-Escobedo (1989).

Generally, cooking reduced the level of reactive lysine in all the beverage types, presumably as a result of Maillard type reactions. However, for most of the beverages, the reduction level was minimal. This could be due to the moist heat cooking conditions used during the beverage preparation in which the samples were boiled (96 °C) for just 10 min. It has been reported that reduction in available lysine as a result of interaction between reducing sugar and ε- amino group of lysine through the Maillard reaction depends largely on the moisture content and also the magnitude of heat treatment (Žilić *et al*., 2006). High moisture content slows down the process of Maillard reaction by obstructing the access of oxygen to the amino acids. All the sorghum-amaranth composite flour beverages had higher reactive lysine content compared with the 100% sorghum flour beverages essentially because of the higher lysine content in amaranth compared to sorghum.

Principal component analysis

The principal component analysis (PCA) (Fig. 1) shows that reactive lysine of all the samples and protein digestibility of sorghum were in the same quadrant as the raw and fermented treatments where there was no cooking involved, and directly opposite to all the treatments that involved cooking. Fermented beverages by back-slopping and lactic acid acidified beverages were also in proximity with protein digestibility and reactive lysine. This shows that fermentation and chemical acidification enhanced *in vitro* protein digestibility and lysine availability. On the other hand, cooking substantially reduced the level of reactive lysine, presumably due to Maillard type reactions (Serrem *et al*., 2011). In addition, it shows that cooking substantially reduced protein digestibility of the sorghum beverages, presumably as a result of protein cross-linking (Duodu *et al*., 2003). However, protein digestibility of amaranth showed strong association with the cooked, cooked and acidified, and acidified and cooked treatments, since all were located in the same quadrant. This indicates that unlike sorghum, cooking improved amaranth protein digestibility, in agreement with what was reported by Schoenlechner *et al*. (2008). This further suggests that the inclusion of amaranth to sorghum-based foods has the potential to enhance the protein digestibility.

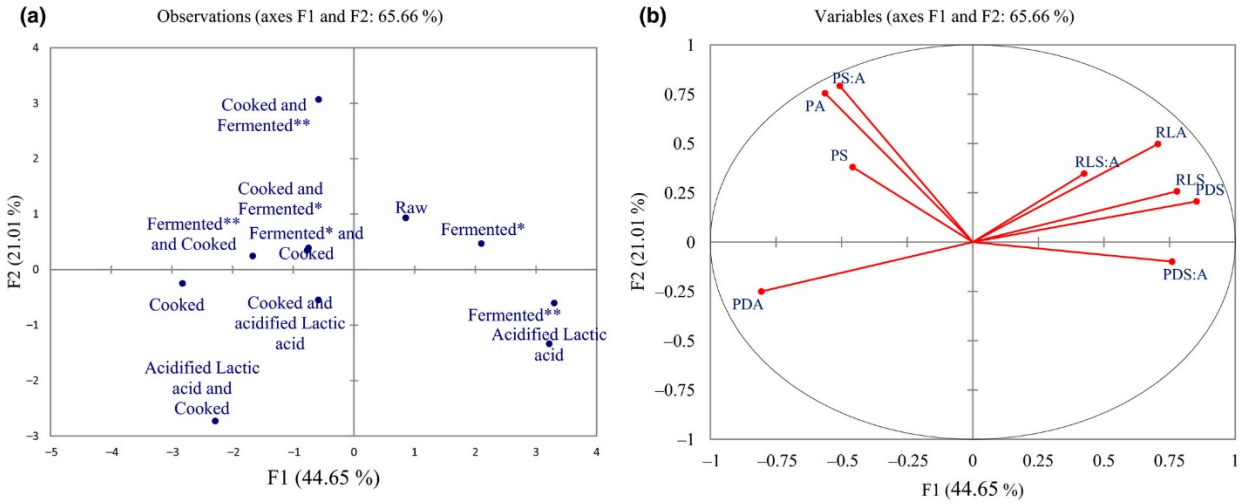

Figure 1. Principal component analysis of treatments and protein quality parameters of beverages prepared from sorghum, amaranth and their composite. (a) Treatments: raw; cooked; fermented* fermentation with *Lactobacillus plantarum*; fermented** fermentation by back-slopping; fermented* and cooked; fermented** and cooked; cooked and fermented*; cooked and fermented**; acidification with lactic acid, acidification with lactic acid and cooked; cooked and acidified with lactic acid. (b) Factor loading: PDA protein digestibility of amaranth; PDS protein digestibility of sorghum; PDS:A protein digestibility of sorghum: amaranth(50:50); RLS reactive lysine sorghum; RLA reactive lysine amaranth; RLS:A reactive lysine sorghum amaranth (50:50); PS protein content sorghum, PA protein content of amaranth, PS:A protein content of sorghum: amaranth (50:50).

