Journal of Food and Nutrition Sciences
2015; 3(5): 180-186
Published online September 2, 2015 (http://www.sciencepublishinggroup.com/j/jfns)
doi: 10.11648/j.jfns.20150305.13
ISSN: 2330-7285 (Print); ISSN: 2330-7293 (Online)

In Vitro Bioaccessibility of Ferrous Sulfate and Ferrous Fumarate from Fortified Cassava *Mahewu*

Elsa Maria Salvador[1, 5, *], Johanita Kruger[2], Cheryl M. E. McCrindle[1], Robert I. McCrindle[3], Vanessa Steenkamp[4]

[1]School of Health Systems and Public Health, Faculty of Health Sciences, University of Pretoria, Pretoria, South Africa
[2]Department of Food Science and Institute for Food, Nutrition and Wellbeing, University of Pretoria, Pretoria, South Africa
[3]Department of Chemistry, Faculty of Science, Tshwane University of Technology, Pretoria, South Africa
[4]Department of Pharmacology, Faculty of Health Sciences, University of Pretoria, Pretoria, South Africa
[5]Department of Biological Sciences, Faculty of Sciences, Eduardo Mondlane University, Maputo, Mozambique

Email address:
elsamariasalvador@gmail.com (E. M. Salvador), esalvador@uem.mz (E. M. Salvador), johanitak@gmail.com (J. Kruger), cheryl.mccrindle@gmail.com (C. M. E. McCrindle), vanessa.steenkamp@up.ac.za (V. Steenkamp), mccrindle@tut.ac.za (R. I. McCrindle)

To cite this article:
Elsa Maria Salvador, Johanita Kruger, Cheryl M. E. McCrindle, Robert I. McCrindle, Vanessa Steenkamp. *In Vitro* Bioaccessibility of Ferrous Sulfate and Ferrous Fumarate from Fortified Cassava *Mahewu*. *Journal of Food and Nutrition Sciences.* Vol. 3, No. 5, 2015, pp. 180-186. doi: 10.11648/j.jfns.20150305.13

Abstract: It has been suggested that iron fortification could reduce dietary anaemia in women of childbearing age and preschool children in Mozambique, where cassava root, the staple carbohydrate, is deficient in iron. *Mahewu* is a traditional non-alcoholic, beverage made from fermented cassava roots (*Manihot esculenta,* Crantz). This study used Luten's *in vitro* dialysis method to compare the bioaccessibility of ferrous sulfate and ferrous fumarate added to *mahewu* made from sweet and bitter cassava varieties, at two different stages of fermentation. Iron concentration was measured using an Inductively Coupled Plasma-Optical Emission Spectrometer. Neither the stage of fortification nor the iron salt used affected the concentration of iron. A significant ($p<0.05$) difference was observed in the proportion of bioaccessible iron in mahewu fortified with either ferrous sulfate or ferrous fumarate. A higher proportion of iron was found to be bioaccessible in *mahewu* fortified with ferrous sulfate. However, the concentration of both total and proportional bioaccessible iron was significantly higher ($p<0.05$) when ferrous sulfate was used, compared to ferrous fumarate. It is recommended that ferrous sulphate be used for both household and commercial fortification of *mahewu* in Mozambique.

Keywords: Bioaccessibility, Cassava *Mahewu*, Ferrous Fumarate, Ferrous Sulfate, Iron Fortification, Mozambique

1. Introduction

The prevalence of under-nutrition in the Mozambican population is approximately 38%; throughout the country it is estimated that 43% of children younger than 5 years of age are moderately undernourished, 20% chronic and 8% acutely undernourished [1]. In Mozambique anaemia due to dietary iron deficiency is of public health concern [2]. Furthermore, the prevalence of anaemia in children younger than five years of age is estimated to be 69%; of which 26% are mildly anaemic, 39% moderately and 4% severely anaemic [1].

Cassava is a staple food in Mozambique, widely cultivated across the country, mainly by small-scale farmers [3]. Approximately 94% of cassava production is consumed by humans, with 4% made available for animal feed and industrial use [4]. While cassava is an excellent source of carbohydrates it contains low levels of protein and the levels of essential micronutrients such as iron are insignificant [5].

Cassava roots are consumed in various forms: stiff porridge (*karakata*); roasted cassava or *rale* [6]; bread and other baked products [7]; cooked with vegetables and peanuts (*xiguinha)* and the non-alcoholic fermented traditional beverage *mahewu* [8]. Cassava *mahewu* is made from the roots of both bitter and sweet varieties and it is often sweetened with sugar just before consumption. Maize *mahewu* has been fortified with micronutrients, including iron, at industrial level in other countries [9].