Effects of different souring methods on beverage phytate content

Table 3 shows that all the beverages had lower phytate contents than their raw flours. The percentage reduction in phytate content (Table 3) in the cooked treatments without fermentation or acidification (by 4–12%) was much lower than in the fermented or acidified treatments (without cooking) (by 37–83%) and in treatments that were cooked after fermentation or acidification (by 54–86%). These findings indicate that fermentation and acidification were more effective at reducing phytate content than cooking on its own. The slight reduction in phytate observed with cooking (without fermentation or acidification) could be as a result of its dephosphorylation by the endogenous phytase in the flour at the early stage of cooking (reviewed by Kumar *et al*., 2010). However, with elevated cooking temperature, the endogenous phytase may be inactivated (reviewed by Kumar *et al*., 2010), which would reduce the extent of phytate reduction. On the other hand, the marked reduction in phytate content with both the fermented and chemically acidified treatments (without cooking) can be attributed to the ability of the endogenous phytase in the flour to hydrolyse the phytate more effectively at low pH (Feil, 2001). Moreover, the further reduction in phytate content of the fermented and chemically acidified beverages by 9–36.7% as a result of cooking suggests that such pre-treatments (fermentation and chemical acidification) could make the phytate more available to dephosphorylation during subsequent heat treatment. A similar reduction in phytate content of fermented flours as a result of cooking during the preparation of thick porridge was reported by Kruger *et al*. (2012).

Table 3 Effects of fermentation and acidification on phytate content (mg g^{-1} db) of beverages from sorghum, amaranth and their composite flour

Treatment	Sorghum flour (100%)	Amaranth flour (100%)	Sorghum:Amaranth (50:50)
Raw flour	$12.48^{d} \pm 0.38$	$19.88^{h} \pm 0.19$	$17.48^{i} \pm 0.39$
Cooked flour	$10.98^{d} \pm 0.37$ (12)	$18.22^{g} \pm 0.18$ (8.4)	$16.69^{i} \pm 0.19$ (4.5)
Fermented using *L. plantarum* (uncooked)	$5.65^{bc} \pm 0.19$(54.7)	$5.31^{c} \pm 0.38$ (73.3)	$4.79^{de} \pm 0.18$(72.6)
Fermented using *L. plantarum* and cooked	$3.61^{ab} \pm 0.36$ (71.1)	$3.46^{a} \pm 0.18$(82.6)	$3.62^{bc} \pm 0.37$(79.3)
Cooked and fermented using *L. plantarum*	$10.37^{d} \pm 1.79$ (16.9)	$19.43^{h} \pm 0.19$(2.3)	$15.25^{h} \pm 0.19$(16.7)
Fermented by back-slopping (uncooked)	$5.09^{ab} \pm 0.38$(59.2)	$5.61^{cd} \pm 0.18$(71.8)	$2.97^{ab} \pm 0.18$ (83.0)
Fermented by back-slopping and cooked	$3.22^{a} \pm 0.18$ (74.2)	$3.83^{ab} \pm 0.19$ (80.7)	$2.48^{a} \pm 0.18$ (85.8)
Cooked and fermented by back-slopping	$4.89^{ab} \pm 0.19$(60.8)	$19.36^{h} \pm 0.19$ (2.6)	$14.56^{h} \pm 0.19$ (16.7)
Acidified with Lactic acid (uncooked)	$5.73^{bc} \pm 0.19$ (54.1)	$6.70^{e} \pm 0.19$ (66.3)	$5.58^{ef} \pm 0.18$ (68.1)
Acidified with Lactic acid and cooked	$3.80^{ab} \pm 0.19$ (69.6)	$4.42^{b} \pm 0.18$ (77.8)	$4.20^{cd} \pm 0.18$(76.0)
Cooked and acidified with lactic acid	$11.04^{d} \pm 0.18$(11.5)	$16.93^{f} \pm 0.19$ (14.8)	$15.20^{h} \pm 0.19$(13.0)
Acidified with HCl (uncooked)	$7.82^{c} \pm 0.17$ (37.3)	$6.13^{de} \pm 0.18$(69.2)	$6.63^{g} \pm 0.18$ (62.1)
Acidified with HCl and cooked	$5.76^{bc} \pm 0.37$ (53.8)	$4.18^{ab} \pm 0.19$ (79.0)	$6.02^{fg} \pm 0.18$ (65.6)
Cooked and acidified with HCl	$11.31^{d} \pm 0.36$ (9.4)	$18.06^{g} \pm 0.18$ (9.2)	$15.07^{h} \pm 0.18$ (13.8)