It has been suggested that communities whose major dietary intake is dependent on plant-derived foods, should consider iron fortification [10]. The World Health Organization (WHO) recommends ferrous sulfate, ferrous fumarate and sodium iron EDTA (NaFeEDTA) for the fortification of wheat and maize [11]. Iron fortification of cassava *mahewu* could alleviate dietary iron deficiency anaemia in vulnerable populations such as women of child-bearing age and young children in Mozambique [8]. Fortification of cassava *mahewu* has been further supported by a report maintaining that populations consuming cassava as their basic energy source are at risk of deficient iron intake [12]. The aim of this study was to compare the *in vitro* bioaccessibility of iron in *mahewu* made from sweet or bitter cassava varieties, fortified with either ferrous sulfate or ferrous fumarate, at two different stages of fermentation.

2. Materials and Methods

2.1. Cassava

Cassava roots were collected in May 2013 from small scale farmers in four Districts of Mozambique. Five samples were collected of each variety per district. Sweet cassava roots (varieties *Calamidade* and *Munhaca*) were collected from Meconta and Zavala Districts located in Nampula and Inhambane provinces, respectively. Bitter cassava roots (varieties *Tomo* and *Incirricano*) were collected from Rapale and Alto Molocue Districts situated in Nampula and Zambézia provinces, respectively.

2.2. Cassava Flour Preparation

To prepare the flour, cassava roots were peeled, washed with tap water, grated and sun-dried, as done traditionally in villages in Mozambique. Flour was made by pounding the dried chips with a pestle and mortar. All instruments and containers used for flour preparation were made from plastic or wood material in order to prevent contamination with metals.

2.3. Mahewu Preparation

Flour from the bitter types of cassava roots were mixed in equal ratios and the same procedure repeated for flour made from sweet cassava roots. The flour was mixed with water, cooked to form a porridge and fermented under controlled conditions, following the method used by Bvochora et al. [13] for maize *mahewu,* with minor modifications. During the pilot study *mahewu* was fermented at 45°C and changes in pH were assessed at different intervals during the fermentation period of 3 days (Figure 1). An end-point of approximately pH 4.5 was consistently reached after 24 h at 45^{o}C.

In the laboratory, the sweet and bitter cassava flours (20 g) were each mixed with distilled water (49 ml) then added to 150 mL boiling water. The mixture was boiled on a magnetic stirrer/hotplate, (FMH Electronics, STR-MH, F1093-0207, 230V ± 1050 HZ 500 watt, Durban, South Africa) for 10 min and then set aside to cool to 25°C. This porridge was then transferred to a 250 mL glass Erlenmeyer flask and 1.25 g of starter culture (freeze-dried cassava *mahewu* previously prepared in the traditional manner), was added and mixed thoroughly. The broth was left to ferment in a Labcon Standard Incubator, (Forced Circulation incubator, FSIM, Maraisburg, South Africa) at 45°C for 24 h. The effect of the 24 h fermentation on the pH, titratable acidity and total solids (% solids after drying) were determined using the methods described by James [14].

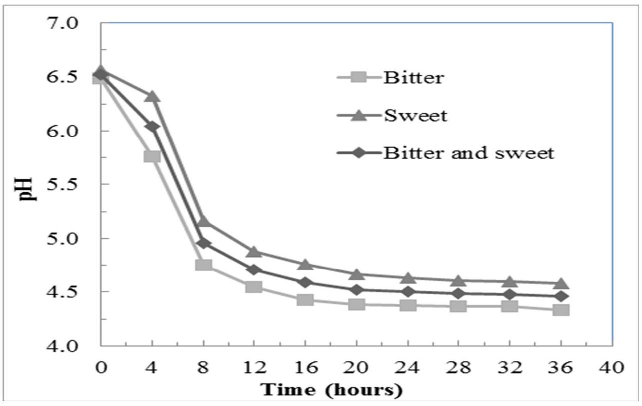

Figure 1. *Changes in pH during the fermentation period.*

2.4. Iron Fortification of Mahewu

Cassava *mahewu* was fortified with two different iron sources, ferrous sulfate ($FeSO_4.7H_2O$), (EMSURE®, CAS-N$_O$ 7782-63-0, Darmstadt, Germany) and ferrous fumarate ($C_4H_2FeO_4$), (ALFA AESAR, LA32XY, UK, England) at two different stages; the beginning (0 hour when the starter culture was added) and end of fermentation (24 hours). The selection of different stages of fortification was to determine whether cassava *mahewu* should be fortified at household or commercial level.