Values are mean ± standard deviation. Mean values in the same column with different superscript letters are significantly different ($P \leq 0.05$) for each sample type. Values in parenthesis represent the percentage reduction in phytate in processed flours compared to their corresponding raw flours.

The percentage reduction in phytate content in the cooked flours before fermentation or acidification (by 2–61%) was lower than the reduction shown when the flours were cooked after fermentation or acidification (by 54–86%). This is illustrated in the PCA (Fig. 2) where phytate showed a strong association with raw flours and flours that were cooked either without or before fermentation or acidification, as they were all in the same quadrant. In fact, these treatments had lower phytate reduction compared with other treatments (Table 3). The application of heat during cooking before fermentation or acidification may have inactivated the endogenous phytases in the grain before attaining the low pH at which the phytase is more active to hydrolyse the phytate.

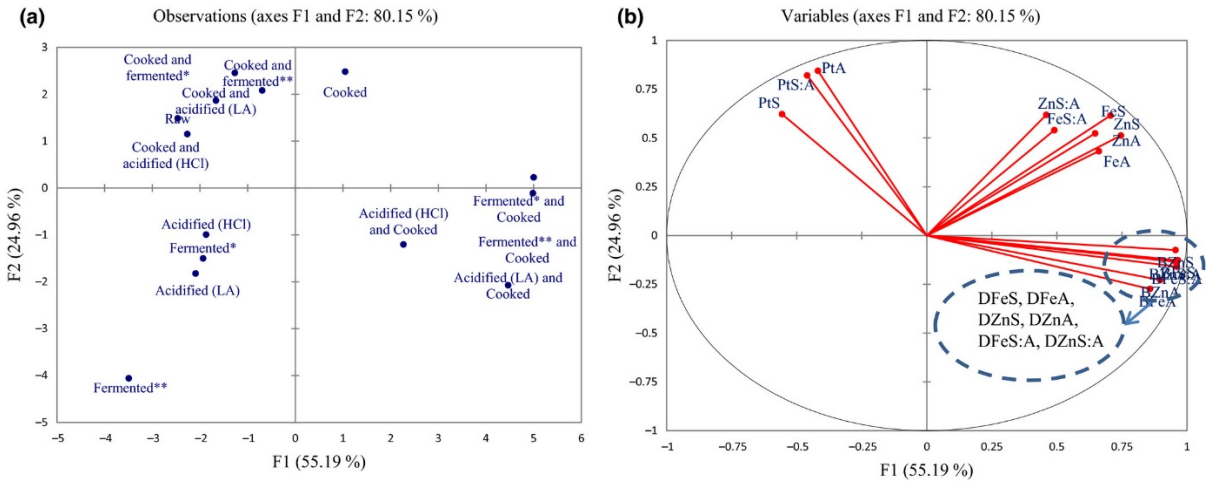

Figure 2. Principal component analysis of treatments and Parameters determined (Phytate, iron and zinc content; dialysable iron and zinc) for beverages prepared from sorghum flour, amaranth flour and their composite. PtSphytate sorghum; PtAphytate Amaranth; PtS:Aphytate sorghum: amaranth composite; ZnS zinc content of sorghum; ZnA zinc content of amaranth; FeS iron content of sorghum; FeA iron content of amaranth; ZnS:A, zinc content of sorghum: amaranth composite; FeS:A, iron content of sorghum:amaranth composite. DFeA dialysable iron amaranth; DZnA dialysable Zinc amaranth; DFeS dialysable iron sorghum; DZnS dialysable zinc sorghum; DFeS:A dialysable iron sorghum:amaranth composite; DZn S: A dialysable zinc sorghum:amaranth composite.

Of particular interest is the observation that chemical acidification either with lactic acid or hydrochloric acid caused significant reduction in phytate content of the raw flour (by 37–69%). This is because the samples were firstly acidified to pH 5 and kept in an incubator for about 2 h at 50 °C before final acidification to pH of around 3.5. During this period, the endogenous phytase in the flour would have had sufficient time to cause the observed reduction in phytate through hydrolysis because the enzyme is more active at a pH around 5 (Reale *et al*., 2007) and temperature of about 50 °C (Kumar *et al*., 2012).

Effects of different souring methods and amaranth inclusion on iron and zinc bioaccessibilities

Generally, fermented and chemically acidified beverages had higher bioaccessible iron and zinc compared to the corresponding beverages without fermentation or acidification (Table 4). For example bioaccessible iron and zinc increased by 8–192% and 24–28%, respectively, when 100% sorghum flour was fermented or chemically acidified compared to the cooked beverage without fermentation or acidification. Similarly, bioaccessible iron and zinc increased by 133–372% and 53–194%, respectively, in the fermented and chemically acidified beverages from 100% amaranth flour compared to the beverage without fermentation or acidification. Bioaccessible iron was higher (by 20–415%) in all the 100% amaranth beverages compared to the corresponding beverages from 100% sorghum. On the other hand, 100% sorghum beverages had higher bioaccessible zinc (by 25–194%) than corresponding beverages from 100% amaranth.

Table 4 Effects of fermentation and acidification on bioaccessible iron and zinc of beverages from sorghum, amaranth and their composite flour

Flour type	Treatment	Dialysable iron (mg/100 g db)	Dialysable zinc (mg/100 g db)
100% Sorghum flour	Cooked	$0.12^{a} \pm 0.01$(3.7)	$0.50^{de} \pm 0.03$ (20.1)
	Fermented (*L. plantarum*) and Cooked	$0.35^{bc} \pm 0.01$ (10)	$0.62^{a} \pm 0.03$ (28.7)
	Fermented (back-slopped) and Cooked	$0.28^{b} \pm 0.01$ (7.7)	$0.62^{a} \pm 0.10$(23.9)
	Acidified lactic acid and Cooked	$0.30^{b} \pm 0.01$ (9.4)	$0.64^{a} \pm 0.01$ (27.1)
	Acidified HCl and Cooked	$0.13^{a} \pm 0.00$ (4.1)	$0.45^{cd} \pm 0.06$ (21.7)
100% Amaranth flour	Cooked	$0.18^{a} \pm 0.04$ (2.2)	$0.17^{a} \pm 0.05$ (5.9)
	Fermented (*L. plantarum*) and Cooked	$0.42^{cd} \pm 0.02$ (4.0)	$0.26^{ab} \pm 0.03$ (9.2)
	Fermented (back-slopped) and Cooked	$0.41^{cd} \pm 0.04$ (4.7)	$0.29^{ab} \pm 0.03$ (8.9)
	Acidified lactic acid and Cooked	$0.85^{f} \pm 0.01$ (11.3)	$0.50^{de} \pm 0.02$ (17.2)
	Acidified HCl and Cooked	$0.67^{e} \pm 0.01$ (8.4)	$0.36^{bcd} \pm 0.00$ (12.04)
Sorghum:Amaranth flour (50:50)	Cooked	$0.10^{a} \pm 0.01$ (1.7)	$0.18^{a} \pm 0.01$ (6.8)
	Fermented (*L. plantarum*) and Cooked	$0.47^{d} \pm 0.00$ (7.6)	$0.26^{ab} \pm 0.01$ (8.7)
	Fermented (back-slopped) and Cooked	$0.46^{d} \pm 0.01$ (7.2)	$0.34^{bc} \pm 0.01$(14.5)
	Acidified lactic acid and Cooked	$0.42^{cd} \pm 0.01$ (8.8)	$0.37^{bcd} \pm 0.01$ (17.3)
	Acidified HCl and Cooked	$0.18^{a} \pm 0.02$ (3.4)	$0.16^{a} \pm 0.00$ (8.6)

Values are mean ± standard deviation; db, dry basis. Mean values in the same column with different superscript letters are significantly different ($P \leq 0.05$).Values in parentheses represent the percentage dialysable iron and zinc.