The amount of iron added to cassava *mahewu* (68 mg/100 g) was based on the average of the prescribed range of iron used to fortify maize meal (2.9 mg/100 g to 5.7 mg/100 g) [15]. The "overage", which is the additional amount of the fortificant added to the flour, to compensate for storage and processing losses, was calculated as 1.2 mg [15]. Ferrous sulfate (6.8 g) or ferrous fumarate (3.8 g) was diluted in 100 mL of distilled water then 1 mL of the solution added to the *mahewu* for the fortification at the beginning of fermentation (0 h). For fortification at the end of the fermentation process (24 h), 6.2 g of ferrous sulfate or 3.45 g of ferrous fumarate, was dissolved in 100 mL distilled water and 1 mL of the solution added to the *mahewu*. Figure 2 represents the schematic flow of cassava *mahewu* preparation and indicates the stage at which iron was added.

2.5. Iron Concentration and In Vitro Iron Bioaccessibility

Iron concentration in the cassava flour and fortified *mahewu* was analyzed using an Inductively Coupled Plasma-Optical Emission Spectrometer (ICP-OES) (Spectro Arcos, Kleve, Germany). *In vitro* bioaccessibility of iron from fortified cassava *mahewu* was determined using the dialysis method described by Luten et al. [16]. The iron concentration in the dialysate was also assessed using ICP-OES. Working multi-element standard solutions were prepared by dilution of the stock standard solutions (1000 mg/L, Merck, Germany) to the desired concentration. The ranges of the calibration standards were selected to match expected concentrations of iron in the samples analyzed by ICP-OES. Digestive enzymes used were pepsin (P-7000), pancreatin (P-1750) and bile extract (B-8631); procured from Sigma (Johannesburg, South Africa). Dialysis tubing used was Spectra/Por 7 (Ø = 20.4 mm) with a molecular weight cut-off (MWCO) of 10 kDa (G.I.C. Scientific, Johannesburg, South Africa).