Compositing sorghum with amaranth increased the bioaccessible iron of the beverages (fermented and chemically acidified) (by 34–64.3%) compared to the corresponding 100% sorghum beverages. However, all the composite beverages had lower bioaccessible zinc compared to the corresponding 100% sorghum beverages. The high level of calcium in amaranth compared to sorghum (122 mg/100 g and 9.7 mg/100 g for amaranth and sorghum respectively; data not presented) could be responsible for the low bioaccessible zinc in 100% amaranth beverages and composite beverages. Calcium has very high tendency

to form insoluble complexes with phytate and zinc, and calcium-zinc-phytate complex is stronger than either calcium-phytate or zinc-phytate complex (Greiner *et al*., 2006; Coulibaly *et al*., 2011). This implies that in the presence of high levels of calcium, zinc bioavailability can be substantially reduced (Kumar *et al*., 2010).

Increased iron and zinc bioaccessibility in all the fermented and chemically acidified beverages corresponded with the significant ($P \leq 0.05$) reduction in phytate content (Table 3). This is illustrated in the PCA (Fig. 2) where fermented and acidified beverages were associated with bioaccessible iron and zinc content, since they were all in the same quadrant. On the other hand, treatments with high phytate content have lower bioaccessible iron and zinc as phytate and bioaccessible iron and zinc are in opposite quadrants to each other (Fig. 2). The increase in bioaccessible iron and zinc due to fermentation has been well documented (Hemalatha *et al*., 2007; Proulx & Reddy, 2007; Kruger *et al*., 2012). The acidic environment created through fermentation, provides a favourable condition for the endogenous phytase in the flour to hydrolyse phytate, thereby reducing its ability to form insoluble complexes with divalent minerals like iron and zinc (Feil, 2001).

It has also been proposed that organic acids such as lactic acid, citric acid and acetic acid, which are produced during lactic acid fermentation, can enhance the bioaccessibility of divalent metals like iron and zinc through the formation of a soluble complex with these metals (Tontisirin *et al*., 2002). This hypothesis is supported by the results of this study. In the chemically acidified samples, lactic acid and hydrochloric acid were used. Acidification with hydrochloric acid was included to determine whether any observed effects on iron and zinc bioaccessibilities were simply due to low pH, or whether lactic acid has some more specific role. The fact that lactic acid acidified beverages exhibited higher bioaccessible iron and zinc compared with beverages acidified with hydrochloric acid suggest possible specific iron and zinc bioaccessibility enhancing ability of lactic acid by way of soluble complex formation with these minerals. In the deprotonated lactic acid, the negatively charged oxygen atoms of the hydroxyl and carboxylate groups could act as potential coordination points for the divalent iron and zinc ions. Such coordination points are not available with the hydrochloric acid.

Conclusions

Compositing sorghum with grain amaranth improves the protein content, protein digestibility, reactive lysine and bioaccessible iron of the beverages compared to sorghum alone. Hence the addition of amaranth to such traditional sorghum foods could be a viable option for improving their quality with respect to these critical nutrients. Phytate content was reduced drastically by both fermentation and chemical acidification with a concomitant improvement in bioaccessible iron and zinc. Significantly, acidification with lactic acid as a means of souring is as good as fermentation either with *Lactobacillus plantarum* starter culture or by back-slopping in terms of improvement in protein digestibility, phytate reduction, and iron and zinc bioaccessibility. Therefore, acidification with lactic acid has great potential as a simple process to improve the nutritional quality of such cereal-based beverages to help combat protein malnutrition and iron and zinc deficiencies.

Acknowledgments

This publication was made possible through support provided by the Office of Agricultural Research and Policy, Bureau for Food Security, U.S. Agency for International Development, under the terms of Cooperative Agreement No. AID-OAA-L-14-00003. The opinions expressed herein are those of the author(s) and do not necessarily reflect the views of the U.S. Agency for International Development.

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