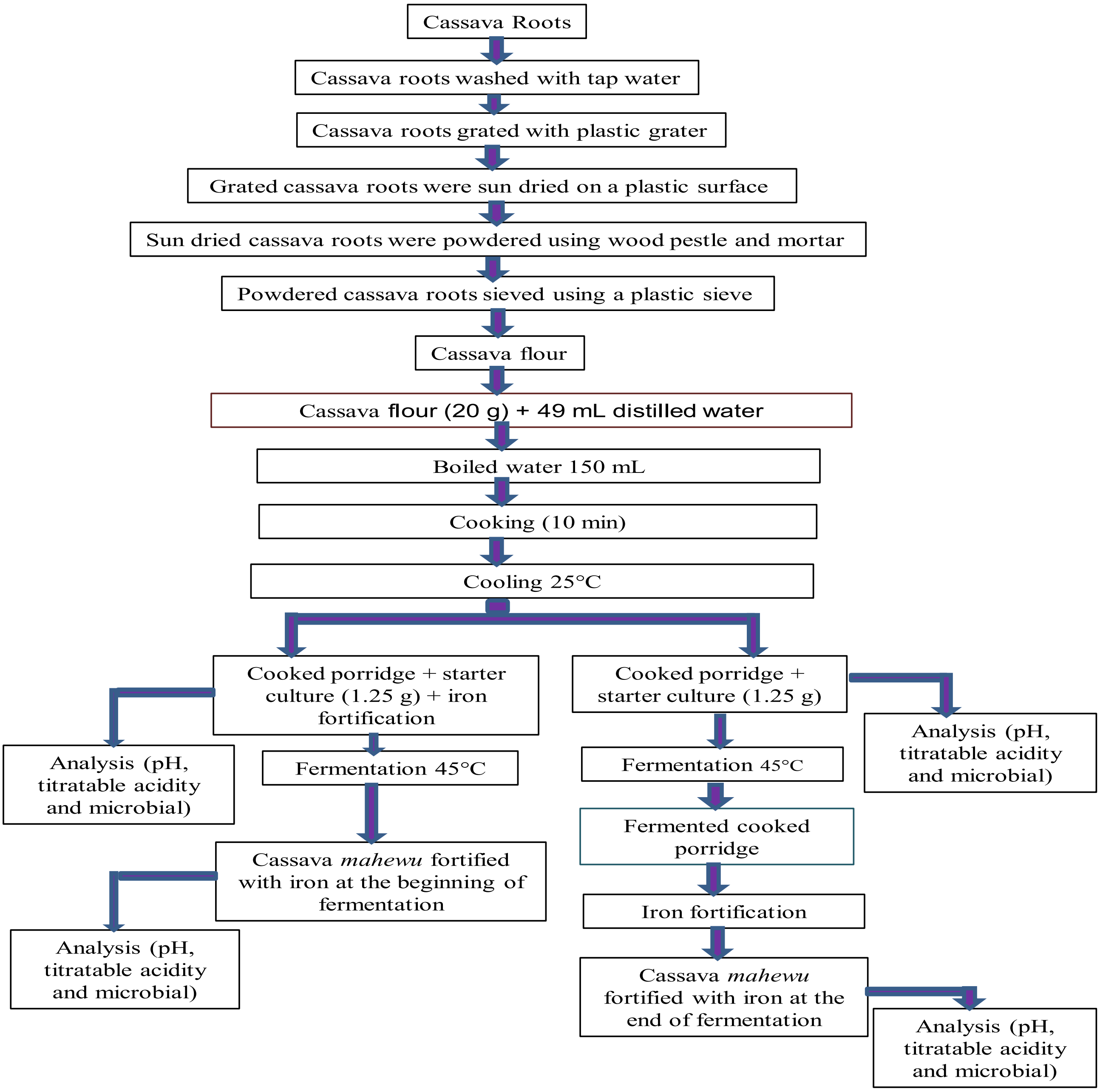

Figure 2. *Schematic presentation of the preparation of cassava mahewu and indication of the stage at which the iron compound was added.*

2.6. Statistical Analysis

Samples of cassava mahewu were homogenized independently and duplicated at the gastric stage. At the intestinal stage, the duplicated homogenized samples were replicated to a final five repetitions. All data was analyzed using STATA version 12 with one way and/or multifactor analysis of variance (ANOVA) at 95% confidence level.

3. Results and Discussion

3.1. Chemical Characteristics of Cassava Mahewu Before and After Fortification with Iron

The chemical characteristics of cassava mahewu before and after fortification with iron are presented in Table 1.

Table 1. *Chemical characteristics of cassava mahewu before and after fortification with iron.*

Independent variables		Acidity (%)	pH	Total solids (%)
Stage of fortification	Beginning	0.06^{a}	6.0^{b}	*
	End	0.30^{b}	4.4^{a}	9.6^{a}
Type of cassava	Bitter	0.30^{a}	4.4^{a}	9.5^{a}
	Sweet	0.30^{a}	4.4^{a}	9.6^{a}
Iron source	Ferrous sulfate	0.30^{a}	4.5^{b}	9.5^{a}
	Ferrous fumarate	0.31^{a}	4.3^{a}	9.6^{a}
	Control	0.31^{a}	4.3^{a}	9.4^{a}

a, b – Values within the same independent variable with different superscripts differ significantly ($p \leq 0.05$)
The % of total solids, was not measured at the beginning of fermentation

A significant (p<0.05) difference was found between the acidity and pH at the beginning and end of fermentation of cassava *mahewu* (Table 1). These findings were similar to those described for maize *mahewu* [13]. The changes were ascribed to multiplication of lactic acid bacteria (LAB) and yeast, which converted the starch into sugars to produce lactic acid, leading to a decrease in pH [17], also confirming fermentation after addition of the starter culture. Although there were some significant changes in the chemical characteristics of cassava *mahewu*, the independent variables (Table 1) did not significantly ($p > 0.05$) affect the acidity, pH or total solid content. A reduction in pH has been reported to result from antimicrobial action in fermented food [18]. The product of fermentation, lactic acid, has also been shown to have antimicrobial activity [19]. Traditionally cassava *mahewu* has been considered safe for consumption, when consumed within two days after fermentation, if kept at room temperature. Pasteurized maize *mahewu* (pH ~3.5) has been reported to be safe for consumption up to 21 days after production if stored at 4°C [20].

3.2. Total Iron Concentration in Fortified Cassava Mahewu

It had been previously determined that iron concentrations in cassava roots from Mozambique contained little or no intrinsic iron; which is in line with findings in other parts of the world [21]. Total and proportional iron concentration and bioaccessibility after fortification of cassava *mahewu* with iron compounds at different stages of fermentation are presented in Table 2.

Table 2. *The effect of iron on fortification process.*

Fortificant	Cassava type	Stage of fortification	Total iron concentration (mg/100 g, db)	Proportion of iron bioaccessibility (%)	Bioaccessible iron concentration (mg/100 g, db)
Ferrous sulfate	Bitter	Beginning of fermentation	$10.0^{c} \pm 0.0$	$26.3^{e} \pm 1.8$	$2.63^{d} \pm 0.2$
		End fermentation	$11.4^{c} \pm 0.7$	$21.4^{d} \pm 1.8$	$2.43^{d} \pm 0.2$
	Sweet	Beginning of fermentation	$18.2^{d} \pm 1.5$	$4.9^{a} \pm 0.4$	$0.88^{c} \pm 0.04$
		End fermentation	$17.9^{d} \pm 2.6$	$5.2^{a} \pm 0.4$	$0.94^{c} \pm 0.04$
Ferrous fumarate	Bitter	Beginning of fermentation	$8.1^{bc} \pm 2$	$7.8^{b} \pm 1.1$	$0.63^{b} \pm 0.1$
		End fermentation	$6.1^{ab} \pm 2.4$	$4.4^{a} \pm 1.7$	$0.26^{a} \pm 0.2$
	Sweet	Beginning of fermentation	$4.0^{a} \pm 1.2$	$12.7^{c} \pm 1.1$	$0.50^{b} \pm 04$
		End of fermentation	$4.5^{a} \pm 2.3$	$10.5^{c} \pm 2.7$	$0.47^{b} \pm 0.3$

a, b, c, d – Values within the same column with different superscripts differ significantly ($p \leq 0.05$), db – dry bases

The total iron concentration of *mahewu* made from either bitter or sweet cassava, fortified with ferrous sulfate or ferrous fumarate, was similar at both stages of fortification, for each iron source (ferrous sulfate or ferrous fumarate) (Table 2). However, a significant (p<0.05) difference in the total iron concentration was observed between *mahewu* made from bitter cassava, fortified with ferrous sulfate and *mahewu* made from the sweet type, fortified with ferrous fumarate; and *vice versa*. The stage of fortification did not have an effect on the total iron concentration of fortified *mahewu,* after fortification with either ferrous sulfate or ferrous fumarate. The difference in total iron concentration between *mahewu* made from bitter or sweet varieties of cassava fortified with ferrous sulfate or ferrous fumarate may be due to the difference in nutritional and ant-nutritional substances found between the varieties [22].

A significant (p<0.05) difference in total iron concentration was found between the *mahewu* fortified with ferrous sulfate and ferrous fumarate (Table 3). Higher levels of total iron concentration were observed in *mahewu* fortified with ferrous sulfate. This difference is likely due to the solubility of ferrous sulfate and ferrous fumarate in water. Ferrous sulfate is reported to be more soluble in water than ferrous fumarate [23]. In the present study fortification was carried out using aqueous solutions of sulfate and fumarate.

The total iron concentration of *mahewu* fortified with ferrous sulfate or fumarate was below 68 mg/100 g, the initial concentration of added iron (Table 2). This finding is in agreement with Ikpeme-Emmanuel et al. [24], where cassava meals (*gari* and *fufu*) fortified with 20 mg/100 g of ferrous sulfate ($FeSO_4$), iron (III) sulfate ($Fe_2(SO_4)_3$) and ferric alum ($NH_4Fe(SO_4)_2 \cdot 12\ H_2O$-FA) were found to have an iron concentration for *gari* of 10.70 mg/100 g ($FeSO_4$), 8.80 mg/100 g ($Fe_2(SO_4)_3$) and 12.40 mg/100 g (FA); and for *fufu* of 13.40 mg/100 g ($FeSO_4$), 14.76 mg/100 g ($Fe_2(SO_4)_3$) and 13.85 mg/100 g (FA) after fortification [24]. This decrease in iron concentration may be ascribed to changes in the oxidation state of iron losses, during the fortification stage or the processing procedure.

3.3. Bioacessibility of Iron in Fortified Cassava Mahewu

Iron bioaccessibility is expressed as the concentration (mg/100 g, db) of bioaccessible (dialysable) iron in the sample and the percentage (%) bioacessibility of iron in the dialysate, relative to respective total iron concentration. A significant (p<0.05) difference was observed in the proportion of bioaccessible iron in *mahewu* fortified with either ferrous sulfate or ferrous fumarate (Table 2). A higher percentage of iron was bioaccessible in *mahewu* fortified with ferrous sulfate. The difference in bioaccessibility of ferrous sulfate and ferrous fumarate is well known [25] and confirms the present results.

With regards to the variety of cassava used to make *mahewu*, the proportion of bioaccessible iron when fortified with ferrous sulfate was significantly (p<0.05) higher in *mahewu* made from the bitter cassava, whereas the percentage of bioaccessible iron after ferrous fumarate fortification was significantly (p<0.05) higher in the *mahewu* made from the sweet cassava (Table 2). This may be due to the fermentation process, as it is known to cause changes in physicochemical and functional characteristics of cassava [26].

Ferrous sulfate in *mahewu* made from the bitter cassava was found to be more bioaccessible (Table 2 and Table 3). The stage of fortification (beginning or end) affected neither the iron concentration in the dialysate, nor the total concentration or proportion of bioaccessible iron (Table 3). Although, the fortification of cassava *mahewu* could be done either at the beginning or at the end of the fermentation process, it is proposed that fortification at the end of fermentation would be better for *mahewu* production at household level. The use of sachets containing micronutrients which could be added to semi-solid foods have been reported previously [27]. *Mahewu* fortification of micronutrients using sachets is a possibility, which could be carried out through partnerships. These partnerships could be between public and private sectors and involve non-government organizations. A public-private partnership for mass fortification of food has recently been introduced in Mozambique [28]. At the commercial level, fortification at the beginning of fermentation is recommended. Fortified cassava flour could be used for *mahewu* preparation or in other cassava foods. Cassava flour has previously been fortified with iron in Brazil, where a reduction in the prevalence of anaemia in school children consuming food products from the fortified cassava flour was reported [29].

***Table 3.** The effect of cassava mahewu iron fortification process.*

Independent variables		Total iron concentration (mg/ 200 g edible portion)	Bioaccessible iron (mg/200 g edible portion)	% bioaccessible iron
Stage of fortification	Beginning of fermentation (0 hr)	1.9^a	0.17^a	11.7^a
	End of fermentation (24 hr)	1.9^a	0.20^a	9.4^a
Type of cassava	Bitter	1.6^a	0.25^b	13.2^b
	Sweet	2.1^a	0.13^a	8.3^a
Iron Source	Ferrous sulfate	2.9^b	0.30^b	12.6^a
	Ferrous fumarate	1.1^a	0.09^a	8.8^a

a, b, c – Values within the same independent variable with different superscripts differ significantly ($p \leq 0.05$)

3.4. Possible Contribution of Fortified Cassava Mahewu to RDA and Absolute Requirement of Vulnerable Populations

In the present study, the size of the edible portion of fortified cassava mahewu was taken as 200 g. The percentage contribution of iron was calculated, based on the recommended daily allowances (RDA) of iron for women and children below five years of age [30, 31]. Results from this study have indicated that fortification of cassava mahewu could be helpful to increase dietary iron.

Based on this, the amount of iron in 200 g of fortified cassava mahewu would be sufficient to provide the RDAl (Tables 3 and 4), with the major benefit being to children (Table 4). In a previous study it was found that 100 g of processed cassava used in food could contribute to between 6 and 14% of RDA iron [32]. The WHO has noted that iron fortification could result in toxicity if the food staple fortified already contains concentrations of iron approaching the RDA [11]. As mentioned previously, the concentration of iron in cassava roots analysed in Mozambique, were below the limit of detection, therefore fortification would not result in consumption of iron in excess of the RDA.

***Table 4.** The possible contribution that bitter and sweet cassava mahewu fortified with ferrous sulfate and ferrous fumarate can make towards the iron RDA and absolute requirements of vulnerable populations.*

Independent variables		% of iron RDA for women	% of iron RDA for children younger 5 Yr	% of highest absolute iron requirement for women	% of highest absolute iron requirement for children younger 5 Yr
Type of cassava	Bitter	9.5^a	15.5^a	9.3^a	39.0^b
	Sweet	11.7^a	19.1^a	19.2^a	18.9^a
Iron Source	Ferrous sulfate	15.2^b	24.8^b	22.5^b	45.6^b
	Ferrous fumarate	6.0^a	9.8^a	6.1^a	12.3^a

a,b – Values within the same dependent variable with different superscripts differ significantly ($p \leq 0.05$).

It was estimated that the difference in the percentage contribution that fortified *mahewu* from bitter and sweet cassava could make to iron RDA for children younger than five years old, was not significant (Table 4). In children younger than five years, the percentage contribution that fortified bitter cassava *mahewu* could make towards absolute iron requirements was higher than that from sweet cassava. M*ahewu* fortified with ferrous sulfate would contribute more to RDA if fortified with ferrous fumarate. It was estimated that *mahewu* fortified with ferrous sulfate would provide approximately 2.5 times more dietary iron to women and children than *mahewu* fortified with ferrous fumarate. This means that it would contribute almost 4 times more to the absolute iron requirements.

4. Conclusions

It is evident that fortification of cassava *mahewu* with iron is feasible. Fortification could take place at either the beginning or end of fermentation. Fortified bitter varieties would deliver more bioaccessible dietary iron to the consumer. Ferrous sulfate seems to be a more suitable source of iron for cassava *mahewu* fortification than ferrous fumarate. Based on this, it is suggested that mass fortification of cassava *mahewu* should be carried out using ferrous sulfate and bitter varieties of cassava. Of importance is that bitter varieties of cassava are more easily cultivated and offer many advantages including resistance to pests and drought, as well as a higher yield when compared to the sweet varieties [3]. Cassava *mahewu* fortified with iron would contribute to iron RDA and absolute iron requirement of the vulnerable women and children. It is recommended that further studies be carried out to determine if cooking and fermentation of *mahewu* denatures cardiac glycosides in bitter cassava.

Acknowledgements

Financial assistance from the Graca Machel Scholarship; the National Research Foundation; the Department of Food Science, University of Pretoria the Department of Chemistry, Tshwane University of Technology; are gratefully acknowledged. Permission to undertake this study was granted by the Research Ethics Committee of the University of Pretoria (67/2013).

References

[1] MISAU/INE/ICF International (ICFI). 2011, (*Ministério da Saúde/Instituto Nacional de Estatística*) Ministry of Health/National Statistics Institutes /International ICF. *Moçambique Inquérito Demográfico e de Saúde* (Mozambique Demographic and Health Survey). Calverton, Maryland, USA: MISAU, INE and ICFI

[2] WHO. 2008, Worldwide prevalence of anemia (1993 – 2005): WHO global database on anemia. Geneva, Switzerland.

[3] MIC (*Ministério de Industria e Comercio*) /FAO/EC. 2004, Analysis of cassava as a target product from Mozambique and in particular to South Africa: External market task force. MIC/FAO/EC. 1-99. Maputo, Mozambique.

[4] FAO/FAOSTAT. 2011, Statistical database: Statistics Division: FAO. Rome. Available online at: www.fao.org/statistics/en/. Accessed 08 May 2012.

[5] Montagnac JA, Davis CR and Tanumihardjo SA. 2009, Nutritional value of cassava for use as a staple food and recent advances for important. Compr Rev Food Sci Saf, 8: 181-194.

[6] Donovan C, Haggblade S, Salrgua AV, Cuambe C, Mudema J and Tomo A. 2011, Cassava commercialization in Mozambique. MSU International development working paper, 120:1-59.

[7] Haggblade S, Djurfeldt AA, Nyirenda DB, Lodin JB, Brimer L, Martin Chiona M, Chitundu M, Chiwona-Karltun L, Cuambe C, Dolislager M, Donovan C, Droppelmann K, Jirström M, Kambewa E, Kambewa P, Mahungu NM, Mkumbira J, Mudema J, Nielson H, Nyembe M, Salegua VA, Tomo A, Weber M, 2012, Cassava commercialization in Southeastern Africa. JADEE, 2(1):4-40.

[8] Salvador EM, McCrindle CME and Steenkamp V. 2014, Production, consumption and nutritional value of cassava (*Manihot esculenta*, Crantz) in Mozambique: An overview. JABSD, 6(3):29-38.

[9] Nout RMJ. 2009, Rich nutrition from the poorest-cereal fermentation in Africa and Asia. Food Microbiol, 26:685-692.

[10] Rehman S, Huma N, Tarar OM and Shah WH. 2010, Efficacy of non-heme iron fortified diets: A Review. Crit Rev Food Sci Nutr, 50(5):403-413.

[11] FFI, WHO Recommendations on wheat flour fortification. UNICEF/FFI Joint workshop, Ankara 12-13 June 2012. Addressing micronutrient deficiencies through flour fortification in the CEE/CIS region. Available at: http://www.ffinetwork.org/plan/standards.html. Accessed April 13, 2015.

[12] Gegios A, Amthor R, Maziya-Dixon B, Egesi C, Mallowa S, Nungo R, Gichuki S, Mbanaso A and Manary MJ. 2010, Children consuming cassava as a staple food are at risk for inadequate Zinc, Iron, and Vitamin A intake. Plant Foods Hum Nutr, 65:64-70.

[13] Bvochora JM, Reed JD, Read JS and Zvauya R. 1999, Effect of fermentation processes on proanthocyanidins in sorghum during preparation of *Mahewu* a non-alcoholic beverage. Process Biochem, 35:21-25.

[14] James CS. 1999, Analytical chemistry of foods, 2nd ed. London, UK: Chapman and Hall.

[15] USA. 1998, Code of Federal Regulations: 197.260. Available online at www.dsm.com/content/dam/dsm/nip/en_US/documents/corn.pd Accessed 20 June 2012

[16] Luten J, Crews H, Flynn A, Van Dael P, Kastenmayer P, Hurrell R, Deelstra H, Shen L-H, Fairweather-Tait S, Hickson K, Farre R, Schlemmer U and Frohlich W. 1996, Inter-laboratory trial on the determination of the *in vitro* iron dialysability from food. J Sci Food Agric, 72:415-424.

[17] Oyewole OB. 2001, Characteristics and significance of yeasts' involvement in cassava fermentation for '*fufu*' production. Int J Food Microbiol, 6:213-218.

[18] Mensah P. 1997, Fermentation:the key to food safety assurance in Africa. Food Control, 8: 271-278.

[19] Berk Z. 1976, Biochemical activity of microorganisms in foods. In Braveman's Introduction to the Biochemistry of Foods, 279-28 I. Elsevier, Amsterdam.

[20] McMaster LDT, Kokott SA, Reid SJ and Abratt VR. 2005, Use of traditional African fermented beverages as delivery vehicles for *Bifidobacterium lactis* DSM 10140. Int J Food Microbiol, 102:231-237.

[21] Maziya-Dixon B, Kling JG, Menkir A and Dixon A. 2000, Genetic variation in total carotene, iron and zinc contents of maize and cassava genotypes. Food Nutr Bul, l 21:419-422.

[22] Sarkiyayi S and Agar TM. 2010, Comparative Analysis on the Nutritional and Anti-Nutritional Contents of the Sweet and Bitter Cassava Varieties. AJFST, 2(6): 328-334.

[23] Hurrell RF. 1999, Iron. In: Hurrell RF, ed. The mineral forti. cation of foods. Surrey: Leatherhead Food RA, Leatherhead Publishing, 54-93.

[24] Ikpeme-Emmanuel CA, Eneji CA and Osuchukwu NC. 2011, Functional and sensory properties of iron fortified West African cassava fermented meals; "*gari*" and "*fufu*." Afr J Food Sci, 5(8): 484-489.

[25] Perez-Exposito AB, Villalpando S, Rivera JA, Griffin IJ and Abrams SA. 2005, Ferrous sulfate is more bioavailable among preschoolers than other forms of iron in a milk-based weaning food distributed by PROGRESA, a national program in Mexico. J Nutr, 135:64-69.

[26] Moorthy SN and Mathew G. 1998, Cassava Fermentation and Associated Changes in Physicochemical and Functional Properties. Crit Rev Food Sci Nutr, 38(2):73-121.

[27] De-Regil LM, Suchdev PS, Vist GE, Walleser S and Peña-Rosas JP. 2013, Home fortification of foods with multiple micronutrient powders for health and nutrition in children under two years of age. Evid Based Child Health, 8(1):112-201.

[28] Sablah M, Klopp L, Steinberg D, Touaoro Z, Laillou A and Baker S. 2012, Thriving public–private partnership to fortify cooking oil in the West African Economic and Monetary Union (UEMOA) to control vitamin A deficiency: Faire Tache d'Huile en Afrique de l'Ouest. Food Nutr Bull, 33(4 Suppl):S310-S320.

[29] Tuma RB, Yuyama LKO, Aguiar JPL and Marque HO. 2003, Impacto da farinha de mandioca fortificada com ferro aminoácido quelato no nível de hemoglobina de pré-escolares (Impact of cassava flour fortified with iron amino acid chelate on the hemoglobin level in pre-schools) Rev Nutr, 16:29-39.

[30] WHO. 2008, Iron deficiency anaemia; assessment, prevention and control. Geneva, Switzerland, WHO.

[31] Adepoju OT, Adekola YG, Mustapha SO and Ogunola SI. 2010, Effect of processing methods on nutrient retention and contribution of cassava *(manihot spp)* to nutrient intake of Nigerian consumers. AJFAND 2010; 10(2):2099-2111.